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# Identification of Bioactive Polyphenolic Compounds and Assessment of Antioxidant Activity of *Origanum acutidens*

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**Abstract.** The main objective of the current work is to search the antioxidant activities of *Origanum acutidens* leaves by using DPPH, FRAP and CUPRAC methods severally. Three well known antioxidant compounds (BHA, BHT and ascorbic acid) were used as standards for comparing the extracts. Also, phenolic compounds of *Origanum acutidens* leaves were identified by UHPLC-ESI-MS/MS. The high concentrations of rosmarinic acid (11158.99 ppb) quinic acid (3200.84 ppb) and naringenin (1238.45 ppb) were detected quantitatively.

## Introduction

Natural plants have been used in pharmaceutical industry for developing new drugs [1]. Also, it is clear that most of fruits, vegetables and green plants are the main sources of natural antioxidants. Exhibition of natural antioxidants in human diet is related to reduce risk of cancers, cardiovascular diseases, cataracts, brain dysfunction [2]. Polyphenols are a large family of compounds found in fruits and vegetables, which exhibit strong antioxidant activity by scavenging different families of reactive oxygen species [3].

*Origanum acutidens* (oregano) is one of the important member of the Lamiaceae that contains approximately 900 species, that extensived all around the world. *Origanum acutidens* is a substantial therapeutic plant belongs to Mentheae (Lamiaceae) family that contain of 42 species and 18 hybrids extensively spread out in Europe, Asia and North Africa [4]. Due to their biological properties aromatic and medicinal plants are still primary health care for almost 80% of the local population. The essential oils of plants are used in alternative therapy and they are useful model for new synthetic products as well. In terms of rich in essential oils and bitter substances these taxa are commonly consumed as diuretic, sedative, antiseptic and degassers as well as they are also use up for constipation, treatment gastrointestinal diseases [5].

In this study, we investigated antioxidant potential of *Origanum acutidens* by three in vitro common methods; DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging, FRAP (Ferric ions Reducing Antioxidant Power) and CUPRAC (Cupric ions Reducing Antioxidant Power) techniques separately. According to the mentioned methods, leaves of *Origanum acutidens* exhibited good potential of radical scavenging and antioxidant activities. Furthermore, we used UHPLC-ESI-MS/MS (Ultra High Pressure Liquid Chromatography-Electrospray Ionisation-Mass Spectrometry/Mass Spectrometry) technique to determine the phenolic contents of the plant material. According to the results, rosmarinic acid, quinic acid, naringenin, apigenin, and kaempferol were detected as the major phenolic components.

## Theory

The substantial antioxidant capacity of the plant material might be related with its rich phenolic content. *Origanum acutidens* leaves might be useful on natural resource industry and

pharmacological process after some additional required studies, due to their high biological activities and phenolic contents.

## Materials and Methods

### Collection and identification of plant material

Dr. Omer Kilic, a plant taxonomist from Technical Vocational College of Bingol University collected the plant samples in vicinity of Saban village, Bingol, Turkey, in July 2014. The taxonomic identification of *Origanum acutidens* was confirmed by him as well.

### Chemicals

Ascorbic acid, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), DPPH, trichloroacetic acid and other chemicals were purchased from Sigma-Aldrich (Germany). Also, standard phenolic compounds of LC-MS/MS were purchased from Sigma-Aldrich (Germany) and Fluka (Germany).

### Sample preparation and extraction

Firstly, the plant leaves were dried on air at room temperature. For preparation of water extracts, 20 g of leaves were powdered by a grinder and mixed with 200 mL distilled water (1/10, w/v). The mixture was homogenized by a magnetic mixer about 12 h at room conditions. The homogeneous mixture filtered with filter paper. The filtrate sample was frozen and lyophilised in a lyophiliser (Labconco, Freezone 1L) at 5 mm Hg pressure and at -50 °C. Then, the lyophilised sample was stored at -30 °C.

For ethanol extract, 20 g of air dried leaves of the plant were powdered by a grinder. The powder was added to 200 mL ethanol (1/10, w/v). The mixture was stirred up by a magnetic mixer for 12 h at room temperature. The mixture filtered with a filter paper. The sample was evaporated with a rotary evaporator (Heidolph 94200, Bioblock Scientific) at 50 °C and sample was stored at -30 °C.

### Determination of phenolic compounds

Quantification and identification of phenolics of the plant sample was analyzed by UHPLC-ESI-MS/MS. Phenolic compounds were quantified by comparison to twenty seven different organic compounds. Initially, extraction for LC-MS/MS system was prepared; plant samples powdered and air-dried. These samples (100 g) was extracted three times with 300 mL of ethanol for 24 h at room conditions. The solvent was evaporated by using vacuum at 30 °C with a rotovap (Yield:15.6 %). Dry filtrate sample was diluted to 1000 mg/L and filtrated with 0.2 μm microfiber filter before LC-MS/MS assay.

LC−MS/MS measurements were carried out with a 8040 model LC-MS triple quadrupole mass spectrometer (Shimadzu) integrated with an ESI source. ESI was used for detection of positive and negative ionization. The LC device was integrated with binary pumps (LC-30AD), degasser (DGU-20A3R), column oven (CTO-10ASvp) and auto sampler (SIL-30AC). Inertsil 4 ODS (C18) reversed-phase analytical column was used for chromatographic isolation and identification of components. A sample volume of 4 μL was injected during analysis. HPLC was runned at 0.5 mL/min flow rate, solvent A was water containing ammonium formate (5 mM) with formic acid (0.1%) and solvent B was methanol containing ammonium formate (5 mM) with formic acid (0.1%). The following flow gradient was used: solvent B (0%) for 40 min, solvent B (20%) for 90 min, solvent B (24%) for 90 min, solvent B (24%) for 40 min, solvent B (29%) for 40 min. The analysis of samples were carried out after two or three transitions for per sample. First transition was for quantitative aim and the other transition was made up for verification. Electrospray ionization (ESI) circumstances were set at 300 °C, 3 L/min nebulizing gas flow and 15 L/min drying gas flow. The temperature was adjusted at 40 °C for column [6].

**FRAP antioxidant assay**

This technique is explained by the reduction ferricyanide. According to this technique, ferric ion reducing power of a sample was fixed by measuring decrease of $Fe^{3+}$ to $Fe^{2+}$. For this reason, 0.75 mL of extracts and standard antioxidants in (10, 20, and 30 μg/mL) concentrations were prepared respectively. Then, 1 mL of potassium ferricyanide (1 %) and 1 mL of sodium phosphate buffer (0.2 M; pH 6.6) were added to each tube. Incubation was carried out at 50 ° C for 20 min. The reaction was completed by adding 1 mL of TCA solution (10 %). After all, 0.25 mL of $FeCl_3$ (0.1 %) was put in each tube. For blank and control distilled water was used. Finally, the absorbance evaluations were acquired at 700 nm by using a UV spectrophotometer [7].

**CUPRAC antioxidant assay**

In the CUPRAC technique, reactive copper (II) neocuproin was used as a oxidizing factor to determine the antioxidant capability of polyphenols. First, 1 mL neocuproine solution ($7.5\times10^{-3}$ M), 1 mL $CuCl_2$ solution (0.01 M) and 1 mL $CH_3COONH_4$ buffer (1 M) were added to each tubes, respectively. After that, different concentrations of extracts (10, 20, and 30 μg/mL) were added to those mixtures. Then, volumes of the mixtures were completed to 4.1 mL with purified water. The samples were left at room conditions for half an hour to reveal their reducing capabilities. Finally, absorbances at 450 nm were reported. Increasing absorbance of a sample was commented as increasing reducing capacity [8].

**DPPH antioxidant assay**

The DPPH technique is based on the reaction of DPPH radicals with antioxidants. Extracts in 10, 20, and 30 μg/mL concentrations and BHA, BHT, and ascorbic acid were prepared with 3 mL of ethanol. Thereafter, 1 mL of DPPH radical solution (0.1 mM) was added to the each sample and left in the dark for half an hour at room conditions. The absorbances of extracts were calculated at 517 nm using a spectrophotometer UV-1800 model (Shimadzu, Japan). The percentages of DPPH free radicals consumed were calculated by comparing with different concentrations of extracts or standards [9].

## Results and Discussion

***In vitro* antioxidant studies**

Antioxidants are considered to carry out a preventive efficacy against free radical damage because of their radical scavenging potential. Antioxidant compounds donate hydrogen atoms to the radical species and form nonradical products. Antioxidants neutralize and quench the free radicals lead to prevent the lipid peroxidation [10]. Also, it is reported that regular exercises positively affect plasma lipid profiles [11].

Plants which have rich antioxidant sources can prevent from cardiovascular, cancer, diabetes, atherosclerosis, arthritis, hepatitis, and asthma disease. In last two decade investigative studies about new natural plants sources of antioxidants became more and more popular. Various plants have been screened and reported to possess antioxidant property [12]. The phenolic components of plants have effective *in vitro* and *in vivo* antioxidant activities. These components, can act as metal chelators and effective scavenging radicals. Also, it is long time known that phenolics possess antiviral, anti-inflammatory, hepatoprotective, antiallergic, antithrombotic activities. Flavonoids are the most known and extensively spreading group among the plant phenolic, exist in all part of plant, in particular the photosynthetic cells [13].

Cupric ion ($Cu^{2+}$) and ferric ion ($Fe^{3+}$) reductions are frequently used as an electron donating [14]. Thus, reducing power potential of the samples were measured by both FRAP and CUPRAC methods. FRAP is one of the methods to detect the antioxidant potential commented as the reducing capacity. Antioxidants cause the reduction of ferric ($Fe^{3+}$) ions to the ferrous ($Fe^{2+}$) due to their reductive abilities. The ferric ions reducing antioxidant capacities of samples and standard antioxidants in (30 μg/mL) concentration decreased in the order of BHA > ascorbic acid > BHT >

ethanol extract > water extract, respectively. Fig. 1A demonstrated low FRAP activity of *Origanum acutidens* extracts than standards (BHA, ascorbic acid, and BHT).

The cupric reducing potentials of *Origanum acutidens* extracts were experimentally measured and compared to BHA, BHT and ascorbic acid. The extracts of *Origanum acutidens* and standard antioxidants demonstrated the high cupric ions reducing antioxidant capacities in the CUPRAC method. Cupric ions reducing capacities of extracts and standard antioxidants in (30 µg/mL) concentration decreased in the order of BHT > BHA > ethanol extract > ascorbic acid > water extract, as shown on Fig. 1B.

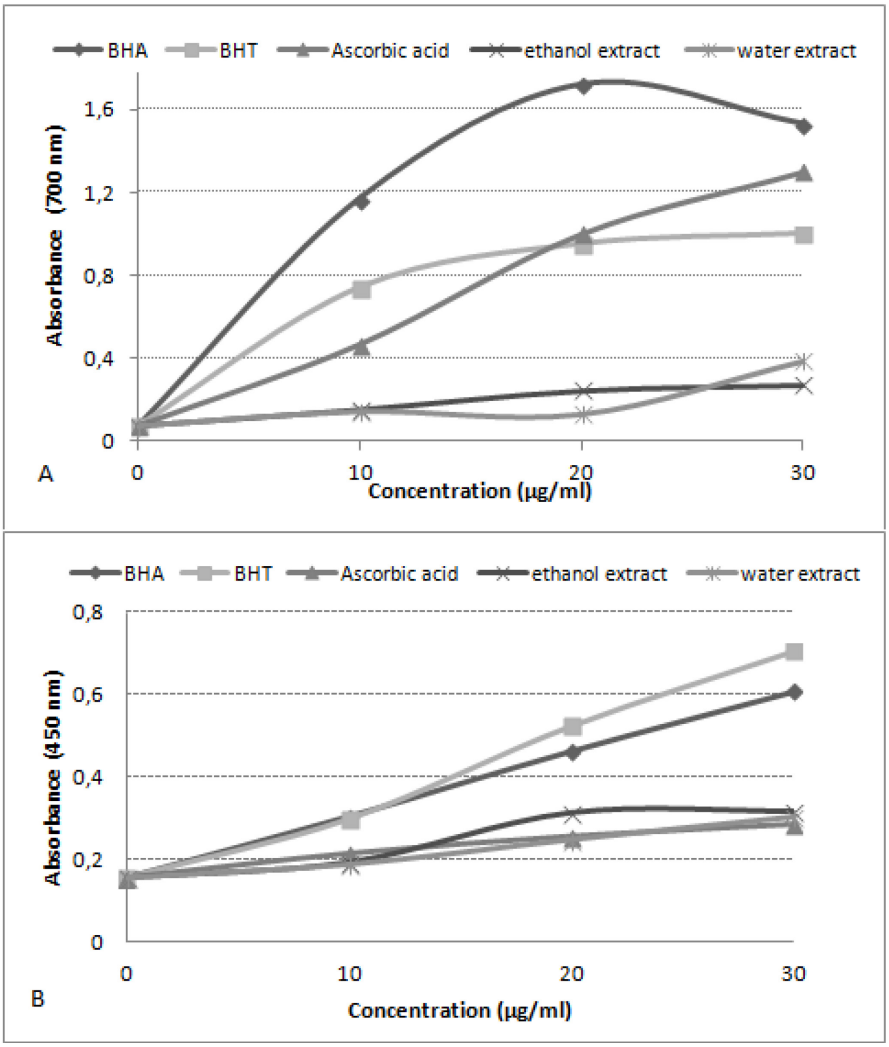


**Figure 1.** Antioxidant activity of *Origanum acutidens* by FRAP method **(A),** CUPRAC method **(B)**.

Radical scavenging level of a sample shows its antioxidant capacity to obviate oxidation chain initiation. DPPH technique describes the reduction of DPPH free radicals. This analysis has been extensively used for determination of free radicals [15, 16]. DPPH free radical scavenging of the extracts and standard antioxidants were investigated, separately. A remarkable correlation between antioxidant potential and concentration was detected for both the standards and the plant extracts. The DPPH free radical scavenging percentages of samples were measured by using equation of the following:

$$\text{Radical scavenging capability}\ (\%) = \left(1 - \frac{A_{\text{Sample}}}{A_{\text{Control}}}\right) \times 100$$

According to the results, the percentages at same concentration (30 µg/mL) decreased in the order of BHA (89.9 %) > ascorbic acid (74.0 %) > water extract (73.6 %) > BHT (54.1 %) > ethanol extract (46.5 %). The high inhibition percentages (73.6 %, 46.5 %) of *Origanum acutidens* extracts showed that the water extract had effective DPPH radical scavenging activity like standard

antioxidants. Also, the ethanol extract demonstrated mid-level DPPH free radicals scavenging activity as shown on Fig. 2.

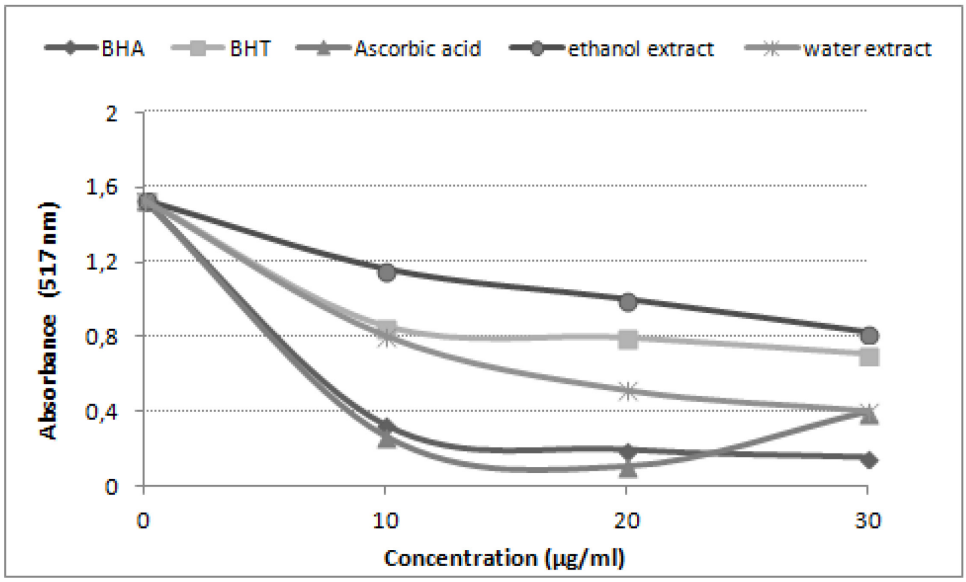


**Figure 2.** Radical scavenging activity of *Origanum acutidens* by DPPH method.

**Determination of phenolic contents**

Flavonoids synthesized by plants are hydroxylated phenolic compounds and are effective against microbial infection. Flavonoids are the most widespread group of phenolic components consumed for human diet. They are plentiful in plants. These components have antimutagenic, antiviral and antioxidant capability [17, 18].

Natural antioxidants also have known as their usefull actions against free radicals in human body. Phenolics are showing an extensive range of physiological feature, such as cardioprotective, vasodilatory, anti-inflammatory, anti-artherogenic and anti-thrombotic effects [19]. Furthermore, these structures have many biologically substantial actions such as antioxidants and antiradical [20]. Phenolic compounds are containing at least one hydroxyl group and they exist in various species of plants [21].

**Table 1.** LC-MS/MS parameters of selected compounds and *Origanum acutidens*.

| No | Analytes | [a]RT | [b]Parent ion (m/z) | Ioniza-tion Mode | [c]$R^2$ | [d]RSD% | Linearity Range (µg/L) | [e]LOD/ LOQ (µg/L) | Reco very (%) | [f]U | [g]Amo-unt |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Quinic acid | 3.32 | 190.95 | Neg | 0.9927 | 0.0388 | 250-10000 | 22.3 / 74.5 | 103.3 | 4.8 | 3200.84 |
| 2 | Malic acid | 3.54 | 133.05 | Neg | 0.9975 | 0.1214 | 250-10000 | 19.2 / 64.1 | 101.4 | 5.3 | |
| 3 | tr-Aconitic acid | 4.13 | 172.85 | Neg | 0.9933 | 0.3908 | 250-10000 | 15.6 / 51.9 | 102.8 | 4.9 | |
| 4 | Gallic acid | 4.29 | 169.05 | Neg | 0.9901 | 0.4734 | 25-1000 | 4.8 / 15.9 | 102.3 | 5.1 | |
| 5 | Chlorogenic acid | 5.43 | 353 | Neg | 0.9932 | 0.1882 | 250-10000 | 7.3 / 24.3 | 99.7 | 4.9 | |
| 6 | Protocatechuic acid | 5.63 | 152.95 | Neg | 0.9991 | 0.5958 | 100-4000 | 25.8 / 85.9 | 100.2 | 5.1 | |
| 7 | Tannic acid | 6.46 | 182.95 | Neg | 0.9955 | 0.9075 | 100-4000 | 10.2 / 34.2 | 97.8 | 5.1 | |
| 8 | tr-caffeic acid | 7.37 | 178.95 | Neg | 0.9942 | 1.0080 | 25-1000 | 4.4 / 14.7 | 98.6 | 5.2 | |
| 9 | Vanillin | 8.77 | 151.05 | Neg | 0.9995 | 0.4094 | 250-10000 | 10.1 / 33.7 | 99.2 | 4.9 | |
| 10 | p-Coumaric acid | 9.53 | 162.95 | Neg | 0.9909 | 1.1358 | 100-4000 | 15.2 / 50.8 | 98.4 | 5.1 | |
| 11 | Rosmarinic acid | 9.57 | 358.9 | Neg | 0.9992 | 0.5220 | 250-10000 | 10.4 / 34.8 | 101.7 | 4.9 | 11158.99 |
| 12 | Rutin | 10.18 | 609.1 | Neg | 0.9971 | 0.8146 | 250-10000 | 17.0 / 56.6 | 102.2 | 5.0 | |
| 13 | Hesperidin | 9.69 | 611.1 | Poz | 0.9973 | 0.1363 | 250-10000 | 21.6 / 71.9 | 100.2 | 4.9 | |
| 14 | Hyperoside | 10.43 | 463.1 | Neg | 0.9549 | 0.2135 | 100-4000 | 12.4 / 41.4 | 98.5 | 4.9 | |
| 15 | 4-OH Benzoic acid | 11.72 | 136.95 | Neg | 0.9925 | 1.4013 | 25-1000 | 3.0 / 10.0 | 106.2 | 5.2 | |
| 16 | Salicylic acid | 11.72 | 136.95 | Neg | 0.9904 | 0.6619 | 25-1000 | 4 / 13.3 | 106.2 | 5.0 | |
| 17 | Myricetin | 11.94 | 317 | Neg | 0.9991 | 2.8247 | 100-4000 | 9.9 / 32.9 | 106.0 | 5.9 | |
| 18 | Fisetin | 12.61 | 284.95 | Neg | 0.9988 | 2.4262 | 100-4000 | 10.7 / 35.6 | 96.9 | 5.5 | |
| 19 | Coumarin | 12.52 | 146.95 | Poz | 0.9924 | 0.4203 | 100-4000 | 9.1 / 30.4 | 104.4 | 4.9 | |
| 20 | Quercetin | 14.48 | 300.9 | Neg | 0.9995 | 4.3149 | 25-1000 | 2.0 / 6.8 | 98.9 | 7.1 | |
| 21 | Naringenin | 14.66 | 270.95 | Neg | 0.9956 | 2.0200 | 25-1000 | 2.6 / 8.8 | 97.0 | 5.5 | 1238.45 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | Hesperetin | 15.29 | 300.95 | Neg | 0.9961 | 1.0164 | 25-1000 | 3.3/ 11.0 | 102.4 | 5.3 | |
| 23 | Luteolin | 15.43 | 284.95 | Neg | 0.9992 | 3.9487 | 25-1000 | 5.8 / 19.4 | 105.4 | 6.9 | |
| 24 | Kaempferol | 15.43 | 284.95 | Neg | 0.9917 | 0.5885 | 25-1000 | 2.0 / 6.6 | 99.1 | 5.2 | 272.91 |
| 25 | Apigenin | 17.31 | 268.95 | Neg | 0.9954 | 0.6782 | 25-1000 | 0.1 / 0.3 | 98.9 | 5.3 | 246.37 |
| 26 | Rhamnetin | 18.94 | 314.95 | Neg | 0.9994 | 2.5678 | 25-1000 | 0.2 / 0.7 | 100.8 | 6.1 | |
| 27 | Chrysin | 21.18 | 253 | Neg | 0.9965 | 1.5530 | 25-1000 | 0.05 / 0.17 | 102.2 | 5.3 | |

[a]RT: Retention time
[b]Parent ion (*m/z):* Molecular ions of the standard compounds (mass to charge ratio)
[c]$R^2$: coefficient of determination
[d]RSD: relative standard deviation
[e]LOD/LOQ (µg/L): Limit of detection/Limit of quantification
[f] U (%): Percent relative uncertainty at 95% confidence level (k=2).
[g]: Quantitative phenolic acid composition of *Origanum acutidens* (ppb; µg analyte/kg extract)

In this study, the quantification and determination of phenolic acids in *Origanum acutidens* was carried out by UHPLC-ESI-MS/MS. Major phenolics in *Origanum acutidens* were detected such as rosmarinic acid (11158.99 ppb), quinic acid (3200.84 ppb), naringenin (1238.45 ppb), apigenin (246.37 ppb) and kaempferol (272.91 ppb). Moreover, small amounts of chlorogenic acid, protocatechuic acid, 4-OH-benzoic acid, rhamnetin, chrysin, luteolin, p-coumaric acid, tr-caffeic acid, vanillin, rutin, and hyperoside were identified and amounts were given on Tab. 1. The chromatograms of standards and *Origanum acutidens* are presented in Fig. 3 (A, B) respectively.

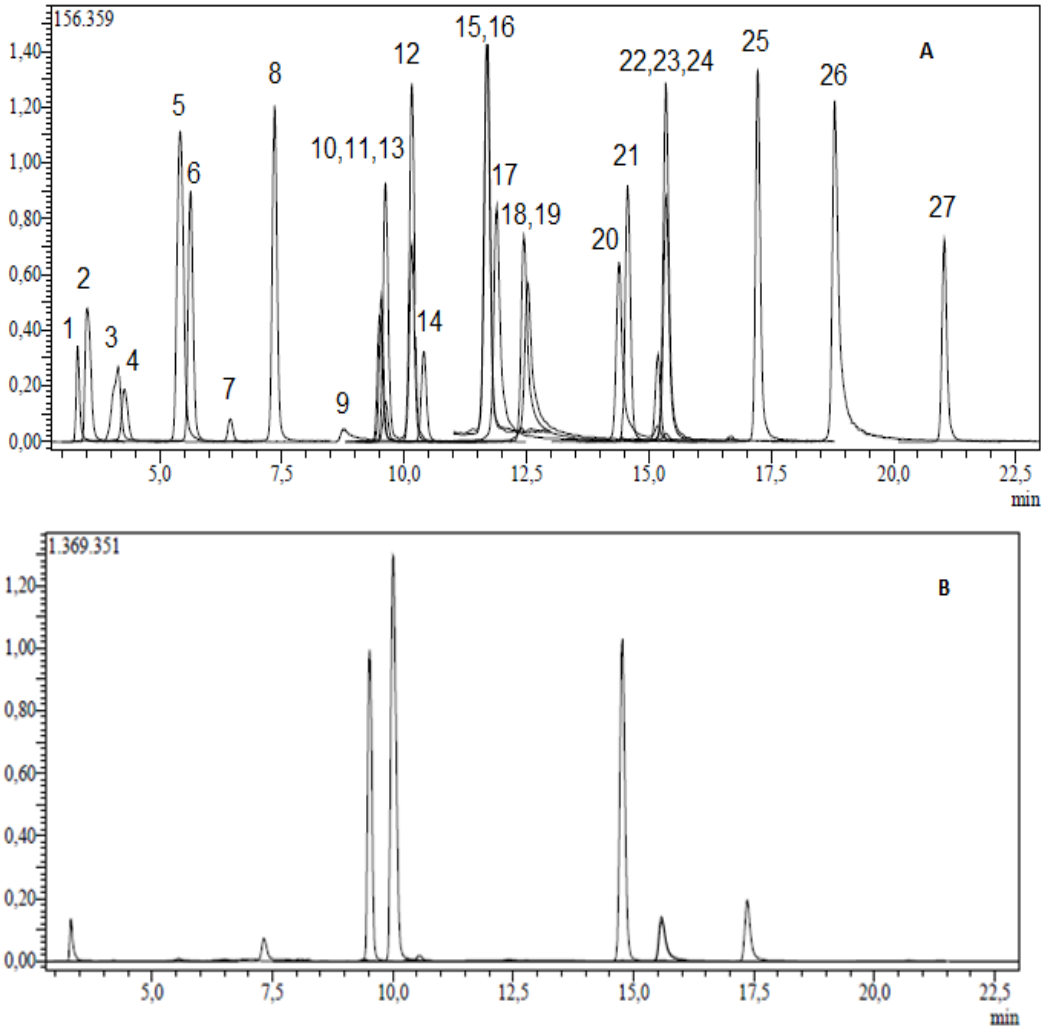


**Figure 3.** UHPLC-ESI-MS/MS chromatograms of 250 ppb standard mix **(A)** and *Origanum acutidens* **(B).**
(1: quinic acid, 2: malic acid, 3: tr-aconitic acid, 4: gallic acid, 5: chlorogenic acid, 6: protocatechuic acid, 7: tannic acid, 8: tr-caffeic acid, 9: vanillin, 10: p-coumaric acid, 11: rosmarinic acid, 12: rutin, 13: hesperidin, 14: hyperoside, 15: 4-OH benzoic acid, 16: salicylic acid, 17: myricetin, 18: fisetin, 19: coumarin, 20: quercetin, 21: naringenin, 22: hesperetin, 23: luteolin, 24: kaempferol, 25: apigenin, 26: rhamnetin, 27: chrysin)

## Conclusions

UHPLC-ESI-MS/MS analysis of phenolic content and antioxidant activities of *Origanum acutidens* were investigated and reported in this study. The results revealed that the plant extracts have high antioxidant capabilities on radical scavenging activity and reducing antioxidant power. In addition, rosmarinic acid, quinic acid and naringenin were detected as the main phenolic compounds. The effective antioxidant and antiradical activity of plant sample might be because of its rich amounts of phenolic compounds. Further studies should be performed in order to investigate possible features of *Origanum acutidens* on food industry and pharmacology.

## Conflict of Interest

No potential conflict of interest was reported.

## References

[1] H. Tohma et al., Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS, J. Food Meas. Charact. 11 (2017) 556-566.

[2] A. Aras, M. Dogru, E. Bursal, Determination of antioxidant potential of *Nepeta nuda* subsp. lydiae, Anal. Chem. Let. 6(6) (2016) 758-765.

[3] V. Sicari, Antioxidant potential of extra virgin olive oils extracted from three different varieties cultivated in the Italian province of Reggio Calabria, J. App. Bot. Food Qual. 90 (2017) 76-82.

[4] H. Duman, K.H.C. Baser, Z. Aytec, Two new species and a new hybrid from Anatolia, Turk. J. Bot. 22 (1998) 51-55.

[5] J. Baranauskaite et al., Optimization of carvacrol, rosmarinic, oleanolic and ursolic acid extraction from oregano herbs (*Origanum onites* L., *Origanum vulgare* spp. *hirtum* and *Origanum vulgare* L.), Nat. Prod. Res. 30(6) (2016) 672-674.

[6] A. Ertas et al., A detailed study on the chemical and biological profiles of essential oil and methanol extract of *Thymus nummularius* (Anzer tea): Rosmarinic acid, Ind. Crop. Prod. 67 (2015) 336-345.

[7] I. Gulcin et al., Polyphenol contents and antioxidant properties of medlar (*Mespilus germanica* L.), Rec. Nat. Prod. 5(3) (2011) 158-175.

[8] R. Apak et al., Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, J. Agric. Food Chem. 52 (2004) 7970-7981.

[9] M. Isik et al., Determination of antioxidant properties of *Gypsophila bitlisensis* bark, Int. J. Pharma. 11(4) (2015) 366-371.

[10] E. Koksal et al., Antioxidant activity and polyphenol content of Turkish thyme (*Thymus vulgaris*) monitored by LCMS/MS, Int. J. Food Prop. 20(3) (2017) 514-525.

[11] N. Kilic Baygutalp et al., Acute effects of training on some biochemical analytes in professional boxers sub-title: Biochemical analytes in boxers, Int. J. Med. Pharmacy. 4(1) (2016) 39-52.

[12] N. Neffati et al., Phytochemical composition and antioxidant activity of medicinal plants collected from the Tunisian flora, Nat. Prod. Res. 31(13) (2017) 1583-1588.

[13] A.R. Tapas, D.M. Sakarkar, R.B. Kakde, Flavonoids as nutraceuticals: A review, Trop. J. Pharm. Res. 7 (2008) 1089-1099.

[14] N. Turan et al., Investigation of synthesis, structural characterization, antioxidant activities and thermal properties of Zn(II), Fe(II) and Mn(II) complexes with thiophene-carboxylate ligand, J. Chem. Biochem. 3(2) (2015) 13-29.

[15] I. Gulcin, Antioxidant properties of resveratrol: A structure-activity insight, Innov. Food Sci. Emerg. 11 (2010) 210-218.

[16] H. Tohma et al., RP-HPLC/MS/MS analysis of the phenolic compounds, antioxidant and antimicrobial activities of *Salvia* L. species, Antioxidants. 5(4) (2016) 38.

[17] J.M. Davis et al., Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance, Am. J. Physiol. Regul. Integr. Comp. Physiol. 296 (2009) 1071-1077.

[18] E. Bursal, Kinetic properties of peroxidase enzyme from chard (*Beta vulgaris* subspecies cicla), Int. J. Food Prop. 16(6) (2013) 1293-1303.

[19] C. Manach et al., Bioavailability and bioefficacy of polyphenols in humans. Review of 97 bioavailability studies, Am. J. Clin. Nutr. 81 (2005) 230-242.

[20] F. Al Juhaimi, K. Ghafoor, Total phenols and antioxidant activities of leaf and stem extracts from coriander, mint and parsley grown in Saudi Arabia, Pak. J. Bot. 43(4) (2011) 2235-2237.

[21] A. Aras, E. Bursal, M. Doğru, UHPLC-ESI-MS/MS analyses for quantification of phenolic compounds of *Nepeta nuda* subsp. Lydiae, J. Applied Pharma. Sci. 6(11) (2016) 9-13.