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Research Article

Quantitative analysis of phenolic compounds of commercial basil cultivars (*Ocimum basilicum* L.) by LC-TOF-MS and their antioxidant effects

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ABSTRACT

Basil (*Ocimum basilicum* L.), an aromatic and medicinal plant, is used for food and pharmaceutical purposes. In this work, quantitative analyses of phenolic compounds for commercial basil cultivars, which are Sweet (1), Purple (2), Lettuce (3), Brush (4) grown in Tokat city in Turkey's ecology, were executed by Liquid Chromatography Time-of-Flight Mass Spectrometry (LC-TOF-MS). Antioxidant activities of related genotypes were determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH)'s radical, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)'s diammonium salt and the ferric reducing antioxidant power (FRAP) assays. The activity-compound relationship was revealed. Brush (**4**) genotype revealed the most DPPH [296 µmole TE (Trolox equivalent/g DW (gram dry weight)], ABTS (706 µmole TE/g DW), and FRAP (650 µmole TE/g DW) activities. It was determined that rosmarinic acid was in the highest amount in all genotypes. Among the genotypes, it was determined that Lettuce contained the most rosmarinic acid with a value of 180460.6 (mg kg^{-1} DW).

Keywords: *Ocimum basilicum* L., antioxidant activity, basil cultivar, quantitative analysis, LC-TOF-MS.

Ticari fesleğen çeşitlerinin (*Ocimum basilicum* L.) fenolik bileşiklerinin LC-TOF-MS ile kantitatif analizi ve antioksidan etkileri

ÖZ

Aromatik ve tıbbi bir bitki olan reyhan (*Ocimum basilicum* L.) gıda ve ilaç amaçlı kullanılmaktadır. Bu çalışmada, Türkiye'nin ekolojisinde Tokat şehrinde yetişen Sweet (1), Purple (2), Lettuce (3), Brush (4) olmak üzere dört ticari fesleğen çeşidinin fenolik bileşiklerinin kantitatif analizleri LC-TOF/MS ile gerçekleştirilmiştir. İlgili genotiplerin antioksidan aktiviteleri, 2,2-Difenil-1-pikrilhidrazil (DPPH) radikali, 2,2-azino-bis(3-etilbenzotiazolin-6-sülfonik asit (ABTS)'in diamonyum tuzu ve indirgenme gücü (FRAP) yöntemleri kullanılarak belirlendi. Aktivite-bileşik ilişkisi gösterildi. Brush **4** genotipi en yüksek DPPH [296 µmol TE (Trolox ekivalent) /g DW (gram kuru ağırlık)], ABTS (706 µmol TE/g DW) ve FRAP (650 µmol TE/g DW) aktivitesi gösterdi. Rosmarinik asidin bütün genotiplerde en yüksek miktarda olduğu belirlendi. Genotipler arasında Lettuce (**3**)'ün, 180460.6' lik (mg kg^{-1} kuru bitki) bir değer ile en fazla rosmarinik asit içerdiği belirlendi.

Anahtar Kelimeler: Reyhan, antioksidan aktivitesi, reyhan kültürü, kantitatif analiz, LC-TOF-MS.

1. INTRODUCTION

Natural products have been consumed extensively for therapeutic properties for centuries.[1,2] Herbal medicines have been accepted as a main basis of crucial health care in many countries.[3] Almost 80% of the world population still depends on folk medicine. Spectroscopic developments have led to the isolation of active compounds from plants in the 19th century. Hence, many organic advances have been inspired by natural products. Synthetic chemists have begun to synthesize natural products with pharmaceutical properties due to the isolation of natural products.[4] Natural compound frameworks have been regarded as

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special structures consist of the basis of effective medicines.[5-9] The importance of natural products is due to the active compounds they contain.[10-18]

Ocimum basilicum L., basil, which is an aromatic and medicinal plant belonging to the Labiatae family consists of more than 150 species. Although this plant is native to Mediterranean, Africa, America and Asia, it is widely grown in many countries.[19] Due to its significance for medicine, it is sold as fresh, dried, or frozen as well.[20] This plant with essential oils has been employed widely in diet, cosmetics, and dental for many years. *Ocimum basilicum* L. has been consumed for the remedy of various illnesses such as viral ocular and hepatic infections, headaches, coughs, diarrhoea, worm, warts, constipation, kidney malfunction.[21] The main essential oil compounds of *Ocimum* species have been identified as linalool, geraniol, camphor and 1,8-cineol, which are classified as monoterpenes.[22] *Ocimum basilicum* L. essential oil consisting of linalool and eugenol as major products has showed an antibacterial activity on *Giardia lamblia.*[23] Phytochemical studies on *Ocimum* genus has revealed that this plant contains some significant secondary metabolites such as flavonoids, steroids and terpenes, anthocyanins.[24] Due to the including bioactive secondary metabolites, *Ocimum* has exhibited a large variety of biological effects such as anticancer, antioxidant, anti-aging, immunity enhancement, antibacterial, diabetes mellitus.[25]

The compounds of essential oil have terpene skeletons with the high volatility that protects plants from external threats. Essential oils are generally terpenoids, a great various class of secondary metabolites. They have been used extensively in flavour and cosmetic applications since ancient times. Due to their high volatility, the essential oils are released into the air space around the plant. Hence, they play a significant role as signaling compounds for pollinators and herbivores.[26-28]

The eighteen Turkish basil essential oils have been studied, and cluster analysis has been carried out by presenting the variety of essential oils in the landraces. So, seven various chemotypes have been elucidated.[29]

Antioxidants reveal a significant role for human health by decreasing oxidative stress. In addition, they are used to prevent food from deterioration. Antioxidants have attracted attention against oxidative stress. Antioxidants are divided into natural and synthetic products. Natural antioxidants are preferred by consumers since they are safe, non-toxic and no side effects.[30]

In this work, the quantitative analyses of phenolic compounds were carried out for 4 commercial basils (Sweet **1**, Purple **2**, Lettuce **3**, Brush **4**) grown in Tokat where Turkey's ecology and antioxidant activity was executed on cultivar basil.

2. MATERIALS AND METHODS

2.1. General

The standard compounds, 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium hexacyanoferrate (III), sodium peroxydisulphate, potassium dihydrogen phosphate, trolox, trichloroacetic acid (TCA), sodium carbonate, sodium hydroxide, iron (III) chloride, iron (II) chloride, ethanol, formic acid, acetonitrile, methanol, dichloromethane were supplied from the Sigma-Aldrich company.

2.2. Plant Materials

Seed of four basil cultivars (*Ocimum basilicum* L.), Sweet (**1**), Purple (**2**), Lettuce (**3**), Brush (**4**) were cultivated at the research field of Tokat Gaziosmanpasa University. The plants were harvested during vegetative period. Plant materials were dried at the shade under room temperature.

2.3. Extraction

Each dried plant sample (0.4 g) was extracted with methanol/dichloromethane (4/1, 20 ml). After vortex stage, each extract solution was kept in the ultrasonic bath at 30ºC for 30 min, then the solution was mixed at room temperature for 24 h. The solvent was evaporated by reduced pressure to yield the crude extract for analysis of antioxidant activity and phenolic compounds. Quantitative analyses of phenolic compounds were performed in 4 commercial basil cultivars (Sweet **1**, Purple **2**, Lettuce **3**, Brush **4**) by Liquid Chromatography Time-of-Flight Mass Spectrometry (LC-TOF-MS).

2.4. Analysis of phenolic compounds

The extract solutions of *O. basilicum* cultivars were prepared (5 mg l^{-1}). The solutions were filtered through 0.22 µm syringe type PTFE filter then injected into the vials. Quantitative analyses were executed by HPLC system equipped with Agilent 1260 infinity LC pump, 6210 TOF-MS detector and Zorbax SB-C18 (4.6 × 100 mm, 3.5 µm, 2.7 mm) (Agilent Technologies) column. The water with 0.1 formic acid (X) and acetonitrile (Y) were applied for mobile phase. The program was adjusted as follow: 0-2 min 10% Y, 2-21 min 45% Y, 21-25 min 75% Y, 25-29 min 15% Y, 29-35 min, 20% Y. The elution was completed for 35 min. Positive ion mode was used for analysis, the temperature of gas was 320°C, the voltage of fragmentary was 175 volts. The

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calibration graph was employed to identify the phenolic compounds. LC-TOF-MS analysis was executed according to retention time and molecular masses of each standart. Concentrations of phenolic compounds were calculated as mg kg^{-1} dry plant.

2.5. Antioxidant assays

2.5.1. DPPH$^{\bullet}$ free radical assay

DPPH$^{\bullet}$ activities of *O. basilicum* cultivars were carried out.[31] 1.0 ml, 0.26 mM of DPPH$^{\bullet}$ solution was treated with *O. basilicum* extract at 20, 40, and 80 µl cconcentrations at room temperature for 30 min. The absorbance was read by a spectrophotometer at 517 nm in which lower absorbance of the reaction product revealed the higher activity. DPPH$^{\bullet}$ scavenging activity was calculated from Eq. (1).

$$\text{DPPH}^{\bullet}\text{ scavenging activity (\%)} = [(A1 - A2)/A2] \times 100 \quad (1)$$

where A1 and A2 are control and sample absorbances.[32]

2.5.2. ABTS$^{+\bullet}$ scavenging assay

The reaction of ABTS (2.0 mM) with $K_2S_2O_8$ (potassium persulfate) (2.45 mM) in phosphate buffer for 6 h in dark at room temperature yielded the formation of ABTS$^{\bullet+}$ radical cation solution. Each *O. basilicum* extract solution (40 and 80 µl) was completed to 3 ml with phosphate buffer (pH 7.4, 1.0 ml). Consequently, 1.0 ml of ABTS$^{\bullet+}$ solution was reacted with *O. basilicum* extract solution (3. 0 ml). After incubation for 10 min at room temperature, absorbance measurement was executed at 734 nm. The ABTS$^{\bullet+}$ scavenging effect was calculated for each *O. basilicum* extract concentration. The absorbance was measured at 734 nm. ABTS$^{\bullet+}$ scavenging activity was calculation from Eq. (2)

$$\text{ABTS}^{\bullet+}\text{ scavenging effect (\%)} = [(A1 - A2)/A2] \times 100 \quad (2)$$

where A1 is the initial concentration of ABTS$^{\bullet+}$, and A2 is remaining concentration.[30]

2.5.3. Reducing power

After preparation of sodium phosphate buffer (0.2 M, pH 6.7), each sample solution was treated with 1.25 ml, 1% of potassium ferricyanide [$K_3Fe(CN)_6$] for thirty min at 45°C. The buffer solution was added to the reaction mixture until the volume reached to 2.5 ml.1.25 ml, 10% of trichloroacetic acid and 0.1%, 0.25 ml of iron (III) chloride were added to the reaction mixture. The absorbance measurement was executed at 700 nm using a spectrophotometer.[32]

2.6. Statistical Analyses

SPSS software (SPSS 15.0) was used for the statistical analysis. The trials were repeated triplicate, and the results were presented as mean value and standard deviation (SD). ANOVA one-way analysis was carried for the evaluation of results. Significant variances in groups were indicated at $p < 0.05$.

3. RESULTS AND DISCUSSION

Quantitative analyses of phenolic compounds from four cultivar basil (*Ocimum basilicum* L.) were presented. Some phenolic compounds were used for the standards (Table 1). Quantitative analysis revealed that rosmarinic acid was the major compound in all genotypes (Table 1). Among the corresponding genotypes, Lettuce (**3**) consisted of the most rosmarinic acid (18460.6 mg kg^{-1} DW). Brush (**4**), Sweet (**1**), Purple (**2**) contained the rosmarinic acid with the quantity of 17880.1 mg kg^{-1} DW, 7805.9 mg kg^{-1} DW, 2610 mg kg^{-1} DW respectively. Besides rosmarinic acid, Sweet (**1**) cultivar consisted of the rutine and chicoric acid as major products with the quantity of 1376.9 mg kg^{-1} DW and 211.0 mg kg^{-1} DW respectively. However, Purple (**2**) consisted of rutin and 4-hydroxybenzoic acid with the value of 669.8 mg kg^{-1} DW and 216.6 mg kg^{-1} DW, respectively. Rutin (3532.4 mg kg^{-1} DW) and 4-hydroxybenzoic acid (228.4 mg kg^{-1} DW) were detected as the second and third main compounds in Lettuce (**3**). Brush (**4**) included the rutin and 4-hydroxybenzoic as the major compounds as well. These cultivars did not contain the chlorogenic acid, apigenin-7-*O*-glucoside and naringenin.

A research was carried out on three purple Basil (*Ocimum basilicum* L.) cultivars, and six standards were used. It was presented that plant maturity influenced the anthocyanin, phenolic concentrations and reducing capacity significantly, while cultivar had a significant effect on anthocyanin concentrations and FRAP antioxidant activity. Rosmarinic acid was found as a major compound.[33] It is also major compound in our study. Another study was executed on *Ocimum basilicum* L. obtained from three different city of Egypt. The variations of the chemical composition of essential oils were determined. Linalool, estragole, methyl cinnamate, bicyclosesquiphellandrene, eucalyptol, alpha bergamotene, eugenol were the major compounds in all essential oil contents. In addition, methanol extract revealed the more antioxidant activity than that of the essential oils.[34] A research was carried out on 15 basil (*Ocimum basilicum* L.) cultivars. It was presented that

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Table 1. Phenolic compounds of Basil (*Ocimum basilicum* L.) Landraces (mg k^{-1} dried plant)

LR	GA	GEA	CA	HA	PA	CFA	HBA	RU	CUA	CCA	FA	RA	SA
1	96.02	40.03	155.38	139.53	0.00	155.61	7.71	1376.94	0.00	211.00	40.49	7805.92	86.46
2	101.77	51.78	153.79	216.59	0.00	140.85	10.23	669.77	11.89	0.00	0.00	2610.15	100.77
3	0.00	44.70	154.03	228.41	0.00	223.17	14.46	3532.35	0.00	144.19	0.00	18460.56	87.85
4	140.15	58.32	264.92	435.83	125.17	277.77	8.49	2026.14	0.00	0.00	44.57	17880.05	91.59

1: Fr sweet, **2**: Fr purple, **3**: Fr lettuce, **4**: Fr brush. GA: gallic acid, GEA: gentisic acid, CA: caftaric acid, CHA: chlorogenic acid, HA: 4-hydroxybenzoic acid, PA: protocatechuic acid, CFA: caffeic acid, HBA: 4-hydroxybenzaldehyde, RU: rutin, CUA: *p*-coumaric acid, CCA: chicoric acid, FA: ferulic acid, HE: hesperidin, AG: apigenin-7-*O*-glucoside, RA: rosmarinic acid, SA: salicylic acid, N: naringenin, LR: landraces. Landraces genotypes do not contain CHA, HE, AG and N.

cultivar influenced the phenolic composition and antioxidant activity. Nine of the cultivars had chicoric acid in high concentrations than rosmarinic acid. Individual phenolic acid composition strongly influenced antioxidant activity.[35]

The antioxidant activities of water, ethanol, and acetone extracts of purple basil were investigated. It was determined that ethanol extract revealed the most antioxidant activity.[36] Our research includes some novelty compared to the corresponding study. Seventeen standards were utilized. The cultivar conditions and basil genotypes were completely different. Hence the results contain novelty in comparison with the previous work.

Antioxidant activities of four basil cultivars (*Ocimum basilicum* L.) were investigated as well. Brush (**4**) showed the most antioxidant activity. DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP activities for Brush (**4**) were assigned as 296.0 µmole TE/g DW, 706.3 µmole TE/g DW, 650.5 µmole TE/g DW, respectively. Lettuce (**3**) cultivar showed the second most activity in all assays including DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and FRAP with the values of 291.8 µmole TE/g DW, 566.9 µmole TE/g DW, 634.9 µmole TE/g DW, respectively. Sweet (**1**) was determined as third active genotype. The activities of DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP for Sweet (**1**) were assigned to 161.5 µmole TE/g DW, 390.0 µmole TE/g DW and 417.0 µmole TE/g DW, respectively. Purple (**2**) genotype revealed the least activity among the investigated genotypes with the values of 93.2 µmole TE/g DW, 203.0 µmole TE/g and 150.6 µmole TE/g corresponding to the DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP assays, respectively (Table 2).

Lettuce (**3**) (22889.7 mg kg^{-1}) and Brush (**4**) (21353.0 mg kg^{-1}) contained the most phenolic content. Hence, it was seen that there was a direct proportion between phenolic content and activity. However, there could be an active compound in the genotype revealing the excellent activity caused the genotype to be high in the activity. All cultivar basil composed rosmarinic acid as a major compound. Rosmarinic acid occurs throughout Boraginaceae and Lamiaceae family. Rosmarinic acid is the bioactive compound revealing a large amount of biological activity such as antioxidant, antiinflammatory, antimutagen, antibacterial, and antiviral. Moreover, it is also utilized in cosmetic and food additive to avoid food decay. Therefore, rosmarinic acid is accepted to be one of the most talented food functional polyphenols.[37] As a consequence, Basil genotypes could be an effective source of rosmarinic acid.

Table 2. Antioxidant activity of Basil (*Ocimum silicum* L.) landraces (µmole TE/g DW)*

Landraces	DPPH$^{\bullet}$	ABTS$^{\bullet+}$	FRAP
Sweet (1)	161.5 ± 14.8b	389.9 ± 6.6c	417.0 ± 13.1c
Purple (2)	93.2 ± 1.5c	202.9 ± 2.0d	150.8 ± 2.6d
Lettuce (3)	291.8 ± 3.9a	566.8 ± 4.0b	634.9 ± 10.1b
Brush (4)	296.0 ± 0.1a	706.3 ± 4.9a	650.2 ± 7.1a

*There is no a significant difference between the mean valuesto the same letter at the column according to the Ducan test ($P < 0.05$).

4. CONCLUSSION

It was determined that Basil (*Ocimum basilicum* L.) included significant phenolic compounds. Hence, this aromatic and medicinal plant could be a source of corresponding compounds. It was seen that all commercial cultivar basil consisted of rosmarinic acid as a major product with a considerable quantity. Therefore, these genotypes could be the source of

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executed to increase the quantity of rosmarinic acid in the *Ocimum basilicum* L.

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Conflict of interests

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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