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Comparison of total phenolic contents and antioxidant capacities in mint genotypes used as spices

Baharat olarak kullanılan nane genotiplerinin toplam fenolik içerikleri ve antioksidan kapasitelerinin karşılaştırılması

Abstract: Objective: Mint (*Mentha* spp.) genotypes used as spices are cultivated in Turkey and used for different purposes including herbal tea, spices, the cosmetics industry, and are used in folk medicine. While mint species have been used in traditional practices during humanity's long history, there is limited research on the comparison of their antioxidant capacity and phenolic contents. This aim of the research is to compare antioxidant capacity and phenolic contents in mint clones to determine superior genotypes for herbal and spice usage and cultivation.

Methods: Antioxidant capacity was evaluated by ferric reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC). Total phenolic content in clones were determined by Folin-Ciocalteu method.

Results: Maximum total phenolic content [28.27±3.95 µg gallic acid equivalent (GAE)/g dry weight (DW)] and FRAP activity 577.09±46.02 µmol Trolox equiv/g DW were obtained from Clone 13 (*M. spicata*), while *M. piperita* clones were higher for TEAC activity (800.02±1.10 µmol Trolox equiv/g DW). The results were first records for *M. villoso nervata*. FRAP and TEAC activities selected clones were correlated with total phenolic content (r=0.77; 0.73 respectively).

Conclusion: According to the results, it can be suggested that Clone 13, Clone 5, (*M. spicata*) Clone 3 and Clone 8 (*M. piperita*) are more suitable for use as spice, herbal tea, and antioxidant agents. The clones must be selected for commercial cultivation by the grower and they can be used as spices and herbal teas.

Keywords: Total phenolic compounds, FRAP, TEAC, antioxidant activity, *Mentha piperita*, *Mentha spicata*, *Mentha villoso nervata*

Özet: Amaç: Baharat olarak kullanılan nane (*Mentha* spp) genotipleri, Türkiye'de kültürü yapılmakta ve biyolojik aktivitelerinden dolayı halk arasında bitki çayı, baharat, kozmetik ve tıbbi amaçlar için kullanılmaktadır. Uzun insanlık tarihi boyunca nane türleri geleneksel olarak kullanılmasına rağmen, bu türlerin antioksidan kapasite ve fenolik bileşikler üzerindeki çalışmalar sınırlıdır. Bu çalışmanın amacı, nane genotiplerinin antioksidan kapasitelerini ve fenolik bileşik miktarlarını karşılaştırarak baharat ve bitkisel çay olarak kullanıma daha uygun ticari genotipleri belirlemektir.

Metod: Antioksidan kapasite; indirme gücü aktivite (FRAP) ve troloxa eşdeğer antioksidan kapasite (TEAC) testleri ile değerlendirildi. Klonlardaki toplam fenolik içeriği Folin-Ciocaltue metodu ile belirlendi.

Bulgular: En yüksek fenolik bileşik miktarı [28.27±3.95 µg gallik aside eşdeğer /g kuru ağırlık (DW)]ve FRAP aktivitesi (577.09±46.02 µmol Trolox eşdeğer/g DW) klon

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13'de (*M. spicata*) bulunurken en yüksek TEAC aktivitesi (800.02±1.10 µmol Trolox equiv/g DW) *M. piperita* klonlarında bulunmuştur. *M. villoso nervata* ile ilgili sonuçlar ilk olarak bu çalışmada yapılmıştır. Seçilen klonlardaki FRAP ve TEAC aktiviteleri toplam fenolik bileşikler ile pozitif bir korelasyona sahiptir (sırasıyla, r=0,77; 0,73).

Sonuç: Bu çalışmada elde edilen sonuçlara göre, klon 3, 5, 8 ve 13'ün daha uygun bir antioksidan ajanı, bitki çayı ve baharat olarak kullanımı uygundur. Bu klonlar baharat ve bitki çayı olarak kullanılabilir ve üreticiler tarafından ticari üretim için seçilmelidir.

Anahtar Kelimeler: Toplam fenolik bileşik, FRAP, TEAC, antioksidan aktivite, *Mentha piperita*, *Mentha spicata*, *Mentha villoso nervata*

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Introduction

Edible plants contain biological active components having antioxidant activity in addition to their food values. Antioxidant activities of plants are a result of their phenolic contents [1–3]. Phenolics are functional compounds synthesized for defensive metabolites in plants. They have a critical role in the human diet for healthy living. Herbs such as mint are an important source of them [4].

Some mint (*Mentha* spp) genotypes have been used in the Mediterranean diet as herbal teas and spices [4–7]. *Mentha spicata*, *M. canadensis*, *M. piperita* etc, have economic importance due to their medicinal and aromatic value [8,9]. Besides essential oil composition, other secondary metabolites composition determines herbal tea and pharmacological properties of the plants [10,11].

While essential oil composition determines quality in cultivated mint, phenolic components and their biological activity (antioxidant, antimicrobial etc.) have vital importance for herbal tea, spice and medical uses of cultivated mint. As results of breeding and characterization research, superior varieties for herb yield and essential oil composition were selected by our group. However, there are no reports on phenol compounds of the selected clones. According to previous reports on *Mentha* species antioxidant activity of *M. piperita* and *M. spicata* species had been carried out by different methodology [12–17]. Four of the selected clones belong to *M. villoso nervata*. Contrary to limited essential oil composition, there are no records on antioxidant capacity of the species, *M. villoso nervata*. There are also limited reports on comparison of the correlation antioxidant capacity with total phenolic content in *Mentha* species. Therefore, the aim of the research is to compare antioxidant capacity and phenolic contents in mint clones to determine superior genotypes for herbal and spice uses and cultivation.

Material and Methods

Chemicals

2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ammonium salt and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals were of analytical grade and obtained from either Sigma-Aldrich or Merck.

Plant material

The selected 13 mint clones of three Turkish cultivated species, *Mentha piperita*, *Mentha spicata* and *Mentha villoso-nervata*, were grown in experimental plot of Agriculture Faculty in Gaziosmanpasa University. Species and origin of selected clones were given Table 1. Rooted samples of each clone were planted experimental plots with three replications. Plants were harvested at the stage of flowering in Mid-July 2009. After, they are dried in drier cabin at 35°C, the leaves were separated from the aerial parts of the plants. Voucher specimens were deposited at the plants herbarium of Science and Arts Faculty at Gaziosmanpasa University (GOPU). Herbarium numbers of voucher specimens are given in Table 1.

Determination of antioxidant capacity in *Mentha* species

Extraction procedures

Plant material, (100 g) was ground into a fine powder in a mill after 10 minutes of storage in liquid nitrogen. The powdered samples were mixed with 500 mL methanol/chloroform (3/1) at room temperatures for 24 hour. The residue was re-extracted under same condition until extraction solvents became colourless. The obtained extracts were filtered over Whatman No.1 paper and the filtrate was collected, then, by extraction the solvent mix was

Table 1: Species, voucher numbers of specimens, clone origins, and extracts yield of selected *Mentha* clones.

Voucher numbers of specimens	Ancestor no	Species	Commercial names	Origin	Yield of extracts (%)
6346	Clone-3	*M. piperita*	Peppermint	Elazığ	23.0
6347	Clone-8	*M. piperita*	Peppermint	Gaziantep	10.5
6348	Clone-4	*M. villoso-nervata*	Spearmint	Amasya	18.0
6349	Clone-7	*M. villoso-nervata*	Spearmint	Antalya	10.5
6350	Clone-9	*M. villoso-nervata*	Spearmint	Osmaniye	11.5
6351	Clone-14	*M. villoso-nervata*	Spearmint	Amasya	11.0
6352	Clone-2	*M. spicata*	Spearmint	Çorum	17.5
6353	Clone-5	*M. spicata*	Spearmint	Manisa	13.5
6354	Clone-10	*M. spicata*	Spearmint	Adana	14.0
6355	Clone-11	*M. spicata*	Spearmint	Karaman	10.5
6356	Clone-12	*M. spicata*	Spearmint	Amasya	10.0
6357	Clone-13	*M. spicata*	Spearmint	Tokat	14.5
6358	Clone-15	*M. spicata*	Spearmint	Nevşehir	12.5

Table 2: Antioxidant capacity and total phenolic content of *Mentha* clones.

Mint. species and clones	Total Phenolic** (µg GAE/g DW)	FRAP** (µmol Trolox equiv/g DW)	TEAC** (µmol Trolox equiv/g DW)
M. piperita			
Clone-3	23.99 ± 4.28^{ab}	558.33 ± 13.52^{ab}	800.02 ± 1.10^{a}
Clone-8	$21.56\pm1.63^{a-d}$	317.60 ± 49.32^{d}	771.58 ± 3.22^{a}
M. villoso-nervata			
Clone-4	9.92 ± 1.61^{e}	314.76 ± 34.01^{d}	550.22 ± 42.61^{bc}
Clone-7	15.84 ± 3.47^{cde}	346.36 ± 56.08^{d}	561.69 ± 56.07^{bc}
Clone-9	17.93 ± 2.71^{bcd}	315.78 ± 50.50^{d}	551.52 ± 70.25^{bc}
Clone-14	$21.17\pm3.84^{a-d}$	374.83 ± 32.08^{d}	593.91 ± 6.56^{bc}
M. spicata			
Clone-2	20.03 ± 2.26^{bcd}	351.18 ± 42.33^{d}	664.59 ± 24.32^{ab}
Clone-5	23.00 ± 3.80^{abc}	425.85 ± 14.89^{bcd}	705.08 ± 16.06^{ab}
Clone-10	19.44 ± 3.18^{bcd}	344.31 ± 61.39^{d}	603.35 ± 16.8^{cd}
Clone-11	14.56 ± 2.88^{de}	280.73 ± 58.76^{d}	490.56 ± 10.72^{de}
Clone-12	17.61 ± 1.95^{bcd}	372.44 ± 45.08^{cd}	549.68 ± 17.25^{bc}
Clone-13	28.27 ± 3.95^{a}	577.09 ± 46.02^{a}	697.25 ± 10.57^{ac}
Clone-15	9.39 ± 1.42^{e}	288.61 ± 1.98^{d}	375.15 ± 18.15^{e}

**$p<0.01$. The same letters (a–e) in the same column indicates no difference statistically.

removed by a rotary evaporator at 50°C. The extracts were used for antioxidant capacity and total phenolic compounds content analysis.

Determination of total phenolic content

The total phenolic contents were determined by Folin–Ciocalteu reagent [18]. 0.1 ml of the extract solution (contains 0.1 mg extract) was mixed with water (46 ml). 1 mL of Folin–Ciocalteu reagent was added and mixed thoroughly. 3 mL of Na_2CO_3 (2%) was added to the mixture. The absorbance was measured at 760 nm. The calibration curve was prepared by 0–100 µg/ml solutions of gallic acid in ethanol. The concentration of total phenolic compounds in the extracts was determined as gallic acid equivalent and calculated as µg gallic acid equivalent/g dry weight (DW) of the plant material using the calibration curve (R^2: 0.99). The data were given as the average of triplicate analyses.

Ferric reducing antioxidant power (FRAP)

Reducing power of mint samples was measured by method of Oyaizu [19] with a slight modification [20]. According to this method the reduction of Fe^{3+} to Fe^{2+} was determined by

measuring absorbance of the Perl's Prussian blue complex. This method is based on the reduction of (Fe^{3+}) ferricyanide in stoichiometric excess relative to the antioxidants. For positive control Trolox was used. Absorbance of these mixtures was measured at 700 nm using a UV spectrophotometer (Hitachi U-2900). Increased absorbance indicates ferric reducing power capability of the samples [21]. Results were given as µmol trolox equiv/g DW. The data were given as the average of triplicate analyses.

Trolox equivalent antioxidant capacity (TEAC)

$ABTS^{\bullet+}$ radical scavenging activity was determined using a modified method of Re et al. [22]. The stock solutions included 5 mL of 7 mM $ABTS^{\bullet+}$ solution and 88 µL of 140 mM $K_2S_2O_8$. The working solution was allowed to react for 16 hours at room temperature in the dark. The ABTS solution was adjusted with distilled water to an absorbance of 0.650±0.050 at 734 nm using the UV-visible spectrophotometer. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. 0.1 mL of *Mentha* species extract was allowed to react with 2.9 mL of the $ABTS^{\bullet+}$ solution in the dark at room temperature for 10 minutes then the absorbance was measured at 734 nm. The data were given as the average of triplicate analyses. Results were given as µmol trolox equiv/g DW [22].

Statistical analysis

The numerical data of total phenolic content and antioxidant activity are presented as mean±standard deviation calculated from triplicate. The values were subject to analysis of variance (ANOVA) using randomized block design. The mean data being significant in variance analysis were grouped with Duncan multiple test. The relationship between total phenolic content and antioxidant activities were also determined by correlation values. The significance threshold was set at 0.01. All statistical calculation were performed using the SPSS 20 statistical software [23].

Results and Discussion

Several methods have been developed to evaluate total antioxidant capacity of food and dietary supplements, herbal extracts, and pure compounds. Nevertheless, few of them have been used widely due to the difficulty of measuring total antioxidant capacity as result of limited methodological protocol and free radical sources [24]. In this study, the antioxidant capacities of the selected clones extracts were evaluated with TEAC and FRAP *in vitro* tests (Table 2). Total phenolic contents were also evaluated and a direct correlation was observed between the total phenolic content of the extracts and their antioxidant capacities (Table 2 and Figure 1).

Total phenolic contents

The amount of total phenolic compound ranged from 9.39 (clone 15) to 28.27 (clone 13) µg GAE/g DW (Table 2) and the variation was statistically significant ($p<0.01$). Total phenolic content were between 28.27 (clone 13) and 9.39 µg GAE/g DW (clone 15) in *M. spicata*, between 21.56 (clone 8) and 23.99 (clone 3) µg GAE/g DW in *M. piperita*, and between 9.92 (clone 4) and 21.17 (clone 14) µg GAE/g DW, in *M. villoso nervata* (Table 2). According to our results, variation in *M. spicata* and *M. villoso nervata* clones was higher than that of *M. piperita* due to number of samples. There are a lot of records on phenolic content in *M. piperita* among other species of *Mentha* genus. Total phenolic content in *M. piperita* ranged between 0.43 and 18570 µg

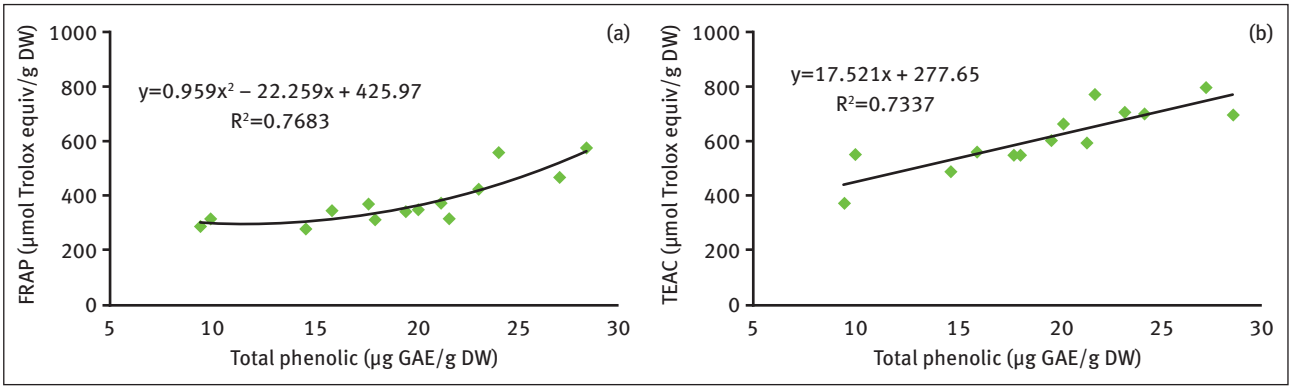


Figure 1: Correlations of total phenolic compounds and antioxidant capacity (a) FRAP and (b) TEAC.

GAE/g plant in previous studies [13,15,25–27]. The content in *M. spicata* was found as 334 mg CAE/g plant, 84 mg GAE/g plant, 80 mg GAE/g plant, 27 mg GAE/g plant and 22.43 mg GAE/g fresh plant by Husseinimehr et al. [28], Elmastas et al. [12], Naidu et al. [29], Conforti et al. [30] and Derakhshani et al. [31] respectively. No investigation of total phenolic content in *M. villoso nervata* has been included in the literature. Phenolic compounds that are synthesized for defending and protecting purpose and have an important role in the human diet varies according to genetic structure, growing, and climate conditions [32]. In the research the change in the phenolic content is due to genetic differences of the clones because the used clones are grown in the same ecologic and field conditions.

The total phenolic content of *M. piperita* in this work was lower than that of some reports [13,15,26] and higher than that of others [27,33]. Total phenolic amounts of *M. spicata* clones were generally higher than that of Ninckavar's result [13] except two clones (Clone 15 and 11). Total phenolic contents in all clones of *M. spicata* in this study were lower than a previous study [12,28,34]. Total phenolic contents in *M. spicata* clones were in agreement with results reported by Derakhshani et al. except clone-11, clone-12 and clone-15 [31]. Those three clone phenolic contents were lower than results of Derakhshani et al. [31]. Total phenolic contents in *M. spicata* clones were higher than that of Naidu et al's result [29]. Although, there are no records on total phenolic amount of *M. villoso nervata*, total phenolic content of the species was similar to the records of *M. spicata* and lower than that of other *mentha* species such as *M. piperita*, *M. longifolia*, *M. pulegium*, *M. roduntifolia* [13]. Even though there was significant variation statistically ($p<0.01$) between clones of the spearmint (*M. spicata* and *M. villoso nervata*) species, *M. piperita* Clones (two clones) had similar data, statistically. Total phenolic of the clones was 23.99 (µg GAE/g DW) in Clone 3 and 21.56 (µg GAE/g DW) in Clone 8. There is dramatic variation in total phenolic amount of *M. spicata* and *M. villoso nervata* (Table 2).

Antioxidant activities

Ferric reducing antioxidant power (FRAP)

FRAP activities varied between 280.73 and 577.09 µmol Trolox equiv/g DW in *M. spicata*. The lowest and the highest FRAP activity of *M. piperita* were 317.6 and 558.33 µmol Trolox equiv/g DW, while 314.76 and 374.83 µmol Trolox equiv/g DW in *M. villoso nervata* (Table 2).

Different studies have indicated that the electron donation capacity of bioactive compounds is associated with antioxidant activity [35,36]. The presence of reductants in the samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [37]. There are a number of assays designed to measure overall antioxidant activity [38]. FRAP assay take advantage of an electron transfer reaction in which a ferric salt is used as an oxidant [39].

In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of an extract may serve as a significant indicator of its potential antioxidant activity. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging [40].

Reduction power of *Mentha* species extracts ranged from 280.7 to 577.1 µmol Trolox equiv./g DW. The highest activity was seen in *Mentha spicata* Clone 13 and the lowest in *Mentha spicata* Clone 11 (Table 2). The second highest FRAP activity of this study was observed *M. piperita* Clone 3. FRAP result of *M. piperita* Clone 3 was agreement with some previous reported results [13,15,16]. According to the results in this study, FRAP activity differences in *Mentha* clones were correlated ($r=0.77$) with total phenolic content (Figure 1a). Chrpova et al. [15] studied antioxidant activities of some herbs belongs Lamiaceae, including *M. piperita*, used for medicinal purposes, and they reported that *M. piperita* had strong free radical scavenging activity and good correlation ($r=0.96$) with total phenolic contents [15].

Studies conducted on free radical scavenging or antioxidant activities of medicinal plant reveal that the efficiency of plant species depends on assay methodology, reflecting the complexity of the mechanisms involved in total antioxidant capacity [41–43].

Trolox equivalent antioxidant capacity (TEAC)

Results of cation radical scavenging activities were given in Table 2. As seen in Table 2, TEAC activity of *Mentha* species extracts ranged from 375.1 to 800.02 µmol Trolox equiv/g DW. The highest activity was seen in *Mentha piperita* clone 3 while the lowest in *Mentha spicata* Clone 15 (Table 2).

Assays based upon the use of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals are among the most popular spectrophotometric methods for determination of the antioxidant capacity of food, beverages, plant extracts and pure compounds

[44,45]. Both chromogen radical compounds can directly react with antioxidants. DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging methods are used to evaluate the antioxidant activity of compounds due to the simple, rapid, sensitive, and reproducible procedure [40].

The reaction of the preformed radical with free-radical scavengers can be easily monitored by following the decay of the sample absorbance at 734 nm. ABTS$^{\bullet+}$ radicals are more reactive than DPPH radicals and unlike the reactions with DPPH$^{\bullet}$ radical which involve H atom transfer, the reactions with ABTS$^{\bullet+}$ radicals involve electron transfer process [46]. Lopez et al. studied antioxidant activities of 5 *Mentha* species. According to their report ABTS$^{\bullet+}$ radical scavenging activities of *M. piperita* showed the best activity when compared to other species [14]. Lopez et al's results also agree with our result (Table 2). Previous antioxidant activity reports on *Mentha* species clearly showed antioxidant activities of *M. piperita* and *M. spicata*. [12–16].

TEAC activity differences in *Mentha* clones were correlated with total phenolic content (r=0.73). It can be said that phenolic compounds are responsible for TEAC activities of *Mentha* species.

Conclusion

Results obtained in the research is summarized as following:

1. Different methods and units in antioxidant activity caused difficulty in comparison of the results. So, a standard unit should be used by researchers for accurate evaluation of antioxidant results in *Mentha* species in future researches.
2. There is a positive correlation with total antioxidant capacity and total phenolic content in *Mentha* clones. In future studies the relationship between antioxidant capacity and phenolic contents on *Mentha* species should be based on antioxidant activities and individual phenolic compound contents in *Mentha* species.
3. According to antioxidant capacity and total phenolic content results it can be suggested that Clone 13, Clone 5 (*M. spicata*) Clone3 and Clone 8 (*M. piperita*) are more suitable for use as spice and herbal tea. The results contain valuable data for breeding studies too.
4. The result of *M. villoso nervata* is the first record on both antioxidant capacity and phenolic content.
5. In future studies the relationship between antioxidant capacity and specific phenolic components of *Mentha* species should be studied in *Mentha* species.

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