

Antioxidative capacities of some GABA_A receptor modulating food plants

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Abstract

Reactive oxygen species cause oxidative stress associated with some health issues such as neurodegenerative diseases. Some antioxidants show neuroprotective activity and regulate neuronal activity through positive modulation of GABA_A receptors. In this study, the antioxidative capacity of some novel GABA_A receptor modulating food plants, including lavender, clove and four *Sideritis* species (*S. arguta*, *S. sipylea*, *S. condensata*, and *S. stricta*) was investigated using ABTS assay. The aqueous clove extract showed the most potent antioxidant effect and its IC₅₀ value was found to be 3.76 µg/mL. The antioxidant capacity of Trolox was lower (IC₅₀: 4.63 µg/mL) than that of the clove extract, but higher than those of the other food extracts. The aqueous lavender extract exhibited less antioxidant activity compared to *Sideritis* species, except *S. sipylea* that had the least antioxidant capacity (IC₅₀: 20.75 µg/mL). In conclusion, these results confirm that GABA_A receptor modulating food plants possess strong antioxidative activity.

Key words: *Sideritis*, lavender, clove, antioxidative capacity

Bazı GABA_A-reseptör modüle edici bitkisel gıdaların antioksidant kapasiteleri

Öz

Reaktif oksijenler nörodejeneratif hastalıklar gibi bazı sağlık sorunlarına yol açan oksidatif strese neden olurlar. Bazı antioksidanlar nöroprotektif aktivite gösterir ve GABA_A reseptörlerinin pozitif modülasyonu ile nöronal aktiviteyi düzenlerler. Bu çalışmada bazı yeni GABA_A reseptör modüle edici

bitkisel gıdalardan lavanta, karanfil ve 4 *Sideritis* türünün (*S. arguta*, *S. sipylea*, *S. condensata*, ve *S. stricta*) ABTS metodu ile antioksidant etkileri araştırılmıştır. Karanfilin sulu ekstraktı en yüksek antioksidant etki göstermiştir ve IC₅₀ değeri 3.76 µg/mL olarak bulunmuştur. Trolox'un antioksidant kapasitesi (IC₅₀: 4.63 µg/mL) karanfilden düşük ancak diğer bitki ekstraktlarından yüksek bulunmuştur. Sulu lavanta ekstraktı, *S. sipylea* hariç diğer *Sideritis* türleri ile karşılaştırıldığında en düşük antioksidant kapasitesi göstermiştir (IC₅₀: 20.75 µg/mL). Sonuç olarak, bu deney sonuçları GABA_A reseptör modüle edici bitkisel gıdaların güçlü antioksidant etkiye de sahip olduklarını doğrulamaktadır.

Anahtar kelimeler: *Sideritis*, lavanta, karanfil, antioksidant kapasite

Introduction

Increase in the reactive oxygen species in the cell causes DNA damage, oxidation of proteins, and lipid degradation (Sesti et al., 2010). Therefore, some chronic and acute diseases, including cardiovascular diseases, cancer, and neurodegenerative diseases, are associated with oxidative stress (Willcox et al., 2004). It has been shown that several antioxidants-e.g. phenolic compounds have neuroprotective effects (Reiter, 1998; Ha et al., 2000; Zbarsky et al., 2005; Silva et al., 2009; Wu et al., 2014). Furthermore, some antioxidants exhibit not only neuroprotective effect but also modulation of the neuronal activity via GABA_A receptors (Ha et al., 2000; Silva et al., 2009; Wu et al., 2014), which are activated by GABA (γ-aminobutyric acid). Physiologically important amino acid GABA is a major neurotransmitter in the brain so that 40 % of

all neurons in the mammalian central nervous system are GABAergic neurons (Granger et al., 2005). GABA_A-receptors are targets for various groups of medications including sedatives, anxiolytics, muscle relaxants, antidepressants and antiepileptics (Möhler, 2006; Sigel and Steinmann, 2012). Some foods and food components which have the antioxidant, antiepileptic, sedative, antidepressant, anxiolytic, or calming effect can modulate GABA_A-receptors (Kessler et al., 2012; Kessler et al., 2014; Sahin et al., 2016; Sahin et al., 2017; Silva et al., 2015). For example, it has been reported that lavender had antioxidant activity and exhibited the sleep-inducing, anxiolytic and sedative activity linked to its modulatory effect on GABA_A-receptors (Alnamer et al., 2012; Aoshima and Hamamoto, 1999; Kessler et al., 2012; Sahin et al., 2016; Shaw et al., 2007; Silva et al., 2015; Woronuk et al., 2011). Some *Sideritis* species demonstrated antioxidant, analgesic and anticonvulsant effects (Charami et al. 2008; Hernandez-Perez et al., 2004; Gonzalez-Burgos et al., 2011) and enhanced the activity of GABA_A-receptors in different cell expression systems (Kessler et al., 2012; Sahin et al., 2016). The clove (*Syzygium aromaticum* L.) acting as antioxidant exhibited topical anesthetic, analgesic, antiepileptic, and anxiolytic effects (Alqareer et al., 2006; Carlsen et al., 2010; Gülçin et al., 2004; Halder et al., 2012; Hosseini et al., 2011) which could be partially mediated by positive modulation of the GABA_A receptor (Sahin et al., 2017). Recently, these three plants –lavender, clove, and some of the *Sideritis* species- have been identified as specific positive modulators for the GABA_A receptors (Sahin et al., 2016; Sahin et al., 2017). Since several antioxidants with neuroprotective properties, also exhibit neurophysiological effect through GABA_A receptors, the aim of this study was to determine the antioxidative capacity of novel GABA_A receptor modulating food plants; lavender, clove and four *Sideritis* species (*S. arguta*, *S. sipylea*, *S. condensata*, and *S. stricta*).

Materials and Methods

Chemicals

Trolox ((±) 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), ABTS, potassium persulfate, sodium dihydrogen phosphate dihydrate (H₂NaO₄P x 2 H₂O), and disodium hydrogen phosphate dihydrate (HNa₂O₄P x 2 H₂O) were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Plant material and preparation of extracts

The three *Sideritis* species (*S. arguta*, *S. condensata*, and *S. stricta*) were obtained from Akdeniz University, Antalya, Turkey while *S. sipylea* was supplied from an online store (enexia.de). Clove buds (*Syzygium aromaticum* L., Hallesche Essig- und Senffabrik, Bad Dürrenberg, Germany) were purchased from a local supermarket. Lavender flowers (*Lavendula officinalis*) were obtained in crushed form from a local pharmacy. The identity of these plants was verified as described before (Sahin et al., 2016). The *Sideritis* species and clove buds were coarsely ground before extraction, whereas the crushed lavender flowers were used directly. Each test material was mixed with boiling water (2.5 g:100 mL). After stirring for 15 min at room temperature, the mixtures were filtered using Whatman filter paper and the filtrates were lyophilized. The lyophilized samples were dissolved in water (1 mg/mL) prior to experiments. Extraction was repeated two times.

ABTS assay for antioxidant activity evaluation

Antioxidative capacity was measured using the ABTS-radical assay according to literature Lee et al. (2015) with some modifications. The ABTS in water (7 mM) was mixed with 2.45 mM of potassium persulfate to prepare the ABTS reagent. 1-20 µL of the plant extracts were added to 180-199 µL of ABTS reagent in a 96-well microplate. After incubation at room temperature for 6 min, the absorbance was measured at 734 nm using a microplate spectrophotometer (µQuant BioTek). The Trolox was used as the antioxidant standard and dissolved in 75 mM of potassium phosphate buffer (pH 7.4). All the samples were analyzed in triplicates. The absorbance of the ABTS reagent without the addition of sample was used as the control. Percent inhibition of absorbance was calculated using the following equation: ABTS scavenging effect (%) = ((A_{control} - A_{sample})/ A_{control}) x100, where "A" represents absorbance.

Data analysis

Linear regression was performed to determine the IC₅₀ values (the half-maximal inhibitory concentration) of the extracts (± 95 % confidence interval [CI]) using OriginPro 2018b (OriginLab Corporation, Northampton, MA 01060 USA). Statistical data analysis was done by Minitab (version 18.1) using one-way ANOVA. Following ANOVA, the Tukey multiple comparisons test was performed with significance levels p < 0.05.

Results and Discussion

The present study evaluated the antioxidative activity of some GABAergic food plants (four *Sideritis* spp., clove, and lavender) using ABTS radical assay based on the measurement of the reduction in the

radical cation as the percentage of inhibition in the absorbance at 734 nm. The Trolox was used at the concentrations of 0.94, 1.88, 2.82, 3.75, and 4.69 $\mu\text{g}/\text{mL}$ to obtain a Trolox dose-response curve (Fig. 1).

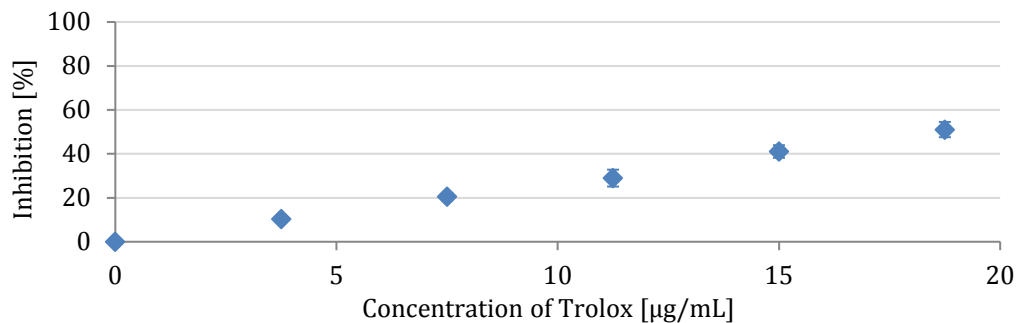


Figure 1. ABTS radical-scavenging activities of Trolox. The results are means of three different experiments, in each of which three measurements were made.

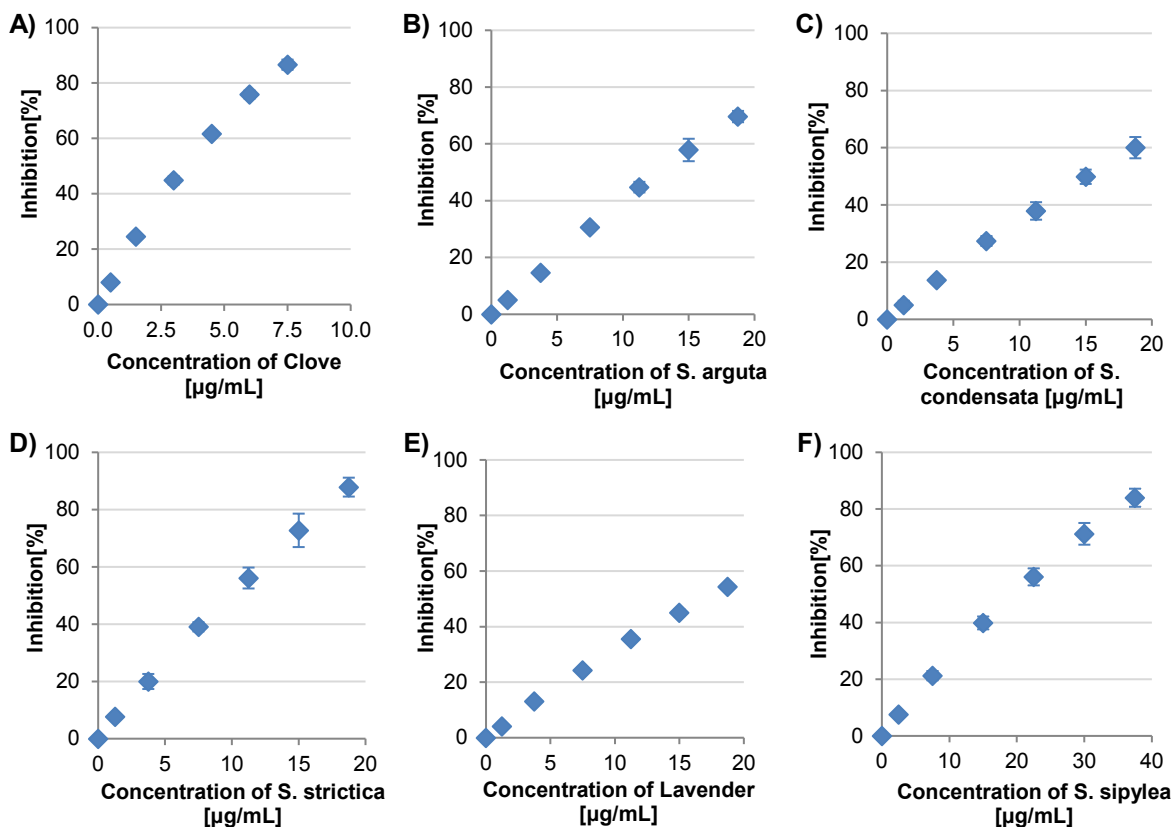


Figure 2. ABTS radical-scavenging activities of the aqueous plant extracts; A) Clove, B) *S. arguta*, C) *S. condensata*, D) *S. strictica*, E) Lavender, F) *S. sipylea*.

Each food extract was tested at various concentrations (0.5 - 37.5 $\mu\text{g}/\text{mL}$) and their inhibitory effect was assessed by determining IC_{50} values (50 % inhibitory concentration). Similar to the Trolox, all tested plant extracts exhibited a

radical scavenging activity in a dose-dependent manner (Fig. 2).

The aqueous extract of clove buds had the highest antioxidant activity with an IC_{50} value of 3.76 $\mu\text{g}/\text{mL}$. To achieve fifty percent inhibition, the required concentration of Trolox (4.63 $\mu\text{g}/\text{mL}$) was higher

than that of aqueous clove extract but lower than that of the other aqueous food extracts (Table 1). When tested at 7.5 µg/mL, the clove extract inhibited ABTS cation formation by 86.6 %, whereas the other food extracts caused approximately 20-40 % inhibition of oxidation of ABTS. Among *Sideritis* species, *S. stricta* was the most active, followed by *S. arguta* and *S. condensata*. The IC₅₀ value of *S. stricta* was 2.7-fold higher than IC₅₀ value of the clove extract. All the tested *Sideritis* species were more potent inhibitors compared to lavender extract, except *S. sipylea* which showed the lowest antioxidant activity with the IC₅₀ value of 20.75 µg/mL (Table 1).

Table 1. IC₅₀ of samples

Samples	IC ₅₀ (with 95 % confidence interval) [µg/mL]
Clove	3.76 (3.33 - 4.19) ^C
Trolox	4.63 (4.17 - 5.10) ^C
<i>S. stricta</i>	10.16 (9.10 - 11.21) ^{BC}
<i>S. arguta</i>	12.70 (11.41 - 13.99) ^B
<i>S. condensata</i>	14.99 (13.44 - 16.54) ^{AB}
Lavender	16.81 (15.04 - 18.57) ^{AB}
<i>S. sipylea</i>	20.75 (18.57 - 22.94) ^A

^{A-C} The values indicated by the different letters differ from each other significantly by the Tukey test (p < 0.05).

Güvenç et al. (2008) tested the aqueous extracts of the 17 lyophilized (50 g plant: 600 mL water) *Sideritis* species including *S. arguta*, *S. condensata*, and *S. sipylea* for their antioxidant activities. They used two methods (DPPH and Thiobarbituric acid assays) to evaluate the antioxidative capacity. They reported that *S. arguta*, *S. condensata*, and *S. sipylea* showed strong antioxidant activities although they only observed the color change under UV light but lacked the absorbance measurements in the DPPH assay. In Thiobarbituric acid (TBA) test, *S. sipylea* exhibited a low antioxidant activity (IC₅₀: 0.71 ± 0.05 mg/mL) as compared with the other two *Sideritis* species (IC₅₀ value of *S. arguta*: 0.44 ± 0.23 mg/mL and IC₅₀ value of *S. condensata*: 0.33 ± 0.04 mg/mL) (Güvenç et al., 2008). This result also supports our current findings where *S. sipylea* was the least potent among tested four *Sideritis* species including *S. arguta* and *S. condensata*.

Lavender (*Lavendula officinalis*), similar to the genus *Sideritis*, belongs to the *Labiatae* family and is commonly used for its sleep-inducing, sedative and calming effects (Woronuk et al., 2011). Lavender owes its GABAergic effect to the positive modulation of GABA_A receptors (Kessler et al., 2012; Şahin et al., 2016). The present findings demonstrated that the

antioxidant activity of aqueous extract of lavender was lower compared to the aqueous extract of clove buds. Similarly, Gülçin et al. (2004) reported that not only the aqueous extract but also the ethanolic extract of lavender had less antioxidant activity than the aqueous and ethanolic extract of clove buds based on the DPPH assay (Gülçin et al., 2004).

Clove (*Syzygium aromaticum* L.) belongs to the *Myrtaceae* family, and its essential oil is used in dental care due to its anesthetic and analgesic effects (Alqareer et al., 2006; Hosseini et al., 2011; Halder et al., 2012). Among tested GABAergic food plants in the present study, it was observed that the aqueous clove bud extract exhibited the most potent antioxidant activity and its activity was higher than the Trolox, which is a water-soluble analogue of α-tocopherol. Both water and ethanol extracts from clove buds showed higher antioxidative capacities than α-tocopherol in thiocyanate and DPPH methods (Gülçin et al., 2004). Using the FRAP assay, it was reported that clove had the highest antioxidant capacity among 3139 different foods tested including beverages, spices, herbs, and supplements (Carlsen et al., 2010). Additionally, the antioxidative capacity of clove buds was compared with the most commonly used antioxidants the butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The ethanolic extract of clove had stronger DPPH radical scavenging activity compared to the synthetic chemicals BHA and BHT at the concentration of 60 µg/mL, whereas the activity of its aqueous extract was higher than BHT but similar to BHA (Gülçin et al., 2004). Previously, eugenol was detected in the aqueous clove extract by UHPLC-DAD and GC-MS analysis and identified as the main clove component predominantly responsible for GABA_A receptor modulation (Şahin et al., 2017). It was shown that eugenol was a strong antioxidant, so that its low concentration (15 µg/mL) had higher antioxidative capacity than BHA, Trolox, and α-tocopherol (Gülçin, 2011).

Conclusion

The results of the present study demonstrate that novel GABA_A receptor modulating food plants (lavender, clove, and four *Sideritis* species -*S. arguta*, *S. sipylea*, *S. condensata*, and *S. stricta*-) possess strong antioxidant potential at low concentrations. The clove, lavender and *Sideritis* species may offer a potential for developing new therapeutic agents which can be used to treat neurological disorders.

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