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RESEARCH ARTICLE

Comparative Bioactivity Studies on Four *Veronica* species

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Summary

It is well known that the excessive production of reactive oxygen species is hazardous for living organisms and damage major cellular constituents such as DNA, lipid and protein. To find new products reducing free radical damage is very important researches in recent pharmaceutical investigations. Considering this information, four Veronica species are decided to be researched in the view point of their antioxidant capacity and the chemical content. The aqueous extracts of the plants were tested for their radical scavenging activity against 2,2-diphenyl-1-picryl hydrazyl (DPPH), superoxide (SO) and nitric oxide (NO) radicals spectroscopically. Dose dependent radical scavenging activity was observed and the results were compared with the known antioxidant compounds BHA (3-t-butyl-4-hydroxyanisole), ascorbic acid and quercetin. In addition, gallic acid equivalent total phenolic contents of the plants were also determined using Folin-Ciocalteau reagent.

Key Words: *Veronica, Plantaginaceae, Radical scavenging effect, DPPH, Nitric oxide, Superoxide.*

Dört Veronica Türü Üzerinde Karşılaştırmalı Biyoaktivite Çalışmaları

Özet

Reaktif oksijen türevlerinin aşırı üretiminin canlı organizmalar için zararlı olduğu ve DNA, lipit ve proteinler gibi temel hücresel bileşenlere hasar verdiği çok iyi bilinmektedir. Serbest radikal hasarı en aza indirecek yeni ürünler bulunması son yıllardaki farmasötik araştırmalarda oldukça önemli bir yer tutmaktadır. Bu bilgiler ışığında dört farklı Veronica türü antioksidan etkinlikleri ve kimyasal içerikleri açısından araştırılmıştır. Bitkilerin sulu ekstrelerinin 2,2-difenil-1-pikril hidrazil (DPPH), süperoksit (SO) ve nitrik oksit (NO) radikallerine karşı süpürücü etkinlileri spektroskopik olarak araştırılmıştır. Doza bağlı antioksidan aktivite tespit edilmiş ve sonuçlar bilinen antioksidan bileşikler BHA (3-t-Butil 4-hidroksianizol), askorbik asit ve kersetin ile karşılaştırılmıştır. Ayrıca bitkilerin gallik asite eşdeğer total fenol içerikleri de Folin-Ciocalteau reaktifi ile tanımlanmıştır.

Anahtar Kelimeler: *Veronica, Plantaginaceae, Radikal süpürücü etki, DPPH, Nitrik oksit, Süperoksit.*

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INTRODUCTION

Scrophulariaceae family is represented by 30 genera in Turkish flora (1). In several recent molecular and phylogenetic studies on the members of Scrophulariaceaea family, a lot of different genera are transferred from Scrophulariaceae to Plantaginaceae family. The genus *Veronica* is one of the transferred genera and its chemotaxonomic relevance is originated from the phytochemical similarity with the genera of Plantaginaceae (2, 3). Previous researches on this genus are resulted to

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the isolation of mainly iridoid glucosides, especially benzoic and cinnamic acid esters of catalpol, some phenylethanoid and flavonoid glycosides (2-6). In addition to the chemotaxonomic importance, the genus *Veronica* also became prominent from the view point of its traditional usage and biological activities. While some of the *Veronica* species are used as diuretic, for wound healing and rheumatic pains in traditional Turkish medicine, several *Veronica* species are used to treat cancer, influenza, hemoptysis, laryngopharyngitis, hernia, cough and respiratory diseases in different countries (8-11). Previously, we have performed a lot of phytochemical and biological studies on the genus *Veronica* (4-6). Our previous researches and recent phylogenetic and pharmacological studies were turned us to focus on the researches on Plantaginaceae family for its phytochemical and biological properties. As a part of this ongoing research project, radical scavenging activity of different *Veronica* species were planned to be tested against DPPH, SO and NO radicals spectroscopically. Their total phenolic contents were also determined using Folin-Ciocalteau reagent.

Extensive studies with different model systems and biological materials have clearly shown that reactive free radicals are able to produce chemical modifications and damage to proteins, lipids, carbohydrates and nucleotides. Many diseases and degenerative processes can be associated with the overproduction of reactive oxygen species (ROS) including inflammation, brain ischemia, mutagenesis, dementia and physiological aging. In addition, the increased amount of free radicals in cancerous cells and tissues is well known (12-14). For these reasons, several methods have been developed to measure antioxidant activities of the herbal extracts *in vitro.* Here we have tested NO, SO and DPPH radical scavenging activity of the aqueous extracts of selected species together with their gallic acid equivalent total phenolic contents. There are no reported bioactivity studies about these species.

MATERIALS AND METHODS

Plant Materials

Veronica peduncularis Bieb., *Veronica baranetzkii* Bordz., *Veronica officinalis* L., and *Veronica orientalis* Miller were collected from different places in Turkey. A voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey. Their location and herbarium numbers are as follows: *Veronica peduncularis* Bieb, Maçka - Trabzon, HUEF 09008; *Veronica baranetzkii* Bordz., Maçka – Trabzon, HUEF 09007; *Veronica officinalis* L.; *Veronica orientalis* Miller, Göle – Kars, 90026).

General

DPPH, nitro blue tetrazolium (NBT), sodium nitroprusside, Folin–Ciocalteu reagent, gallic acid, ascorbic acid were obtained from Sigma-Aldrich Chem Co (St. Louis, MO). 3-*t*-butyl-4-hydroxyanisole (BHA) was purchased from Nacalai Tesque Co. (Kyoto, Japan). Sulfanilamide and napthylethylenediamine dihydrochloride were obtained from Merck Co. (Darmstadt, Germany).

Preparation of herbal extracts

The air-dried aerial parts of the plants were extracted with MeOH at 40°C for three times. The combined MeOH extracts were evaporated under *vacuum* to give crude MeOH extract. MeOH extract was dissolved in water and partitioned with petroleum ether to remove chlorophylls. The aqueous extracts were tested for the activity studies.

DPPH radical scavenging effect

DPPH radical scavenging effect of the *aqueous extracts* was assessed by the decoloration of methanol solution of DPPH spectroscopically; BHA and ascorbic acid were used as standard compounds. MeOH solution (100 μl) of the extract at various concentrations was added to DPPH/MeOH (80 μg/ml) solution. The reaction mixture was shaken vigorously and the absorbance of the remaining DPPH was measured at 520 nm after 30 min. The radical scavenging activity was determined by comparing the absorbance with that of blank (100%) containing only DPPH and solvent. All the analyses were done in 3 replicates (4, 5, 15, 16).

SO radical scavenging activity by alkaline DMSO method

The method of Elizabeth and Rao (1990) was used for the detection of SO radical scavenging activity

with slight modification. Briefly, SO radical was generated in non-enzymatic system. To the reaction mixture containing 10 μL of NBT (1 mg/mL solution in DMSO) and 30 μL of the extract or standard compounds dissolved in DMSO, 100 μL of alkaline DMSO (1 mL DMSO containing, 5 mM NaOH in 0.1 mL water) was added to give a final volume of 140 μL and the absorbance was measured at 560 nm using microplate reader (17, 18).

NO radical scavenging activity

In order to determine NO radical scavenging activity of the extracts, 60 μl of serial diluted sample were added into a 96-well flat-bottomed plate. Following this, 60 μl of 10 mM sodium nitroprusside, dissolved in phosphate buffered saline (PBS), were added to each well and the plate was incubated under light at room temperature for 150 min. Finally, an equal volume of Griess reagent (1% sulfanilamide, 0.1% napthylethylenediamine dihydrochloride, 2.5% H_3PO_4) was added into each well in order to measure the nitrite content. After chromofore was formed at room temperature in 10 min, absorbance at 577 nm was measured in a microplate reader (4, 19, 20).

Estimation of total phenol content

Antioxidant compounds generally contain phenolic group(s) and the amount of phenolic compounds in the extract was estimated by using Folin–Ciocalteu reagent. Briefly, 10 μL sample or standard (50-500 mg/L gallic acid) plus 150 μL diluted Folin–Ciocalteu reagent (1:4 reagent:water) was placed in each well of a 96-well plate, and incubated at room temperature for 3 min. Following the addition of 50 μL sodium carbonate (2:3, saturated sodium carbonate: water) and a further incubation of 2 h at room temperature, absorbance was read at 725 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram extract. All tests were conducted in triplicate (21, 22).

RESULTS and DISCUSSION

Involvement of free radical mediated cell damage in many different diseases has led us to determine radical scavenging activity of four *Veronica* extracts. Radical scavenging activity of the aqueous extracts was screened against DPPH, NO and SO radicals. All the tested extracts were found to show dose dependent DPPH radial scavenging ability from the concentration of 50 μg/mL (Fig. 1). While they show strong radical scavenging activity against DPPH radical, their activities are not stronger than those of standard compounds BHA and ascorbic acid. Their IC_{50} values were found as follows: 37.68 μg/mL for *V. officinalis*, 54.19 μg/mL for *V. peduncularis*, 85.1 μg/mL for *V. orientalis*, 99.03 μg/mL for *V. baranetzkii*. The

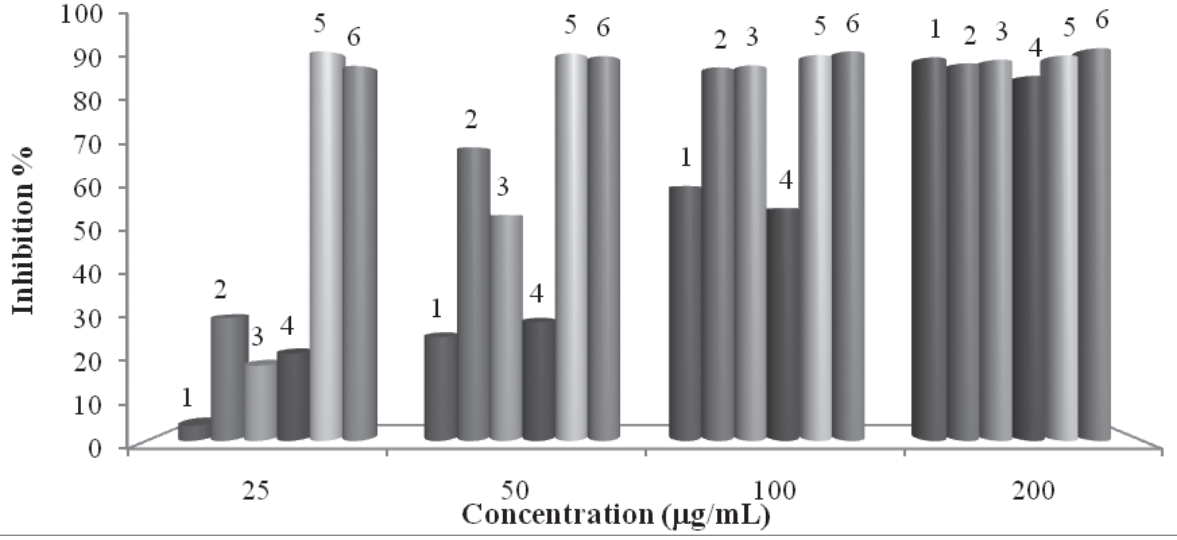

Figure 1. DPPH radical scavenging activity of four *Veronica* species, ascorbic acid (AA) and BHA (1- *V. orientalis*, 2- *V. officinalis*, 3- *V. baranetzkii*, 4- *V. peduncularis*, 5- AA, 6- BHA)

strongest radical scavenging activity was recognized for the aqueous extract of *V. officinalis* among the tested samples (Fig. 1).

Figure 2 has represented NO radical scavenging activities of the extracts and the standard compounds, ascorbic acid (AA), quercetin and BHA. Nitric oxide is a very unstable species under aerobic condition. It reacts with O_2 to produce its stable product nitrate and nitrite through intermediates NO_2, N_2O_4 and N_3O_4 (19). NO scavenging effect of the aqueous extracts determined using Griess reagent. All the tested extracts showed dose dependent NO radical scavenging activity and when the activity of each extracts compared to each other, very similar results were observed (Fig. 2). While high IC_{50} values (400 ~ 800 µg/mL) for the extracts indicated the only moderate NO scavenging activity, close inhibition between the extracts and the standard compounds had given chance to determine activity in different radical scavenging experiments.

On the other hand, NBT assay was carried out to test whether the extracts scavenged superoxide anions or not. Alkaline DMSO was used as a superoxide generating system reacts with NBT to give colored diformazan. SO radical scavenging activity of the extracts was observed at 25 µg/mL concentration and *V. peduncularis* showed the highest activity with 27.4 % inhibition. In addition, SO radical scavenging activity of BHA was found to be lower than those of the extracts in each tested concentrations (Fig. 3). Since superoxide anions are the most common free radicals *in vivo,* the concentration of superoxide anions is very important under conditions of oxidative stress.

Concerning DPPH, NO and SO radical scavenging activities of the tested extracts, DPPH radicals were found to be the most scavenged radicals (Fig 1-3). Radical scavenging activity of the extracts indicated the presence of high phenolic constituents of the extracts. It is obvious that the total phenolic content measured by the Folin–Ciocalteu procedure does not give a full picture of the quality or quantity of the phenolic constituents in the extracts. However, correlation between the radical scavenging activity and the total phenolic contents of the different extracts was shown in several different studies (22, 23). Therefore, total polyphenols in the extracts

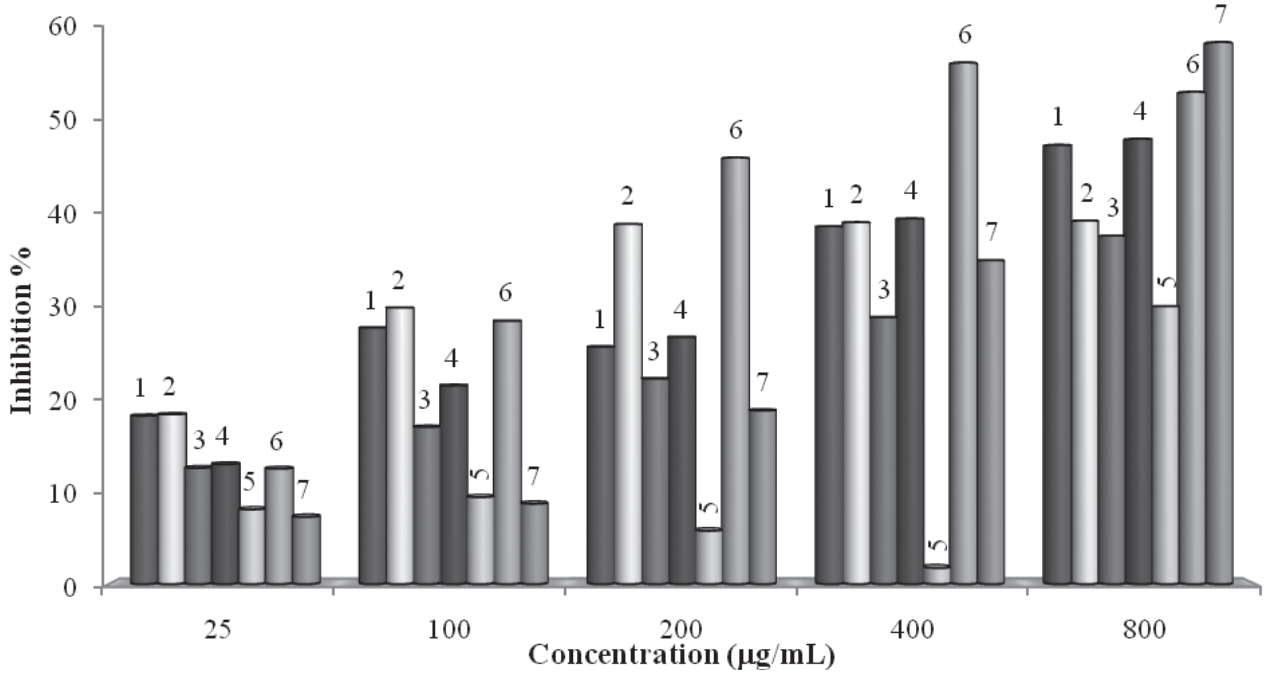

Figure 2. Nitric oxide radical scavenging activity of four *Veronica* species, ascorbic acid (AA), BHA and quercetin (1- *V. orientalis*, 2- *V. officinalis*, 3- *V. baranetzkii*, 4- *V. peduncularis*, 5- AA, 6- BHA, 7- Quercetin)

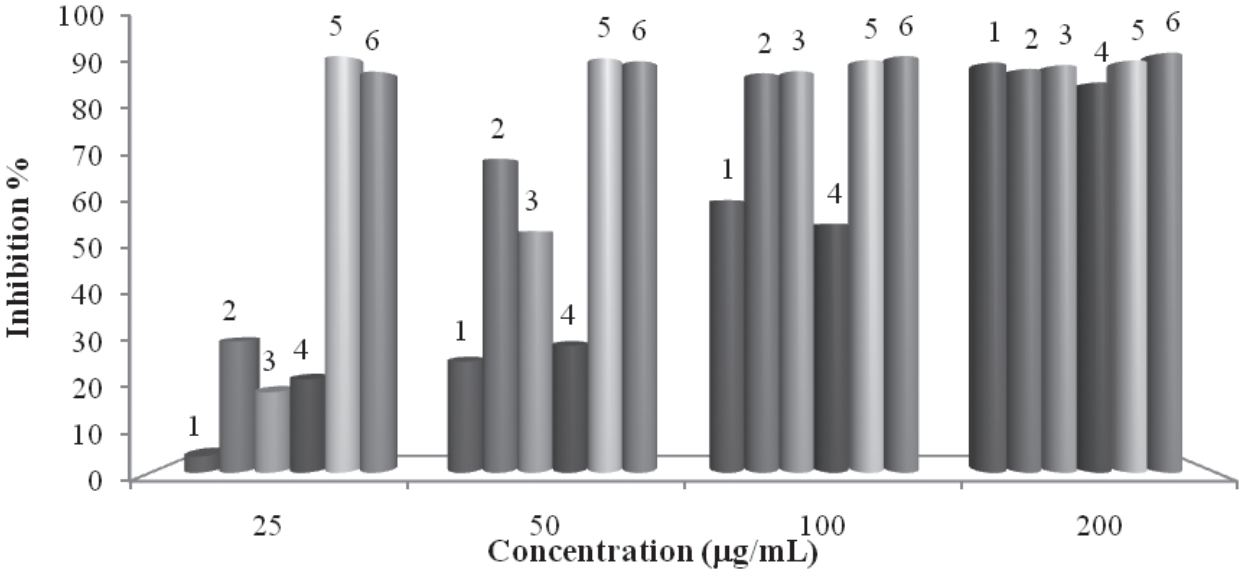

Figure 3. Superoxide radical scavenging activity of four *Veronica* species, ascorbic acid (AA), BHA and quercetin (1- *V. orientalis*, 2- *V. officinalis*, 3- *V. baranetzkii*, 4- *V. peduncularis*, 5- AA, 6- BHA)

were determined by Folin-Ciocalteu reagent. This assay has been extensively used to measure the total phenolics in plant materials for many years and based on electron transfer reaction and actually measures a sample's reducing capacity. Therefore, it is accepted as a routine assay for rough estimation of the antioxidant capacity of herbal samples. Here, total phenolic content of the aqueous extracts was expressed as gallic acid equivalent in mg/g dry extract. As shown in Table 1, the highest phenolic content was determined for *V. officinalis*, 200.20 mg/g dry extract. Since *V. officinalis* has been found to exhibit strongest DPPH radical scavenging activity, direct correlation between the high phenolic contents of the plant and its radical scavenging activity indicated that radical scavenging activity of *V. officinalis* was arising from phenolic content of the plant. On the other hand, radical scavenging activity of each species was found very similar to each other against NO and SO radicals. Well known high ester-iridoid and phenylethanoid contents for *Veronica* species, and the above mentioned results showed us the importance of phenolic constituents for the scavenging activity (4-6). In addition, since *V. officinalis* is found to be the most scavenging extract against DPPH radical, bioactivity guided isolation studies will be performed on this species to find the active compounds. Our phytochemical and biological researches on the member species of Plantaginaceae family are still continuing and further researches are needed to clarify chemotaxinomic and biologic significance of these species.

Table 1. Gallic acid equivalent total phenolic content of the tested species (mg/g dry extract)

Tested species	Phenolic content
1. ***V. orientalis***	127.64 mg/g
2. ***V. officinalis***	200.20 mg/g
3. ***V. baranetzkii***	83.15 mg/g
4. ***V. peduncularis***	139.92 mg/g

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