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Research article

Total Phenolic Content and Antioxydant Activity of *Two Rhododendron* Species Collected from the Rize Province (Turkey)

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Six Rhododendron species, one of which (*R. smirnowii*) is endemic, are growing naturally in Turkey, especially in Northeastern Anatolia (Eastern Part of Black Sea Region). Total phenolic content and antioxidant activity of two Rhododendron species collected in Rize at different altitudes from their natural habitats were determined. Samples obtained from Rize province (Turkey) were screened for total phenolic content by the modified Folin–Ciocalteu method, for potential antioxidant activity using phospho-molybdenum assay and by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) and FRAP method for antiradical activity. Total phenolic content and antioxidant activity of plant parts (flower, leaf) of two *Rhododenron* sepecies, namely *R. luteum* L. and *R. ponticum* L. were determined. The total phenolic content of leaves of *R. luteum* L. collected from different altitudes ranged between 112,363 and 219,071 mg GAE/gr DW. Total phenolic content of flower parts of the same species ranged between 82,275 and 201,642 mg GAE/gr DW. The total phenolic content of different parts of *R. ponticum* L. were higher compared with *R. luteum* L. On the other hand, the antioxidant activity values of leaf parts of *R. luteum* L. were lower compared with flower parts and like the phenolic content the antioxidant activity values of different plant parts of *R. ponticum* L. were higher compared with that of *R. lutem* L. İn the present study it was determined that leaf and flower parts of two *Rhododendron* species could be diffrentiated regarding their total phenolic content and antioxidant activity.

Keywords: *Rhododendron, phenols, antioxidant activity*

INTRODUCTION

Rhododendron spp. (azaleas) are deciduous or evergreen shrubs commonly used as garden plants worldwide. According to Metcalfe and Chalk (1950) the Ericaceae, particularly *Rhododendron* L. are widely distributed, but numerous species are present especially in China and South Africa. *Rhododendron* species are generally distribu-ted in China, Tibet, Burma, Nepal, New Guinea, Tropical Asia, Europe and North America. There are about 700 species in these regions (Heywood, 1978; Davidian, 1989). Suzuki and Ohba (1988) stated that habitats of *Rhododendron* species show a wide range, from low montain forests to alpine regions more than 4000 m high. These species are usually shrubs and tall trees about 30 m high and 100 cm in diameter in Nepal.

In Turkey, *Rhododendrons* distributed from sea level to about 3200 m, are usually large shrubs (*R. ponticum* L., *R. ungernii* Trautv.), shrubs (*R. smirnowii* Trautv., *R. luteum* Sweet.) or dwarf shrubs (*R. caucasicum* Pallas). *R. luteum* is deciduous, and the others are evergreen shrubs. *R. caucasicum* is alpine type, and the others are forest type.

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Table 1: Collection definition of Rhododendron spp.

Species	Definition	Altitude	Analysed part
R. luteum	SK- 1 (L)	749 m	Leaf (L)
R. luteum	SK-1 (F)	749 m	Flower (F)
R. luteum	SK-2 (L)	968 m	Leaf (L)
R. luteum	SK-2 (F)	968 m	Flower (F)
R. luteum	SK-3 (L)	980 m	Leaf (L)
R.luteum	SK-3(F)	980 m	Flower (F)
R. luteum	SK-4 (L)	1062 m	Leaf (L)
R. luteum	SK-4 (F)	1062 m	Flower (F)
R. ponticum	MK-1 (L)	740 m	Leaf (L)
R. ponticum	MK-1 (F)	740 m	Flower (F)
R. ponticum	MK-2 (L)	980 m	Leaf (L)
R. ponticum	MK-2 (F)	980 m	Flower (F)
R. ponticum	MK-3 (L)	1107 m	Leaf (L)
R. ponticum	MK-3 (F)	1107 m	Flower (F)

All samples were collected from Çat district, Rize/Turkey; SK = Sarı Komar (*R. luteum*), MK = Mor Komar (*R. ponticum*)

Rhododendrons (*R. ponticum* L. and *R. luteum*) dominate the understories of the mesic environments of the Black Sea Region Forests in Turkey (Eşen *et al*., 2006). They are deciduous short trees with green leaves and have flowers of different colours and an aesthetically important role in landscape.

Six *Rhododendron* species, one of which (*R. smirnovii*) is endemic, grow naturally in Turkey, especially Northeastern Anatolia (Black Sea Region) (Stevens, 1978). Some members of this genus, such as *R. ponticum* and *R. luteum* are well known for being poisonous (Baytop, 1999; Onat *et al*. 1991; Sütlüpınar *et al*. 1993). The consumption of "mad honey" (deli bal in Turkish) produced from the nectar of these plants still causes intoxications in humans in the Eastern Black Sea Region of Turkey. Serious Rhododendron poisonings are also common in livestock, particularly in sheep and goats fed with the young leaves or flowers of these species (Baytop, 1999; Puschner *et al*., 2001). The toxic effects of these plants have been attributed to grayanine type tetracyclic diterpenes (grayanatoxins = andromedotoxins) that bind to sodium channels in cell membranes to increase the permeability of sodium ions in excitable membranes (Onat *et al*. 1991; Sütlüpınar *et al*. 1993). Interestingly, toxic *Rhododendron* species, particularly *R. ponticum*, are common folk medicines of the Black Sea Region. *R. ponticum* is widely used as analgesic for the treatment of rheumatic or dental pain, common colds and edema, both internally and externally (baytop, 1999). The other Turkish *Rhododendron* species have no reputation for being toxic. Instead, flowers of some species are eaten or their nectars are sucked by the local people (Stevens, 1978; Taşdemir et., 2003).

Specially, *R. luteum* and *R. ponticum* contain a poisonous principle, andromedotoxin, in their leaves and flowers. Sheep and goats fed on their young shoots or flowers are poisoned. A honey made up of their flowers is poisonous due to andromedotoxin. This honey which is locally known as mad honey (deli bal) has hypotensive properties. When consumed consciousness disorders similar to drunkennes are observed. Over dose is lethal. Leaves of *R. luteum* contain tanin, essential oil, erikolin, arbutin and andromedol derivatives. Although used as diuretic and analgesic in rheumatic pains its infusion can be dangerous due to andromedol derivatives (Alan et., 2010). A decoction of *R. luteum* and *R. ponticum* leaves is externally used to treat fungal foot infectious in Giresun province (Dereli, Çalca Eğriambar). *R. luteum* is also used as ornamental due to its showy flowers (Baytop, 1984; Baytop, 1991; Zeybek and Zeybek, 1994; Acartürk, 1997; Tuzlacı, 2006).

Many species of the genus *Rhododendron* contain a large number of phenolic compounds and antioxidant activities that could be developed into pharmaceutical products (Qiang *et al*., 2011). In addition, some members of the genus are already used in traditional medicine for several ailments, especially arthritis, acute and chronic bronchitis, asthma, pain, inflammation, rheumatism, hypertension, and muscle and metabolic diseases (Popescu and Kopp, 2013; Iwata *et al*., 2004; Li *et al*., 2011; Wu and li, 2011; Fu *et al*., 2012). The main aim of this study was to determine the total phenolic content and antioxidant activity of flower and leaf parts of *R. luteum* and *R. ponticum* collected from different altitudes in Çat/Rize.

MATERIALS AND METHODS

The research material were collected during 2016 in the Çat district of the Rize province (Turkey). Branches carrying leaves and flowers were cutted with pruning shears and leaves and flowers were separated thereafter. The information of investigated material is given in Table 1. The study area Çat Valley (Fig. 1) is located in the Kaçkar National Park of Rize at the Black Sea Region.

Chemical Analysis

The determination of metabolites and following statistics are as given below.

Fig. 1: Location of study area

Antioxidant activity

A modified version of the FRAP assay described by Izzreen and Fadezelly (2013) was used to determine the antioxidant activity of collected samples as mg FeSO4/gr DW.

For the determination of antioxidant content of the samples as pretreatment, 0.1 g of each dried sample was completed with methanol (80 %) to reach 10 ml volume. Samples were mixed first in the water bath (50 °C) for a duration of 20 minutes and and the samples were keep waiting after this procedure for 1 h in the dark. The mixture was centrifuged after that for a 20 min, 4000 cycle/min process for obtaining the extracts, which are used for the determination of phenolic content and antioxidant activity of the investigated samples.

Collected samples were analyzed regarding their antioxidant activity values. Green tea leaves were collectes at two shooting periods and the leaves were dried in the drying oven at 40 ^oC and its antioxidant activity was determined using the UV-spectrophotometer by the FRAP method. The determination of antioxidant capacity of investigated samples (pretreatments completed) was done using the FRAP method. The FRAP method bases on the colourization after the degradation of the Fe^{+3} ion, bounded to TPTZ in an acid environment, to Fe^{+2}. 300 mM acetate buffer (pH 3,6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3.6H_2O$ solutions were mixed at a proportion of 10:1:1 as FRAP (ferric reducing / antioxidant power) reactives to obtain a buffer solution. A $FeSO_4.H_2O$ solution was used to prepare different standart probes to obtain a calibration curve. The final samples were obtained with a mix of 1980 µl FRAP dispersive + 20 µl sample and keep waiting after that for 3 min n a ultrasonic shaker (50°C). The measurements were done using a UV Spectrophotometer device at a wave lenght of 595 nm to obtain the final absorbance values

Total Phenolic content

The total phenolic content of collected samples were determined using UV-Vis spectrophotometer as mg GAE/gr DW. The pretreatment of samples was the same as descryibed in the FRAP method. Gallic acid was used as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. Sample extract as 1/10 of the total volume and 300 µl Na_2CO_3 was added to tubes containing water involving Folin reagent and all tubes were keep waiting in a ultrasonic shaker (50°C) for 15 min. The measuremnet were done using a UV spectrophotometer device at a wawe lenght of 765 nm to obtain the absorbance values.

Statistical analysis

Principal component and Biplot analysis were used to distinguish the collected samples regarding analysed characteristics (Backhaus *et al*., 1989). Principal component and Biplot analysis was performed using XLSTAT2016 Trial Version. The principal components represent the axes which are the orthogonal projections for the alues representing the highest possible variances in the case of PC1 and PC2. The obtained data were used to create scatter plot diagrams. Therefore, a factor analysis was performed, whereby each variable was used to calculate relationships between sample and investigated trait. Based on the obtained data, the Biplot diagramme was created showing the relationship of investigated samples regarding to their chemical composition.

RESULTS AND DISCUSSION

The phenolic content of collected *R. luteum* leaves changed according to altitude. While phenolic content increased with altitude, leaves of *R. luteu*m collected from 980 m altitude displayed lower phenolic content. In general, phenolic contents of *R. ponticum* leaves were higher than *R. luteum* leaves (212,823 – 230,580 mg GAE/gr DW). Considering the phenolic content of *R. luteum* flowers, it changed depending on altitude (82,275 - 201,642 GAE/gr DW). In *R. ponticum*, the phenolic contents of flowers were again higher than *R. luteum*.

The antioxidant activity values of *R. luteum* leaves generally increased with altitude, exceptly *R. luteum*

Table 2: Total phenolic contents and antioxidant activity values of collected samples

Species	Definition	Altitude	Total phenolic content (mg GAE/gr DW)	Antioxidant activity (mg FSO4/gr DW)
R. luteum	SK- 1 (L)	749 m	188,228	22,191
R. luteum	SK-1 (F)	749 m	98,223	505,628
R. luteum	SK-2 (L)	968 m	196,874	42,400
R. luteum	SK-2 (F)	968 m	82,275	561,896
R. luteum	SK-3 (L)	980 m	112,363	1 6,990
R. luteum	SK-3(F)	980 m	146,398	201,300
R. luteum	SK-4 (L)	1062 m	219,071	463,624
R. luteum	SK-4 (F)	1062 m	201,642	768,744
R. ponticum	MK-1 (L)	740 m	230,580	933,192
R. ponticum	MK-1 (F)	740 m	213,974	1062,769
R. ponticum	MK-2 (L)	980 m	232,882	941,513
R. ponticum	MK-2 (F)	980 m	216,933	1061,184
R. ponticum	MK-3 (L)	1107 m	212,823	834,523
R. ponticum	MK-3 (F)	1107 m	185,201	826,598

As can be seen in Fig. 2 the investigated samples of *Rhododendron luteum* could be clearly seperated from the leave and flower samples of *Rhododendron ponticum*. Especially the higher total phenolic contents and higher antioxidant values of *Rhododendron ponticum* samples led to this clear diversification.

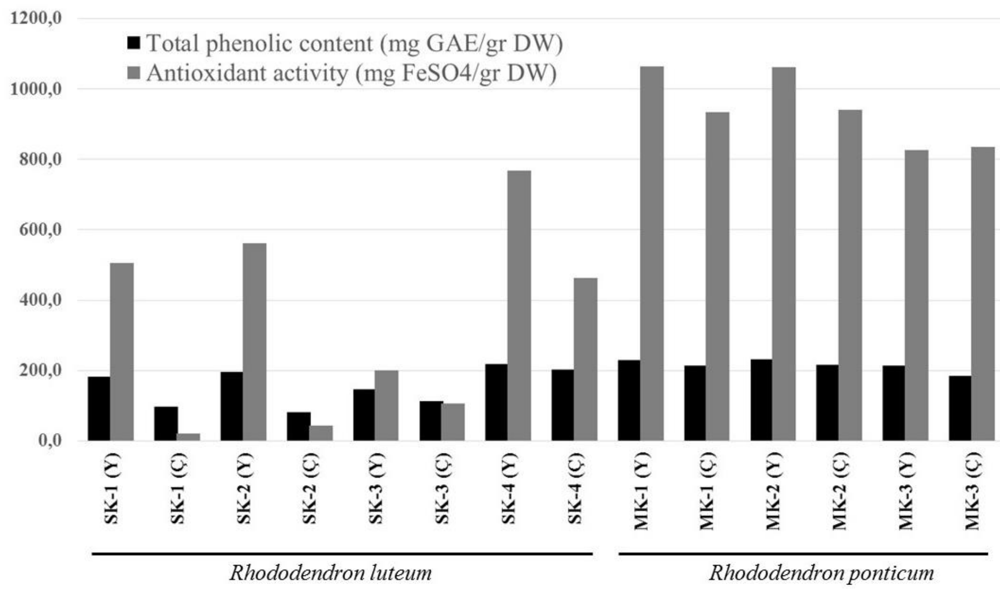


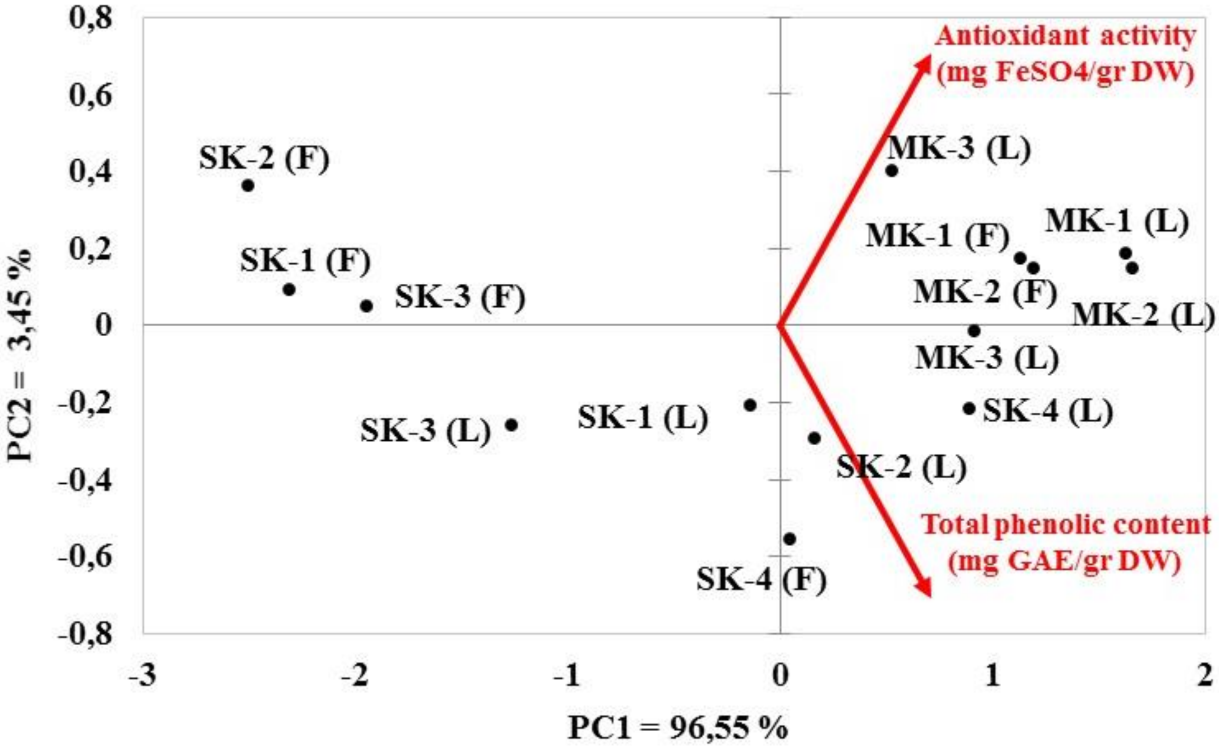
Fig. 2. Comparison of total phenolic contents and antioxidant activity values of collected samples

leaves collected from 980 m altitude. If we consider antioxidant activity of flowers, we can see the same situation (Table 2, Fig. 2). In *R. ponticum*, antioxidant activity values changed based on collection sites. Generally, antioxidant activity values of leaves were higher compared with flowers, exceptly *R. ponticum* leaves collected from 1107 altitude.

The obtained data was used to differentiate the collected material based on phenolic and antioxidant activity content (Fig. 3). The first two principal components corresponded to 100 % of the present variation regarding investigated traits in present study material. Specially, *R. ponticum* material differed clearly from *R. luteum* material regarding antioxidant activity and total phenolic content.

Malkoç *et al*. (2016) determined that in *R. ponticum* phenolic contents were higher in flowers compared with leaves, opposite results compared with our study. On the other side Bhandari and Rajbhandari (2014) investigated plant extracts from different parts of *R. arboreum* and stated that total phenolic content and antoioxidant activity values of investigated leaves were higher than flowers.

Environmental factors, such as soil composition, temperature, rainfall and ultraviolet radiation incidence can affect the concentrations of phenolic compounds (Monteiro *et al*., 2006). Among phenolic compounds, the tannins can be influenced by development of the plant and by environmental changes (Hatano *et al*., 1986; Salminen *et al*., 2001). Thus, phenolic compounds and others secondary metabolites represent a chemical interface between plants and environment (Gobbo-Neto and Lopes, 2007). Changes in phenols amounts influence directly the quality of the plant for medicinal application (Santos *et al*.,



SK-1 (L) =	*Rhododendron luteum* L.	749 m	MK-1 (L) =	*Rhododendron ponticum* L.	740 m
SK-1 (F) =	*Rhododendron luteum*	749 m	MK-1 (F) =	*Rhododendron ponticum* L.	740 m
SK-2 (L) =	*Rhododendron luteum*	968 m	MK-2 (L) =	*Rhododendron ponticum* L.	980 m
SK-2 (F) =	*Rhododendron luteum*	968 m	MK-2 (F) =	*Rhododendron ponticum* L.	980 m
SK-3 (L) =	*Rhododendron luteum*	980 m	MK-3 (L) =	*Rhododendron ponticum* L.	1107 m
SK-3 (F) =	*Rhododendron luteum*	980 m	MK-3 (F) =	*Rhododendron ponticum* L.	1107 m
SK-4 (L) =	*Rhododendron luteum*	1062 m			
SK-4 (F) =	*Rhododendron luteum*	1062 m			

Fig. 3: Biplot analysis of different parts of Rhododendron species based on their phenolic contents and antioxidant activity values

2006). Therefore, it is not surprising that the collected samples differed in their total phenolic content and antioxidant activity.

In folk medicine, the tincture prepared from Rhododendron plant leaves are used to treat gout, epilepsy, headaches, insomnia, intimate and diuretic conditions, rheumatism, dysentery, sharp and chronic colic. Broth prepared from rhododendron is recommended by folk medicine as a cure against syndrome of oxygen insufficiency. Also in general and in the study area present *Rhododendron* plants are present as deciduous short trees with green leaves and have flowers of different colours and an aesthetically important role in landscape.

In the present study only two species and two traits were analysed. Further studies should involve different species from different locations and determination of compounds which could be helpful in future studies.

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