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Research Article

Chemical content profile and antioxidant activity of *Rhododendron ponticum* L. (Ericaceae) extracts

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Abstract: *Rhododendron* species (Ericaceae) is considered the most diverse group, with more than 1200 species famous for their colorful flowers. *Rhododendron*, also known as azalea, is a flowering tree in the Ericaceae family. Herein, *in vitro* antioxidant activities of acetone and methanol extracts of *Rhododendron ponticum* leaves were studied along with their phenolic contents using High-Performance Liquid Chromatography with a Diode-Array Detector (HPLC-DAD). Antioxidant activity was performed spectrophotometrically using $ABTS^{\cdot+}$, DPPH·, CUPRAC, and *β*-carotene/linoleic acid assays. Acetone extract showed better antioxidant activity than methanol extract in all tests. The HPLC-DAD analysis revealed fifteen phenolic compounds, of which seven were common for both extracts. Catechin (25.80 and 33.08 mg/g extract, respectively) and epicatechin (31.15 and 26.54 mg/g extract, respectively) were calculated as major phenolic components in acetone and methanol extracts.

1. INTRODUCTION

Medicinal plants are the richest biological source of drugs for traditional medicine systems, modern drugs applied in clinical therapy, nutraceuticals, food supplements, folk remedies, cosmetics, nutricosmetics, pharmaceutical intermediates, and chemical assets for synthetic drugs. One of the essential areas in which developed countries contribute to the economy is the bulk trade of medicinal and aromatic plants. The first and most crucial step in adding value to natural products is the production of herbal medicine extracts using various methods, from traditional approaches to advanced technological extraction techniques. Together with the increasing demand for natural products that have medicinal properties for health all over the world, manufacturers of medicinal plant extracts have started to use the most appropriate extraction technologies to produce extracts of defined quality.

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In traditional medicines used around the world, different *Rhododendron* species are also known to have applications against various inflammatory conditions and pain (Chosson *et al*., 1998; Baytop, 1999; Li *et al*., 2000; Erdemoğlu *et al*., 2008). *Rhododendron ponticum* L., which is distributed in the northern parts of Türkiye, is a large evergreen deciduous shrub. It is a member of the Ericaceae family. There are five Rhododendron species and four hybrids in the Flora of Türkiye (Stevens, 1978; Sales and Milne, 2000). The sap obtained from the freshly cut branch of *R. ponticum* is used in Turkish folk medicine by dripping into the tooth cavity against toothache. In addition, after the plant's fresh leaves are dried in the fire, it is applied externally to the affected area for the treatment of back pain or joint edema. (Yeşilada *et al*., 1999). Oral treatment of 2% leaf infusion of *R. ponticum* alleviates rheumatic pains (Baytop, 1999). It has been reported that *Rhododendron* species exhibit many biological activities such as anti-inflammatory, antidiabetic, analgesic, antimicrobial, insecticidal and antioxidant upon its plant extracts and pure components (Oztasan *et al*., 2005; Erdemoğlu *et al*., 2008; Prakash *et al*., 2008; Jing *et al*., 2009; Silici *et al*., 2010; Yarlıoğlues *et al*., 2011).

It will direct our country, which is rich in medicinal and aromatic plants, to domestic production in the world health sector by contributing to the country's economy by encouraging the local people to grow medicinal and aromatic plants by bringing the plants whose extracts have been identified to be usable in the health sector to a better position in the country. In many studies, antioxidants can prevent or delay the oxidation of an oxidizable substrate in a chain reaction. Hence, they have a significant place in preventing many diseases. Antioxidant supplements, which have the possible potential to fight conditions linked to oxygen species by scavenging free radicals, are such as nutraceuticals, medicinal drinks, and syrup.

The antioxidant activities of phenolic compounds are associated with several different mechanisms, including free radical inhibition, hydrogen transfer, singlet oxygen removal, and metal ion chelation, and they act as a substrate for radicals such as superoxide and hydroxyl. A direct relationship was found between the phenolic content of plants and their antioxidant capacity (Robards *et al*., 1999; Al-Mamary *et al*., 2002; Caliskan *et al*., 2022).

In this study, phenolic component analyses of the acetone and methanol extracts of *R. ponticum* leaves were screened against 42 reference materials using the HPLC-DAD instrument. Acetone and methanol extracts of *R. ponticum* leaves were also tested for antioxidants using four complementary methods (*β*-carotene/linoleic acid, $DPPH^{\bullet}$ scavenging, $ABTS^{\bullet+}$ scavenging, CUPRAC reducing assays). The relationship between phenolic contents and antioxidant activity between *R. ponticum* methanol and acetone extracts was investigated.

2. MATERIAL and METHODS

2.1. Plant Material Collection and Extraction

R. ponticum was obtained from Trabzon Akçaabat in September 2020 and compared with herbarium samples. *R. ponticum* leaves were dried in the laboratory under shade. Acetone and methanol extracts of *R. ponticum* were obtained using the maceration technique. Then, the solvents were removed using a rotary evaporator under vacuum to obtain crude extracts. The visual of *R. ponticum* is given in Figure 1.

Figure 1. The visual of *R. ponticum*

2.2. Chemical Content

2.2.1. *Determination of phenolic profiles by HPLC-DAD*

2.2.1.1. Preparation samples for HPLC-DAD analysis: 8 mg extracts dissolved in 1 mL methanol and was homogenized in an ultrasonic bath at 20°C for 5 min and filtered through 0.45 µm PTFE filters.

2.2.1.2. HPLC-DAD analysis: The phenolic profile of *R. ponticum* leaf extracts was screened with a modification of the method described by Tokul-Ölmez et al. 2020. In this study, methanol and acetone extracts were investigated against 42 standard compounds (fumaric acid, gallic acid, protocatechuic acid, theobromine, theophylline, catechin, 4-hydroxy benzoic acid, 6,7-dihydroxycoumarine, methyl-1,4-benzoquinone, vanillic acid, caffeic acid, vanillin, chlorogenic acid, *p*-coumaric acid, ferrulic acid, cynarin, coumarine, prophylgallate, rutin, *trans*-2-hydroxycinnamic acid, ellagic acid, myricetin, fisetin, quercetin, *trans*-cinnamic acid, luteoline, rosmarinic acid, kaempherol, apigenin, chrysin, 4-hydroxy resorcinol, 1,4-dichlorobenzene, pyrocatechol, 4-hydroxybenzaldehyde, epicatechin, 2,4-dihydroxybenzaldehyde, hesperedin, oleuropein, naringenin, hesperetin, genistein, curcumin) using a Shimadzu high-performance liquid chromatography (Shimadzu Cooperation, Japan) system that consists of a Shimadzu model LC-20AT. The column temperature was set at 35 ºC. The chromatographic separation was performed on a C_{18} (5 µm, 4.6 mm x 250 mm) reverse phase column and an Inertsil C_{18} guard column (Tokul-Ölmez *et al*., 2020).

2.3. *Antioxidant activity*

2.3.1. *Determination of the antioxidant activity with the β-carotene bleaching method*

The antioxidant activity of *R. ponticum* leaf extracts was evaluated using a *β*-carotene-linoleic acid assay (Miller, 1971). *β*-carotene (0.5 mg) in 1 mL of $CHCl_3$ was added to 25 µL of linoleic acid and 200 mg of Tween 40. As soon as $CHCl_3$ was evaporated under vacuum, distilled H_2O saturated with O_2 was added and shaken vigorously. 160 µL of this prepared mixture was added to 40 µL of extracts at different concentrations in a 96-well plate. After adding the emulsion to each, the zero-time absorbance was measured at 470 nm. The mixture was incubated for two hours at 37 °C. A blank, devoid of *β*-carotene, was prepared for background subtraction. BHT and α-TOC were used as standards.

2.3.2. *DPPH• scavenging activity assay*

The DPPH• scavenging activity of *R. ponticum* leaf extracts was determined according to Blois (1958). Briefly, 160 mL of 0.1 mM DPPH, prepared in MeOH, was added to 40 mL of extract solutions in MeOH at various concentrations. The absorbance of each extract was measured at 517 nm after 30 min.

2.3.3. *$ABTS^{+\bullet}$ scavenging activity assay*

$ABTS^{+\bullet}$ scavenging activities assay was used to determine the analysis samples (Re *et al.*, 1999). In this method, the lightening of the $ABTS^{+\bullet}$ solution was measured. Before using the $ABTS^{+\bullet}$ solution, it was diluted with EtOH. 160 µL of $ABTS^{+\bullet}$ solution was added to 40 µL of extract solution at different concentrations in a 96-well microplate. Then, the mixture was incubated for 10 min at 20°C. The absorbance of extracts was measured at 734 nm.

2.3.4. *Cupric reducing antioxidant capacity (CUPRAC)*

The cupric-reducing antioxidant capacity of *R. ponticum* leaves extracts was determined according to the method described by Apak *et al.* (2004). To each well, 40 µL extract in various concentrations, aqueous solutions including 50 µL $CuCl_2.2H_2O$ (10 mM), 50 µL neocuproine (7.5 mM), and 60 µL NH_4Ac buffer (1 M, pH 7.0) were added. After one hour, the absorbances of the extracts were measured. BHT and α-TOC were used as antioxidant standards to compare the activity results. The results were given as $A_{0.5,}$ which corresponded to the concentration versus 0.500 absorbance.

3. RESULTS

The HPLC-DAD results of acetone and methanol extracts of *R. ponticum* leaves are given in Table 1. According to the results, 14 phenolic compounds, namely, epicatechin (31.15 µg/mL), catechin (25.80 µg/mL), myricetin (8.02 µg/mL), pyrocatechol (5.77 µg/mL), rutin (5.49 µg/mL), theobromine (5.21 µg/mL), *p*-coumaric acid (4.87 µg/mL), 4-hydroxy benzaldehyde (3.08 µg/mL), fisetin (2.48 µg/mL), taxifolin (2.29 µg/mL), theophylline (1.63 µg/mL), fumaric acid (1.53 µg/mL), gallic acid (0.61 µg/mL), genistein (0.34 µg/mL) were elucidated in acetone extract. Among them, the major phenolics quantified were epicatechin (31.15 µg/mL) and catechin (25.80 µg/mL).

Table 1. Phenolic component analysis results of *R. ponticum* extracts with the HPLC-DAD (mg/g).

Phenolic compound	RT (min)	Acetone extract	Methanol extract
Fumaric acid	14.014	1.53± 0.04	-
Gallic acid	15.225	0.61±0.01	0.92±0.02
Pyrocatechol	24.658	5.77±0.14	7.69±0.19
Theobromine	25.967	5.21±0.13	-
Theophylline	29.449	1.63±0.04	-
Catechin	30.274	25.80±0.64	33.08±0.82
4-hydroxy benzaldehyde	33.367	3.08±0.08	-
Epicatechin	35.278	31.15±0.78	26.54±0.66
p-coumaric acid	40.874	4.87±0.12	2.02±0.05
Taxifolin	41.200	2.29±0.06	-
Rutin	47.527	5.49±0,14	4.44±0.11
Myricetin	50.368	8.02±0.20	6.13±0.15
Fisetin	51.243	2.48±0.06	-
Genistein	57.739	0.34±0.01	-
Luteolin	57.872	-	0.24±0.01

In methanol extract, however, eight phenolic compounds, namely, catechin (33.08 µg/mL), epicatechin (26.54 µg/mL), pyrocatechol (7.69 µg/mL), myricetin (6.13 µg/mL), rutin (4.44 µg/mL), *p*-coumaric acid (2.02 µg/mL), gallic acid (0.92 µg/mL), luteolin (0.24 µg/mL) were elucidated. Methanol extract of catechin (33.08 µg/mL) and epicatechin (26.54 µg/mL) were also quantified as major phenolics.

The antioxidant activity results of extracts are given in Table 2. The acetone extract (IC_{50}: 1.29±0.76 µg/mL) exhibited higher lipid peroxidation activity than the methanol extract. It also demonstrated higher activity than *α*-Tocopherol (IC_{50}: 4.50±0.09 µg/mL) and BHT (IC_{50}: 2.34±0.09 µg/mL). The methanol extract also indicated higher lipid peroxidation inhibitory activity (IC_{50}: 4.64±0.83 µg/mL), which is comparable with *α*-tocopherol and BHT.

The $DPPH^{\bullet}$ scavenging activity of acetone and methanol extracts are compared with *α*-Tocopherol (IC_{50}: 12.26±0.07 µg/mL) and BHT (IC_{50}: 54.97±0.99 µg/mL) (Table 2). The acetone extract (IC_{50}: 10.21±0.56 µg/mL) exhibited excellent radical scavenging activity. On the other hand, the methanol extract (IC_{50}: 19.03±0.09 µg/mL) also demonstrated superior activity. Similarly, the $ABTS^{+\bullet}$ scavenging activity of acetone. (IC_{50}: 2.11±0.22 µg/mL) and methanol (IC_{50}: 2.67±0.64 µg/mL) extracts indicated excellent cation radical scavenging activity. In the same conditions, *α*-Tocopherol and BHT demonstrated4.87±0.45µg/mL and IC_{50}: 2.91±0.55 µg/mL IC_{50} values. The CUPRAC assay also provided results similar to those of the $ABTS^{+\bullet}$ and $DPPH^{\bullet}$ assays. The acetone extract ($A_{0.5}$: 9.34±0.00 µg/mL) also exhibited higher activity than the methanol extract ($A_{0.5}$: 10.28±0.01 µg/mL). Both extracts competed with the positive standards BHT ($A_{0.5}$: 4.00±0.04 µg/mL) and α-Tocopherol ($A_{0.5}$: 25.55±0.04 µg/mL).

Table 2. Antioxidant activity of *R. ponticum* extracts.

Extract	*β*-carotene/linoleic acid assay IC_{50} (µg/mL)	$DPPH^{\bullet}$ assay IC_{50} (µg/mL)	$ABTS^{+\bullet}$ assay IC_{50} (µg/mL)	CUPRAC assay $A_{0.5}$ (µg/mL)
Methanol	4.64±0.83	19.03±0.09	2.67±0.64	10.28±0.01
Acetone	1.29±0.76	10.21±0.56	2.11±0.22	9.34±0.00
α-TOC*	4.50±0.09	12.26±0.07	4.87±0.45	25.55±0.04
BHT*	2.34±0.09	54.97±0.99	2.91±0.55	4.00±0.04

*α-TOC: *α*-tocopherol and *BHT: butylated hydroxytoluene were used as standards.
* Values expressed are the mean ± SEM of three parallel measurements (p<0.05).

4. DISCUSSION and CONCLUSION

In our study, the phenolic profile and antioxidant activity of the acetone and methanol extracts of the leaves of *R. ponticum* collected from Trabzon Akçaabat, Türkiye were studied. Fourteen phenolic compounds were detected in the acetone extract, while eight were in the methanol extract. Considering the phenolic contents of both extracts, catechin and epicatechin were major compounds. According to the antioxidant activity test results, acetone extract showed excellent activity, which is superior to positive standards and methanol extract in all antioxidant assays. The higher amount of phenolic compounds in acetone extract is responsible for the activity. This study also reveals that *Rhododendron ponticum* contains more amount of catechin and epicatechin. In order to make maximum use of the rich catechin and epicatechin content of *R. ponticum* in the food and pharmaceutical industries, further studies are required to optimize these compounds.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Yusuf Sıcak: Investigation, Finding Materials, Extraction and Writing. **İrfan Öztürk**: Investigation, Activity experiments. **Bihter Şahin**: Interpretation of Results, Writing. **Dilaycan Çam**: Extraction, Activity Experiments. **Cansel Çakır**: Extraction, Activity Experiments. **Mehmet Öztürk**: Supervision, Interpretation of Results.

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REFERENCES

Al-Mamary, M., Al-Meeri, A., & Al-Habori, M. (2002). Antioxidant activities and total phenolics of different types of honey. *Nutrition Research*, *22*, 1041-1047.

Apak, R., Güçlü, K., Özyürek, M., & Karademir, S.E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method. *Journal of Agricultural and Food Chemistry*, *52*, 7970-7981.

Baytop, T. (1999). *Therapy with Medicinal Plants in Turkey: Past and Present (Second edition)*. Nobel Tıp Kitabevleri.

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*, 1199-1200.

Caliskan, H., Argon, M., & Sabudak, T. (2022). The genus Isatis L.: A review on its flavonoid and phenolic compound profile. *Records of Agricultural and Food Chemistry*, *2*(2), 75-83.

Chosson, E., Chaboud, A., Chulia, A.J., & Raynoud, J. (1998). Dihydroflavonol glycosides from *Rhododendron ferrugineum*. *Phytochemistry*, *49*, 1431-1433.

Erdemoglu, N., Akkol, E. K., Yesilada, E., & Calış, I. (2008). Bioassay-guided isolation of anti-inflammatory and antinociceptive principles from a folk remedy, *Rhododendron ponticum* L. leaves. *Journal of Ethnopharmacology*, *119*(1), 172-178.

Jing, X., Zhonghua, Z., Jianshe, L., & Yang, W. (2009). The effect of root of Rhododendron on the activation of NF-κ B in a chronic glomerulonephritis rat model. *Journal of Nanjing Medical University*, *23*, 73.

Li, C.-J., Wang, L.-Q., Chen, S.-N., & Qin, G.-W. (2000). Diterpenoids from the fruits of *Rhododendron molle*. *Journal of Natural Products*, *63*, 1214-1217.

Mahomoodally, M.F., Sieniawska, E., Sinan, K.I., Nancy, Picot-Allain, M.C., Yerlikaya, S., Cengiz Baloglu, M., & Zengin, G. (2019). Utilisation of *Rhododendron luteum* Sweet bioactive compounds as valuable source of enzymes inhibitors, antioxidant, and anticancer agents. *Food and Chemical Toxicology*, 111052.

Miller, H.M. (1971). A simplified method for the evaluation of antioxidants. *Journal of the American Oil Chemists Society*, 48, 91.

Oztasan, N., Altinkaynak, K., Akcay, F., Gocer, F., & Dane, S. (2005). Effect of mad honey on blood glucose and lipid levels in rats with streptozocin-induced diabetes. *Turkish Journal of Veterinary and Animal Science*, *29*, 1093-1096.

Prakash, T.F., Snehal, D.S., Uday R.S., & Diyakar. (2008). Hepatoprotective activity of leaves of *Rhododendron arboreum* in CCl_4 induced hepatotoxicity in rats. *Journal of Medical Research*, *2*(11), 315-320.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*, 1231-1237.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*, *66*, 401-436.

Sales, F., & Milne, R. Güner, A., Özhatay, N., Ekim, T., & Başer, K.H.C. (Eds.) (2000). Rhododendron L. *Flora of Turkey and the East Aegean Islands*, University Press, Edinburgh, 11, 181-183.

Shrestha, A., Hakeem Said, I., Grimbs, A., Thielen, N., Lansing, L., Schepker, H., & Kuhnert, N. (2017). Determination of hydroxycinnamic acids present in *Rhododendron* species. *Phytochemistry*, 144, 216-225.

Silici, S., Sagdic O., & Ekici, L. (2010). Total phenolic content, antiradical, antioxidant, and antimicrobial activities of *Rhododendron* honeys. *Food Chemistry*, *121*(1), 238-243.

Stevens, P., & Fernandez, J.W. (1978). Fang Architectonics. *African Arts*, *11*(2), 90-92. https://doi.org/10.2307/3335466

Tokul Ölmez, Ö., Şahin, B., Çakır, C., & Öztürk, M. (2020). Rapid and easy method for simultaneous measurement of widespread 27 compounds in natural products and foods. *Journal of Chemical Metrology*, *14*(1), 1-11.

Yarlioglues, M., Akpek, M., Ardic, I., Elcik, D., Sahin, O., & Kaya, M.G. (2011). Mad-honey sexual activity and acute interior myocardial infarctions in a married couple. *Texas Heart Institute Journal*, *38*, 577-580.

Yeşil, T., & Akgül, Y. (2022). Major components of *Rhododendron luteum* leaves. *Natural Product Research*, *37*(15), 2608-2612. https://doi.org/10.1080/14786419.2022.2055015