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Study of Phenolic Compounds of *Rhododendron ponticum* Distributed in Adjara

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Abstract: *Rhododendron ponticum L.* represents an important relict species within the flora of Georgia, including the Adjara region, and is characterized by significant ecological value and a rich composition of biologically active metabolites. According to the available scientific literature, this species is particularly rich in polyphenolic compounds, especially flavanol-type catechins, which are widely recognized for their pronounced antioxidant, antimicrobial, and cytoprotective properties. Nevertheless, the phenolic composition and quantitative distribution of phenolic metabolites in *R. ponticum* populations growing in Georgia remain insufficiently investigated.He present study aimed to identify and characterize the phenolic compounds present in the leaves of R. ponticum distributed in Georgia using qualitative and quantitative analytical approaches. The conducted analysis revealed the presence of several major phenolic constituents. Gallocatechin was detected, demonstrating the strong antioxidant potential of the species. In addition, catechin dimers, namely proanthocyanidins (Procyanidin B1, B2, and B3), were identified, which are known to contribute significantly to free radical scavenging mechanisms.Furthermore, several other phenolic metabolites were identified, including gallic acid, quercetin-3-O-glucoside (isoquercitrin), quercetin-3-O-rutinoside (rutin), kaempferol-3-O-glucoside, and kaempferol-3-O-rutinoside. The obtained findings confirm that R. ponticum growing in Georgia represents a valuable natural source of phenolic compounds and demonstrates considerable potential for further pharmacological and biochemical investigations.

Keywords: *Rhododendron ponticum*; Polyphenols; Proanthocyanidins; Antioxidant activity.

Introduction

Rhododendron ponticum L. belongs to the family (Ericaceae) and is a widely distributed evergreen plant known for both its ornamental and medicinal importance. The genus *Rhododendron* includes more than 1,200 species and is characterized by a rich phytochemical composition, which determines its diverse biological activity (Kumar, V., 2018). In traditional medicine, the leaves of *R. ponticum* are used to treat inflammatory processes, rheumatic pain, and toothache, indicating its potential pharmacological effects (Erdemoglu, 2019).

In this species, multiple groups of bioactive compounds have been identified, including phenolic acids, flavonoids, tannins, and diterpenes, which are responsible for its antioxidant, antimicrobial, and anti-inflammatory properties (Gürbüz et al., 2021; Kula et al., 2022). Phenolic compounds are particularly important, as they play a significant role in reducing oxidative stress and are actively used in the production of functional foods and pharmaceutical preparations (Shahidi & Yeo, 2018).

The isolation of bioactive components and the evaluation of their biological activity yield significant results. Phytochemical studies show that *R. ponticum* contains numerous bioactive compounds, especially phenolic compounds and flavonoids. Based on HPLC-DAD analysis, at least 15 phenolic components have been identified, among which catechin and epicatechin are predominant (Sıcak, Y., 2024). These compounds are known for their strong antioxidant properties and play an important role in neutralizing free radicals. In addition, phenolic acids (gallic, ferulic, caffeic), flavonoids (quercetin, apigenin), and terpenoids have been identified in other *Rhododendron* species, indicating a general biochemical similarity within the genus (Shootha, D., 2022). *Rhododendron ponticum* is distinguished by a high content of flavonoids and phenolic compounds, which constitute the main group of bioactive components in this plant. Studies have confirmed the presence of compounds such as quercetin, kaempferol, catechins and chlorogenic acid, which are characterized by strong antioxidant activity and are found in the leaves and flowers of the plant (Demir et al., 2019; Popescu et al., 2020). In addition to simple phenolic compounds, *R. ponticum* contains complex flavonoid compounds that contribute to its anti-inflammatory and antimicrobial activity (Gürbüz et al., 2021; Kula et al., 2022). A high concentration of phenolic compounds is also associated with the plant's adaptive ability under environmental stress conditions, particularly in regions with high humidity such as Western Georgia (Kandelaki, M., 2021).

Catechins in *Rhododendron ponticum* represent an important group of flavonoid compounds found mainly in the leaves and flowers. They are characterized by strong antioxidant activity and play a significant role in neutralizing free radicals and protecting the plant from oxidative stress. Catechins are also associated with antimicrobial and anti-inflammatory effects, which determine the biological activity of this species (Demir et al., 2019; Gürbüz et al., 2021). Various forms of catechins have been identified in the leaves of *Rhododendron ponticum*, including epicatechin.

Phenolic carboxylic acids represent one of the important groups of bioactive components in *Rhododendron ponticum*. Various studies have shown that chlorogenic acid, caffeic acid, and gallic acid are present in the leaves and flowers of the plant, all of which are distinguished by strong antioxidant activity (Demir et al., 2019; Popescu et al., 2020).

These compounds effectively neutralize free radicals and protect cells from oxidative damage, playing an important role in the prevention of chronic diseases (Shahidi & Yeo, 2018).Over the past decade, research has increasingly focused on the detailed analysis of the phenolic profile of *Rhododendron ponticum*, highlighting its potential as an alternative source of natural antioxidants (Demir et al., 2019). At the same time, special attention is paid to the variability of its chemical composition under the influence of ecological factors such as climate, soil type, and altitude above sea level, which determine the quantitative and qualitative characteristics of bioactive substances (Popescu et al., 2020).

Despite existing studies, the phenolic composition of *Rhododendron ponticum* and its variability under the ecological conditions of the Adjara region have not yet been sufficiently studied. This issue is particularly relevant, as Adjara is characterized by diverse climatic and geographical conditions, which may significantly affect the profile of bioactive compounds in the plant. Therefore, the present topic is important both from a fundamental scientific perspective and from a practical point of view, in terms of identifying new local sources of natural antioxidants and their rational use.

Plant Material and Sample Preparation

The object of the study was rhododendron leaves (*Rhododendron ponticum L.*). The samples were collected in Western Georgia, specifically in the Adjara region, from three different locations: Keda Municipality, village Tskhmorisi (41°38′20″ N, 42°02′47″ E), at an altitude of 532 m above sea level; Keda Municipality, village Kharaula (41°36′00″ N, 41°58′00″ E), at an altitude of 880 m above sea level; and Machakhela Valley (41°31′32″ N, 41°44′51″ E), at an altitude of 350 m above sea level.

Figure 1. *Rhododendron ponticum L.*

For the extraction of rhododendron samples, 50 g of sample was taken; 20% ethanol was used as the extractant. The sample was homogenized (1–2 min) using a homogenizer, then placed in an ultrasonic bath for 30 minutes, and finally filtered under vacuum conditions. This process was repeated until the solvent became colorless (complete extraction).

Research Methods

Quantitative determination of total phenols - by the Folin–Ciocalteu method (recalculated to chlorogenic acid). For the determination of total phenolic compounds, a spectrophotometric method using the Folin Ciocalteu reagent was applied. To 1 mL of plant extract, 1 mL of Folin–Ciocalteu reagent was added; after 3 minutes, 1 mL of 10% Na_2CO_3 was added, and the volume was adjusted to 10 mL with water. After 90 minutes, the optical density was measured at 725 nm. The obtained results are expressed as chlorogenic acid equivalents - mg/kg (Datuashvili, 2025).

Quantitative determination of catechins - by a spectrophotometric method: Extraction of the sample taken for analysis was carried out using 80% ethyl alcohol at a temperature of 70–75°C. From the total volume of the extract, 1 mL was taken, to which 3 mL of vanillin reagent was added, and after 3 minutes, the optical density of the red-colored sample was measured at 500 nm. As a control, 1 mL of the corresponding extractant and 3 mL of vanillin reagent were used. The obtained data were recalculated using a (+)-catechin calibration curve (Putkaradze, 2026).

Determination of antioxidant activity by the DPPH method: To determine total antioxidant activity, reactions proceeding via a radical mechanism are used, between a colored radical and an extract possessing antioxidant activity. The optical density of the solution is measured spectrophotometrically, allowing the evaluation of both individual substances and the total antioxidant activity of compounds. DPPH ($C_{18}H_{12}N_5O_6$, M = 394.33) is a free radical with maximum absorption at 515–517 nm. To determine antioxidant activity (radical scavenging activity), 3 mL of alcoholic DPPH solution was added to 1 mL of the analyzed extract, and after 30 minutes, the optical density of the test sample was measured at 515 nm. The control solution was 70% ethyl alcohol. The inhibition of free radical (DPPH) activity is calculated using the following formula: In% = (AC − AS) / AC × 100 (Datuashvili, 2025).

Identification of Compounds by Ultra-High-Performance Liquid Chromatography with a Mass Spectrometric Detector (UPLC-MS-PDA)

Identification of compounds by ultra-high-performance liquid chromatography with a mass spectrometric detector (UPLC-MS-PDA). For chromatographic analysis, the liquids obtained as a result of extraction were used. Before chromatography, the sample was prepared: from the total volume, 20 mL was taken for quantitative analysis, and the remaining part (150 mL) was concentrated. The obtained concentrate was filtered through a 0.45 µm membrane filter and transferred into so-called vials. For the identification and quantitative determination of catechins and other phenolic compounds, an ultra-high-performance liquid chromatography system Waters ACQUITY UPLC with a mass spectrometric detector (QDa, ESI) was used. Chromatographic analysis was performed on an ACQUITY BEH C18 column (2.1 × 100 mm, 1.7 µm). The mobile phase consisted of: eluent A – 0.1% formic acid in water, and eluent B – acetonitrile. Gradient elution was applied under the following conditions: 0–2.0 min – 5% B; 2.0–8.0 min – 20% B; 8.0–10.0 min – 35% B; 10.0–11.0 min – 95% B; 12.0 min – 5% B; 15.0 min – 5% B. The flow rate was 0.3 mL/min, the injection volume was 2 µL, and the column temperature was 40°C. Mass spectrometric detection was carried out in negative ionization

mode (ESI^-). The applied parameters were: capillary voltage – 0.8 kV, cone voltage – 15 V, source temperature – 120°C, desolvation temperature – 500°C. Data acquisition was performed in SIR mode, with a scanning range of m/z 100–1000. For quantitative determination, a calibration curve with 5–7 points in the concentration range of 0.1–100 µg/mL was used, with a correlation coefficient ($R^2 \geq 0.995$).

Results and Discussion

Table 1. Quantitative determination of biologically active compounds of rhododendron and evaluation of their antioxidant activity.

Sampling collection site	Extraction method	Phenols (mg/g dry weight)	Catechins (mg/g dry weight	Amount of sample (mg) required for 50% inhibition of 0.1 Mm DPPH
	US bath	88,92	12,84	20,56
Tskhmorisi	US probe	59,65	8,00	41,23
Kharaula	US probe	81,12	18,00	19,65
	US bath	100,58	8,97	20,56
Machakhela	US probe	120,98	19,68	6,59

According to the research results, the total phenolic content in *Rhododendron ponticum* samples is relatively higher at the Machakhela location, particularly in the extract obtained by the US probe method (120.98 mg/g), while in other samples it ranges between 59.65–100.58 mg/g. The catechin content is also highest in the Machakhela sample (US probe) - 19.68 mg/g, followed by Kharaula - 18.00 mg/g, while in Tskhmorisi samples it is 12.84 mg/g (US bath) and 8.00 mg/g (US probe). According to antioxidant activity (IC_{50}), the highest activity was observed in the Machakhela sample (US probe) - 6.59 mg, while in other samples the values range between 19.65-41.23 mg, with the lowest activity recorded in the Tskhmorisi sample (US probe) - 41.23 mg. The obtained results indicate that Machakhela samples, particularly in the case of US probe extraction, are distinguished by both a high content of phenolic compounds and strong antioxidant activity.

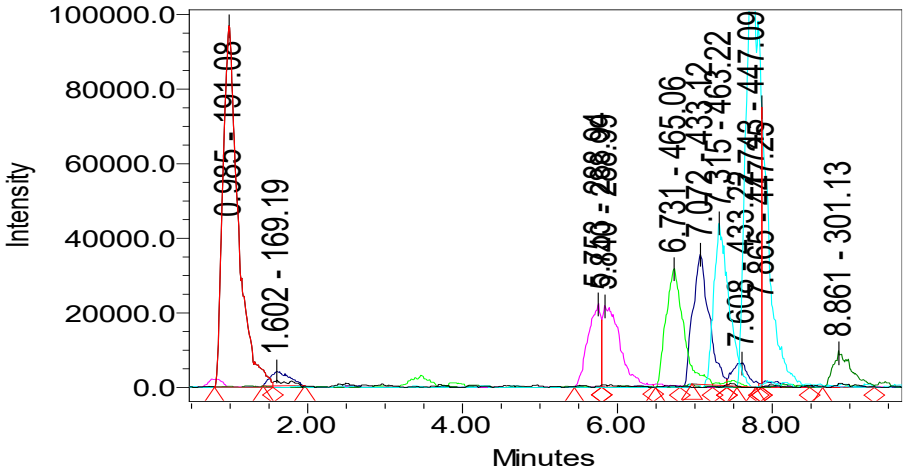

Figure 2. Chromatographic profile of phenolic compounds of rhododendron (*Rhododendron ponticum* L.) leaf extract (UPLC-MS analysis)

Table 1. LC–ESI–MS Markers – *Rhododendron ponticum L.*

Compound	RT (min)	m/z (ESI−) $[M-H]^-$	Fragments (MS/MS)	Compound Class
Quinic acid	0.985	191.08	—	Phenolic acid
Gallic acid	1.566	169	125	Phenolic acid
Quercetin-3-O-glucoside (Isoquercitrin)	7.269	463	301	Flavonol glycoside
Quercetin	1.098	301	179, 151	Flavonol aglycone
Kaempferol-3-O-glucoside	7.676	447	285	Flavonol glycoside
Catechin	5.897	289	245, 205	Flavan-3-ols
Epicatechin	6.161	289	179, 576	Flavan-3-ols
Procyanidin B-type	5.531	577	425, 289	Dimers

As a result of the study, quinic acid was identified in *Rhododendron ponticum* $[M-H]^-$ m/z 191.08 (RT 0.985 min). In addition, catechin dimers, proanthocyanidins (Procyanidin B1, B2, B3) $[M-H]^-$ m/z 577 (RT 5.531

min) were detected. The following compounds were also identified: gallic acid $[M–H]^-$ m/z 169 (RT 1.566 min); quercetin-3-O-glucoside (isoquercitrin) $[M–H]^-$ m/z 301 (RT 1.098 min); quercetin $[M–H]^-$ m/z 191.08 (RT 0.985 min); kaempferol-3-O-glucoside $[M–H]^-$ m/z 447 (RT 7.676 min). Among flavanol compounds, catechin $[M–H]^-$ m/z 289 (RT 5.897 min) and epicatechin $[M–H]^-$ m/z 289 (RT 6.161 min) were detected, which indicates the high antioxidant activity of the plant.

Conclusion

As a result of the study, a high concentration of phenolic compounds was detected in the leaves of *Rhododendron ponticum L.,* distributed in Adjara, which emphasizes the significant biological activity of this endemic species. Analysis conducted by ultra-high performance liquid chromatography with mass spectrometry detector (UPLC-MS-PDA) revealed phenolic compounds that provide high antioxidant activity of the plant, which is confirmed by the evaluation of DPPH radical inhibition and also demonstrates its potential as a source of natural antioxidants and a regenerative resource of bioactive components. The data presented in the table show that flavonol compounds (quercetin, kaempferol, catechins) and proanthocyanidins play an important role in the neutralization of free radicals, which emphasizes the potential of R. ponticum for medicinal and nutraceutical purposes.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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