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Pharmacochemistry

Biologically Active Polyphenols from Leaves and Grape Pomace of Georgian Grapevine Cultivars

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Abstract. Winery by-products represent a potential source of bioactive polyphenols. In this study, polyphenol-rich extracts were obtained from grape pomace and leaves of two indigenous Georgian white cultivars, Mtsvane Kakhuri and Khikhvi; and their phenolic composition and biological activities were evaluated. Pomace extracts showed higher total phenolic and flavonoid contents (TPC up to 84.32 mg GAE/g dw; TFC up to 37.94 mg QE/g dw) than leaf extracts. Antioxidant activity, assessed using DPPH, ORAC, and DCFH-DA assays, was stronger in pomace samples (DPPH IC50 37.42-43.75 μg/mL; ORAC up to 1.5 μmol TE/mg). The extracts moderately inhibited nitric oxide production without detectable toxicity at tested concentrations. These results indicate that Georgian grapevine by-products are promising sources of polyphenols with antioxidant and anti-inflammatory potential. © *2026 Bull. Natl. Acad. Sci. Georg.*

Keywords: grapevine leaves, grape pomace, polyphenols, antioxidant activity, anti-inflammatory activity

Introduction

Viticulture and winemaking constitute a major sector of the global agro-industrial system. According to the International Organization of Vine and Wine (OIV), the global vineyard area currently covers approximately 7.1 million hectares (2024-2025) (International Organization of Vine and Wine, 2025). The expansion of vineyard cultivation and wine production is associated with the generation of substantial amounts of residues, including leaves, shoots, seeds, skins, stems, and grape pomace, the latter consisting of approximately 50% skins, 25% seeds, and 25% pulp (Di Lorenzo et al., 2023). Globally, around 20 million tons of grapevine-related residues are produced annually (Kojić & Prodanović, 2024; Ratto et al., 2025). A significant proportion of these by-products remains underutilized, contributing to environmental challenges such as soil and water contamination and greenhouse gas emissions (Evtuguin et al., 2023; Georgiev & Yankova, 2021). However, grapevine residues represent a valuable source of bioactive compounds, particularly polyphenols (flavonoids, stilbenes,

phenolic acids, and tannins). During winemaking, only 30-40% of total polyphenols are transferred into wine, whereas 60-70% remain in solid residues (Constantin et al., 2024; Di Lorenzo et al., 2023). These compounds have been reported to exhibit antioxidant, anti-inflammatory, cytotoxic, cardio-protective and neuroprotective properties, supporting their potential application in pharmaceutical and nutraceutical fields (Kojić & Prodanović, 2024; Olszowy, 2019). Georgia, recognized as one of the primary centers of grapevine domestication and characterized by remarkable genetic diversity (more than 525 identified cultivars, predominantly indigenous) (Mittova et al., 2026; Sargolzaei et al., 2021), represents a unique platform for investigating the phytochemical profile and biological potential of grapevine by-products. In 2024, national wine production reached approximately 2.4 million hectoliters, reflecting steady sectoral development (Georgian Wine Association, n.d.; National Wine Agency Georgia, n.d.). Among indigenous white cultivars, Mtsvane Kakhuri and Khikhvi are widely cultivated in the Kakheti region, generating considerable amounts of leaves and pomace during vineyard management and winemaking (Georgian Wine Association, n.d.; National Wine Agency Georgia, n.d.). Despite this diversity and production scale, systematic studies on the preparation of polyphenol-rich extracts from Georgian grapevine by-products, particularly leaves and pomace, remain limited. Therefore, the present study evaluates the phenolic composition and antioxidant and anti-inflammatory activities of extracts obtained from these materials, providing a scientific basis for their further valorization.

Materials and Methods

Chemicals. Methanol and ethanol (analytical grade) were obtained from Scharlau (Spain). The Folin–Ciocalteu reagent, DPPH, AAPH, DCFH-DA, tert-butyl hydroperoxide, lipopolysaccharide (LPS), N(G)-nitro-L-arginine methyl ester (L-NAME), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Germany). Gallic acid, quercetin, cyanidin-3-glucoside chloride, Trolox, and ascorbic acid were used as reference standards for spectrophotometric and antioxidant assays. Hanks' Balanced Salt Solution (HBSS) and RAW 264.7 murine macrophages were obtained from commercial suppliers. All reagents were of analytical grade and used without further purification.

Plant material. Grapevine leaves and grape pomace were obtained from two indigenous Georgian white cultivars, Vitis vinifera L. cv. Mtsvane Kakhuri and cv. Khikhvi, cultivated in the Kakheti wine-growing region (Alvani village, eastern Georgia). Grape pomace was collected in October 2025, three weeks after grape pressing and completion of alcoholic fermentation performed according to the traditional Kakhetian method (fermentation in contact with solid parts). The material was dried in a temperature-controlled oven at ≤45°C. Grapevine leaves were collected in July 2025 after the completion of the flowering stage and air-dried in a well-ventilated area at room temperature (18-23°C). Prior to extraction dried samples were finely ground.

Extraction. Ultrasound-assisted extraction (UAE) was applied to grapevine leaves and grape pomace. Dried materials were ground (leaves: 1.5-2.0 mm; pomace: 0.3-0.5 mm) and extracted with 70% ethanol (1:20 w/v) at 60°C for 30 min in an ultrasonic bath (40 kHz). The process was performed in two consecutive cycles to improve extraction efficiency. Filtrates were combined and used for subsequent analyses. All samples were extracted under identical conditions.

Determination of total phenolic content. The total phenolic content of the extracts was determined spectrophotometrically using the Folin-Ciocalteu method (Singleton & Rossi, 1965). Absorbance was measured at 765 nm with a UV-Vis spectrophotometer (I9, Hanon Instruments, China). Results were expressed as milligrams of

gallic acid equivalents per gram of dry weight (mg GAE/g dw).

Determination of total flavonoid content. Total flavonoids were determined using the aluminum chloride spectrophotometric method (Ferioli et al., 2020). After incubation with aluminum chloride reagent, absorbance was measured at 425 nm. Quantification was performed using a quercetin calibration curve (10-50 μg/mL; R2 = 0.9957), and results were expressed as mg quercetin equivalents per gram of dry weight (mg QE/g dw). All measurements were carried out in triplicate.

Determination of total anthocyanin content. Total anthocyanin content was determined using the pH differential method (Shen et al., 2020). Absorbance was measured at 520 and 700 nm with a UV-Vis spectrophotometer. Results were expressed on a dry weight basis as milligrams of cyanidin-3-glucoside equivalents per gram of dry material (mg C3G/g dw).

Antioxidant Activity Assays

DPPH radical scavenging assay. Free radical scavenging activity was evaluated using the DPPH method (Blois, 1958). Sample solutions were mixed with DPPH reagent and incubated in the dark for 60 min at room temperature. Absorbance was measured at 517 nm, and IC50 values were calculated from concentration-response curves and expressed in μg/mL.

Oxygen radical absorbance capacity (ORAC) assay. The ORAC assay was performed using AAPH-generated peroxyl radicals and a fluorescent probe as previously described (Dufour et al., 2007; Ou et al., 2001). Measurements were conducted in black 384-well microplates at 37.5°C using a fluorescence microplate reader. Extracts and Trolox standards were prepared at multiple concentrations in dimethyl sulfoxide. Antioxidant capacity was calculated from the net area under the fluorescence decay curve and expressed as μmol Trolox equivalents per milligram of extract (μmol TE/mg).

Cellular Antioxidant Activity (DCFH-DA Assay). Cellular antioxidant activity was evaluated in WS1 human skin fibroblasts using the DCFH-DA assay (Dufour et al., 2007; Grenier et al., 2021). Cells (10,000 cells/well) were seeded in 96-well plates and incubated for 24 h at 37°C (5% CO_2). After loading with DCFH-DA (5 μM), cells were treated with extracts and exposed to tert-butyl hydroperoxide (200 μM) to induce oxidative stress. Fluorescence was measured (Ex 485 nm, Em 530 nm), and results were expressed as IC50 values for the inhibition of DCFH oxidation relative to untreated control.

Anti-inflammatory activity assay. Anti-inflammatory activity was assessed by evaluating the inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 murine macrophages (Dufour et al., 2007; Ou et al., 2001; Oueslati et al., 2012). After treatment with different concentrations of extracts, nitrite accumulation in culture supernatants was quantified using the Griess reaction, and absorbance was measured at 540 nm. Nitrite levels were calculated from a sodium nitrite standard curve. L-NAME was used as a positive control. Results were expressed as percentage inhibition relative to LPS-treated cells and as IC50 values.

Statistical analysis. All experiments were performed in triplicate and results are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test (SigmaStat 4.0). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

The investigated Georgian grapevine by-products represent a meaningful reservoir of polyphenolic constituents, with grape pomace generally showing

a richer phenolic profile than leaves. This trend is plausible, as pomace consists of the solid winery fraction (skins/seeds/pulp residues) in which a substantial portion of polyphenols remains even after fermentation and pressing (Di Lorenzo et al., 2023). Notably, Khikhvi pomace exhibited the highest TPC (84.32 mg GAE/g dw), exceeding that of Mtsvane Kakhuri (73.45 mg GAE/g dw), indicating cultivar-dependent variability even among white grape varieties.

Table 1. Total phenolic (TPC), flavonoid (TFC), and anthocyanin (TAC) contents of grape pomace and leaves extracts (cv. Mtsvane Kakhuri and cv. Khikhvi)

Samples	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg C3GE/g)
Pomace - Mtsvane Kakhuri	73.45 ± 1.31	31.33 ± 0.83	0.947 ± 0.07
Pomace - Khikhvi	84.32 ± 1.54	37.94 ± 0.97	1.031 ± 0.12
Leaves - Mtsvane Kakhuri	47.75 ± 0.68	17.13 ± 0.43	0.318 ± 0.02
Leaves - Khikhvi	56.23 ± 1.22	19.52 ± 0.5	0.505 ± 0.04

Values are expressed on a dry weight (dw) basis as mean ± standard deviation (n = 5).

Since the investigated cultivars are white grape varieties, their phenolic content was expected to be lower than that typically reported for red cultivars, in which pigmented tissues are known to accumulate higher levels of extractable polyphenols (Samah et al., 2012; Tatanashvili et al., 2025). This tendency is consistent with our previous findings, where Saperavi (a red Georgian cultivar) seeds exhibited a TPC of 121.43 ± 0.21 mg GAE/g dw (Tatanashvili et al., 2025), exceeding the values observed in the present white pomace samples. Nevertheless, the relatively high TPC values recorded for Khikhvi and Mtsvane Kakhuri pomace (>70 mg GAE/g dw) indicate that these white cultivars also represent considerable phenolic sources. Because antioxidant activity may vary depending on the reaction mechanism and assay system, multiple complementary models were applied (Huang et al., 2005). Antioxidant potential was evaluated using DPPH radical scavenging, ORAC (peroxyl radical-based kinetic assay), and a cell-based oxidative stress model (DCFH-DA).

Table 2. Antioxidant activity of grape pomace and leaf extracts (cv. Mtsvane Kakhuri and cv. Khikhvi)

Samples	DPPH (μg/mL)	ORAC (μmol /mg)	DCFH-DA (μg/mL)
Ascorbic acid	3.0 ± 0.2	-	-
Trolox	-	5.3±0.5	-
Quercetin	-	-	0.49 ± 0.05
Pomace - Mtsvane Kakhuri	43.75 ± 0.93	0.9 ± 0.1	53.0 ± 0.3
Pomace - Khikhvi	37.42 ± 0.81	1.5 ± 0.3	49.0 ± 0.3
Leaves - Mtsvane Kakhuri	69.86 ± 1.23	0.2 ± 0.1	70.0 ± 0.5
Leaves - Khikhvi	61.13 ± 1.18	0.6 ± 0.1	77.0 ± 0.6

Values are expressed as mean ± standard deviation (n = 5).

Khikhvi pomace, which showed the highest TPC and TFC, also exhibited the strongest antioxidant activity (DPPH IC_{50} = 37.42 μg/mL; ORAC = 1.5 μmol TE/mg), whereas leaf extracts with lower phenolic content demonstrated weaker effects (Table 2). The inverse relationship between DPPH IC_{50} values and phenolic concentration supports the contribution of flavonoids and related polyphenols to hydrogen- and electron-transfer mechanisms. However, the smaller differences observed in the ORAC assay indicate that antioxidant capacity is influenced not only by total phenolic content but also by structural characteristics and synergistic interactions among phenolic subclasses. The cellular antioxidant assay (DCFH-DA) showed a similar trend, although with less pronounced differences between samples. This suggests that intracellular antioxidant effects depend not only on chemical reactivity but also on membrane permeability, cellular stability, and interactions with endogenous defense systems, highlighting the multifactorial nature of phenolic action.

Table 3. Anti-inflammatory activity of grape pomace and leaf extracts (cv. Mtsvane Kakhuri and cv. Khikhvi)

Samples	IC_{50} (μg/mL)	Inhibition maximum nontoxic concentration (%)	Toxicity μg/mL (>20% mortality)
L-NAME (250 μM)	-	58 ± 0.7	-
L-NAME (1 mM)	-	78 ± 0.4	-
Pomace - Mtsvane Kakhuri	> 160	21.2 ± 0.2	ND
Pomace - Khikhvi	> 160	16.3 ± 0.14	ND
Leaves - Mtsvane Kakhuri	> 160	45.7 ± 0.5	ND
Leaves - Khikhvi	> 160	40.1 ± 0.3	ND

Values are expressed as mean ± standard deviation (n = 5). L-NAME: N(G)-nitro-L-arginine methyl ester. ND: not detected

In contrast to antioxidant effects, the anti-nflammatory activity was moderate. Although the IC_{50} values exceeded 160 μg/mL, leaf extracts produced measurable NO inhibition at non-toxic concentrations (up to 45.7% for Mtsvane Kakhuri leaves). Interestingly, this effect did not strictly parallel TPC levels, suggesting that specific phenolic subclasses – rather than the total quantity – may be responsible for modulation of inflammatory signaling.

Conclusion

The present study demonstrates that grape pomace and leaf by-products of two Georgian white cultivars (cv. Mtsvane Kakhuri and cv. Khikhvi) are valuable sources of bioactive polyphenols. The pomace extracts showed higher phenolic and flavonoid contents, corresponding to stronger antioxidant activity in chemical and cell-based assays. The extracts moderately inhibited nitric oxide production without detectable toxicity. These findings highlight the biological potential of Georgian grapevine by-products and support their further development as nutraceutical or functional ingredients.

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ზოგიერთი ქართული აბორიგენული ვაზის ფოთლებისა და გადამუშავებული ნაყოფების ბიოლოგიურად აქტიური პოლიფენოლები

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ღვინის წარმოების ნარჩენები წარმოადგენს ბიოლოგიურად აქტიური პოლიფენოლების მნიშვნელოვან წყაროს. წინამდებარე კვლევაში მიღებულ იქნა პოლიფენოლებით მდიდარი ექსტრაქტები ქართული ვაზის ორი ავტოქტონური თეთრი ჯიშის – კახური მწვანისა და ხიხვის – გადამუშავებული ნაყოფებისა და ფოთლებიდან, შეფასდა მათი ფენოლური შემადგენლობა და ბიოლოგიური აქტივობა. გადამუშავებული ნაყოფების ექსტრაქტები გამოირჩეოდა ფენოლური ნაერთებისა და ფლავონოიდების მაღალი შემცველობით (ფენოლები – 84.32 მგ გმე/გ მშრალ წონაზე; ფლავონოიდები – 37.94 მგ ქე/გ მშრალ წონაზე) ფოთლის ექსტრაქტებთან შედარებით. ანტიოქსიდანტური აქტივობა შეფასდა DPPH, ORAC და DCFH-DA მეთოდებით, გადამუშავებული ნაყოფების ექსტრაქტებში გამოვლინდა ანტიოქსიდანტური პოტენციალი (DPPH IC_{50} – 37.42-43.75 მკგ/მლ; ORAC – 1.5 მკმოლ ტროლოქსის ეკვივალენტი/მგ-მდე). შესწავლილ კონცენტრაციებში ექსტრაქტებმა ზომიერად შეამცირეს აზოტის ოქსიდის წარმოქმნა ტოქსიკური ეფექტის გარეშე. მიღებული შედეგები ხაზს უსვამს ქართული ვაზის წარმოების ნარჩენების პოტენციალს, როგორც მრავალფუნქციური პოლიფენოლების წყაროს, რომლებიც შეიძლება მნიშვნელოვან როლს ასრულებდნენ ოქსიდაციური სტრესის მოდულაციისა და ანთებითი პროცესების რეგულაციის კონტექსტში, რაც საფუძველს ქმნის მათი შემდგომი შესწავლისათვის საკვები და სამედიცინო დანიშნულებით გამოყენების მიზნით.

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