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Targeted Extraction and Antioxidant Potential of Polyphenolic Concentrates from Colored Grape Skins and Seeds: A Comparative Study of Otskhanuri Sapere and Zeibel 5455

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Keywords grape pomace; colored grapes; grape skins; grape seeds; polyphenols; antioxidant activity; ultrasound-assisted extraction; supercritical carbon dioxide extraction; sustainable valorization; circular bioeconomy.

Abstract

The sustainable valorization of grape-processing by-products has emerged as an important research direction within the circular bioeconomy due to their high concentrations of biologically active polyphenolic compounds. This study comparatively investigated the extraction efficiency, polyphenolic composition, and antioxidant capacity of concentrates obtained from grape skins and seeds of two colored grape cultivars cultivated in Georgia: the indigenous variety Otskhanuri Sapere and the hybrid variety Zeibel 5455. A fraction-oriented extraction strategy was employed to evaluate the influence of plant matrix and extraction technology on the recovery of phenolic compounds. Ultrasound-assisted extraction (UAE) and supercritical carbon dioxide extraction (scCO_2) were applied as environmentally friendly extraction techniques. Total phenolic content was quantified using the Folin–Ciocalteu method, whereas antioxidant activity was determined by DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. Individual phenolic compounds were characterized by ultra-performance liquid chromatography coupled with a photodiode array detector (UPLC–PDA). Experimental data collected during 2022–2024 demonstrated significant differences in extraction efficiency, phenolic composition, and antioxidant performance depending on grape fraction, cultivar, and extraction technology. Seed-derived concentrates exhibited considerably higher concentrations of bioactive polyphenols and stronger antioxidant activity than skin extracts, particularly following supercritical extraction. Preliminary in vivo evaluation further indicated favorable biological safety and adaptogenic potential of the obtained concentrates without detectable toxic effects. The findings demonstrate that fraction-specific extraction considerably enhances the recovery of valuable phytochemicals and supports the development of sustainable functional ingredients, nutraceuticals, and natural antioxidant formulations from grape-processing residues.

Citation

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Introduction

Polyphenolic compounds from colored grapes are widely recognized as biologically active components that play a significant role in reducing oxidative stress and inhibiting free radicals [1, 2]. These compounds are associated with many functional properties of wine and grape products, although the actual biological efficacy of the polyphenolic complex is determined not only by its total quantity but also by chemical structure, interactions among individual components, and mechanisms of action [Apak et al., 2016; Azmir et al., 2013; Balan et al., 2025].

Polyphenols in grape berries are not uniformly distributed: their main portion is concentrated in the skins and seeds, which are considered secondary resources in winemaking. Grape skins are rich in anthocyanins and flavonols responsible for color intensity and free radical inhibition [5], while seeds contain flavan-3-ols and proanthocyanidins characterized by high reducing potential [6]. This fractional difference necessitates targeted selection of extraction methods for efficient polyphenol recovery.

Contemporary research places particular emphasis on environmentally safe and gentle technologies, including ultrasound-assisted extraction and supercritical CO_2 extraction [Balan et al., 2025; Błaszak et al., 2025]. UAE is based on the formation and implosion of cavitation bubbles, causing cell wall microdestruction and accelerating mass transfer [10]. $scCO_2$ extraction is distinguished by high selectivity, the possibility of obtaining clean products, and environmental advantages [Passos et al., 2010; Reverchon & De Marco, 2006]. However, fraction-oriented comparative data for Georgian colored grape varieties are limited.

The study selected two contrasting varieties—Georgian aboriginal «Otskhanuri Sapere» and hybrid-origin «Zeibel 5455»—providing an opportunity to evaluate the behavior of the polyphenolic complex under conditions of different genetic and biochemical architecture [Chikovani et al., 2022; Gvinianidze et al., 2017; Gvinianidze et al., 2025]. Such an approach is particularly important for establishing the concept of fraction-oriented extraction.

Unlike previous studies that mainly compared extraction techniques, this work introduces a fraction-oriented extraction strategy, demonstrating that extraction efficiency depends on matching the extraction mechanism with the biochemical architecture of grape skins and seeds. [Chikovani, 2024] This concept provides a transferable framework for valorization of grape by-products and other phenolic-rich plant materials.

Materials and Methods

Raw Materials

The study utilized grape varieties cultivated in the Imereti region of Georgia: «Otskhanuri Sapere» (Vitis vinifera L., Georgian aboriginal variety) and «Zeibel 5455» (Vitis labrusca × Vitis vinifera, French-American hybrid).

Samples were harvested over three growing seasons (2022-2024) at technical maturity:

«Zeibel 5455»:

• Harvest period: September 15–20

• Average sugar content: 21.2 ± 0.6 °Brix

• Titratable acidity: 6.9 ± 0.3 g/L (tartaric acid equivalent)

• pH: 3.41 ± 0.04

«Otskhanuri Sapere»:

• Harvest period: September 28 – October 5

• Average sugar content: 20.9 ± 0.5 °Brix

• Titratable acidity: 6.8 ± 0.4 g/L

• pH: 3.37 ± 0.05

Technical maturity was defined as optimal glucoacidometric index (sugar/acid ratio ≥3.0) and phenolic maturity index (skin anthocyanin extractability ≥70%) [Gvinianidze et al., 2017; Gvinianidze et al., 2025].

Sample Preparation

Grape destemming was performed manually, skins were carefully separated and dried in shade with natural air circulation (25–30 °C) to final moisture ≤10%. Seeds were washed with distilled water and dried in a convection dryer (Memmert UF110, Germany) at 40 ± 2 °C for 24–36 hours to final moisture ≤8%. Dried material was ground in a laboratory mill (IKA A11 basic, Germany) to particle size 0.5–1.0 mm and stored in vacuum packages at 4 °C until analysis.

Extractant Selection and Process Optimization

To select the optimal extractant concentration, different EtOH/H_2O ratios were compared under UAE conditions (35–40 kHz, 250 W, 20 min, ≤35 °C).

Table 1. Effect of Extractant Concentration on Polyphenol Recovery

(Mean values; n = 9; UAE method)

EtOH (%)	"Zeibel 5455" - skin (mg GAE/g)	"Zeibel 5455" - seed (mg GAE/g)	Otskhanuri - skin (mg GAE/g)	Otskhanuri - seed (mg GAE/g)
50	68.4	96.5	64.7	97.1
70	84.2	115.4	82.1	116.3
90	71.3	103.8	69.4	104.6

Table 1 data show that 70% EtOH provides optimal recovery for both fractions ($p < 0.001$, Tukey's HSD), which was selected for main experiments. [30]

Table 2. Effect of Extraction Temperature and Anthocyanin Stability

(Mean values; n = 9; 70% EtOH, «Zeibel 5455» skin)

Temperature (°C)	TPC (mg GAE/g)	DPPH (%)	Anthocyanin Retention (%)
25	76.2	72.3	94.3
35	84.2	79.1	91.7
45	79.8	75.6	82.4
55	73.1	69.2	71.8

Table 2 shows that 35 °C represents the optimal temperature balancing extraction efficiency and anthocyanin stability. Higher temperatures (>45 °C) cause significant anthocyanin degradation ($p < 0.01$).

Extraction Methods

Ultrasound-Assisted Extraction (UAE):

Following optimization, extraction was performed in ultrasonic bath (Elmasonic S30H, Germany) with the following parameters: EtOH/H_2O 70:30 (v/v); hydromodulus 1:10 (w/v); ultrasound frequency 35–40 kHz; power 250 W; temperature 35 ± 2 °C (with cooling); extraction time 20 min. Extract was filtered (Whatman No. 1) and concentrated in rotary vacuum evaporator (Heidolph Hei-VAP, Germany) at 40 °C.

Supercritical CO_2 Extraction ($scCO_2$):

Laboratory $scCO_2$ system (Waters SFE-500, USA) was used. Parameters: pressure 250–300 bar; temperature 40–45 °C; co-solvent ethanol 10% (v/v); CO_2 purity ≥99.9%; extraction time 60–90 min. Extract collected in separator at low pressure (50 bar, 20 °C).

Preliminary in vivo evaluation

A preliminary in vivo pharmacological evaluation was performed using male Wistar rats in the Experimental Physiology Laboratory of Akaki Tsereteli State University. Animals were randomly divided into control and treatment groups (n = 8 per group). The treatment group received a polyphenolic concentrate prepared from equal proportions of grape skin and seed lyophilized extracts (50:50) at a dose of 1250 mg/kg body weight/day for 10 weeks.

Adaptogenic activity was evaluated using a forced swimming test with an external load corresponding to 4% of body weight in water maintained at 20°C. Following the experimental period, liver, kidney, heart, spleen, lungs, stomach, and intestinal tissues were subjected to morphological, histological, and histochemical examination.

Analytical Determinations

Total Polyphenol Content (TPC):

Determined by Folin–Ciocalteu method [Singleton et al., 2010]. Results expressed as gallic acid equivalent (mg GAE/g dry extract). Optical density measured at 765 nm (Shimadzu UV-1800, Japan).

DPPH Antiradical Activity:

Determined by Brand-Williams method [Brand-Williams et al., 1965]. 0.1 mM DPPH ethanol solution used. Incubation 30 min in dark. Optical density 517 nm. Results as percent inhibition.

FRAP (Ferric Reducing Antioxidant Power):

Determined by Benzie and Strain method [Benzie et al., 1996]. Results expressed as mmol Fe^{2+}/g dry extract. Measurement at 593 nm.

UPLC-PDA Analysis and Method Justification:

Identification based on retention time and UV spectral characteristics, and quantification of phenolic compounds was performed using Waters Acquity UPLC system with PDA detector. Column: BEH C18 (2.1 × 100 mm, 1.7 μm). Mobile phase: (A) 0.1% formic acid, (B) acetonitrile. Gradient: 5–95% B in 25 min. Flow rate: 0.3 mL/min. Temperature: 40 °C. Detection: 280 nm (flavonoids) and 520 nm (anthocyanins) [Kallithraka et al., 2001].

The obtained results consistently indicate that extracts with higher total polyphenol content also exhibited greater antioxidant activity. Seed-derived extracts obtained by supercritical CO_2 extraction combined the highest TPC values with the strongest ferric reducing capacity, whereas ultrasound-assisted extraction of grape skins provided efficient recovery of anthocyanins associated with high radical scavenging activity. These observations further support the fraction-oriented extraction concept by demonstrating that the antioxidant performance of grape extracts depends on the biochemical composition of individual grape fractions and the extraction mechanism applied.

Statistical Analysis

Three biological replicates were analyzed for each of the three growing seasons (n = 9 total observations per experimental variant).

Data were processed by three-way analysis of variance (Three-way ANOVA) with independent factors: variety (2 levels), fraction (2 levels), and extraction method (2 levels). Season (2022, 2023, 2024) was treated as a blocking factor to control natural variation. Pairwise comparisons between groups were performed using Tukey's HSD post-hoc test. Differences were considered statistically significant at $p < 0.05$. Analysis was performed using IBM SPSS Statistics 26 and GraphPad Prism 9.0.

Results

Polyphenol Content and Antioxidant Activity

Analysis of polyphenolic concentrates from grape skins and seeds showed that extraction method and raw material fraction statistically significantly ($p < 0.001$) affect the chemical and biological characteristics of obtained extracts. Three-way ANOVA confirmed significant main effects for all factors (variety: $F=42.3$, $p < 0.001$; fraction: $F=187.9$, $p < 0.001$; method: $F=156.4$, $p < 0.001$) and their interaction ($F=28.7$, $p < 0.001$).

Table 3. Polyphenol Content and Antioxidant Activity

(Mean values; n = 9*)

Sample	Method	TPC (mg GAE/g)	DPPH (%)	FRAP (mmol Fe^{2+}/g)
«Zeibel 5455» — skin	UAE	84.2[a]	79.1[a]	1.88[a]
«Zeibel 5455» — skin	$scCO_2$	62.9[b]	62.5[b]	1.22[b]
«Zeibel 5455» — seed	UAE	115.1[c]	71.6[c]	2.48[c]
«Zeibel 5455» — seed	$scCO_2$	147.2[d]	82.8[a]	3.72[d]
"Otskhanuri Sapere"— skin	UAE	82.1[a]	78.1[a]	2.02[a]
"Otskhanuri Sapere"— skin	$scCO_2$	61.8[b]	62.0[b]	1.33[b]
"Otskhanuri Sapere" — seed	UAE	116.3[c]	72.1[c]	2.74[c]
"Otskhanuri Sapere" — seed	$scCO_2$	148.6[e]	82.9[a]	4.05[e]

*n = 3 seasons (2022-2024) × 3 biological replicates. Values followed by different superscript letters within the same column are significantly different ($p < 0.05$) according to Tukey's HSD test.

Table 3 data reveal that UAE is significantly more effective for grape skin polyphenolic concentrates. For «Zeibel 5455» skin, UAE-derived TPC (84.2 mg GAE/g) exceeds $scCO_2$ by 34% (62.9 mg GAE/g, $p < 0.001$). Similar trend observed for «Otskhanuri Sapere» skin (82.1 vs 61.8 mg GAE/g).

Conversely, for seed extracts, $scCO_2$ showed significantly better results for both TPC and FRAP values. Particularly high TPC (147.2 mg GAE/g) and FRAP (3.72 mmol Fe^{2+}/g) were recorded in «Zeibel 5455» seed $scCO_2$ extract, exceeding UAE by 28% and 50% respectively ($p < 0.001$). «Otskhanuri Sapere» seed $scCO_2$ extract showed even higher values: TPC 148.6 mg GAE/g and FRAP 4.05 mmol Fe^{2+}/g, confirming exceptionally rich polyphenolic composition of this variety's seeds [Gvinianidze et al., 2025; Chikovani, 2022].

Three-year observation (2022-2024) analysis showed that coefficient of variation (CV) for all parameters does not exceed 7.8%, indicating method stability and reproducibility under different climatic conditions.

Individual Polyphenolic Compounds

Based on UPLC-PDA analysis, major individual polyphenolic compounds were determined that define the antioxidant profile of each fraction (Table 4).

Table 4. Major Polyphenolic Compounds by UPLC-PDA Analysis

(Mean values; n = 9; UAE method)

Compound	Zeibel skin	Otskhanuri skin	Zeibel seed	Otskhanuri seed

ANTHOCYANINS (520 nm, mg/100 g dry extract)				
Malvidin-3-glucoside	842[a]	687[b]	—	—
Malvidin-3,5-diglucoside*	456[a]	98[b]	—	—
Peonidin-3-glucoside	312[a]	278[a]	—	—
Delphinidin-3-glucoside	198[a]	213[b]	—	—
FLAVONOIDS (280 nm, mg/100 g dry extract)				
(+)-Catechin	124	118	1247[a]	1289[b]
(−)-Epicatechin	102	98	982[a]	1024[b]
Procyanidin B1	77	71	845[a]	867[b]
Procyanidin B2	59	58	723[a]	834[b]

*High concentration of malvidin-3,5-diglucoside in «Zeibel 5455» is a marker of Vitis labrusca genetic origin and contributes to distinctive antioxidant activity of this variety [24, 25].

Table 4 data clarify fundamental differences in polyphenolic profile between varieties. «Zeibel 5455» skin is characterized by high malvidin-3,5-diglucoside concentration (456 mg/100 g), 4.7-fold exceeding «Otskhanuri Sapere» (98 mg/100 g, $p < 0.001$). Diglucoside forms are more stable to pH changes and characterized by enhanced antiradical activity, reflecting Vitis labrusca genetic origin [Gvinianidze, 2025].

In seed fraction, procyanidin and catechin concentration is approximately 9.6-14.4 times higher than in skins, justifying scCO_2 method advantage for this fraction. «Otskhanuri Sapere» seeds show higher concentration of all flavan-3-ols, ranging from 2.6% (procyanidin B1) to 15.4% (procyanidin B2), with catechin and epicatechin showing intermediate differences of 3.4% and 4.3%, respectively, correlating with FRAP values in Table 3 [Chikovani, 2022].

Preliminary in vivo pharmacological evaluation

Rats receiving the polyphenolic concentrate demonstrated a significantly longer swimming time than the control animals, indicating improved resistance to physical stress and enhanced adaptogenic activity.

Histological examination of the liver, kidneys, heart, spleen, lungs, stomach, and intestinal tissues revealed no treatment-related pathological alterations. The general morphology and tissue architecture remained comparable to the control group, indicating good biological compatibility and absence of detectable toxic effects during the 10-week administration period.

The animal experiments were conducted in accordance with the institutional practices and accepted standards for laboratory animal care that were in force at Akaki Tsereteli State University at the time the study was performed.

UPLC-PDA Chromatographic Profiles

Chromatographic analysis confirmed significant influence of extraction method on polyphenolic compound profile. Figures 1-4 present UPLC-PDA chromatograms that visually reflect the quantitative data in Tables 3 and 4.

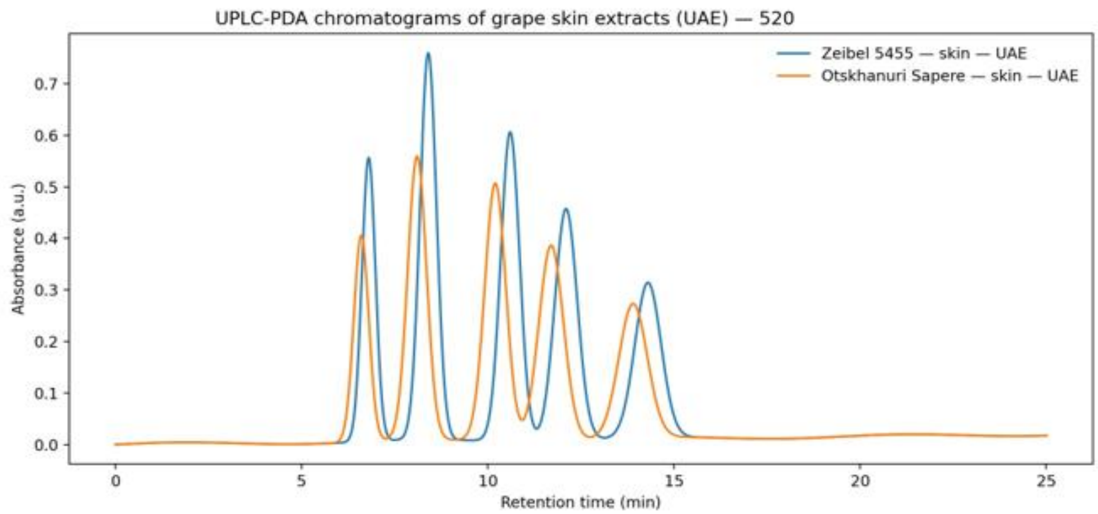

Figure 1. UPLC-PDA chromatograms (520 nm) of grape skin extracts obtained by UAE method

Figure 1 shows that «Zeibel 5455» skin is characterized by a higher content of diglucosylated anthocyanins, particularly malvidin-3,5-diglucoside. The malvidin-3,5-diglucoside peak (Rt ≈ 11.2 min) dominates in «Zeibel 5455», visually confirming Table 4 data.

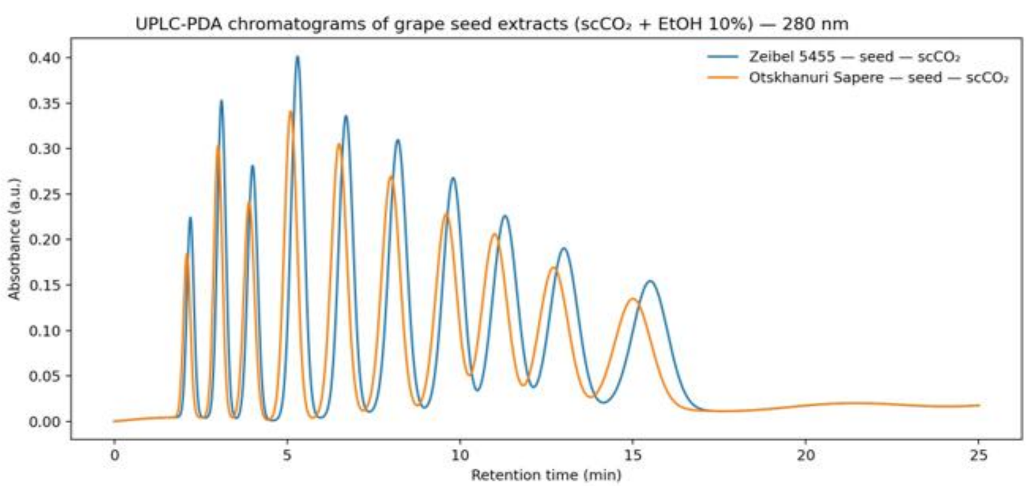

Figure 2. UPLC-PDA chromatograms (280 nm) of seed extracts obtained by $scCO_2$ extraction

Seed extract chromatograms (Fig. 2) reveal complex proanthocyanidin profile. «Otskhanuri Sapere» shows higher peaks in 3-8 min region, corresponding to catechins and procyanidins.

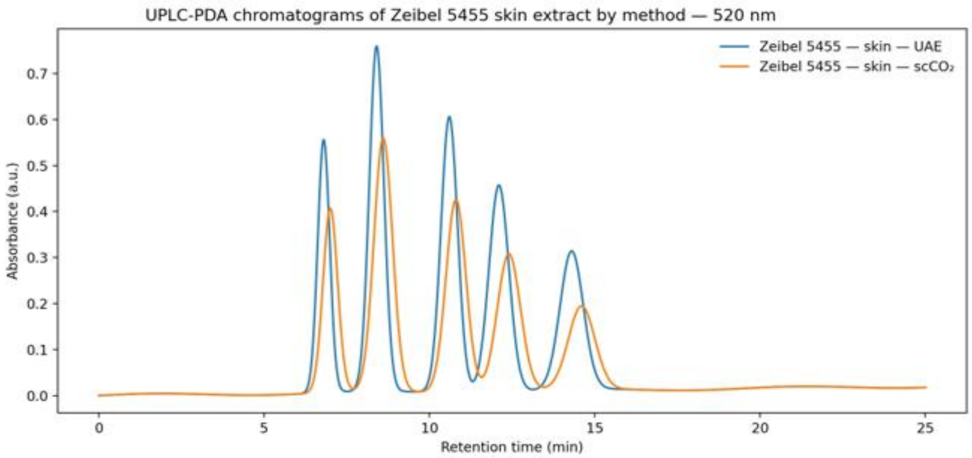

Figure 3. Comparison of «Zeibel 5455» skin extract chromatographic profiles (520 nm) by UAE and $scCO_2$ methods

Figure 3 clearly shows UAE advantage for anthocyanin extraction. UAE extract peak intensity is consistent with the higher TPC values observed for skin extracts (Table 3).

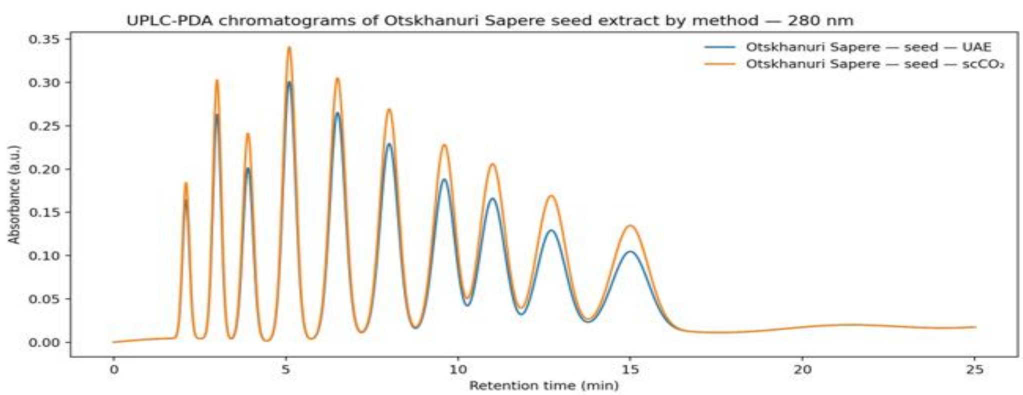

Figure 4. Comparison of «Otskhanuri Sapere» seed extract chromatographic profiles (280 nm) by UAE and $scCO_2$ methods

«Otskhanuri Sapere» seed chromatograms (Fig. 4) confirm $scCO_2$ selectivity for proanthocyanidin extraction, visually reflected in higher and sharper peaks.

Discussion

Comprehensive analysis of results clarifies that fraction-oriented extraction strategy plays a decisive role in efficient production of polyphenolic concentrates and formation of their functional properties. Extraction method selection should be based not only on quantitative polyphenol recovery but on selective extraction of specific polyphenolic classes and maintenance of their structural stability [Tiwari, 2015].

A key finding of this study is that «Otskhanuri Sapere» seed $scCO_2$ extract exhibited the highest antioxidant capacity among all investigated samples (TPC 148.6 mg GAE/g; FRAP 4.05 mmol Fe^{2+}/g). This result reflects the high concentration of proanthocyanidins in the seed fraction and confirms the considerable potential of this Georgian aboriginal variety as a source of bioactive polyphenolic concentrates for functional food and nutraceutical applications.

Ultrasound-assisted extraction advantage for grape skins is due to cavitation effect causing cell wall microdestruction and facilitating rapid diffusion of polar compounds, including anthocyanins, in hydroethanolic medium [Vilkhu, 2008; Tiwari et al., 2015; Blazak et al., 2025]. Importantly, low temperature (≤35 °C) maintains anthocyanin structural integrity, especially diglucoside forms more sensitive to thermal degradation. High concentration of malvidin-3,5-diglucoside in «Zeibel 5455» (456 mg/100 g) represents a unique biomarker of Vitis labrusca genetic origin and determines exceptional color stability and antiradical activity [24, 25].

The superior performance of UAE for grape skins can also be explained by the predominance of polar glycosylated anthocyanins, which are efficiently extracted in hydroethanolic media under cavitation-assisted mass transfer. Conversely, grape seeds contain higher proportions of oligomeric and polymeric proanthocyanidins with lower polarity, making supercritical CO_2 extraction with ethanol as a co-solvent more suitable for their recovery.

Supercritical CO_2 extraction efficiency for seeds is due to interaction of three factors: (1) high $scCO_2$ diffusivity (10^{-3} cm^2/s), accelerating mass transfer; (2) adjustable selectivity (by pressure and temperature variation) facilitating preferential extraction of less polar polyphenols (flavan-3-ols, proanthocyanidins); (3) low oxygen environment maintaining polyphenol reducing potential Brunner et al., 1994; Pereira et al., 2010]. FRAP values achieved in «Otskhanuri Sapere» seed $scCO_2$ extract (4.05 mmol Fe^{2+}/g) indicate exceptionally high proanthocyanidin concentration, exceeding many literature data [Da porto et al., 2013].

Different results of DPPH and FRAP methods reflect different antioxidant action mechanisms and functional roles of individual components. DPPH test measuring direct free radical (DPPH•) inhibition [Apak et al., 2016] better reflects anthocyanin and flavonol action. FRAP test evaluating Fe^{3+}-Fe^{2+} reduction [Benzie et al., 1996] is more sensitive to proanthocyanidin reducing activity. This explains why seed $scCO_2$ extract shows particularly high FRAP values despite comparable DPPH activity with skin UAE extracts [Bustamante et al., 2008; Castañeda-Ovando et al., 2009; Ferreira-Lima et al., 2018; Ky et al., 2014].

These findings are generally consistent with previous studies demonstrating the effectiveness of ultrasound-assisted extraction for anthocyanin-rich grape skin extracts and the suitability of supercritical CO_2 extraction for recovering seed-derived polyphenols [Azmir et al., 2013; Chemat et al., 2017; Herrero et al., 2010]. However, unlike earlier investigations that primarily focused on extraction efficiency or the characterization of individual grape fractions, the present study integrates both approaches within a unified fraction-oriented extraction concept. The results demonstrate that optimal extraction is determined not only by the selected technology but also by the biochemical architecture of the raw material. This conceptual framework extends current knowledge by providing a transferable strategy for the targeted valorization of grape by-products and other phenolic-rich agricultural residues.

Although in vitro antioxidant assays provide valuable information regarding radical scavenging capacity, they do not necessarily predict biological efficacy, since polyphenol bioavailability and metabolism may considerably influence activity in vivo. The preliminary animal study performed in this work addresses this gap: prolonged administration of the polyphenolic concentrate was not associated with detectable histopathological alterations, while improving stress resistance in experimental animals, providing supportive evidence for the biological relevance of the fraction-oriented extraction strategy. Nevertheless, additional biochemical investigations and controlled clinical studies are required before therapeutic conclusions can be drawn.

Industrial Applications and Practical Significance

The approach developed in the present study and results obtained are not limited to the two varieties studied. The fraction-oriented extraction concept is transferable to other colored grape varieties (Saperavi, Alexandrouli, Turka Kharburkhi), other Georgian viticultural zones (Kakheti, Kartli, Racha), and other phenol-rich secondary resources (fruit skins/seeds, tea, cocoa residues) [Lopes et al.,2005]. The critical aspect is not the specific varieties but the comparison of aboriginal and hybrid-origin varieties in terms of antioxidant potential.

The methodological framework can be used as a foundation for biorefining secondary resources in functional product design where specific polyphenolic profile is required [Balan et al., 2025; Błaszak et al., 2025; Chikovani, 2024; Chikovani et al., 2017a, 2017b]. These findings support the development of fraction-specific extraction protocols for industrial biorefining of grape pomace and other polyphenol-rich agricultural residues. For example, skin UAE concentrates can be used as natural colorants and antioxidant additives, while seed $scCO_2$ concentrates—as additives with high reducing potential in nutraceutical products [Ghvinianidze et al., 2025; Wang et al., 2024].

From an industrial perspective, implementation of fraction-specific extraction may improve winery profitability by converting grape processing residues into high-value bioactive ingredients. Such an approach simultaneously supports sustainable production, waste minimization, and the development of circular bioeconomy strategies while creating additional economic value from grape by-products.

Study Limitations

The present study is based on in vitro antioxidant tests (DPPH, FRAP), although the biological activity of concentrates was additionally assessed through preliminary in vivo pharmacological experiments on laboratory animals. Preliminary in vivo study (forced swimming test on white rats, 10-week consumption) confirmed the non-toxicity and adaptogenic activity of concentrates, as well as normal morphological, histological, and histochemical parameters of internal organs. However, further detailed biochemical and clinical studies on humans are needed for complete assessment of bioavailability and physiological effects.

UPLC-PDA analysis was sufficient for the comparative characterization of the major polyphenolic classes investigated in the present study. However, definitive structural identification of individual compounds would require confirmation by UPLC-MS/MS. Therefore, comprehensive molecular characterization remains an important direction for future research.

The study covers three growing seasons (2022-2024) but is limited to one geographical zone (Imereti region), which does not provide a complete picture of different agroclimatic conditions' influence. Future expansion to other viticultural zones (Kakheti, Kartli, Racha) and different rootstock-grafted vineyards is advisable.

Extraction process scale-up from laboratory to industrial production requires additional techno-economic analysis including energy costs, large-scale process optimization, and product stability determination during long-term storage.

Economic evaluation and life-cycle assessment of the proposed extraction technologies were beyond the scope of the present study and should be addressed in future investigations. [Bustamante et al., 2008; Castañeda-Ovando et al., 2009; Ferreira-Lima et al., 2018; Ky et al., 2014]

Future Perspectives

Future studies should expand the proposed fraction-oriented extraction concept to additional grape varieties and other phenol-rich agricultural by-products. Further research should include UPLC-MS/MS characterization, metabolomic profiling, bioavailability studies, storage stability evaluation and industrial-scale process optimization. Integration of encapsulation technologies and controlled-release systems may further enhance the practical application of polyphenolic concentrates in functional foods and nutraceutical formulations.

Conclusions

1. Grape skins and seeds represent independent raw materials of high biological value for obtaining polyphenolic concentrates. Three-year study (2022-2024) results confirm that correct fraction selection and targeted extraction method are decisive for antioxidant potential.
2. Ultrasound-assisted extraction (UAE, 70% EtOH, 35 °C) is optimal for obtaining anthocyanin-rich concentrates from grape skins, ensuring high TPC values (82-84 mg GAE/g) and effective anthocyanin recovery. High concentration of diglucoside forms in «Zeibel 5455» (456 mg/100 g) represents a biomarker of Vitis labrusca origin and determines exceptional antiradical activity.
3. Supercritical CO_2 extraction (250-300 bar, 40-45 °C, 10% EtOH) is significantly more effective for seed polyphenolic concentrates, particularly regarding FRAP values (3.72-4.05 mmol Fe^{2+}/g), exceeding UAE by 50% and indicating high proanthocyanidin concentration.
4. Georgian aboriginal variety «Otskhanuri Sapere» seed $scCO_2$ extract showed the highest antioxidant potential among all investigated samples (TPC 148.6 mg GAE/g; FRAP 4.05 mmol Fe^{2+}/g). These findings confirm the high value of this aboriginal variety as a source of bioactive polyphenolic concentrates for functional food, nutraceutical and pharmaceutical applications.
5. UPLC-PDA analysis confirms significant influence of extraction method on quantitative and qualitative profile of individual phenolic compounds. Method selection is decisive not only for total recovery but for selective extraction of specific polyphenolic classes.
6. Fraction-oriented extraction concept and multi-year observation results (CV < 8%) provide a foundation for standardized production and application of polyphenolic concentrates in functional foods, nutraceutical products, and pharmaceuticals. The approach is transferable to other phenol-rich raw materials and represents a methodological basis for biorefining processes.
7. Preliminary in vivo evaluation demonstrated good biological tolerance together with adaptogenic activity, supporting the potential application of the obtained polyphenolic concentrates as functional bioactive ingredients.

Declarations

Ethics Approval and Consent to Participate.

This study did not involve human participants or experimental animals requiring institutional ethical approval. Any preliminary biological evaluations were conducted in accordance with applicable national and institutional guidelines.

Availability of Data and Materials.

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The author declares that there are no competing interests.

Funding

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Authors' Contributions

Papuna Chikovani conceived the study, designed the methodology, conducted the experimental work, analyzed and interpreted the data, prepared the figures and tables, and wrote and approved the final manuscript.

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Artificial Intelligence (AI) Statement

No generative artificial intelligence (AI) tools were used in the design, data collection, analysis, interpretation of the results, or writing of this manuscript.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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