GEORGIAN BIOMEDICAL NEWS

VOLUME 3 ISSUE 1. JAN-MAR 2025

Influence of Extraction Parameters on the Yield of Total Phenolic Compounds from Grape (*Vitis vinifera L.*) Seeds

Mariam Tatanashvili[1,ID], Malkhaz Jokhadze[2,ID], Koba Sivsivadze[1], Tamaz Murtazashvili[1], Sopio Gokadze[2,ID]

DOI: 10.52340/GBMN.2025.01.01.103

ABSTRACT

Background: The scientific literature has developed general approaches to assess the content of phenols in raw plant materials. However, the conditions for extracting and analyzing these compounds can vary significantly between plant species. Using suboptimal extraction conditions may lead to the degradation of target substances and reduced extraction efficiency. Therefore, selecting optimal methods for extracting and analyzing polyphenols is crucial to ensure accuracy and efficiency.

Objectives: This study aimed to investigate the influence of various factors on the extraction of phenolic compounds from Georgian grape (*Vitis vinifera L.*) seeds, with the goal of developing an optimized extraction method.

Methods: Phenolic compounds were extracted from the raw material using ultrasound-assisted extraction (UAE). The total phenolic content was determined using the Folin-Ciocalteu reagent, which forms a blue complex with phenols in an alkaline medium. The solutions' absorbance was measured spectrophotometrically at the appropriate wavelength for quantification.

Results: The maximum yield of phenolic compounds (95.969 mg/g GAE) was achieved under the following extraction conditions: solvent—70% ethyl alcohol, particle size: 0.3-0.5 mm, raw material-to-solvent ratio: 1:20, temperature: 60°C, duration: 30 minutes, and double extraction cycle.

Conclusions: These findings provide a solid foundation for efficiently extracting phenolic compounds from grape seeds. This optimized method has potential applications in the food, pharmaceutical, and cosmetic industries, contributing to the valorization of residues from wine production.

Keywords: Extraction; Folin-Ciocalteu reagent; grape; residues; optimal conditions; phenolic compounds.

BACKGROUND

The grapevine (*Vitis vinifera L.*) is one of the most economically significant plants globally, valued for its fruit and the products, such as wine, raisins, and grape juice.[1] In Georgia, viticulture holds profound historical and cultural importance, with the country recognized as one of the oldest wine-producing regions in the world. Georgian vineyards thrive in various climates, producing high-quality grapes that are the foundation of its internationally renowned wine industry.[2,3] However, vineyard cultivation and winemaking generate significant by-products, including grape seeds, which remain underutilized despite their valuable bioactive compounds.[4]

Grape seeds are rich in biologically active compounds, including phenolic compounds known for their antioxidant, anti-inflammatory, and anticancer properties.[5-9] These compounds are of great interest due to their potential applications in the pharmaceutical and cosmetic industries.[10]

Certain polyphenols have demonstrated pharmacological benefits in breast cancer treatment, including their ability to inhibit the aromatase enzyme involved in estrogen biosynthesis among the diverse phenolic compounds in grape-derived materials.[8,9] This finding has spurred interest in exploring grape seeds as a sustainable source of phenolic compounds for developing phytopreparations that may serve as complementary or alternative treatments for hormone-related conditions.

The efficient extraction of phenolic compounds is a critical step in their isolation and application.[11,12] Transferring biologically active components from the plant matrix to a solvent is essential to achieve the goal. The efficiency of this process depends on the extraction method employed and various extraction parameters, including temperature, solvent type and concentration, extraction time, and the physical characteristics of the raw material.[11,12] Higher temperatures can increase the permeability of cell walls and the solubility of phenolic compounds, thereby enhancing the extraction yield. However, excessive heat may lead to the degradation of sensitive phenolic compounds, emphasizing the need for careful optimization.[11-16] Similarly, the choice of solvent and its concentration play a crucial role, as different phenolic compounds exhibit varying solubility profiles. For instance, aqueous ethanol is commonly used because it dissolves hydrophilic and lipophilic phenolic compounds.[11,12,16-18] Particle size and solvent-to-solid ratio are also significant, as smaller particle sizes increase the surface area available for mass transfer. In contrast, the optimal solvent-to-solid ratio ensures effective diffusion without excess solvent usage.[11,12,16,19,20]

Given the influence of these conditions on extraction efficiency, their correct selection and optimization are essential to maximize the yield of phenolic compounds and ensure the quality of the extract.

Therefore, this study aims to optimize the extraction conditions for phenolic compounds from grape seeds to maximize their yield. By addressing these factors, the research aims to develop a sustainable approach to utilizing vineyard residues and creating valuable products with potential applications in the pharmaceutical and cosmetic industries.

METHODS

Kisi grape seeds were selected as the research object. They were collected in October 2023 from the village of Alvani, Kakheti, after a two-week fermentation period following wine pressing. The raw material was dried and processed for analysis (Fig.1).

FIGURE 1. Grape seeds of the Kisi variety

Ultrasound-assisted extraction (UAE) was used to prepare the extracts. UAE is an innovative technique that enhances extraction using high-frequency sound waves (20–100 kHz). These sound waves generate cavitation bubbles in the solvent. As the bubbles collapse, they release significant energy through high pressure and temperature, breaking plant cell walls and releasing phenolic compounds into the solvent. UAE offers several advantages over traditional methods, including shorter extraction times, higher yields, and reduced solvent consumption. Moreover, it is an environmentally friendly technique, allowing the use of non-toxic, food-grade solvents, such as ethanol. UAE is also cost-effective, requiring less energy and simplifying the extraction process compared to conventional methods.[11,12]

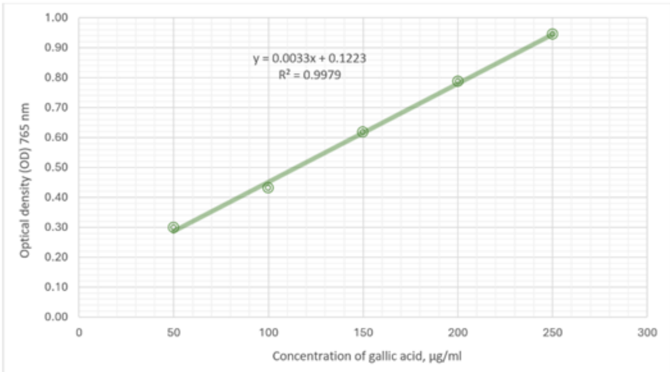
A series of experiments with variations in alcohol concentration, particle size, temperature, solid-to-solvent ratio, extraction duration, and the number of extraction cycles were conducted to study the influence of these factors on the extraction quality of phenolic compounds from grape seeds and identify the optimal conditions.

To determine the influence of the first parameter (ethyl alcohol concentration), extraction was performed under the following conditions: particle size, 0.7-0.9 mm; solid-to-solvent ratio, 1:10; temperature, 50°C; duration, 30 min; and extraction repetitions, 1.

In each subsequent series, the raw material was extracted using the optimal value of the studied parameter. Three parallel experiments were conducted at each experimental point, with the exact weight of the raw material being 1 ± 0.001 g.

The total phenolic content of the extracts was determined spectrophotometrically using the Folin-Ciocalteu colorimetric method.[21] For this, 1 ml of the extracts obtained from the seeds was placed in test tubes, to which 5 ml of 10% Folin-Ciocalteu reagent (Sigma-Aldrich, Germany) was added. After shaking, the tubes were left at room temperature for 8 minutes. Then, 4 ml of 7.5% Na_2CO_3 solution was added, and the resulting solutions were kept in the dark for 40 minutes. The optical density was then determined at a wavelength of 765 nm. The total phenolic content of the extracts was determined using a gallic acid calibration curve, prepared with serial solutions of gallic acid (Carl Roth GmbH & Co., Germany) at concentrations of 50, 100, 150, 200, and 250 µg/mL, based on the optical density-concentration relationship. The results were expressed as milligrams of gallic acid equivalent (mg/g GAE) per gram of raw material (Fig.2).

FIGURE 2. Gallic acid calibration curve for the Folin-Ciocalteu method



RESULTS

Ethyl alcohol is widely used as a solvent due to its bacteriostatic activity, relative cost, and capacity to inactivate enzymes.[11,12,16-18]

Table 1 shows that the yield of phenolic compounds increases with increased ethanol concentration, reaching a peak at 70% ethanol, then decreasing as the concentration approaches 95%.

The extraction rate is also affected by the particle size of the raw material. The greater the degree of grinding of the material, the faster the transition of the target components into the extragent occurs.[11,12,16,20] Table 2 presents the influence of particle size.

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

TABLE 1. Effect of alcohol concentration on the extraction of phenolic compounds from Grape (*Vitis vinifera L.*) seeds

Alcohol concentration, %	Phenolic compound yield (mg/g GAE)
0	12.597
30	26.839
50	36.536
70	65.718
95	49.082

TABLE 2. Effect of the degree of grinding of raw materials on the extraction of phenolic compounds from Grape (*Vitis vinifera L.*) seeds

Particle size (mm)	Phenolic compound yield (mg/g GAE)
0.05-0.2	69.255
0.3-0.5	67.257
0.7-0.9	65.861
1.0-1.5	48.770

The solid-to-solvent ratio also played a crucial role in extraction efficiency. Table 3 shows that the yield of phenolic compounds increased with higher solvent availability, peaking at a 1:20 ratio, beyond which the yield slightly declined.

TABLE 3. The effect of the solid-to-solvent ratio on the extraction of phenolic compounds from Grape (*Vitis vinifera L.*) seeds

Solid-to-solvent ratio	Phenolic compound yield (mg/g GAE)
1:5	64.860
1:10	68.045
1:20	86.406
1:30	75.678

Temperature is another significant factor that influences the yield of biologically active compounds, causing rapid swelling of the plant material, which contributes to cell damage, enzyme inactivation, microbiota death, and a decrease in the solvent's viscosity, thereby increasing the solubility of the target components.[11] As shown in Table 4, an increase in temperature generally enhances the extraction yield of phenolic compounds, with the highest yield observed at 60°C, followed by a slight decline at 70°C.

TABLE 4. The effect of temperature on the extraction of phenolic compounds from Grape (*Vitis vinifera L.*) seeds

Temperature, °C	Phenolic compound yield (mg/g GAE)
25	53.544
40	59.887
50	86.732
60	95.765
70	93.541

Table 5 shows the effect of the extraction process duration. The yield increased to 30 minutes, beyond which further extraction did not significantly improve the yield.

Single, double, and triple extractions were performed under selected conditions. The influence of the number of extraction cycles on the yield of phenolic compounds was minimal. A double extraction yielded 95.969 mg/g GAE, and subsequent cycles provided negligible increases. Given the insignificant difference, a double cycle is sufficient to achieve near-maximum extraction efficiency.

TABLE 5. The effect of the extraction process duration on the phenolic compounds from Grape (*Vitis vinifera L.*) seeds

Extraction process duration (min)	Phenolic compound yield (mg/g GAE)
10	58.368
20	73.567
30	95.825
60	95.903

DISCUSSION

The findings suggest that optimizing extraction parameters has a significant impact on the yield of phenolic compounds. The results align with previous studies, underscoring the significance of ethanol concentration, temperature, and particle size in maximizing phenolic compound recovery.[11-20] The choice of ethanol concentration played a critical role in determining extraction efficiency. The highest yield was obtained at 70% ethanol concentration, supporting earlier findings that ethanol-water mixtures enhance the solubility of both hydrophilic glycosides and less polar aglycones. The decline in yield at 95% ethanol is likely due to reduced polarity, which limits the dissolution of hydrophilic compounds and reduces overall extraction efficiency.[11,12,16-18] Maintaining an appropriate ethanol-water ratio is essential for maximizing the recovery of phenolic compounds while ensuring an effective extraction process.

Particle size played a crucial role in determining extraction efficiency. The highest phenolic yield was observed with particles sized 0.05-0.2 mm. Smaller particles increase the surface area for solvent penetration and extraction, resulting in the enhanced release of phenolic compounds.[11,12,16,20] However, excellent particles caused operational issues such as agglomeration and filtration difficulties, which limited efficiency. Larger particles (1.0-1.5 mm) had lower yields due to reduced surface area for interaction with the solvent. A particle size of 0.3-0.5 mm was chosen as an optimal, balancing efficiency and practicality.

The solid-to-solvent ratio significantly affected the yield of phenolic compounds, with the highest efficiency observed at a ratio of 1:20. Excess solvent (e.g., 1:30) did not substantially enhance the yield, likely due to saturation effects, which aligns with previous studies on extraction efficiency.[11,12,19]

Increasing the temperature from 25°C to 60°C resulted in a steady rise in phenolic yield, with a maximum of 60°C. Similar findings have been reported in the literature, where moderate heating improves mass transfer and phenolic extraction efficiency.[14-16] Above 60°C, the yield declined slightly. This can be attributed to the thermal degradation of phenolic compounds or the extraction of undesirable ballast substances, which reduce the quality of the extract, as observed in previous studies.[11-15] Thus, controlling the thermal

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

conditions during extraction is crucial for maintaining compound stability.

The optimal extraction duration was 30 minutes, beyond which additional extraction did not significantly improve the yield. This aligns with findings that prolonged extraction can lead to the reabsorption or degradation of phenolic compounds.[11,14] The number of extraction cycles played a minimal role, with a double extraction yielding near-maximal phenolic recovery, making further cycles inefficient.[11]

The study results emphasize the importance of selecting suitable extraction conditions to optimize the yield of phenolic compounds.

CONCLUSIONS

The influence of key extraction parameters, ethyl alcohol concentration, raw material particle size, solvent-to-solid ratio, temperature, duration, and extraction cycles on the yield of phenolic compounds from grape seeds was systematically studied. Optimal conditions were identified: solvent, 70% ethyl alcohol; particle size, 0.3–0.5 mm; raw material-to-solvent ratio, 1:20; temperature, 60°C; duration, 30 minutes; and a double extraction cycle. These conditions yielded a maximum phenolic compound concentration of 95.969 mg/g GAE. The findings establish a solid foundation for efficiently extracting phenolic compounds from grape seeds, with potential applications in the food, pharmaceutical, and cosmetic industries.

AUTHOR AFFILIATIONS

[1]Department of Pharmaceutical and Toxicological Chemistry, Faculty of Pharmacy, Tbilisi State Medical University, Tbilisi, Georgia;
[2]Direction of Pharmacognosy and Pharmaceutical Botany, Tbilisi State Medical University, Tbilisi, Georgia.

ACKNOWLEDGEMENTS

This work was supported by the Shota Rustaveli National Science Foundation of Georgia, Ph.D. Educational Programs Funding № Grant PHDF-22-323.

REFERENCES

1. Torregrosa L, Vialet S, Adivèze A, Iocco-Corena P, Thomas MR. Grapevine (Vitis vinifera L.). In: Agrobacterium Protocols: Volume 2. Springer; 2015:177-194.
2. Chilashvili L. The vine, wine and the Georgians. GWS; 2004.
3. Kighuradze T. Georgia, the cradle of viticulture and winemaking. J Vazi Da Ghvino. 2000;27-29.
4. del Mar Contreras M, Romero-García JM, López-Linares JC, Romero I, Castro E. Residues from grapevine and wine production as feedstock for a biorefinery. Food Bioproducts Process. 2022;134:56-79. https://doi.org/10.1016/j.fbp.2022.05.005.
5. Baroi AM, Popitiu M, Fierascu I, Sărdărescu ID, Fierascu RC. Grapevine wastes: A rich source of antioxidants and other biologically active compounds. Antioxidants. 2022;11(2):393. https://doi.org/10.3390/antiox11020393.
6. Goufo P, Singh RK, Cortez I. A reference list of phenolic compounds (including stilbenes) in grapevine (Vitis vinifera L.) roots, woods, canes, stems, and leaves. Antioxidants. 2020;9(5):398. doi:10.3390/antiox9050398.
7. Perestrelo R, Silva C, Pereira J, Câmara JS. Healthy effects of bioactive metabolites from Vitis vinifera L. grapes: A review. In: Grapes: Production, Phenolic Composition and Potential Biomedical Effects. 2014:38.
8. Dayem AA, Choi HY, Yang GM, Kim K, Saha SK, Cho SG. The anti-cancer effect of polyphenols against breast cancer and cancer stem cells: Molecular mechanisms. Nutrients. 2016;8(9):581. doi:10.3390/nu8090581.
9. Lephart ED. Modulation of aromatase by phytoestrogens. Enzyme Res. 2015;2015:594656. doi:10.1155/2015/594656.
10. Oksana S, Marian B, Mahendra R, Bo SH. Plant phenolic compounds for food, pharmaceutical, and cosmetics production. J Med Plants Res. 2012;6(13):2526-2539. doi:10.5897/JMPR11.1695.
11. Santos-Buelga C, Gonzalez-Manzano S, Dueñas M, Gonzalez-Paramas AM. Extraction and isolation of phenolic compounds. In: Natural Products Isolation. Springer; 2012:427-464.
12. Alara OR, Abdurahman NH, Ukaegbu CI. Extraction of phenolic compounds: A review. Curr Res Food Sci. 2021;4:200-214. https://doi.org/10.1016/j.crfs.2021.03.011.
13. Seidel V. Initial and bulk extraction of natural products isolation. Natural products isolation. 2012:27-41.
14. Dent M, Dragović-Uzelac V, Penić M, Bosiljkov T, Levaj B. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in Dalmatian wild sage (Salvia officinalis L.) extracts. Food technology and biotechnology. 2013 Mar 29;51(1):84-91.
15. Hernández-Carranza P, Ávila-Sosa R, Guerrero-Beltrán JA, Navarro-Cruz AR, Corona-Jiménez E, Ochoa-Velasco CE. Optimization of antioxidant compounds extraction from fruit by-products: Apple pomace, orange and banana peel. Journal of food processing and preservation. 2016 Feb;40(1):103-15.
16. Dulo B, De Somer T, Moyo M, Nakyese E, Githaiga J, Raes K, De Meester S. Kinetic modeling of phenolic compounds extraction from nutshells: influence of particle size, temperature and solvent ratio. Biomass Conversion and Biorefinery. 2024 Oct;14(19):23565-79.
17. Metrouh-Amir H, Duarte CM, Maiza F. Solvent effect on total phenolic contents, antioxidant, and antibacterial activities of Matricaria pubescens. Industrial crops and products. 2015 May 1;67:249-56.
18. Iloki-Assanga SB, Lewis-Luján LM, Lara-Espinoza CL, Gil-Salido AA, Fernandez-Angulo D, Rubio-Pino JL, Haines DD. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of Bucida buceras L. and Phoradendron californicum. BMC research notes. 2015 Dec;8:1-4.
19. Mokaizh AA, Nour AH, Kerboua K. Ultrasonic-assisted extraction to enhance the recovery of bioactive phenolic compounds from Commiphora gileadensis leaves. Ultrasonics Sonochemistry. 2024 May 1;105:106852.
20. Pătrăuanu OA, Lazăr L, Popa VI, Volf I. Influence of particle size and size distribution on kinetic mechanism of spruce bark polyphenols extraction. Dev Food Sci. 2019;8:11.
21. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144-158.

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994