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trans-Resveratrol and ε-viniferin recovery from grapevine pruning residues: process optimization and varietal screening towards industrial valorisation

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Abstract

Vine shoots are abundant viticultural by-products and promising renewable sources of high value stilbenoids for sustainable biorefinery applications. Stilbenoids are phytoalexins bearing phenolic moieties produced by Vitaceae under biotic and abiotic stress, and endowed with nutraceutical, cosmeceutical, and agrochemical potential. This study optimized the recovery of *trans*-resveratrol and ε-viniferin from vine shoots using Box–Behnken response surface methodology by evaluating extraction time (t), solvent mixture (S), and matrix-to-solvent ratio (R) as initial step of the circular economy task of European LIFE - Natural Agro project. The optimized conditions were then applied to conventional solid–liquid extraction (SLE), ultrasound-assisted extraction (UAE), and Soxhlet extraction. The optimal conditions were 100% ethanol, R = 0.12 g mL^{-1}, and t = 35 min. Although Soxhlet extraction gave the highest extraction yield (EY%), UAE produced the most stilbene-enriched extract, with 9.50 mg g^{-1} dry extract of *trans*-resveratrol and 25.66 mg g^{-1} dry extract of ε-viniferin, while reducing extraction time and prolonged thermal exposure. The optimized UAE protocol was subsequently applied to grapevine varieties from Italy, Portugal, and France, revealing marked genotype-dependent differences in stilbene composition. ε-Viniferin was generally more abundant than *trans*-resveratrol, and Pinot Meunier, Grechetto Gentile, Moscato, and Moscatel Graudo were identified as promising raw materials, depending on the target compound. Spectrophotometric assays further confirmed variety-dependent differences in antioxidant activity, total phenolic content, and total flavonoid content. Overall, the proposed UAE strategy offers a simple, food-grade, and scalable approach for valorising vine-pruning residues as sources of bioactive ingredients.

1. Introduction

The grape and wine sector generates substantial quantities of agricultural residues and processing by-products, including vine shoots, grape stalks, grape pomace, seeds, and wine lees. Among these materials, vine shoots, also referred to as grape canes or vine-pruning residues, constitute one of the largest lignocellulosic biomass streams generated directly in vineyards. Depending on the cultivar, vine vigour, planting density, training system, climatic conditions and pruning intensity, grapevines may generate approximately 0.9–5.0 tonnes of pruning residues per hectare annually [1–3]. Based on the global vineyard area and the biomass coefficients applied, the annual generation of vine-pruning residues has been estimated at several million tonnes worldwide, with reported estimates ranging from approximately 6.7 to 18.6 million tonnes and, in some assessments, up to 14.8–37 million tonnes [1, 2]. Despite this abundance, vine shoots remain underutilized and are commonly shredded and incorporated into the soil, composted, burned or used for low-value energy generation [3, 4].

Although soil incorporation may contribute organic matter and nutrients, these conventional management practices do not exploit the high-value extractable compounds present in vine shoots. Open-field burning may also contribute to particulate and greenhouse-gas emissions, whereas uncontrolled field retention can create logistical or phytosanitary concerns depending on local conditions [3, 4]. Consequently, vine shoots are increasingly regarded as renewable feedstocks for circular-bioeconomy and biorefinery processes rather than merely as agricultural waste. Their composition, which includes cellulose, hemicellulose, lignin and a chemically diverse extractive fraction, enables the sequential recovery of high-value phytochemicals followed by the conversion of the remaining lignocellulosic material into fermentable sugars, bioethanol, biochar, solid fuels, lignin-derived products or biomaterials [4–7].

The extractive fraction of vine shoots is particularly relevant because it contains phenolic acids, flavonoids and a broad range of stilbenoids. A comprehensive characterization of grapevine woody tissues has identified numerous monomeric and oligomeric stilbenes, while pilot-scale studies have confirmed that stilbenoids can represent the predominant phenolic class in grape-cane extracts [8, 9]. Among these compounds, *trans*-resveratrol and ε-viniferin are of particular interest. *trans*-Resveratrol is a monomeric stilbene widely used as a chemical and commercial marker of grape-derived bioactive ingredients, whereas ε-viniferin is an oxidative resveratrol dimer that can occur at high concentrations in grapevine woody tissues [8, 10, 11]. Other relevant compounds include *trans*-piceid, polydatin, piceatannol, δ-viniferin, vitisin A, vitisin B, hopeaphenol and additional oligomeric stilbenoids [9, 10].

In grapevine, stilbenes function mainly as phytoalexins and are synthesized or accumulated in response to fungal infection, ultraviolet radiation, mechanical injury and other biotic or abiotic stresses [9, 10]. Their biological significance for the plant is complemented by their potential technological and health-related applications. *trans*-Resveratrol and ε-viniferin have demonstrated antioxidant, anti-inflammatory and antimicrobial properties in experimental systems [10–13]. ε-Viniferin has also been investigated for potential cardiometabolic, vascular, neuroprotective and anticancer-related activities, although much of this evidence remains preclinical and does not yet establish therapeutic efficacy in humans [10, 12, 14]. Furthermore, the antioxidant behaviour of vine-derived extracts cannot always be attributed to a single compound, because *trans*-resveratrol, ε-viniferin, vitisin B and other stilbenes may produce additive, antagonistic or synergistic interactions [13].

The recovery of these compounds creates opportunities for the development of natural antioxidants, food and beverage preservatives, nutraceutical ingredients, cosmetic formulations, biocides, and other bio-based products. Pilot-scale extraction has demonstrated that stilbene-rich fractions can be recovered from grape canes, while purified grapevine-shoot extracts have shown potential as antimicrobial wine preservatives [8, 15]. However, the industrial exploitation of vine shoots requires extraction processes that provide adequate recovery and selectivity while minimizing solvent consumption, energy demand, processing time and degradation of thermolabile compounds.

Extraction efficiency is affected by the lignified structure of vine shoots and by operational variables such as solvent composition, temperature, particle size, and matrix-to-solvent ratio (R). Conventional maceration and Soxhlet extraction can provide useful reference conditions but generally involve long processing times, substantial solvent consumption or prolonged heating. Ultrasound-assisted extraction (UAE) offers an alternative based on acoustic cavitation, which promotes cell-wall disruption, solvent penetration and mass transfer. This technology can reduce extraction time (t) and solvent requirements and can be operated under relatively mild temperatures, making it suitable for recovering oxidation- and temperature-sensitive phenolic compounds [11, 16]. Nevertheless, its performance depends on ultrasound configuration, energy density and process conditions, and probe-based systems require careful optimization to avoid localized overheating and compound degradation.

Accordingly, this study aimed to optimize the recovery of *trans*-resveratrol and ε-viniferin from vine shoots as an abundant and underutilized grapevine residue. A Box–Behnken experimental design was used to determine the individual and interactive effects of t, solvent composition (S) and R. The optimized conditions were subsequently applied to UAE and Soxhlet extraction to compare their effectiveness in recovering the target stilbenes. The study therefore addresses the development of a statistically supported extraction strategy for converting discarded vine-shoot biomass into a source of high-value bioactive compounds. This research work is the starting point of the circular economy task of European LIFE - Natural Agro project, which aims to develop a new treatment without copper, against grapevines diseases (LIFE Programme's 2022 call for Standard Action Projects co-financed by the CINEA LIFE-2022-SAP-ENV-ENVIRONMENT).

2. Materials and methods

2.1 Vine shoot Samples

A total of 18 vine shoot samples from 18 grape varieties were collected from different vineyards in three European countries, as detailed in Table 1. All samples were collected the day after pruning, then promptly powdered with a grinder (Albrigi, Stallavena, Verona, Italy, mod. E0585) using a 1.5 mm sieve and then frozen and freeze-dried (BUCHI Lyovapor™ L-200 freeze-dryer, Büchi Labortechnik AG, Flawil, Switzerland) until constant weight. Dried samples were transferred to containers and kept at room temperature until extraction.

Table 1
Grapevine shoots samples information.

1	Grape variety	Country	GPS	Altitude (m a.s.l.)	Date of sampling
	Mourvedre	**France**	**44° 11' 8.88" N, 4° 57' 18.36" E**	**116**	**Feb-26**
2	Verdicchio	Italy	43° 28' 37" N 13° 05' 54" E	196	Feb-26
3	Pinot Grigio	Italy	44° 22' 15.81" N, 11° 45' 5.6" E	32	Jan-26
4	Grechetto Gentile	Italy	44° 20' 9.56" N 11° 41' 35.11" E	45	Dec-25
5	Malvasia Di Candia	Italy	44° 23' 48.05" N, 11° 59' 33.36" E	9	Dec-25
6	Pinot Meunier	France	49° 14' 19" N 4° 2' 56" E	70	Dec-25
7	Teroldego	Italy	46° 4' 44.3" N, 11° 8' 4.43" E	350	Dec-25
8	Trebbiano romagnolo	Italy	44° 17' 16" N 11° 46' 47" E	63	Dec-25
9	Bonarda	Italy	44° 43' 42.3" N 8° 29' 29.28" E	240	Nov-25
10	Chardonnay	Italy	45°34'55.5"N 10°01'08.6"E	195	Nov-25
11	Corvina	Italy	45°31'55.4" N 10°52'59.9"E	160	Nov-25
12	Friulano	Italy	46°01'42.2" N 13°28'42.5"E	85	Nov-25
13	Glera	Italy	45° 56' 36.97" N 12° 17' 50.18" E	145	Nov-25
14	Merlot	Italy	45° 57' 6.31" N 13° 27' 21.6" E	50	Nov-25
15	Moscatel Graudo	Portugal	39° 16' 58" N 9° 06' 39" W	110	Nov-25
16	Moscato	Italy	44° 43' 45" N 8° 29' 25.72" E	235	Nov-25
17	Sangiovese	Italy	43° 31' 09" N 11° 08' 59" E	120	Nov-25
18	Touriga National	Portugal	39° 4' 12.01" N 9° 7' 1.13" W	115	Nov-25

2.2 Experimental design for the optimization of extraction parameters

To determine the optimal conditions (S, R, and t) for the extraction of ε-viniferin and *trans*-resveratrol from vine shoots a Design of Experiment (DoE) tool of XLSTAT software (version 2023.1.4.1408) was used in Box-Behnken design mode to prepare the experimental plan. Extraction time (t; from 10 to 60 min), matrix/solvent ratio (R; from 0.02 to 0.12 g/mL), and solvent mixture (S; water/ethanol mixtures ranging from 0-100% v/v; ethanol absolute, Carlo Erba Reagents Srl, Cornaredo, Milan, Italy) were set as variables, while extraction yield (EY%), ε-viniferin, and *trans*-resveratrol concentrations were monitored as dependent variables (responses). These extreme points were extrapolated from scientific literature [17, 18]. A total of 13 experiments were suggested and performed on a pooled sample (prepared using the same quantity of each of the 18 samples) using a solid-liquid extraction (SLE) method in duplicate according to the run order (**Table S1, Online Resource 1**). Samples and solvent were poured into Erlenmeyer flask and left to steer with a shaken frequency of 100 rpm. The flasks were covered to prevent solvent loss and to protect the samples from the light. Then, mixtures were filtered and dried using a rotary evaporator or the freeze-drier to completely remove water traces. The extracts were then stored at − 20°C until analysis.

2.3 Stilbenoids quantification through HPLC-DAD

Dried extracts were dissolved in 10 mL of HPLC-grade acetonitrile, then filtered through a 0.45 µm PTFE filter. The analysis was performed using a 1260 Infinity High-Performance Liquid Chromatography (HPLC) apparatus (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (DAD). The chromatographic separation was achieved with a Synergy Polar-RP C18 (4.6 mm× 150 mm, 4 µm) from Phenomenex (Torrance, CA, USA) set at

40°C. The mobile phase consisted of Milli-Q water (A) (resistivity 58 M) obtained via a Milli-Q SP system (Millipore, Bedford, MA) and acetonitrile (B) (HPLC-grade, Sigma-Aldrich) with a flow rate of 0.8 mL/min. Elution gradient was set as follow: 0–10 min 20% B, 10–20 min 70% B and a 20%B reconditioning time of 12 min for a total run time of 32 minutes. The injection volume was 5 µL. *trans*-Resveratrol and ε-viniferin were identified and quantified using analytical standards (analytical standard and phyproof® Reference Substance, respectively) supplied by Sigma Aldrich (Milan, Italy) (**Fig. S1, Supplementary material**). Standard solutions with concentrations ranging from 5 to100 mg/L for *trans*-resveratrol and ε-viniferin were prepared to plot the calibration curves. The ε-viniferin was monitored at 236 nm while *trans*-resveratrol at 304 nm. The standard calibration curves, reporting a good linearity ($R^2 > 0.99$), were used for quantification and results were reported as µg/g of dry extract (DE). Limit of Detection (LOD) and Limit of Quantification (LOQ) were experimentally estimated injecting low concentrations of the analyte standard solutions and measuring the signal-to-noise (S/N) ratio. A concentration giving a S/N ratio (height of peak/height of noise) of three was assigned to LOD while that of ten was LOQ. LOD were 0.18 mg/L and 0.37 mg/L while LOQ were 0.59 mg/L and 1.22 mg/L for *trans*-resveratrol and ε-viniferin respectively.

2.4 Optimization of the extraction procedure

The extraction parameters (t; R; S) selected based on the SLE DoE results were applied to other two techniques: UAE and Soxhlet extraction. For UAE, a 9 L temperature-controlled water ultrasonic bath (ARGOLAB AU-220) was used. According to DoE optimized parameters, 3 g of pooled sample were extracted with 25 mL of solvent for 35 min at a frequency of 45 kHz at room temperature. The temperature was kept lower than 25°C by adding ice in the bath. The Soxhlet extraction procedure was performed using a 6-channels Universal Extractor in Soxhlet mode (Büchi E-800). A total of 24 g of pooled sample was placed inside extraction bags and then positioned in the equipment chamber. According to optimum R parameter, 200 mL of solvent were added. The extraction was carried out at 70°C and 35 min. Afterwards, both UAE and Soxhlet extracts were filtered and dried to calculate the EY% before reconstitution in acetonitrile and HPLC-DAD injection. Each extraction experiment was performed in triplicate. The optimized extraction parameters and technique were applied to the 18 single samples monitoring EY%, *trans*-resveratrol and ε-viniferin concentrations.

2.5 Total Phenolic and Flavonoids content and radical scavenging activity of the extracts

For the determination of total phenolic content, a procedure previously reported by Corsetti et al. [19] was involved. Folin-Ciocalteau working solution was prepared by diluting 5 mL of Folin-Ciocalteau reagent (Sigma–Aldrich, Milan, Italy) with 45 mL of distilled water. Samples were prepared by mixing 2.5 mL of Folin-Ciocalteau solution, 0.5 mL of grapevine shoots extract and 7 mL of sodium carbonate solution (from Sodium carbonate anhydrous ≥ 99 %, a_2CO_3; Sigma–Aldrich, Milan, Italy). The blank solution was prepared by mixing 2.5 mL of Folin-Ciocalteau solution with 0.5 mL of ethanol (EtOH, Carlo Erba Reagents Srl, Cornaredo, Milan, Italy). All solutions were then stored in the dark for 2 hours, then the absorbance was measured at 765 nm with Cary 8454 UV–visible spectrophotometer (Agilent Technologies, Woburn, MA, USA). Gallic acid was used to prepare the calibration curve, and results are expressed as mg of gallic acid equivalents (GAE)/kg of DE.

The total flavonoid content was determined using a spectrophotometric colorimetric method previously reported by Alessandroni et al. [20]. Specifically, 0.5 mL of each sample were mixed with 0.15 mL of a 0.5 M sodium nitrite solution (≥ 99%, $NaNO_2$, Sigma-Aldrich, Milan, Italy) and 3.2 mL of methanol (Carlo Erba Reagents Srl, Cornaredo, Milan, Italy). After 5 min, 0.15 mL of 0.3 M aluminium chloride solution (≥ 99%, $AlCl_3$, Sigma-Aldrich, Milan, Italy) were added. The mixture was allowed to react for additional 5 min, so 1 mL of 1 M sodium hydroxide solution (≥ 98%, NaOH, Sigma-Aldrich, Milan, Italy) was added. A blank sample was prepared using methanol. All samples were then stored in the dark for 30 min. The absorbance was monitored at 506 nm through the spectrophotometer and results were calculated by comparing the samples absorbance with a rutin (≥ 94%, $C_{27}H_{30}O_{16}$, Sigma-Aldrich, Milan, Italy) calibration curve and were expressed as mg of rutin equivalents (RE)/kg of DE.

The antioxidant activity was evaluated using the DPPH assay following the procedure reported by Alessandroni et al. [20]. The DPPH working solution was prepared by dissolving 3.94 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl radical, Sigma–Aldrich, Milan, Italy) in 100 mL of ethanol. Subsequently, 0.5 mL of each sample were mixed with 4.5 mL of DPPH solution. For the control sample, 0.5 mL of ethanol were added to 4.5 mL of DPPH solution, while 100% ethanol was used as blank. All solutions were then stored in the dark for 30 min, then the absorbance was read at 517 nm using the UV–visible spectrophotometer. The Trolox (6-hydroxy-2,5,7,8 tetramethylchromane-2-carboxylic acid, Sigma–Aldrich, Milan, Italy) was used to set the calibration curve and the results were reported as the percentage of DPPH inhibition. The described procedures were repeated in triplicate for each of the 18-grapevine variety extract obtained using the optimized extraction method.

2.6 Statistical analysis

To optimize extraction parameters the Design of Experiment was prepared using Box Behnken designs. The independent variables were t, R, and S, while EY%, *trans*-resveratrol and ε-viniferin concentrations were monitored as dependent variables or responses. Data were analyzed using the “analysis of surface response design” tool in XLSTAT software (version 2023.1.4.1408). After the DoE parameters optimization, the EY%, *trans*-resveratrol and ε-viniferin concentrations from SLE, UAE, and Soxhlet extractions were compared using ANOVA tests to determine the most effective technique. Same tool was used to compare results from the 18 grapevine varieties.

3. Results

3.1 Optimization of extraction parameters

The extraction parameters were investigated on the pooled sample according to the experimental plan proposed by the Box-Behnken design. This mode was chosen because it contains fewer design points compared to the widely utilized central composite designs, making it less costly and quicker, while maintaining the same number of factors. The maximum t was set at 60 min for applicability reasons, aiming to scale-up processes. The influence of the main process parameters, being t, R, and S, were evaluated on the EY%, ε-viniferin and *trans*-resveratrol concentrations in the extracts. The experimental data for

each response were fitted to a second-order polynomial regression model (**Eqs. 1, 2 and 3** for EY%, *trans*-resveratrol and ε-viniferin concentrations respectively), whose adequacy was confirmed by ANOVA, the goodness of fit statistics values is reported in Table 2. These results indicate a good agreement between experimental and predicted values, confirming the reliability of the model.

$$EY\% = 9.43 - 2.094*R - 1.55*S + 0.52*t + 0.30*R^2 - 0.31*R*S - 1.04*R*t - 2.96*S^2 - 0.79*S*t - 1.19*t^2$$ **Eq. 1**

$$Trans - resveratrol\left(\frac{mg}{Kg}DE\right) = = 726.02 + 125.13*R + 1608.76*S + 159.71*t - 245.36*R^2 + 340.18*R*S + 81.51*R*t + 1108.08*S$$

Eq. 2

$$\epsilon - viniferin\left(\frac{mg}{Kg}DE\right) = 2352.78 + 635.77*R + 6338.49*S + 700.79*t - 405.53*R^2 + 1508.90*R*S + 97.84*R*t + 4514.87*S^2 + 14$$

Eq. 3

in which R is the matrix/solvent ratio, S is the H_2O/EtOH proportion in solvent mixture expressed as percentage of EtOH, and t is the extraction time.

Table 2
Box-Behnken experimental design goodness of fit statistics ANOVA results for the three response variables

Response	R^2	Adjusted R^2	F	*p*-value
EY%	0.883	0.817	13.414	< 0.0001
trans-resveratrol (mg/kg DE)	0.925	0.883	22.011	< 0.0001
ε-viniferin (mg/kg DE)	0.930	0.891	23.697	< 0.0001

EY%, extraction yield (%). DE, dry extract.

To better visualize the obtained results of the DoE performed experiments, surface plots were reported in Fig. 1.

The solvent choice (variable S) was the most influential factor, with the use of 100% ethanol as the solvent consistently resulting in the highest EY% and concentrations for both target stilbenoids. For example, observations 3, 4, 10, 12, 17, 18, 24, and 26, all using pure ethanol, consistently produced *trans*-resveratrol and ε-viniferin concentrations exceeding 800 mg/kg and 1400 mg/kg, respectively, with peak values exceeding 2400 mg/kg and 4800 mg/kg (Obs18). Response surface plots in Fig. 1 also confirmed these trends, showing a significant maximum at the highest ethanol level. A higher ratio of 0.12 g/mL, especially when combined with pure ethanol, resulted in a significant increase in yield and stilbene content. This effect was synergistic; R increased with extraction and enhanced with 100% ethanol, while lower ethanol percentage attenuated this effect. Time appeared to be a secondary factor, as longer t (60 min) often yielded better results than shorter t (10 min), although its effect was less than that of solvent strength [21]. The center point results (Obs13 and Obs14 in 50% EtOH, 35 min) showed high variability, especially for Obs14, suggesting that this intermediate range may be a sensitive region or that the process is strongly influenced by interactions between factors. Therefore, the optimal parameters to maximize the extraction of both stilbenes are high ethanol concentration (100%), high matrix/solvent ratio (0.12 g/mL), and 35 min for time. The optimal duration of 35 min represented a balance between sufficient contact time for mass transfer and avoidance of compound degradation. This finding is consistent with studies on similar polyphenolic compounds, where t of 30 to 60 min typically provides optimal yields [17]. Longer extraction periods may result in thermal degradation of sensitive stilbenes or extraction of interfering compounds [18, 22].

3.2 Effects of the extraction variables on extraction yield and stilbene recovery

For an in-depth comprehension of the independent variables' impact on the responses (t, R, and S) Pareto charts were prepared and graphically presented in Fig. 2.

The relative influence of the independent variables and their interactions on EY%, *trans*-resveratrol, and ε-viniferin recoveries was evaluated using Pareto charts of standardized effects (Fig. 2). The magnitude of each bar represents the relative contribution of the corresponding model term, while positive and negative effects are indicated in green and red, respectively. The Lorenz curves show the cumulative contribution of the model terms ranked according to their standardized effects.

For EY% (Fig. 2A), R was the most influential factor and created a positive effect, accounting for 33.8% of the cumulative standardized effect. This result indicates that the proportion between plant material and solvent was a major determinant of the overall mass recovered from vine shoots. Within the investigated experimental range, the higher R value appeared to favour extract recovery, probably because it increased the amount of extractable material available in the extraction system while maintaining sufficient solvent capacity for mass transfer. However, the negative effects associated with the linear and quadratic terms of ethanol concentration indicate that EY% did not increase continuously with increasing ethanol percentage. Instead, the response suggests that an intermediate ethanol/water composition was more appropriate for recovering the chemically heterogeneous extractive fraction of vine shoots.

The factor-response pattern differed for *trans*-resveratrol (Fig. 2B). t was the dominant positive term and accounted for 39.9% of the cumulative standardized effect, indicating that *trans*-resveratrol recovery was primarily governed by extraction kinetics. Although ultrasound can rapidly disrupt plant tissues, sufficient

contact time remains necessary for *trans*-resveratrol to diffuse from the lignified vine-shoot structure and dissolve in the extraction solvent. Previous optimization of UAE from grape canes similarly identified t, ethanol concentration, and temperature as important determinants of stilbene recovery, with aqueous ethanol providing efficient extraction of the principal stilbenes [23].

For ε-viniferin (Fig. 2C), the quadratic effect of the matrix-to-solvent ratio (R^2) was the dominant model term, contributing 41.2% of the cumulative standardized effect. The predominance of the quadratic rather than the linear term indicates a pronounced curvature in the response and suggests the existence of an optimum R within the experimental domain. Below this optimum, insufficient solvent may restrict wetting, diffusion, and solubilization, whereas an excessive solvent volume may dilute the extract without producing a proportional increase in ε-viniferin recovery.

Several interaction terms negatively affected ε-viniferin recovery, indicating that simultaneous changes in t, ethanol concentration, and R could reduce extraction efficiency even when individual factors exerted positive effects. The greater importance of quadratic and interaction terms for ε-viniferin demonstrates that its recovery was governed by a more complex response surface than that of *trans*-resveratrol. Consequently, optimization based solely on individual factor effects would be inadequate for identifying the most favourable conditions.

Overall, the Pareto analysis showed that the extraction variables affected the three responses differently: EY% was primarily controlled by the linear effect of R, trans-resveratrol recovery was mainly governed by t, and ε-viniferin recovery was dominated by the quadratic effect of R. These response-specific patterns confirmed that the condition associated with the highest total extraction yield was not necessarily the condition providing the highest recovery of the target stilbenes.

3.3 Extraction process optimization

The optimized extraction conditions were applied to SLE, UAE, and Soxhlet extraction, with each experiment performed in triplicate. The extraction method significantly affected the overall EY% and the concentration of the target stilbenes in the resulting dry extracts (Table 3). Soxhlet extraction produced the highest EY% (3.95 ± 0.10%), followed by UAE (3.13 ± 0.13%) and SLE (2.11 ± 0.096%), with significant differences among all three methods. However, the higher total EY% obtained by Soxhlet did not correspond to a proportionally higher recovery of *trans*-resveratrol or ε-viniferin.

Table 3
Extraction methods comparison. Different letters represent statistically significant differences in concentration values between the three tested methods

EY%	**SLE**	**UAE**	**Soxhlet**
	2.11 ± 0.096 a	**3.13 ± 0.13 b**	**3.95 ± 0.10 c**
trans-resveratrol (mg/g DE)	4.61 ± 0.46 a	9.50 ± 1.03 b	7.62 ± 0.25 b
ε-viniferin (mg/g DE)	16.14 ± 3.05 a	25.66 ± 1.16 b	17.14 ± 1.22 ac
(EY%: extraction yield percentage; SLE: Solid Liquid Extraction; UAE: Ultrasound Assisted Extraction; DE: dry extract)			

The UAE extract contained the highest concentrations of both *trans*-resveratrol and ε-viniferin, reaching 9.50 ± 1.03 and 25.66 ± 1.16 mg/g DE, respectively. *trans*-Resveratrol concentration did not differ significantly between UAE and Soxhlet extraction, although both methods produced significantly higher values than SLE. In contrast, ε-viniferin concentration was significantly higher in the UAE extract than in the Soxhlet and SLE extracts. These findings indicate that UAE provided a more stilbene-enriched extract, whereas the higher EY% achieved by Soxhlet was partly attributable to the recovery of additional non-target extractable compounds.

To account for differences in total DE yield, the concentrations expressed as mg/g DE were converted into the absolute recovery of each stilbene per 100 g of vine-shoot matrix. UAE produced 3.13 g of DE per 100 g of matrix, corresponding to 29.74 mg of *trans*-resveratrol and 80.32 mg of ε-viniferin. Soxhlet extraction produced 3.95 g of DE, corresponding to 30.10 mg of *trans*-resveratrol and 67.70 mg of ε-viniferin. Therefore, the two methods provided practically identical *trans*-resveratrol recoveries, equivalent to approximately 0.030% of the original matrix. However, UAE recovered approximately 18.6% more ε-viniferin than Soxhlet, corresponding to 0.080 and 0.068% of the matrix, respectively.

3.4 Grapevine shoots application and extract characterization

trans-Resveratrol and ε-viniferin were identified by HPLC-DAD at retention times of 10.8 and 11.3 min, respectively. The calibration curves showed adequate linearity over the investigated analytical range, with coefficients of determination of 0.9937 for *trans*-resveratrol and 0.9992 for ε-viniferin. The 18 vine-shoot samples from different varieties (Table 1) were extracted in triplicate by UAE and analysed for both target compounds. Across the complete sample set, *trans*-resveratrol concentrations ranged from 10.81 ± 3.18 to 696.93 ± 80.28 mg/kg freeze-dried matrix, whereas ε-viniferin ranged from 197.91 ± 64.63 to 1179.52 ± 153.11 mg/kg freeze-dried matrix. The generally higher ε-viniferin values indicate that the dimeric stilbene constitutes an important component of the vine-shoot phenolic fraction.

The EY% and stilbene concentrations obtained for the 18 grape varieties are reported in Table 4. EY% varied from 2.22 ± 0.19% in Moscatel Graudo to 5.56 ± 0.84% in Grechetto Gentile. Relatively high EY% were also obtained for Trebbiano romagnolo and Pinot Grigio, reaching 5.11 ± 1.39% and 4.89 ± 0.84%, respectively. In contrast, Bonarda and Touriga Nacional both showed EY% of 2.44 ± 0.38%, while Moscatel Graudo exhibited the lowest value. These differences indicate that the total amount of ethanol-soluble material recovered from vine shoots was strongly dependent on grape variety. Nevertheless, EY% alone did not reflect the degree of enrichment in the target stilbenes. For example, Grechetto Gentile produced the highest EY% and contained relatively high concentrations of both *trans*-resveratrol and ε-viniferin, whereas Moscatel Graudo produced the lowest EY% but was one of the most *trans*-resveratrol-enriched extracts. Conversely, Pinot Grigio showed a comparatively high EY% but a low *trans*-resveratrol concentration. Therefore, the gravimetric yield

included stilbenes together with other ethanol-soluble constituents, such as phenolic acids, flavonoids, sugars, organic acids, and additional extractives. This finding demonstrates that total EY% and target-compound selectivity should be considered as complementary, rather than interchangeable, indicators of extraction performance.

Varietal differences were observed in the *trans*-resveratrol concentration of the DEs. Values ranged from 1.78 ± 0.15 mg/g DE in Sangiovese to 35.07 ± 5.07 mg/g DE in Pinot Meunier. Pinot Meunier was followed by Moscatel Graudo, Moscato and Bonarda, which contained 26.14 ± 0.10, 15.37 ± 0.27 and 13.82 ± 1.18 mg/g DE, respectively. Mourvèdre and Touriga Nacional also exhibited appreciable concentrations, reaching 10.10 ± 0.18 and 9.60 ± 0.42 mg/g DE. In contrast, *trans*-resveratrol concentrations below 3 mg/g DE were observed in Sangiovese, Verdicchio, Friulano and Pinot Grigio.

The ε-viniferin concentration was higher than that of *trans*-resveratrol in every variety evaluated. It ranged from 15.89 ± 1.53 mg/g DE in Verdicchio to 65.65 ± 8.75 mg/g DE in Pinot Meunier. Grechetto Gentile showed a similarly high ε-viniferin concentration of 64.73 ± 1.30 mg/g DE, followed by Moscato and Bonarda at 57.88 ± 0.91 and 51.16 ± 0.82 mg/g DE, respectively. Intermediate-to-high values were measured in Teroldego, Corvina, Friulano and Trebbiano romagnolo, whereas Verdicchio, Malvasia di Candia, Chardonnay, and Touriga Nacional contained the lowest ε-viniferin concentrations. Pinot Meunier was therefore the most promising variety in terms of simultaneous enrichment in both target compounds, presenting the highest *trans*-resveratrol and ε-viniferin concentrations in DE. However, the results also revealed compound-specific varietal patterns. Moscatel Graudo was strongly enriched in *trans*-resveratrol but contained only an intermediate level of ε-viniferin, whereas Grechetto Gentile contained substantially more ε-viniferin than *trans*-resveratrol. These differences indicate that a variety suitable for *trans*-resveratrol recovery is not necessarily the most suitable source of ε-viniferin. Similar genotype-dependent variations have been reported previously in grapevine canes and shoots, confirming that cultivar is one of the principal determinants of stilbene composition [11, 24–26].

Because the concentrations in Table 4 are expressed per g of DE, they describe extract enrichment but not necessarily the total recovery from the starting matrix. To account for differences in EY%, the amount of each compound recovered per 100 g of vine-shoot matrix was estimated by multiplying EY% by concentration in the DE. On this basis, Pinot Meunier provided the highest *trans*-resveratrol recovery, corresponding to approximately 105.6 mg/100 g matrix, followed by Moscato and Moscatel Graudo at approximately 63.2 and 58.0 mg/100 g matrix, respectively. Grechetto Gentile yielded approximately 49.6 mg *trans*-resveratrol/100 g matrix, whereas Sangiovese and Verdicchio provided only approximately 5.9 and 6.1 mg/100 g matrix.

The matrix-based ranking differed for ε-viniferin. Grechetto Gentile provided the highest estimated recovery, at approximately 359.9 mg/100 g matrix, because of combining the highest EY% with a high ε-viniferin concentration. Moscato and Pinot Meunier followed, with approximately 237.9 and 197.6 mg/100 g matrix, respectively. Trebbiano romagnolo, Teroldego, Corvina and Pinot Grigio also provided relatively high estimated ε-viniferin recoveries, ranging from approximately 151 to 168 mg/100 g matrix. In contrast, Touriga Nacional, Verdicchio and Chardonnay provided less than approximately 56 mg/100 g matrix.

Table 4
Extraction yield (EY%) and concentrations of trans-resveratrol and ε-viniferin (mg/g DE) in dry extracts obtained by ultrasound-assisted extraction from vine shoots of 18 grape varieties. Results are expressed as mean ± standard deviation.

Grape variety	EY%	*trans*-resveratrol (mg/g DE)	ε-viniferin (mg/g DE)
Bonarda	2.44 ± 0.38%	13.82 ± 1.18	51.16 ± 0.82
Chardonnay	2.89 ± 0.38%	3.03 ± 0.46	19.14 ± 2.81
Corvina	4.22 ± 0.51%	4.46 ± 0.34	37.34 ± 1.95
Friulano	3.56 ± 0.51%	2.40 ± 0.20	33.20 ± 1.55
Glera	3.50 ± 0.17%	3.03 ± 0.11	26.85 ± 1.95
Grechetto Gentile	5.56 ± 0.84%	8.92 ± 0.66	64.73 ± 1.30
Malvasia Di Candia	3.33 ± 0.33%	6.06 ± 1.02	18.06 ± 1.60
Merlot	4.11 ± 0.69%	8.60 ± 0.69	32.63 ± 4.10
Moscato	4.11 ± 0.69%	15.37 ± 0.27	57.88 ± 0.91
Mourvedre	4.56 ± 0.38%	10.10 ± 0.18	24.42 ± 0.50
Pinot Grigio	4.89 ± 0.84%	2.84 ± 0.22	30.88 ± 2.38
Pinot Meunier	3.01 ± 0.02%	35.07 ± 5.07	65.65 ± 8.75
Sangiovese	3.33 ± 0.67%	1.78 ± 0.15	23.81 ± 2.76
Teroldego	4.11 ± 0.69%	4.36 ± 0.40	39.99 ± 1.66
Trebbiano romagnolo	5.11 ± 1.39%	6.28 ± 0.11	32.80 ± 0.95
Verdicchio	3.33 ± 0.33%	1.84 ± 0.12	15.89 ± 1.53
Touriga National	2.44 ± 0.38%	9.60 ± 0.42	19.36 ± 2.31
Moscatel Graudo	2.22 ± 0.19%	26.14 ± 0.10	28.50 ± 3.14

The spectrophotometric assays showed clear differences among the 18 vine-shoot varieties in DPPH radical-scavenging activity, total phenolic content (TPC), and total flavonoid content (TFC) (Fig. 3). The DPPH results indicated that the antioxidant capacity of the extracts was strongly variety-dependent, with Merlot samples showing markedly higher radical-scavenging activity than the other varieties, reaching approximately 96 mg TE/g DE. A similar trend was observed for TPC, where only a limited number of varieties showed high phenolic accumulation. Merlot exhibited the highest total phenolic content, with a value of 17.33 mg GAE/kg DE. These results confirm that vine shoots are not a homogeneous by-product, but rather a variable source of bioactive compounds whose composition may depend on genotype, vineyard conditions, and post-harvest factors [11, 27].

4. Discussion

The experimental findings demonstrate that extraction performance depended on the combined effects of solvent composition, matrix-to-solvent ratio, extraction time, and extraction technique, while the varietal screening highlighted substantial differences in stilbene enrichment and antioxidant characteristics among grapevine shoots.

4.1 Influence of extraction parameters

The findings indicate that ethanol concentration is the most important extraction parameter for stilbene recovery, which is consistent with the extensive literature documenting the superior performance of alcoholic solvents for polyphenolic compounds [28]. The observed superiority of pure ethanol (100%) is consistent with reports indicating that the polarity properties of ethanol make it particularly effective for the extraction of phenolic compounds, including stilbenes [29–32].

R emerged as the third most influential parameter, and higher ratios (0.12 g/mL) consistently yielded higher extraction efficiencies and stilbenoids concentrations. This finding is consistent with the basic principles of solid-liquid extraction, where the solvent volume directly affects the concentration gradient that drives mass transfer. The superior performance at higher ratios (0.12 g/mL) can be explained by several interconnected mechanisms that collectively increase the extraction efficiency [33].

Hydroalcoholic mixtures are generally more effective than pure ethanol or water for extracting grapevine phenolics because water promotes matrix swelling and facilitates solvent penetration, whereas ethanol improves the solubilization of less polar phenolic compounds [23, 34]. Although intermediate ethanol/water mixtures may favour the recovery of the heterogeneous extractive fraction, 100% ethanol was more suitable for selective recovery of the target stilbenes under the optimized conditions. The negative quadratic effect of ethanol concentration therefore reflects the loss of extraction efficiency at solvent compositions located away from the optimum region. The negative R × S interaction further indicates that the influence of ethanol concentration depended

on the R. Thus, increasing ethanol concentration was not equally effective at all solvent volumes, confirming that solvent polarity and availability should be optimized simultaneously rather than independently.

The t had a comparatively limited positive effect on total EY%, whereas its quadratic contribution was negative. This pattern suggests that most readily extractable compounds were released during the initial extraction period and that extending the process produced progressively smaller improvements. Similar behaviour has been reported for UAE of grapevine stilbenes, in which efficient recovery was achieved within relatively short extraction periods because acoustic cavitation accelerated solvent penetration and mass transfer [23]. Prolonged extraction may therefore offer limited benefits while increasing energy consumption and the exposure of phenolic compounds to oxygen, light, and temperature.

4.2 Interpretation of factor effects on trans-resveratrol and ε-viniferin recovery

The positive R × t interaction demonstrates that the effect of t depended on solvent availability. Under a suitable R, prolonged contact may maintain a stronger concentration gradient and reduce saturation of the liquid phase, thereby favouring continued transfer of *trans*-resveratrol from the solid matrix. Conversely, the negative interaction between R and ethanol concentration indicates that some combinations of high or low factor levels were unfavourable. This finding confirms that increasing solvent volume or ethanol percentage individually does not necessarily improve *trans*-resveratrol recovery when both variables are modified simultaneously.

The linear effect of ethanol concentration on *trans*-resveratrol was positive, consistent with the moderate polarity of this stilbene and its favourable solubility in hydroalcoholic solvents. Nevertheless, the contribution of the interaction terms indicates that solvent composition should be selected in relation to t and matrix loading. Studies on grapevine shoots and canes have commonly used ethanol/water mixtures for the recovery of *trans*-resveratrol and ε-viniferin, supporting the suitability of food-compatible hydroalcoholic media for stilbene extraction [11, 23, 34].

The t and the quadratic effect of ethanol concentration also exerted positive effects on ε-viniferin recovery. The relevance of S^2 indicated that ε-viniferin required a more narrowly defined solvent composition than total EY% or *trans*-resveratrol. This distinction may be attributed to the dimeric structure of ε-viniferin, which results in different polarity, molecular size, and solvent affinity compared with monomeric *trans*-resveratrol. Vine-shoot and grape-cane studies have shown that *trans*-resveratrol and ε-viniferin respond differently to solvent composition and extraction conditions, confirming that the two stilbenes cannot be assumed to have identical extraction behaviour [11, 23, 35].

4.3 Comparison of extraction techniques

These calculations clarify why UAE can be considered more favourable despite its lower overall EY%. Soxhlet extraction recovered a greater total mass of soluble material, but the resulting extract was less concentrated in the target stilbenes. In contrast, UAE showed greater selectivity, particularly toward ε-viniferin, and produced a DE containing approximately 25% more *trans*-resveratrol and 50% more ε-viniferin per g than the Soxhlet extract. Thus, EY% alone was not an adequate indicator of process efficiency, because it did not distinguish between the recovery of the target stilbenes and the co-extraction of other matrix constituents.

The improved performance of UAE can be attributed to acoustic cavitation, which promotes particle erosion, cell-wall disruption, solvent penetration, and rapid mass transfer from the lignified vine-shoot matrix. Previous studies have demonstrated that UAE can efficiently recover *trans*-resveratrol, ε-viniferin, and other stilbenes from grape canes within relatively short extraction periods [11, 23]. Although elevated temperatures may enhance the release and solubility of stilbenes, prolonged thermal exposure can also promote oxidation, isomerization, or degradation, depending on the compound, temperature, exposure time, light, and solvent conditions [36]. Soxhlet extraction continuously exposes the sample and extract to refluxing ethanol, whereas the UAE treatment used in the present study was performed without intentional external heating. Therefore, UAE reduced prolonged thermal exposure while maintaining *trans*-resveratrol recovery and improving that of ε-viniferin.

It should be noted that ultrasound extraction is not inherently non-thermal, because cavitation can produce localized heating and the bulk temperature may increase during prolonged sonication. Temperature monitoring is therefore necessary to ensure reproducibility and prevent unwanted degradation. Moreover, recent studies have shown that short, controlled high-temperature treatments, including microwave-assisted extraction, can achieve high stilbene recovery when exposure time is carefully optimized [16]. Accordingly, the advantage of UAE in the present study was not simply the absence of heat, but the combination of rapid mass transfer, limited processing time, reduced prolonged thermal exposure, and greater selectivity toward ε-viniferin.

From a process perspective, UAE also offers advantages over Soxhlet extraction in terms of shorter t, lower energy demand, reduced solvent recirculation, and easier adaptation to intensified extraction systems. Considering the comparable recovery of *trans*-resveratrol, the higher recovery and enrichment of ε-viniferin, and the milder and more time-efficient operating conditions, UAE was selected as the most appropriate extraction method for the subsequent experimental phases.

4.4 Varietal differences and antioxidant characteristics

These calculations emphasize the importance of reporting both stilbene concentration in the extract and recovery relative to the original biomass. An extract may be highly concentrated because only a limited quantity of total material was recovered, whereas another variety may generate a less concentrated extract but a higher absolute amount of the target compound because of its greater EY%. For industrial raw-material selection, matrix-based recovery is therefore more informative for estimating production capacity, while concentration in the dry extract remains relevant for downstream purification, formulation and transportation costs.

The predominance of ε-viniferin over *trans*-resveratrol in the present extracts is consistent with previous characterizations of grapevine woody tissues. Vine shoots and mature canes contain complex mixtures of monomeric, dimeric and oligomeric stilbenes, and ε-viniferin is frequently among the major constituents. It is generated through oxidative coupling of resveratrol units and may accumulate as part of the grapevine defence response. Consequently, its

concentration depends not only on resveratrol biosynthesis but also on the extent of oxidative transformation and oligomerization within the tissue. This helps explaining why the two compounds did not follow identical varietal patterns.

Noviello et al. [11] reported substantial variation among 23 Italian grape varieties, with total *trans*-resveratrol plus ε-viniferin contents ranging approximately from 2700 to 6400 mg/kg dry weight. Their results showed that cultivars affected stilbene concentration more strongly than the tested thermal pretreatments. Guerrero et al. [24] similarly demonstrated that stilbene profiles differed among 13 Vitis materials and varied across three consecutive years, indicating that genotype interacts with climatic and seasonal factors. Loupit et al. [25] also observed highly diverse polyphenolic profiles among cultivated and wild Vitis canes collected immediately after pruning. Together, these studies support the strong varietal dependence observed in the present investigation.

Direct quantitative comparison among studies should nevertheless be made cautiously. Reported values are influenced by cultivar, geographical origin, vintage, vine age, tissue maturity, post-pruning storage, drying conditions, particle size, solvent composition, extraction technology, and analytical procedure. In addition, concentrations may be expressed as mg/g DE, mg/kg dry matrix or amount recovered per mass of initial biomass. The values reported by Noviello et al. [11] were expressed on a dry-matrix basis, whereas Table 3 reports stilbene enrichment in the DE. Conversion between these units requires the corresponding EY%, as demonstrated above.

Duarte et al. [37] reported that the recovery of *trans*-resveratrol and trans-ε-viniferin from vine shoots varied according to grape variety, solvent composition and extraction conditions, further confirming that both biological and technological factors determine the measured concentration. Pilot-scale characterization of grape-cane extracts has likewise identified stilbenoids as a major phenolic group, with ε-viniferin representing an important component of the recovered fraction [8]. These observations are consistent with the present results and reinforce the suitability of vine shoots as a source of stilbene-rich extracts.

From an industrial perspective, the present findings support cultivar-specific rather than indiscriminate processing of mixed pruning residues. Pinot Meunier appears particularly suitable when simultaneous recovery of *trans*-resveratrol and ε-viniferin is desired. Grechetto Gentile represents the most promising matrix for maximizing ε-viniferin recovery per unit of starting biomass, whereas Moscatel Graudo may be advantageous for producing extracts enriched in trans-resveratrol. However, industrial selection should not rely on a single harvest or location because stilbene biosynthesis is also affected by environmental conditions, pathogen pressure, post-harvest handling and storage. Multi-year and multi-location validation would therefore be required before defining stable high-stilbene varieties for commercial supply chains.

The partial agreement between DPPH activity and TPC suggests that phenolic compounds made a substantial contribution to the antioxidant potential of the extracts [38]. However, the TFC profile showed a narrower distribution among varieties, with Moscato and Chardonnay exhibiting the highest flavonoid contents, at 1.55 and 1.34 mg RE/g DE, respectively. This indicates that flavonoids contributed to the antioxidant response but were unlikely to be the only compounds responsible for the observed activity. This is particularly relevant for vine shoots, which are known to contain high levels of stilbenes, especially *trans*-resveratrol and ε-viniferin. These compounds can contribute to antioxidant activity either individually or through interactions with other phenolic constituents [11, 13].

To sum up, these results support the use of vine shoots as a promising source of antioxidant-rich extracts for pruning residue valorisation. Nevertheless, the differences among DPPH, TPC, and TFC profiles indicate that spectrophotometric assays should be considered as preliminary screening tools rather than complete indicators of extract quality. Therefore, their interpretation should be combined with chromatographic quantification of key stilbenes, such as *trans*-resveratrol and ε-viniferin, to better identify the compounds mainly responsible for the bioactive potential of each variety [13, 16].

5. Conclusion

This study demonstrated that vine shoots are a valuable and chemically variable source of stilbene-rich extracts, particularly *trans*-resveratrol and ε-viniferin. The Box–Behnken response surface approach allowed the optimization of the main extraction parameters, identifying 100% ethanol, a R of 0.12 g mL^{-1}, and 35 min extraction time as suitable conditions for recovering the target compounds. Among the tested extraction techniques, UAE provided the best compromise between extraction efficiency, stilbene enrichment, reduced processing time, and limited prolonged thermal exposure. Although Soxhlet extraction gave the highest total EY%, UAE showed greater selectivity toward the target stilbenes, especially ε-viniferin, confirming that EY% alone is not sufficient to evaluate process performance.

The application of the optimized UAE protocol to grapevine varieties revealed pronounced varietal diversity in EY%, extract composition, and biomass-based stilbene recovery. ε-Viniferin was the predominant target compound in all evaluated varieties, although its relationship with *trans*-resveratrol was not uniform. The combined evaluation of DE concentration and matrix-based recovery identified Pinot Meunier, Grechetto Gentile, Moscato, and Moscatel Graudo as particularly valuable vine-shoot sources, depending on whether the objective is simultaneous stilbene recovery, selective *trans*-resveratrol enrichment, or maximum ε-viniferin production. Spectrophotometric characterization further supported the antioxidant potential of vine-shoot extracts, while confirming that total phenolic and flavonoid contents alone cannot fully explain the observed bioactivity.

Some limitations should be acknowledged. The study was performed at laboratory scale and on vine-shoot samples collected from a single pruning season, therefore, multi-year validation would be necessary to confirm the stability of the observed varietal differences. In addition, cultivar effects could not be completely separated from vineyard, geographical, and post-harvest factors. Further pilot-scale studies, including solvent recycling, energy demand, extract stability, and application-oriented biological evaluation, are required before industrial implementation.

In conclusion, the optimized UAE protocol represents a simple, food-grade, and scalable strategy for converting vine-pruning residues into high-value bioactive extracts. These results support the integration of vine shoots into circular biorefinery schemes and highlight the importance of cultivar-specific raw-material selection for future food, nutraceutical, cosmetic, and bio-based applications.

Declarations

Ethics statement

This study did not involve human participants or experimental animals; therefore, ethical approval was not required.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

G.N.: Formal analysis, Investigation, Writing – original draft; L.A.: Methodology, Validation, Visualization. L.A. and E.S: Conceptualization, Data curation, Software; F.M. and G.S.: Funding acquisition, Resources, Supervision; V.B. and M.M. Project administration. L.A., E.S., F.M., G.S. and V.B.: Writing – review and editing

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Author Contribution

G.N.: Formal analysis, Investigation, Writing – original draft; L.A.: Methodology, Validation, Visualization. L.A. and E.S: Conceptualization, Data curation, Software; F.M. and G.S.: Funding acquisition, Resources, Supervision; V.B. and M.M. Project administration. L.A., E.S., F.M., G.S. and V.B.: Writing – review and editing

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Anđelini D, Cvitan D, Prelac M et al (2023) Biochar from Grapevine-Pruning Residues Is Affected by Grapevine Rootstock and Pyrolysis Temperature. Sustain (Switzerland) 15. https://doi.org/10.3390/su15064851
2. Ašonja A, Vještica S, Bošković A et al (2025) Research into the Energy Potential of Vine Pruning Residues in Western Serbia. Energies (Basel) 18. https://doi.org/10.3390/en18246384
3. Wei M, Ma T, Ge Q et al (2022) Challenges and opportunities of winter vine pruning for global grape and wine industries. J Clean Prod 380:135086. https://doi.org/10.1016/J.JCLEPRO.2022.135086
4. Jesus M, Romaní A, Mata F, Domingues L (2022) Current Options in the Valorisation of Vine Pruning Residue for the Production of Biofuels, Biopolymers, Antioxidants, and Bio-Composites following the Concept of Biorefinery: A Review. Polymers (Basel). 14
5. Cardoza D, Gomes-Dias JS, Pereira SG et al (2025) Bioconversion of vine shoots into renewable products using ohmic heating extraction and autohydrolysis. Sustain Chem Pharm 47:102155. https://doi.org/10.1016/J.SCP.2025.102155
6. Dávila I, Gullón B, Labidi J, Gullón P (2019) Multiproduct biorefinery from vine shoots: Bio-ethanol and lignin production. Renew Energy 142:612–623. https://doi.org/10.1016/J.RENENE.2019.04.131
7. Pereira AR, Costa C, Mateus N et al (2023) Exploring the Potential of Vine Shoots as a Source of Valuable Extracts and Stable Lignin Nanoparticles for Multiple Applications. Int J Mol Sci 24. https://doi.org/10.3390/ijms24065165
8. Escobar-Avello D, Mardones C, Saéz V et al (2021) Pilot-plant scale extraction of phenolic compounds from grape canes: Comprehensive characterization by LC-ESI-LTQ-Orbitrap-MS. Food Res Int 143:110265. https://doi.org/10.1016/J.FOODRES.2021.110265
9. Goufo P, Singh RK, Cortez I (2020) A reference list of phenolic compounds (Including stilbenes) in grapevine (vitis vinifera l.) roots, woods, canes, stems, and leaves. Antioxidants 9. https://doi.org/10.3390/antiox9050398
10. Fuloria S, Sekar M, Khattulanuar FS et al (2022) Chemistry, Biosynthesis and Pharmacology of Viniferin: Potential Resveratrol-Derived Molecules for New Drug Discovery, Development and Therapy. Molecules 27

11. Noviello M, Caputi AF, Squeo G et al (2022) Vine Shoots as a Source of Trans-Resveratrol and ε-Viniferin: A Study of 23 Italian Varieties. Foods 11:. https://doi.org/10.3390/foods11040553
12. Beaumont P, Courtois A, Atgié C et al (2022) In the shadow of resveratrol: biological activities of epsilon-viniferin. J Physiol Biochem 78:465–484
13. Sy B, Krisa S, Richard T, Courtois A (2023) Resveratrol, ε-Viniferin, and Vitisin B from Vine: Comparison of Their In Vitro Antioxidant Activities and Study of Their Interactions. Molecules 28:. https://doi.org/10.3390/molecules28227521
14. Gómez-Zorita S, Milton-Laskibar I, Eseberri I et al (2023) Beneficial Effects of ε-Viniferin on Obesity and Related Health Alterations. Nutrients 15
15. Gutiérrez-Escobar R, Fernández-Marín MI, Richard T et al (2021) Development and characterization of a pure stilbene extract from grapevine shoots for use as a preservative in wine. Food Control 121. https://doi.org/10.1016/j.foodcont.2020.107684
16. Muñoz-Realpe CC, Contreras M del, Vidal M AM, et al (2025) Green extraction strategies to obtain bioactive compounds from 'Charelo' vine shoots. Sustain Chem Pharm 43. https://doi.org/10.1016/j.scp.2024.101899
17. Šikuten I, Štambuk P, Andabaka Ž et al (2020) Grapevine as a rich source of polyphenolic compounds. Molecules 25
18. Wang B, Chen P, Zhang H et al (2025) Optimization of polyphenols extraction by deep eutectic solvent from broccoli stem and characterization of their composition and antioxidative effects. Sci Rep 15. https://doi.org/10.1038/s41598-025-00632-z
19. Corsetti S, Alessandroni L, Coppin JC et al (2026) Cross-supply-chain by-product valorization: optimization of Sorrento lemon peel aromatization of refined olive and grape seed oils via Ultrasound-Assisted Extraction. Applied Food Research 6:. https://doi.org/10.1016/j.afres.2026.101854
20. Alessandroni L, Bellabarba L, Corsetti S, Sagratini G (2024) Valorization of Cynara cardunculus L. var. scolymus Processing By-Products of Typical Landrace Carciofo Di Montelupone from Marche Region (Italy). Gastronomy 2:129–140. https://doi.org/10.3390/gastronomy2040010
21. Gao Q, Zheng R, Lu J et al (2024) Trends in the Potential of Stilbenes to Improve Plant Stress Tolerance: Insights of Plant Defense Mechanisms in Response to Biotic and Abiotic Stressors. J Agric Food Chem 72:7655–7671
22. Suprun AR, Dubrovina AS, Aleynova OA, Kiselev KV (2021) The bark of the spruce picea jezoensis is a rich source of stilbenes. Metabolites 11:. https://doi.org/10.3390/metabo11110714
23. Piñeiro Z, Marrufo-Curtido A, Serrano MJ, Palma M (2016) Ultrasound-assisted extraction of stilbenes from grape canes. Molecules 21. https://doi.org/10.3390/molecules21060784
24. Guerrero RF, Aliaño-González MJ, Puertas B et al (2020) Comparative analysis of stilbene concentration in grapevine shoots of thirteen Vitis during a three-year study. Ind Crops Prod 156. https://doi.org/10.1016/j.indcrop.2020.112852
25. Loupit G, Prigent S, Franc C et al (2020) Polyphenol Profiles of Just Pruned Grapevine Canes from Wild Vitis Accessions and Vitis vinifera Cultivars. J Agric Food Chem 68:13397–13407. https://doi.org/10.1021/acs.jafc.9b08099
26. Vergara C, Von Baer D, Mardones C et al (2012) Stilbene levels in grape cane of different cultivars in southern Chile: Determination by HPLC-DAD-MS/MS method. J Agric Food Chem 60:929–933. https://doi.org/10.1021/jf204482c
27. Acquadro S, Appleton S, Marengo A et al (2020) Grapevine green pruning residues as a promising and sustainable source of bioactive phenolic compounds. Molecules 25. https://doi.org/10.3390/molecules25030464
28. Bzainia A, Igrejas G, Pereira MJV et al (2024) Purification of stilbenes from grape stems in a continuous process based on photo-molecularly imprinted adsorbents and hydroalcoholic solvents. Sep Purif Technol 349. https://doi.org/10.1016/j.seppur.2024.127798
29. Krambeck K, Oliveira A, Santos D et al (2020) Identification and quantification of stilbenes (Piceatannol and resveratrol) in passiflora edulis by-products. Pharmaceuticals 13. https://doi.org/10.3390/ph13040073
30. Billet K, Houillé B, Besseau S et al (2018) Mechanical stress rapidly induces E-resveratrol and E-piceatannol biosynthesis in grape canes stored as a freshly-pruned byproduct. Food Chem 240:1022–1027. https://doi.org/10.1016/j.foodchem.2017.07.105
31. Billet K, Unlubayir M, Munsch T et al (2021) Postharvest Treatment of Wood Biomass from a Large Collection of European Grape Varieties: Impact on the Selection of Polyphenol-Rich Byproducts. ACS Sustain Chem Eng 9:3509–3517. https://doi.org/10.1021/acssuschemeng.0c07875
32. Ewald P, Delker U, Winterhalter P (2017) Quantification of stilbenoids in grapevine canes and grape cluster stems with a focus on long-term storage effects on stilbenoid concentration in grapevine canes. Food Res Int 100:326–331. https://doi.org/10.1016/j.foodres.2017.08.003
33. Brahmi F, Mateos-Aparicio I, Garcia-Alonso A et al (2022) Optimization of Conventional Extraction Parameters for Recovering Phenolic Compounds from Potato (Solanum tuberosum L.) Peels and Their Application as an Antioxidant in Yogurt Formulation. Antioxidants 11:. https://doi.org/10.3390/antiox11071401
34. Sun H, WW LQ et al (2018) Ultrasound-assisted extraction of resveratrol from grape leaves and its purification using mesoporous carbon. Food Sci Biotechnol 27:1353–1359
35. Tříska J, Vrchotová N, Balík J et al (2017) Variability in the content of trans-resveratrol, trans-ε-viniferin and r2-viniferin in grape cane of seven Vitis vinifera L. varieties during a three-year study. Molecules 22. https://doi.org/10.3390/molecules22060928
36. Kosović E, Topiař M, Cuřínová P, Sajfrtová M (2020) Stability testing of resveratrol and viniferin obtained from Vitis vinifera L. by various extraction methods considering the industrial viewpoint. Sci Rep 10. https://doi.org/10.1038/s41598-020-62603-w
37. Duarte H, Aliaño-González MJ, Cantos-Villar E et al (2024) Sustainable extraction of polyphenols from vine shoots using deep eutectic solvents: Influence of the solvent, Vitis sp., and extraction technique. Talanta 267:. https://doi.org/10.1016/j.talanta.2023.125135
38. Contreras Mdel, Feriani M, Gómez-Cruz A et al (2023) I, Grapevine Shoot Extract Rich in Trans-Resveratrol and Trans-ε-Viniferin: Evaluation of Their Potential Use for Cardiac Health. Foods 12:. https://doi.org/10.3390/foods12234351

Figures

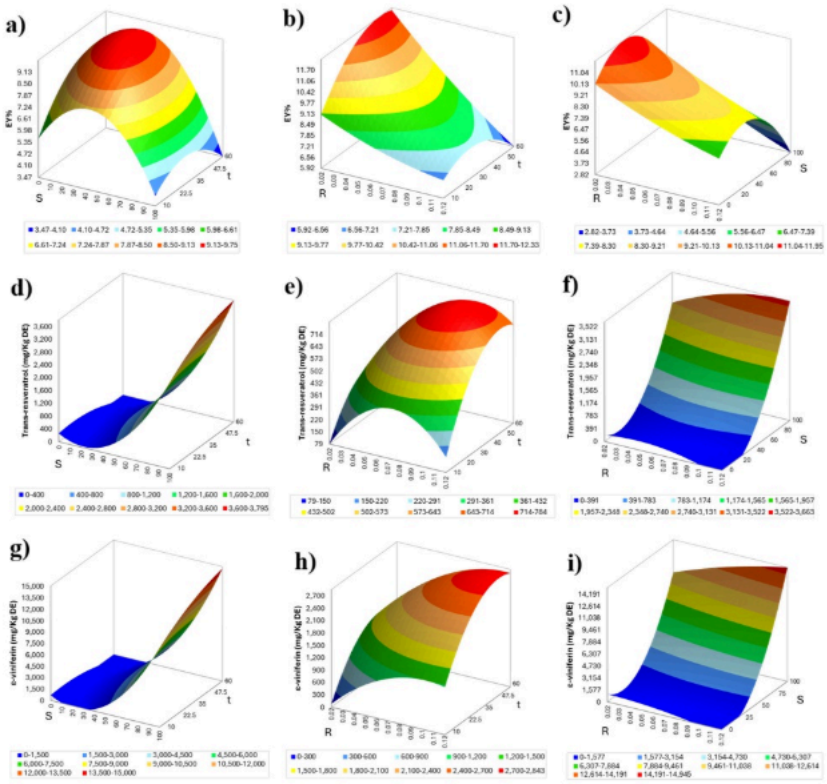


Figure 1

Response surface plots showing the effect of extraction parameters on extraction yield (a, b, c), trans-resveratrol (d, e, f) and ε-viniferin (g, h, i) concentrations. Plots report the data correlation of S vs. t (a, d, g), R vs. t (b, e, h), and R vs. S (c, f, i). (t: extraction time; R: matrix/solvent ratio; S: H_2O: EtOH mixtures)

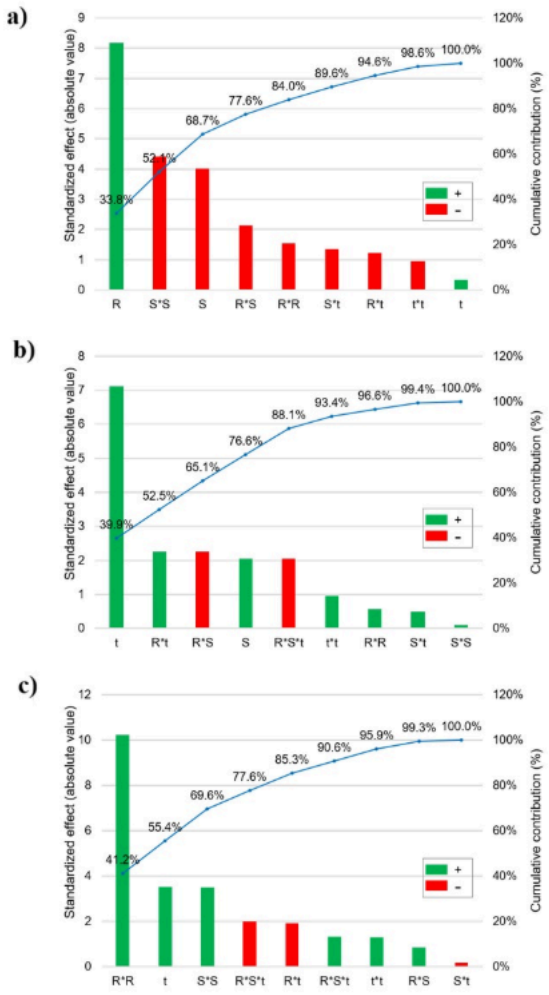


Figure 2

Pareto chart of standardized effects for a) extraction yield, b) trans-resveratrol and c) ε-viniferin (R: matrix/solvent ratio; t: time; S: percentage of EtOH in the EtOH/H_2O mixture). Green bars indicate terms with positive and red bars negative effect on the extraction yield. The Lorenz curve represents the cumulative contribution (percentage) of each term

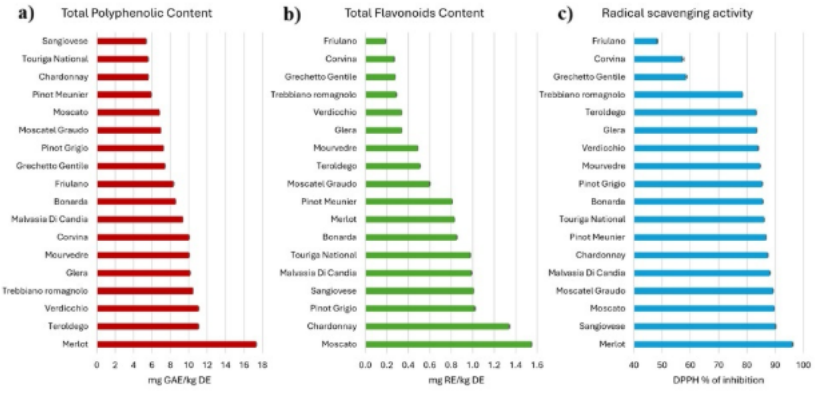


Figure 3

Extracts characterization through spectrophotometric assays: a) TPC, b) TFC and c) DPPH assays results. (DE: dry extract)

Supplementary Files

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- Supplementarymaterial.docx