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ORIGINAL ARTICLE

Unveiling potential functional applications of grape pomace extracts based on their phenolic profiling, bioactivities, and circular bioeconomy

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Abstract
Grape pomace (GP), a by-residue from the wine industry, contains bioactive molecules such as phenolic compounds, and anthocyanins, among others, with potential health benefits. In the current study, these bioactive molecules were extracted from GP of different *Vitis vinifera L.* varieties (Tinta Negra, Complexa, Malvasia Roxa, Malvasia, Sercial, Verdelho, Boal, Terrantez) using the micro quick, easy, cheap, effective, rugged, and safe (μQuEChERS) procedure. The GP extracts were investigated using ultra-high performance liquid chromatography (UHPLC-PDA) to establish the phenolic fingerprint, and by in vitro assays to assess the antibacterial, anti-inflammatory, and antioxidant activities. Nine phenolic compounds were identified and quantified in GP extracts, with gallic acid (ranging from 10.4 to 12.9 g/100 g), catechin (2.97 to 5.08 g/100 g), quercetin (2.17 to 2.85 g/100 g), and *trans*-resveratrol (0.28 to 1.82 g/100 g) being the most prominent. GP from the Complexa variety exhibited the highest levels of total anthocyanin content (TAC, 6.67 mgCGE/100 g), total phenolic compounds (TPC, 4727 mgGAE/100 g), and antioxidant activity (DPPH, 9472 mgTE/100 g), while the Tinta Negra variety had the highest total catechin content (TCC, 947 mgCATE/100 g). A strong correlation ($p < 0.001$) was observed between the TPC-TAC, TPC-DPPH, DPPH-TAC, and TAC-TCC. Moreover, o-coumaric acid and quercetin are significantly ($p < 0.001$) correlated with TPC, TAC, TCC, and DPPH assays. The investigated GP extracts, at a concentration of 100 μg/mL, showed promising inhibition of albumin protein denaturation compared to aspirin (reference standard). The findings showed that the GP extracts were more useful at inhibiting *Staphylococcus aureus* compared to *Escherichia coli*. It is important to emphasise that the GP extracts demonstrated antioxidant, anti-inflammatory, and antibacterial properties, positioning it as an agro-waste with promising potential for use in the development of innovative functional foods, dietary supplements, and cosmetics, aligning with the circular bioeconomy model for its valorisation.

Keywords Grape pomace · Phenolic fingerprint · μQuECHERS · UHPLC-PDA · Circular economy · Valorisation

1 Introduction

Grape pomace (GP) is a biodegradable agro-waste that represents 20—25% of the total grape mass [1], which amounts to approximately 8.49 million tonnes per year worldwide [2]. The management of winemaking by-residues like GP is a significant challenge for the wine industry. Direct disposal of GP in landfills leads to serious environmental concerns, including soil and groundwater contamination, microbial pollution, the attraction of plant disease vectors, and various health risks to human and aquatic populations. These issues stem from GP's high chemical oxygen demand and its biodegradable organic content, such as tannins, among other factors [3]. However, recent shifts towards sustainability and resource efficiency have prompted a revaluation of

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GP as a potential source of value-added molecules, namely phenolic compounds, anthocyanins, fibres, volatile organic compounds, vitamins, tannins, lipids, lignocellulosic compounds, and minerals (e.g. potassium, iron), which can promote human health and serve various industrial applications [4]. The valorisation of GP through the use of value-added molecules represents a multifaceted approach aligned with circular economy principles, aiming to minimise waste and promote more sustainable and efficient resource use [5, 6].

Phenolic compounds, a varied group of secondary metabolites present in plants, are plentiful in GP and have garnered considerable attention for their various health benefits and industrial applications. This chemical family can be divided into two primary classes: flavonoids and non-flavonoids, with flavonoids being the most prevalent class. The non-flavonoids class includes stilbenes and phenolic acids, whereas the flavonoid class includes anthocyanins, flavanones, flavonols, and flavan-3-ols [7]. The concentration of phenolic compounds depends on genetic factors, environmental conditions, and the plant's stage of maturity [8]. Numerous studies over the past ten years have investigated the effects of grape phenolic consumption on human health, highlighting their properties, including cardioprotective [9], antidiabetic, neuroprotective [10], and protective of the gut microbiota [11]. As a result, they exhibit preservative properties by inhibiting lipid oxidation and suppressing the growth of certain bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutans* [12, 13]. These compounds act as natural antioxidants by neutralising free radicals and reactive oxygen species that contribute to lipid oxidation and spoilage in foods. In addition, phenolic compounds possess antimicrobial activity against pathogenic bacteria, making them effective preservatives for prolonging the shelf life of foods and inhibiting the growth of foodborne pathogens, as well as anticancer properties, making them valuable assets in various industries, including food, pharmaceuticals, and cosmetics [8, 14–17]. Ferreira-Santos et al. [18] extracted phenolic compounds from GP of the Croatina variety using ohmic heating technology. The GP extracts showed high antioxidant activity (58.1 µmolTE/g), total phenolic content (TPC, 89.3 ± 0.68), effective reactive oxygen species prevention, effective against human colorectal cell (Caco-2, $IC_{50} = 234.1$ mg/mL) and human cervix adenocarcinoma cells (HeLa, $IC_{50} = 122.7$ mg/mL). Xu et al. [19] evaluated phenolic profile, antioxidant and antibacterial activities of GP from Viognier, Vidal Blanc, Carbernet Franc and Chambourcin varieties. Total phenolic (TPC, ranging from 55.5 to 154 mg GAE/g), total flavonoid (TFC, 32.8 to 91 mg CATE/g extract), total anthocyanins (0.02 to 1.7 mgG3GE/g) and antioxidant activity (3.54 to 28.2 µmolTE/g) differed significantly among the four GP studied. In addition, these GP exhibited antibacterial activity against *Listeria monocytogenes* ATCC 7644 (inhibition diameters ranging from 14.0 to 25.1 mm) and *S. aureus* ATCC 29213 (1.2 to 11.7 mm), but not against *E. coli* O157:H7 ATCC 3510 and *Salmonella typhimurium* ATCC 14028. On the other hand, Ozkan et al. [13] observed that GP from Emir and Kalecik karasi varieties exhibited antibacterial activity against a pool of bacterial strains, including *E coli* O157:H7 (inhibition diameters ranging from 7.67 to 23.4 mm). Bucić-Kojić et al. [20] evaluated the anti-inflammatory activity of GP extract based on their potential to inhibit 5-lipoxygenase. The GP extract inhibited 5-LOX (IC_{50} ranging from 37 to 240 µg/mL) more efficiently compared to quercetin ($IC_{50} = 3$ µg/mL). This data indicates that the phenolic compounds identified in GP extract, namely gallic acid, *p*-hydroxybenzoic acid, rutin, as well as the potential synergistic influence of all extract components might promote the inhibition of 5-LOX.

Microextraction techniques, including solid-phase microextraction (SPME) [21], dispersive liquid–liquid microextraction (DLLME) [22, 23], micro-solid phase extraction (µ-SPE) [24], micro quick, easy, cheap, effective, rugged, and safe (µQuEChERS) [25, 26], among others, offer advantages such as lowered solvent volume, reduced extraction times, and increased sensitivity compared to traditional extraction techniques, thus representing valuable procedures for extracting phenolic compounds from GP. Moreover, these microextraction techniques are amenable to automation and miniaturisation, enabling high-throughput analysis and reducing the overall environmental impact of the extraction procedure [27]. These microextraction techniques could be combined with advanced analytical methods to establish the phenolic fingerprint in a variety of foods, including high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) spectrophotometry [23] or diode array detector (DAD) [21, 22], gas chromatography-mass spectrometry (GC–MS) [21], liquid chromatography-tandem mass spectrometry (LC–MS/MS) [25], and electrophoretic techniques coupled with diverse detection systems [28]. Ultra-high-pressure liquid chromatography equipped with a photodiode array detection system (UHPLC-PDA) compared to other analytical methods offers better resolution, sensitivity, solvent consumption, compatibility with MS detection, reproducibility, cost-effectiveness, ease of use, and maintenance compared to other analytical methods, making it well-suited for comprehensive phenolic profiling and quantitative analysis.

The current research aims to determine the antioxidant, anti-inflammatory, and antibacterial activities of GP extracts obtained by µQuEChERS using in vitro assays. To our knowledge, this is the first time that µQuEChERS has been utilised to extract phenolic compounds from GP, leveraging its enhanced efficiency and lower solvent consumption to create a more sustainable and effective alternative to traditional extraction techniques. Recent improvements in

the technique have reduced test portion sizes to just 0.5–2 g while maintaining effectiveness, enabling the process of larger sample batches. In addition, the technique covers a wider range of analytes, allowing the analysis of multiple compounds from a single sample preparation, reducing the number of methods needed, improving the laboratory throughput and sustainability. Moreover, the versatility of the technique, can cover over several hundreds of organic compounds across various food matrices, makes it adaptable to different industrial needs. Therefore, it is highly suitable for scale-up in large-scale industrial applications. In addition, the individual phenolic compounds and vitamin C in GP extracts were analysed by UHPLC-PDA. The methods were fully validated for selectivity, sensitivity, precision, and accuracy. Furthermore, the phenolic compounds and vitamin C were correlated with their antioxidant activities using Pearson correlation. Further discussion also encompasses the scalability of μQuEChERS and the industrial uses of the identified phenolic compounds, since they possess potential industrial usage in food, pharmaceuticals, and cosmetics due to their antioxidant, anti-inflammatory, and antimicrobial activities.

2 Materials and methods

2.1 Chemicals

All chemicals and reagents were of analytical grade. HPLC grade acetonitrile, formic acid (FA), dimethyl sulfoxide (DMSO), and ethyl acetate were purchased from Fischer Scientific (Loughborough, UK). Gallic acid (purity ≥ 99%), catechin (99%), sinapic acid (≥ 98%), *o*-coumaric acid (≥ 98%), *trans*-resveratrol (≥ 98%), quercetin (≥ 98%), cinnamic acid (≥ 98%), vanillin (99%), kaempferol (≥ 98%), taxifolin (≥ 85%) and L-ascorbic acid (99%) were purchased from Fluka (Buchs, Switzerland). These standards were utilised to identify the phenolic compounds in GP. Stock solutions of each phenolic standard were prepared by dissolving them in acetonitrile at a concentration of 500 mg/L and were stored at −80 ºC. Sodium chloride (NaCl), anhydrous magnesium sulphate ($MgSO_4$), sodium potassium (KCl), sodium acetate (CH_3COONa), sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), hydrochloric acid (HCl, 37% v/v), sodium citrate dibasic sesquihydrate ($C_6H_5Na_2O_7 \cdot 1.5\ H_2O$) and albumin (≥ 99%) were supplied from Sigma-Aldrich (St. Louis, MO, USA). The Folin-Ciocalteu reagent (FR, 2 N), trolox ($C_{14}H_{18}O_4$, 98%), and 1,1-diphenyl-2-picrylhydrazyl (DPPH·≈ 90%) in free radical form were obtained from Fluka (Buchs, Switzerland), whereas anhydrous sodium carbonate (Na_2CO_3, 99.8%), ethylendiaminetetraacetic acid disodium salt (EDTA) and metaphosphoric acid (MPA) were purchased from Panreac (Barcelona, Spain). Ultrapure water (18 MΩ cm) for the mobile phase and other aqueous solutions was supplied by the Milli-Q water purification system (Millipore, Milford, MA, USA).

2.2 Samples

The grapes were harvested at ripeness during the 2023 vintage, and the pomace was generously supplied by the Justino's Madeira Wines S.A (coordinates 32° 39′ 04″ North latitude and 16° 51′ 45″ West longitude) as a by-residue of the winemaking process. Five *V. vinifera* L. white grape varieties (Boal, Malvasia, Terrantez, Verdelho, Sercial), categorised as Noble varieties, and three red grape varieties (Complexa, Malvasia Roxa, Tinta Negra) used in the Madeira wine production, were considered for the current investigation. Three samples per typology were obtained, except for the GP from Malvasia Roxa, which had only one sample. The GP from these different varieties was collected instantly afterwards pressing and transported to the laboratory in refrigerated containers (approximately 2—5 °C). The GP was then lyophilised (Telstar, Cryodos, Spain) for 10 h, ground in a laboratory mill (Grindomix GM200, Rech, Germany) to achieve a fine and uniform powder, and kept at −80 °C until analysis.

2.3 μQuEChERS procedure to extract phenolic compounds

The μQuEChERS procedure was adapted from the technique reported by Izacara et al. [26]. Briefly, 0.5 g of lyophilised GP and 0.5 g of a partitioning salt mixture ($MgSO_4$, NaCl, $C_6H_5Na_3O_7 \cdot 2H_2O$ and $C_6H_5Na_2O_7 \cdot 1.5\ H_2O$ maintaining the original QuEChERS ratio 4:1:1:0.5) were measured into a 2 mL screw-capped centrifuge tube. Then, 2 mL of 1:1 (v/v) acetonitrile: ethyl acetate acidified with 0.1% FA was put. The tube was vortexed for 30 s, sonicated for 5 min, and then centrifuged for 5 min at 5000 rpm. The supernatants were collected and transferred to a PTFE dSPE clean-up tube of 2 mL having 150 mg of $MgSO_4$ and 25 mg of PSA (primary secondary amines). The mixture was vortexed for 30 s and centrifuged for 5 min at 5000 rpm. The supernatant was collected and evaporated under a mild nitrogen stream at 22 ± 1ºC. The dried residue was redissolved in 250 μL of acetonitrile and filtered through a 0.22 μm PTFE filter membrane for UHPLC analysis and in vitro assays as described in Sect. 2.7. The μQuEChERS procedure was done in triplicate.

2.4 Solid–liquid extraction (SLE) to extract vitamin C

The extraction procedure for vitamin C from GP was adapted from Spínola et al. [29], with some modifications. Briefly, 0.5 g of lyophilised GP was placed into a 15 mL

screw-capped centrifuge tube. Then, 5 mL of extraction solution (3% MPA, 8% acetic acid, 1 mM EDTA) was added. The tube was vortexed for 30 s, followed by centrifugation at 5000 rpm for 10 min. The supernatants were collected and filtered using a PTFE filter membrane of 0.22 µm for UHPLC analysis.

2.5 UHPLC conditions

UHPLC analyses of phenolic compounds and vitamin C were performed on a Waters Acquity H-Class system (Milford, MA, USA) equipped with a sample manager, quaternary solvent manager, column heater, a degassing system, and a 2996 PDA detector. An Acquity UPLC® CSH™ C18 analytical column (2.1 mm × 150 mm, 1.7 µm particle size, Waters, Milford, MA, USA) was maintained at a temperature of 40 °C. The gradient elution for phenolic compounds was performed following the method outlined by Izcara et al. [26]. The mobile phase comprised water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B), under the following conditions: 80% A (0 min), 60% A (3 min), 55% A (6 min), 30% A (7 min), 20% A (7.5 min), and 80% A (8 min), with a 2 min re-equilibration to initial conditions earlier the subsequent injection, totalling 10 min for the analysis. An isocratic mobile phase of water containing 0.1% formic acid was used for determining vitamin C in GP extracts for 5 min, with a 2 min re-equilibration to starting conditions before the subsequent injection, totalling 7 min for the analysis. The flow rate was set at 250 µL/min, with an injection volume was 5 µL, and the samples were stored at 20 °C throughout the analysis. The UV detection wavelength was tuned to the highest absorbance for the target analytes (Table 1) and 245 nm for vitamin C. The chromatographic data were collected and integrated using Empower 2 software (Milford, MA, USA). The identification of phenolic compounds and vitamin C was performed by comparing the retention times (RT) and UV spectra obtained for each analyte with standards under similar experimental conditions.

2.6 Method validation

The µQuEChERS/UHPLC-PDA methodology for identifying and quantifying phenolic compounds and SLE/UHPLC-PDA for quantifying vitamin C in GP extracts were validated for selectivity, linearity, sensitivity (limit of detection (LOD), limit of quantification (LOQ)), precision (intra- and inter-day) and accuracy (% recovery). The method selectivity was evaluated by comparing the PDA spectra, RT, and peak purity generated for the target analytes in the studied GP extracts with standards. Linearity was determined by creating a calibration curve for each analyte with seven calibration points ($n = 7$) at values ranging from 0.5 to 400 mg/L. These ranges were chosen based on the sensitivity of the UHPLC-PDA system for each analyte and the range of phenolic compound and vitamin C concentrations commonly observed in GP extracts. Calibration curves were constructed by plotting the average peak area of each analyte against its concentration for both methods, and they were fitted using linear least-squares regression. The LOD of each analyte was determined at a signal-to-noise (S/N) ratio of 3 and the LOQ was estimated at an S/N ratio of 10, using the smallest concentration from the calibration curve. The method accuracy was reported as the recovery percentage (%), determined using GP extracts from red (Tinta Negra) and white (Boal) grape extracts that were spiked in triplicate at three concentration levels: low (LL), medium (ML), and high (HL), which corresponded to the linear range of each phenolic compound and vitamin C. Intra-day (repeatability) and inter-day (reproducibility) precision were obtained by injecting the same spiked levels used in the accuracy assays within a single day ($n = 6$) and over five serial days ($n = 30$), respectively. Each assay was analysed in triplicate.

2.7 *Assessment of the antioxidant, anti-inflammatory, and antibacterial activities of GP extracts*

Total phenolic content (TPC), total catechin content (TCC), total anthocyanin content (TAC), antioxidant, anti-inflammatory, and antibacterial activity were determined in GP extracts obtained by µQuEChERS. All spectrophotometric assays were performed in triplicate.

2.7.1 Total phenolic content

TPC was measured spectrophotometrically utilising the Folin-Ciocalteu assay, as outlined by Abreu et al. [30]. Gallic acid was applied as a reference standard at concentrations ranging from 30 to 150 mg/L to construct the calibration curve for determining TPC in GP extracts. The results were stated as milligrams of gallic acid equivalent per 100 g dry sample [mg(GAE)/ 100 g DW]. Spectrophotometric measurements were performed using a UV–Vis spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA) at 765 nm.

2.7.2 Total anthocyanin content

The TAC of the GP extracts was assessed by the pH differential procedure as outlined by Ribeiro et al. [31], applying a spectroscopic assay in which the absorbance of the GP extracts was assessed at 510 and 700 nm for pH 1.0 (KCl, 0.025 mol/L) and pH 4.5 (CH_3COONa, 0.40 mol/L). For the assay, 500 µL of GP extract was placed in a 5 mL volumetric flask to produce two dilutions of the extract, one

adjusted with buffer pH 1.0 and the other to pH 4.5. The absorbance values were transformed by applying the molar absorption coefficient of 26,900 L/mol·cm, and the results were reported as total milligrams of cyanidin-3-glucoside per 100 g dry weight of GP, mg(CGE)/100 g DW. Water was used as the control.

2.7.3 Total catechin content

TCC was calculated following the method described by Onach et al. [32]. Briefly, 2 mL of GP extract was combined with 2 mL HCl 12 N and 1 mL 1% vanillin. The absorbance of the GP extracts was determined at 500 nm, after 20 min of reaction. Catechin was applied as a reference standard in a concentration range of 100 to 800 mg/L to construct the calibration curve for determining the TCC in GP extracts. The results were reported as milligrams of catechin equivalent per 100 g of dry sample [mg(CATE)/ 100 g DW].

2.7.4 Antioxidant activity

The antioxidant activity of the DPPH$^{\bullet}$ free radical-scavenging activity (A_{AR}) was assessed using the method outlined by Abreu et al. [30]. In brief, a stock solution of DPPH$^{\bullet}$ radical in methanol (400 µM) was prepared and maintained at 25 ± 1 ºC in the dark. For the assay, this stock solution was diluted in methanol to give a working solution with an absorbance of 0.900(± 0.030) at 515 nm. Trolox, applied as standard, was tested at concentrations ranging from 30 to 600 mg/L to construct the calibration curve. Results were reported as mg of trolox equivalents (TE) per 100 g of dry sample [mg(TE)/ 100 g DW]. Methanol was used as a control.

2.7.5 Anti-inflammatory activity

The protein denaturation assay was conducted following the method outlined by Gunathilake et al. [33]. For the assay, 0.5 mL of 1% bovine albumin solution in phosphate-buffered saline (PBS) at pH 6.4 and 0.5 mL of GP extract were added to a 5 mL screw-capped centrifuge tube. The reaction mixture was vortex for 30 s and incubated at 37 ºC for 15 min. Following this, the mixture was heated to 70 ºC for 5 min. After cooling, turbidity was determined at 660 nm using a UV/VIS spectrometer. PBS was utilized as a control, and aspirin as a standard. Results were reported as %inhibition of albumin denaturation. The %inhibition of albumin denaturation was calculated by the following equation: $\%\text{Inhibition} = (A_C - A_S/A_C) \times 100$, where A_C represents the absorbance of the control and A_S represents the absorbance of the sample.

2.7.6 Antibacterial activity

The Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria strains were isolated from samples obtained at Hospital Dr. Nélio Mendonça, Funchal. Before each assay, an isolated colony was picked and grown in Mueller–Hinton agar (Thermofisher, Waltham, Massachusetts) incubated at 37 ± 2 ºC for 24 h.

The antibacterial activity of GP extracts was assessed using the Kirby-Bauer disc diffusion method, against *S. aureus* and *E. coli* seeded in Mueller–Hinton agar for at least 24 h. Briefly, the suspensions were prepared from a pure culture in 0.9% NaCl sterile solution, whose turbidity was adjusted to 0.5 in the McFarland scale. The inoculum of each bacterium was evenly spread over the entire surface of the agar of Petri dishes using a sterile spreader. The GP extract was diluted in DMSO to achieve a final concentration of 100 µg/mL, and 100 µL of this dilution was applied to sterile blank discs (6 mm in diameter) before being added to the inoculated agar. Thereafter, the plates were incubated at 37 ± 2 ºC for 24 h. After incubation, the zones of growth inhibition around each disc containing GP extract were measured. The results were expressed as mean ± standard deviation.

2.8 Statistical analysis

Statistical analysis was conducted by the MetaboAnalyst 6.0 web-based tool [34]. The raw UHPLC-PDA data underwent pre-processing, which included normalisation through cubic root transformation and autoscaling. Subsequently, one-way analysis of variance (ANOVA) was performed, followed by Tukey's HSD (Honestly Significant Difference) test for post-hoc multiple comparisons of means from the data of GP varieties, with a significance threshold set at p-value < 0.001 to recognise significant differences.

3 Results and discussion

3.1 Method validation

The effectiveness of the proposed methods for quantifying phenolic compounds and vitamin C in GP extracts was assessed based on selectivity, linearity, sensitivity, precision, and accuracy. The findings summarised in Table 1.

The method selectivity was evaluated through the analysis of both blank and spiked GP extracts according to the control of the RT and the maximum wavelengths (λ_{max}) of the target analytes studied. The UHPLC-PDA chromatograms obtained from a spiked GP of the Verdelho variety at the medium level (Fig. 1) revealed the lack of interference peaks in the RT of target analytes. Regression lines were

Table 1 Figures of merit of the μQuEChERS and SLE/UHPLC-PDA methodologies for the determination of phenolic compounds and vitamin C, respectively, in GP extracts of a red (Tinta Negra) and white (Boal) grape varieties

RT (min)	Bioactive compound	λ_{max} (nm)	Linear range (mg/L)	Calibration curve		LOQ (mg/L)		Precision (%RSD)			Recovery (%) ± SD	
								Intra-day		Inter-day	Tinta Negra	
1.49	Gallic acid	280	0.5 – 400	Equation	Y = 2046x324	0.140	0.424	LL	4.24	10.6	98 ± 7	81 ± 7
				R^2	0.990			ML	1.03	3.96	110 ± 6	104 ± 3
								ML	1.03	3.99	84 ± 1	109 ± 1
3.78	Sinapic acid	320	1 – 30	Equation	Y = 2042 x + 3067	0.132	0.399	LL	3.99	6.36	108 ± 3	96 ± 4
				R^2	0.999			ML	9.47	12.0	100 ± 10	84 ± 5
								HL	11.5	15.8	94 ± 3	105.8 ± 0.4
4.49	o-Coumaric acid	320	1.5 – 15	Equation	Y = 11531x + 6907	0.085	0.258	LL	2.58	13.6	94 ± 6	98 ± 3
				R^2	0.999			ML	1.33	2.24	88 ± 3	103.2 ± 0.4
								HL	3.77	6.52	95 ± 5	91 ± 8
5.19	*trans*-Resveratrol	320	2 – 50	Equation	Y = 25017x + 19181	0.023	0.071	LL	0.71	2.64	94 ± 3	92.7 ± 0.5
				R^2	0.995			ML	0.94	0.86	99 ± 6	91.7 ± 0.2
								HL	5.06	6.29	105 ± 5	95 ± 5
5.37	Quercetin	360	1 – 16	Equation	Y = 4119x—2180	0.096	0.29	LL	2.90	9.25	93 ± 7	108 ± 4
				R^2	0.999			ML	1.92	3.95	103 ± 5	99.7 ± 0.5
								HL	4.27	5.07	93 ± 4	93.6 ± 0.1
5.88	Cinnamic acid	280	5 – 400	Equation	Y = 34289x - 216,420	0.008	0.023	LL	0.52	4.42	116 ± 2	90.2 ± 0.2
				R^2	0.999			ML	0.23	0.77	93.3 ± 0.1	87.8 ± 0.1
								HL	2.83	2.91	96 ± 4	93 ± 3
6.39	Kaempferol	360	4 – 50	Equation	Y = 14133x + 2363	0.100	0.303	LL	3.03	8.07	99 ± 5	82 ± 4
				R^2	0.997			ML	0.99	0.91	106 ± 1	92.8 ± 0.5
								HL	4.35	5.56	108 ± 6	90 ± 3
2.20	Vitamin C	245	20 – 400	Equation	Y = 10443x - 273,553	0.111	0.335	LL	3.35	3.66	110 ± 7	99 ± 3
				R^2	0.997			ML	0.91	6.88	94 ± 5	97 ± 2
								HL	0.69	7.74	97 ± 4	102 ± 1

Abbreviation: *LOD* Limit of detection, *LOQ* Limit of quantification, R^2 Coefficient of determination, *LL, ML, and HL* Low, medium, and high level (mg/L) corresponding to the linear range of each phenolic compound, respectively

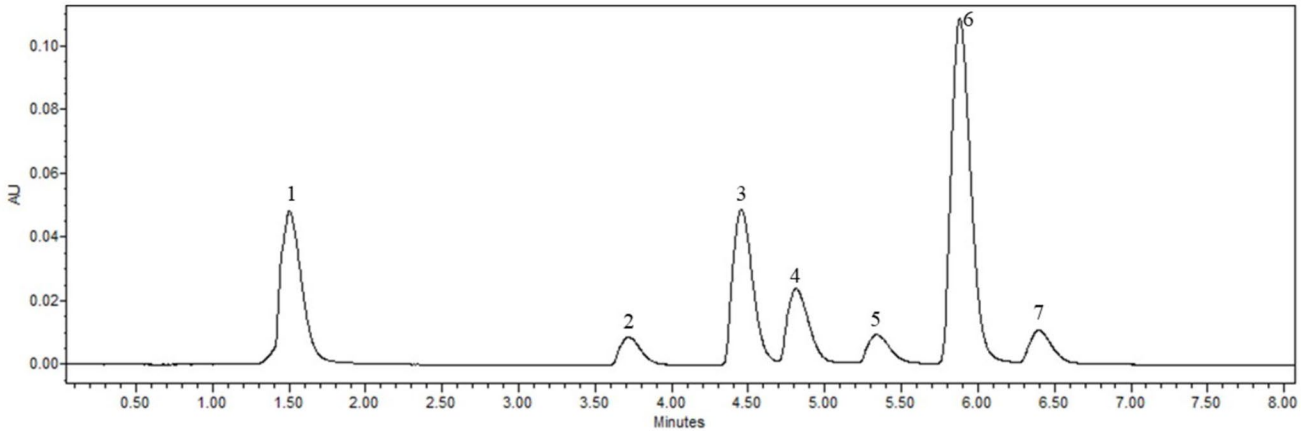

Fig. 1 Typical chromatogram ($\lambda_{acquisition} = 280$ nm) of phenolic compounds from GP of Verdelho variety, spiked with target analytes at medium level, by μQuEChERS/UHPLC-PDA methodology. Peak number identification: 1: gallic acid; 2: sinapic acid; 3: *o*-coumaric acid; 4: *trans*-resveratrol; 5: quercetin; 6: cinnamic acid; 7: kaempferol

established through least-squares linear regression analysis of the data, resulting in coefficients of determination (R^2) ranging from 0.990 to 0.999 for all target analytes. The linearity of the calibration curve was deemed acceptable when the R^2 values were equal to or higher than 0.990. Residual analysis revealed that the errors were regularly distributed around the respective curves. The determination of LOD and LOQ allowed the evaluation of the method's sensitivity. For phenolic compounds, the LODs and LOQs ranged from 0.008 (cinnamic acid) mg/L to 0.140 (gallic acid) mg/L and from 0.023 (cinnamic acid) mg/L to 0.424 (gallic acid) mg/L, respectively. The LOD and LOQ were 0.111 and 0.335 mg/L for vitamin C, respectively.

Recovery and precision were evaluated by spiking the target analytes in GP extracts at different concentrations (low, medium, and high) (Table 1), according to the linear range of the analytical curve. The recovery of phenolics ranged from 81 to 116%, whereas for vitamin C the recovery ranged from 94 to 110%. For all target analytes, the intra-day precision varied from 0.23% to 11.5%, while the inter-day precision varied from 0.77% to 15.8%. The average recoveries, between 70 and 120%, and the RSD values, less than 20%, demonstrated that the proposed methods are appropriate for quantifying phenolic compounds and vitamin C in GP extracts, as reported in the literature [35]. In addition, the proposed μQuEChERS/UHPLC-PDA method showed numerous benefits compared to others reported in the literature: low sample amount, low solvent consumption, and low LODs and LOQs values [36, 37]. Globally, the validation results indicated that both methods, μQuEChERS/UHPLC-PDA and SLE/UHPLC-PDA, were effective for extracting and quantifying phenolic compounds and vitamin C, respectively, from GP derived from red and white grape varieties, contributing to cost-effective valorisation strategies in industries such as nutraceuticals, cosmetics, and functional foods Nevertheless, future research should be conducted to investigate the scalability of the current methods for industrial applications, namely exploring greener extraction techniques, and assessing the bioavailability and functional properties of the extracted phenolics in product formulations, as well as method automatization (Fig. 2).

3.2 Quantitative determination of phenolic compounds and vitamin C in GP extracts

After demonstrating the suitability of the μQuEChERS/UHPLC-PDA method for quantifying phenolic compounds, it was applied to GP from different *V. vinifera* L varieties. Table 2 presents the concentration of the identified phenolic compounds in the various GP extracts, reported as milligrams of the phenolic compound per 100 g of dry weight (mg/100 g DW). A typical chromatogram of GP extract from red varieties obtained using μQuEChERS/UHPLC-PDA method was illustrated in Fig. 3.

The concentration of phenolic compounds in the studied grape extracts ranged from 18.4 mg/100 g DW (Verdelho) to 21.7 mg/100 g DW (Tinta Negra). On average, the phenolic compound content in the GP of the red varieties studied (21.5 ± 0.02 mg/100 g DW) is very close (20.3 ± 0.02 mg/100 g DW) to that determined in the GP of the white varieties. Except for gallic acid, the other phenolic compounds identified in the studied GPs showed the highest concentration in GP extracts from red varieties compared to GPs from white varieties. A comparable pattern was noted by Onache et al. [32], where gallic acid was predominant in GP from white grape varieties, whereas quercetin was more abundant in GP from red grape varieties.

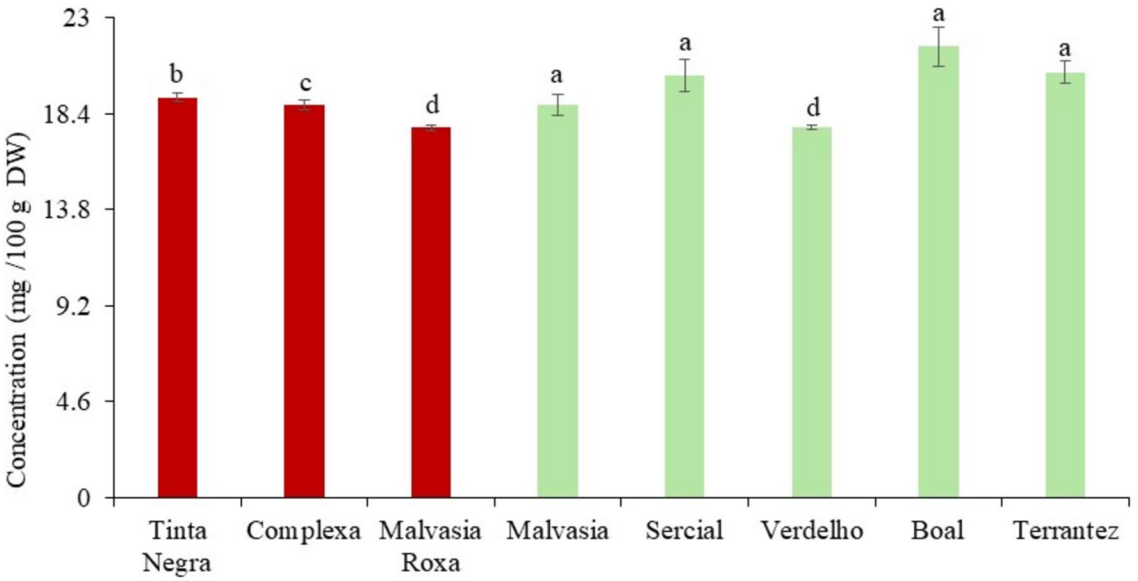

Fig. 2 Concentration (mg/ 100 g DW) of vitamin C in the analysed GP determined by SLE/UHPLC-PDA methodology. Values followed by the same letters did not differ by Tukey's HSD test ($p > 0.001$)

Phenolic acids were identified as the most prevalent chemical family in the GP extracts investigated, accounting for an average of 61.7% of the total phenolic compounds fraction. GP from Boal seemed to be the richest in phenolic acids (14.6 mg /100 g DW), followed by Sercial (13.9 mg /100 g DW), Complexa (13.2 mg /100 g DW), Malvasia Roxa (13.1 mg /100 g DW), Tinta Negra (12.4 mg /100 g DW), Terrantez (11.9 mg /100 g DW), Malvasia (11.8 mg /100 g DW) and Verdelho (11.6 mg /100 g DW). Gallic acid was identified as the most prevalent phenolic acid across all grape pomace (GP) extracts examined, with the highest concentration observed in the GP of the Boal variety (12.9 mg/100 g DW) and the smallest in the Tinta Negra variety (10.4 mg/100 g DW). There were no significant differences ($p > 0.001$) in gallic acid concentrations among the GP from Tinta Negra, Malvasia, Verdelho, and Terrantez. Onache et al. [32] reported similar concentrations of gallic acid in the GP of Muscat Ottonel, Tamaioasa Romaneasca, Cabernet Sauvignon, and Feteasca Neagra grape varieties, with concentrations ranging from 6.44 to 14.1 mg/100 g DW. Scientific research suggested that gallic acid has great potential as a dietary supplement due to its health benefits, including antioxidant, antimicrobial, anti-inflammatory, neuroprotective, cardioprotective, and anticancer activities. After ingestion, gallic acid is quickly absorbed and metabolised, leading to limited bioavailability. This bioavailability is affected by various factors, including intestinal bacteria, transport proteins, and the metabolism of galloyl derivatives [38, 39]. Finally, o-coumaric acid was the only phenolic compound quantified in GP from red grape varieties, with concentrations ranging from 0.12 to 0.33 mg/100 g DW, while the other GP extracts had levels of this compound that fell below the LOQs.

A significant difference in the content of *trans*-resveratrol was found among all the GP extracts, with the highest content detected in GP from the Complexa grape variety (1.82 mg/100 g DW), while the content in GP from the Verdelho grape variety was significantly lower (0.28 mg/100 g DW). The average content of *trans*-resveratrol in GP from red grape varieties (1.27 ± 0.02 mg/100 g DW) was 1.47 times higher than in GP from white grape varieties (0.86 ± 0.01 mg/100 g DW). The concentration range of *trans*-resveratrol aligns with findings from other researchers regarding GP extracts from red grape varieties (*V. vinifera* L. and *V. labrusca* L.) [40]. As far as flavonols were concerned, the GP of the Complexa grape variety provided the richest extract (3.45 mg/100 g DW), whereas the GP of the Sercial variety was the least rich (2.51 mg/100 g DW). Quercetin was the predominant flavonol found in all the examined GP extracts, with its concentration being 1.19 times greater in GP from red varieties (2.72 ± 0.02 mg/100 g) compared to GP from white varieties (2.29 ± 0.01 mg/100 g DW). Contrarily, catechin, a flavan-3-ol, showed a slightly superior concentration in GP from red grape varieties investigated (4.10 ± 0.02 mg/100 g DW) compared to its content found in GP from white grape varieties (4.04 ± 0.03 mg/100 g DW). The GP extract from Tinta Negra showed the greatest concentration of catechin (5.08 mg/100 g DW), while the lowest concentration was found in GP from Complexa variety (2.97 mg/100 g DW). *Trans*-resveratrol, quercetin, and catechin provide numerous health advantages, particularly in terms of antioxidant, antiangiogenic, immunomodulatory, antimicrobial,

Table 2 Concentration (mg/ 100 g DW) ± standard deviation (SD) of phenolic compounds found in GP analysed by the µQuEChERS/UHPLC-PDA methodology

RT (min)	Phenolic compound	λ_{max} (nm)	Concentration (mg/100 g DW) ± SD							
			Tinta Negra	Complexa	Malvasia Roxa	Malvasia	Sercial	Verdelho	Boal	Terrantez
1.49	Gallic acid	280	10.4 ± 0.03^{d}	11.5 ± 0.18^{b}	10.8 ± 0.03^{c}	$10.6 \pm 0.01^{c,d}$	12.7 ± 0.02^{a}	$10.6 \pm 0.01^{c,d}$	12.9 ± 0.04^{a}	$10.6 \pm 0.01^{c,d}$
2.18	Catechin[A]	280	5.08 ± 0.04^{a}	2.97 ± 0.03^{f}	4.25 ± 0.01^{c}	4.11 ± 0.02^{c}	4.13 ± 0.01^{c}	3.81 ± 0.02^{d}	3.71 ± 0.01^{e}	4.42 ± 0.04^{b}
3.78	Sinapic acid	320	0.78 ± 0.02^{b}	0.66 ± 0.02^{c}	0.94 ± 0.02^{a}	0.43 ± 0.01^{d}	0.32 ± 0.01^{e}	0.19 ± 0.01^{f}	0.89 ± 0.01^{b}	0.24 ± 0.02^{f}
4.49	o-Coumaric acid	320	0.12 ± 0.01^{b}	0.31 ± 0.01^{a}	0.33 ± 0.01^{a}	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4.95	Taxifolin[A]	280	0.38 ± 0.01^{b}	0.42 ± 0.02^{a}	0.27 ± 0.02^{c}	0.20 ± 0.01^{e}	0.11 ± 0.01^{g}	0.18 ± 0.01^{f}	0.23 ± 0.01^{d}	0.18 ± 0.01^{f}
5.19	*trans*-Resveratrol	320	1.12 ± 0.01^{c}	1.82 ± 0.02^{a}	0.87 ± 0.03^{f}	0.95 ± 0.01^{e}	0.43 ± 0.02^{g}	0.28 ± 0.01^{h}	1.62 ± 0.01^{b}	1.03 ± 0.01^{d}
5.37	Quercetin	360	2.60 ± 0.01^{c}	2.85 ± 0.01^{a}	2.70 ± 0.03^{b}	2.36 ± 0.01^{d}	2.27 ± 0.02^{e}	2.38 ± 0.02^{d}	2.17 ± 0.01^{f}	2.25 ± 0.01^{e}
5.88	Cinnamic acid	280	1.12 ± 0.01^{a}	0.66 ± 0.02^{f}	1.02 ± 0.02^{b}	0.72 ± 0.03^{e}	0.86 ± 0.03^{c}	0.81 ± 0.03^{d}	0.82 ± 0.01^{d}	1.02 ± 0.01^{b}
6.39	Kaempferol	360	0.19 ± 0.01^{b}	0.18 ± 0.01^{b}	0.23 ± 0.01^{a}	0.21 ± 0.02^{a}	0.13 ± 0.01^{c}	0.21 ± 0.01^{a}	0.13 ± 0.01^{c}	0.17 ± 0.01^{b}

LOQ – lower than the limit of quantification; Values followed by the same letters on the same line did not differ by Tukey's HSD test ($p > 0.001$); [A] Expressed in equivalents of quercetin

neuroprotective, and cardioprotective effects. However, their poor bioavailability poses a challenge to their effective use. Ongoing research and development of novel delivery methods aim to overcome these limitations and maximise the therapeutic potential of these phenolic compounds [41–43].

Figure 2 illustrates the vitamin C concentration in the GP of the eight grape varieties. The concentration of vitamin C ranged from 17.7 mg/100 g DW (Malvasia Roxa) to 21.6 mg/100 g DW (Boal). There was no significant difference ($p > 0.001$) in vitamin C levels between the GP of Boal, Terrantez, and Sercial varieties, or between Malvasia Roxa and Verdelho. However, the GP from Tinta Negra and Complexa displayed significantly different ($p < 0.001$) vitamin C concentrations compared to the other varieties studied. The range of vitamin C concentration in the studied GP was slightly lower than those reported for the Benitaka variety, which had 26.25 mg/100 g DW [44]. Vitamin C was a highly potent antioxidant and free radical scavenger. It has been intensively explored in recent years due to growing data supporting its immunomodulatory capabilities and effects on the gut microbiota, particularly under conditions of oxidative stress. In addition, vitamin C enhanced iron absorption and aided in the production of collagen, hormones, and carnitine. Vitamin C is absorbed in the small intestine via active transport. Absorption efficiency decreased with increasing dose, being virtually completed at low doses (30–180 mg/day) but less efficient at high doses (> 1,000 mg/day)) [45].

3.3 Assessment of the antioxidant activity of GP extracts

The antioxidant activity of GP extracts obtained by the µQuEChERS extraction procedure was assessed using spectrophotometric assays (TPC, TAC, TCC, and DPPH). Phenolic compounds are well-known for their strong antioxidant properties, which are largely attributed to their redox characteristics, allowing them to effectively scavenge free radicals. Table 3 shows the TPC, TAC, and DPPH of the studied GP extracts.

The highest TPC level (4727 mgGAE/100 g DW), assessed by the Folin–Ciocalteu test, was found in GP from Complexa variety, while the lowest TPC (1746 mgGAE/100 g DW) was found in GP from Boal. There was no significant difference ($p > 0.001$) in TPC between the GP extracts of Tinta Negra and Terrantez varieties. The data obtained are quite like those obtained for GP from red varieties Tempranillo (2680 – 7180 mg GAE/ 100 g DW) [46], Georgian red GPs (2790 mg GAE/100 g DW) [47], and Italian white grape varieties Riesling (4794 mgGAE/100 g DW) [48]. On average, the TPC levels in GP from red grape varieties (3492 ± 10.1 mgGAE/100 g DW) were 1.51 times higher than those determined in GP from white grape varieties (2306 ± 11.4 mgGAE/100 g DW).

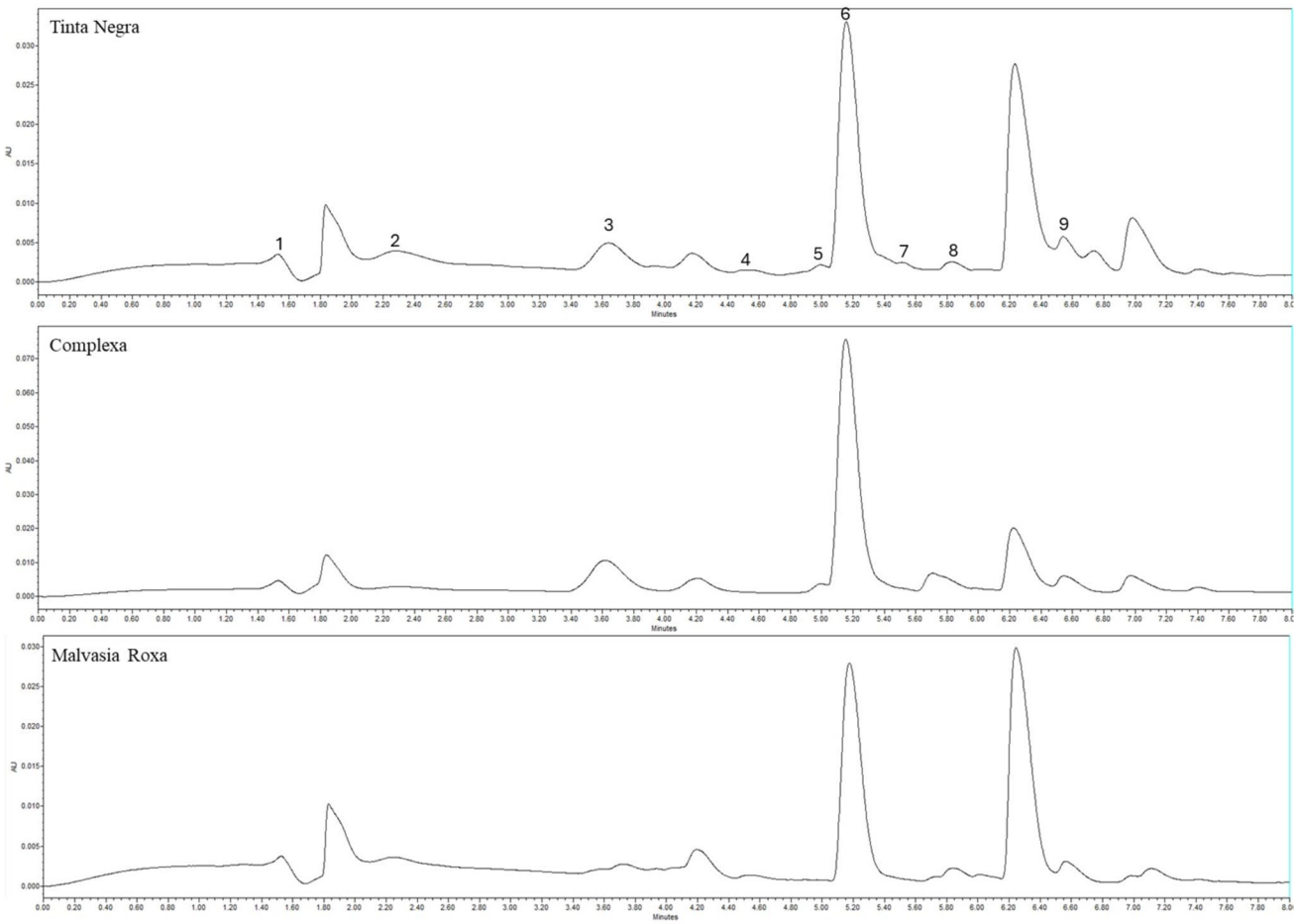

Fig. 3 Typical chromatogram ($\lambda_{acquisition} = 320$ nm) of phenolic compounds from GP of red variety by µQuEChERS/UHPLC-PDA methodogy. Peak number identification: 1: gallic acid; 2: catechin; 3: sinapic acid; 4: o-coumaric acid; 5: taxifolin; 6: *trans*-resveratrol; 7: quercetin; 8: cinnamic acid; 9: kaempferol

Table 3 Total phenolic content (TPC), total anthocyanins content (TAC), total catechin content (TCC), and antioxidant capacity (DPPH) determined in the GP of the investigated varieties

GP extracts	TPC mgGAE/100 g	TAC mgCGE/100 g	TCC mgCATE/100 g	DPPH mgTE/100 g
Tinta Negra	2515 ± 9^{d}	6.67 ± 0.03^{b}	947 ± 1^{a}	4175 ± 28^{e}
Complexa	4727 ± 12^{a}	10.6 ± 0.01^{a}	688.7 ± 0.4^{b}	9472 ± 67^{a}
Malvasia Roxa	3234 ± 9^{b}	0.08 ± 0.01^{c}	550 ± 18^{c}	6464 ± 22^{b}
Malvasia	1838 ± 13^{f}	nd	313 ± 9^{d}	3117 ± 50^{g}
Sercial	3083 ± 12^{c}	nd	246 ± 7^{e}	6278 ± 21^{c}
Verdelho	2307 ± 15^{e}	nd	242 ± 5^{e}	4468 ± 19^{d}
Boal	1746 ± 7^{g}	nd	122 ± 3^{g}	2547 ± 13^{h}
Terrantez	2554 ± 10^{d}	nd	183.4 ± 0.4^{f}	4085 ± 23^{f}

Values expressed as mean ± standard deviation (SD) per 100 g dry weight (DW) (n = 3)

nd not detected, *GAE* Gallic acid equivalent, *CATE* Catechin equivalent, *CGE* Cyanidin-3-glucoside equivalent, *TE* Trolox equivalent, Values followed by the same letters on the same column did not differ by Tukey's HSD test ($p > 0.001$)

The TAC concentrations of the GP investigated ranged from 0.08 to 10.6 mgCGE/100 g DW, with the lower value corresponding to Malvasia Roxa and the higher value to the GP of the Complexa variety. The TAC of GP extracts from white grape varieties (Malvasia, Sercial, Verdelho, Boal, Terrantez) was null, as expected (Table 4).

Table 4 Pearson correlation between the bioactive compounds (phenolic compounds and vitamin C), antioxidant activity, and levels of TPC, TAC and TCC for the studied GP (* significant difference at $p < 0.001$; ** significant difference at $p < 0.05$)

	TPC	TAC	DPPH	TCC	Vitamin C	Gallic acid	Catechin	Sinapic acid	o-Coumaric acid	Trans-resveratrol	Taxifolin	Quercetin	Cinnamic acid	Kaempferol
TPC	1.00													
TAC	0.71*	1.00												
DPPH	0.98*	0.63*	1.00											
TCC	0.49**	0.78*	0.44*	1.00										
Vitamin C	-0.28	-0.15	-0.35	-0.40	1.00									
Gallic acid	0.05	-0.12	0.07	-0.42**	0.63*	1.00								
Catechin	-0.34	-0.33	-0.32	0.10	0.14	-0.07	1.00							
Sinapic acid	0.14	0.30	0.10	0.48**	0.05	0.19	-0.14	1.00						
o-Coumaric acid	0.76*	0.59*	0.75*	0.68*	-0.47**	-0.18	-0.33	0.62**	1.00					
Trans-resveratrol	0.31	0.60*	0.19	0.29	0.35	0.24	-0.55**	0.60**	0.40**	1.00				
Taxifolin	0.54*	0.89*	0.45**	0.84*	-0.23	-0.25	-0.38	0.62**	0.73*	0.70**	1.00			
Quercetin	0.77*	0.76*	0.76*	0.82*	-0.60**	-0.37	-0.32	0.44**	0.93*	0.32*	0.82*	1.00		
Cinnamic acid	-0.04	0.02	-0.11	0.44**	0.00	-0.36	0.63*	0.26	0.19	-0.11	0.18	0.13	1.00	
Kaempferol	0.11	0.08	0.13	0.40	-0.74*	-0.76*	-0.15	0.13	0.48**	-0.16	0.30	0.56**	0.15	1.00

The TCC of the studied GP ranged from 122 to 947 mgCATE/100 g DW, with the lowest values found in the GP of the Boal variety (122 mgCATE/100 g DW) and Terrantez (183 mgCATE/100 g DW) and the highest values in GP of the Tinta Negra grapes (947 mgCATE/100 g DW) and Complexa varieties (689 mgCATE/100 g DW). The results achieved were lower than those stated in the literature for different white and red grapes [32]. Additionally, there was no significant difference ($p > 0.001$) in the TCC between the GP from Sercial and Verdelho varieties.

The antioxidant activity of GP from Complexa variety (9472 mgTE/100 g DW) and Malvasia Roxa (6464 mgTE/100 g DW) was meaningfully superior compared to the antioxidant activity of GP from the other grape varieties studied. Boal (2547 mgTE/100 g DW) had the smallest antioxidant activity among the grape varieties studied. The antioxidant activity was significantly different ($p < 0.001$) among all the GPs studied, Table 4. On average, the antioxidant activity in red GP (6704 ± 39.2 mgTE/100 g DW) was 1.6 times higher than in GP from white grape varieties (4099 ± 25.2 mgTE/100 g DW). A previous study on Italian red GP reported antioxidant activity ranging from 3204 to 7484 mgTE/100 g DW [49], closely aligning with the current findings, while Putnik et al. [50] reported lower values (1279 to 2056 mgTE/100 g DW).

3.4 Pearson correlation between phenolic compounds and biological activities

To elucidate whether the phenolic compounds contributed to the antioxidant activities of the analysed GP, Pearson correlations were performed to compare the different data obtained. The results obtained presented in Table 4. The interpretation of the correlation was based on the correlation coefficient (r), with values greater and lower than 0 being positive and negative correlations, respectively. A strong correlation was defined as r values between 0.7 and 1, a moderate correlation between 0.3 and 0.7, and a weak correlation as values between 0 and 0.3.

A strong positive and significant ($p < 0.001$) correlation was observed between TPC-DPPH (r = 0.98), TPC-TAC (r = 0.71), and TCC-TAC (r = 0.78). Additionally, a moderate positive correlation was attained between DPPH-TAC (r = 0.63), TPC-TCC (r = 0.49) and TCC-DPPH (r = 0.44). This finding suggests that phenolic compounds, such as phenolic acids and flavonoids, played a significant role in the antioxidant activity of GP, especially in red GP. Yu et al. [19] reported a positive correlation (r = 0.99, $p < 0.05$) between TAC and DPPH when analysing four different GP.

The phenolic compounds that showed a significant ($p < 0.001$) correlation with TPC, TCC, and DPPH were

quercetin (r = 0.77, r = 0.82, and r = 0.76, respectively), and o-coumaric acid (r = 0.76, r = 0.68, and r = 0.75, respectively). Contrarily, o-coumaric acid (r = 0.59), *trans*-resveratrol (r = 0.60), taxifolin (r = 0.89), and quercetin (0.76) were significantly correlated ($p < 0.001$) with TAC. It is possible that only a few phenolic components were correlated with antioxidant activity, and shouldtake into account the chemical interactions (synergism, antagonism) that occur between the different phenolic compounds present in GP extracts. Furthermore, a strong correlation ($p < 0.001$) was obtained for quercetin with *trans*-resveratrol (r = 0.93), for quercetin with taxifolin (r = 0.82), and for taxifolin with o-coumaric acid (r = 0.73). A moderate correlation ($p < 0.05$) was attained for sinapic acid with o-coumaric acid (r = 0.62), taxifolin (r = 0.62), and *trans*-resveratrol (r = 0.60). The findings suggest that the GP extracts investigated could be considered as an appropriate source of phenolic compounds (e.g., *trans*-resveratrol, o-coumaric acid, quercetin) with favourable health effects, such as antioxidant activity. Taxifolin, quercetin, and *trans*-resveratrol are effective at neutralising free radicals, the antioxidant properties of these bioactive molecules have been shown to help prevent several malignancies in humans, including cancers, diabetes, and cardiovascular disease, among others [42, 51–53]. However, their mechanisms and efficacy depend on the biological system and the type of oxidative stress. Topal et al. [54] confirmed taxifolin's strong antioxidant, radical-scavenging, and metal-chelating properties, highlighting its noteworthy potential as an antioxidant and providing insights into its structure–activity relationship [55]. The antioxidant action of quercetin affects ROS, signalling pathways, enzyme function, and glutathione levels. By suppressing cytokine production, lowering the expression of cyclooxygenase and lipoxygenase, and preserving mast cell integrity, quercetin also has potent anti-inflammatory properties [56]. *Trans*-resveratrol, a popular dietary supplement with well-known antioxidant properties, works by altering several cellular processes, such as the Nrf2/ARE pathway, which increases the production of antioxidant enzymes. Nevertheless, Santana et al. [57] observed that the antioxidant effect of *trans*-resveratrol in the liver may not be linked to Nrf2 activation or inflammatory cytokine changes, prompting further investigation into alternative mechanisms, such as direct radical scavenging. *Trans*-resveratrol can reduce oxidative damage from metal-catalyzed processes by chelating metal ions. Additionally, it preserves mitochondrial function, which is necessary for the synthesis and preservation of cellular energy and inhibits the action of several oxidative enzymes [58].

3.5 Anti-inflammatory activity

The anti-inflammatory activity of GP extracts was assessed by the egg albumin denaturation assay. This process involves the alteration of albumin structure, which can be caused by external factors such as acids, bases, heat, or organic solvents that disrupt the protein's tertiary and secondary structures. Table 5 shows the percentage of inhibition of aspirin (a non-steroidal anti-inflammatory drug, reference) and GP extracts obtained by μQuEChERS at different concentrations (20 to 100 μg/mL). In the concentration ranging of 20 to 100 μg/mL, the percentage inhibition of protein denaturation for the studied GP ranged from 10.0% to 70.1%, while aspirin showed its inhibitory effect in the range of 43.4% to 63.5%. It was verified that the percentage inhibition of protein denaturation improved with the concentration. According to the literature, promising anti-inflammatory agents must suppress protein denaturation by at least 20% [59]. In this sense, at a concentration of 20 μg/mL, GP from Tinta Negra, Complexa, and Malvasia cannot be regarded as probable anti-inflammatory agents, since their protein denaturation was lower than 20%. At 20 μg/mL, the GP from Boal (43.1%) and Verdelho (41.7%) showed an inhibition percentage of protein denaturation that was not significantly different ($p > 0.001$) when compared to the reference drug aspirin (43.4%). At 50 μg/mL, the reference drug aspirin (51.32%) was more effective than GP from Tinta Negra, but not significantly different ($p > 0.001$) when compared to all studied GP extracts. At the highest concentration (100 μg/mL), the GP from Terrantez and Malvasia showed the highest activity against protein denaturation with an inhibition percentage of 70.1% and 68.6%, respectively, while Tinta Negra showed the lowest (41.3%). Their effect was significantly different from the reference drug aspirin (63.5%; $p < 0.001$). Quercetin and catechin, identified in GP, may regulate inflammatory pathways through several mechanisms, such as inhibition of NF-κB and MAPK signalling pathways, reduction in the production of pro-inflammatory cytokines and enzymes, and

Table 5 Inhibition percentage (%) of albumin denaturation at different concentrations of GP extracts from different *Vitis vinifera* L. relatively to the aspirin (anti-inflammatory positive control)

GP extracts	Concentration (μg/mL)		
	20	50	100
Aspirin	43.4 ± 0.1^{a}	51.5 ± 0.2^{a}	63.5 ± 0.1^{c}
Tinta Negra	10 ± 2^{f}	15 ± 2^{b}	41.3 ± 0.6^{f}
Complexa	14 ± 1	53.3 ± 0.6^{a}	57.9 ± 0.4^{e}
Malvasia Roxa	35.6 ± 0.2^{b}	53 ± 2^{a}	66.8 ± 0.5^{b}
Malvasia	18.5 ± 0.2^{d}	43 ± 1^{a}	$68.6 \pm 0.2^{a,b}$
Sercial	29.9 ± 0.9^{c}	50.9 ± 0.6^{a}	61.1 ± 0.9^{d}
Verdelho	41.7 ± 0.6^{a}	52 ± 1^{a}	63.5 ± 0.1^{c}
Boal	43.1 ± 0.2^{a}	51 ± 5^{a}	66.2 ± 0.3^{b}
Terrantez	34.1 ± 0.6^{b}	45 ± 4^{a}	70.1 ± 0.7^{a}

Values followed by the same letters on the same column did not differ by Tukey's HSD test ($p > 0.001$)

scavenging of reactive oxygen species to reduce oxidative stress [42].

3.6 Antibacterial activity

Table 6 displays the diameters of the inhibition zones (in mm) related to the antibacterial activities of the GP extracts. The results against *S. aureus* and *E. coli* were reported as mean ± standard deviation. At 100 µg/mL, GP extracts from Sercial and Complexa exhibited the biggest inhibition zones against *S. aureus* (11.5 and 11.3 mm, respectively) and *E. coli* (7.50 and 7.10 mm, respectively), whereas GP extracts from Terrantez showed the lowest inhibition zones, 8.78 and 5.50 mm, respectively. The most sensitive and resistant bacteria were *S. aureus* (average, 10.3 ± 0.97 mm) and *E. coli* (average, 6.30 ± 0.48 mm), respectively. There was a significant difference ($p < 0.001$) against *S. aureus* between the GP extracts of Terrantez and Complexa, as well as between Terrantez and Sercial. On the other hand, no significant differences ($p > 0.001$) were observed for GP extracts against *E. coli***.** These variations may be attributed to the lipopolysaccharide cell wall found in Gram-negative bacteria, which restricts the ability of phenolic compounds to enter the cell. Similar results with phenolic GP extracts were obtained by Fereira & Santos [17] and Gerardi et al. [60]. The antibacterial effect of the extract may be due to its phenolic compounds, especially gallic acid and quercetin, which are the most effective in inhibiting bacterial growth. Gallic acid exhibits antibacterial properties by damaging cell membranes and interfering with energy production within bacterial cells, while quercetin can disrupt cell walls and membranes and inhibit nucleic acid synthesis [61]. Using the checkerboard technique, Sanhueza and colleagues [62] investigated the synergistic interactions between GP extracts and a variety of commonly applied antibiotics against *S. aureus* and *E. coli*. GP phenolic compounds (e.g., quercetin, gallic acid, protocatechuic acid) combined with several antibiotic classes (amphenicol, β-lactam, fluoroquinolone, tetracycline) effectively reduced the growth of *S. aureus* and *E. coli*. However, the precise mechanisms through which phenolic compounds exert their antibacterial effects are not completely understood. It is believed that these bioactive substances may interact with various components of bacterial cells, hinder biofilm formation, disrupt the proton gradient necessary for oxidative phosphorylation, alter the balance of bacterial metabolites and ions, and interfere with nucleic acid synthesis and gene regulation [63]. In sum, GP extracts exhibited bacterial activities against both Gram-negative and Gram-positive bacteria, suggesting that this underutilised by-residue may be the ideal source of antimicrobials for further use in food processing companies that monitor or deny food-borne infections.

Table 6 Antibacterial activities of the investigated GP

Grape pomace extracts	Zone of inhibition (mm)	
	Staphylococcus aureus	*Escherichia coli*
Tinta Negra	$10.5 \pm 0.5^{a,b}$	6.5 ± 0.5^{a}
Complexa	11.3 ± 0.9^{a}	7.1 ± 0.8^{a}
Malvasia Roxa	$10.8 \pm 0.3^{a,b}$	6.9 ± 0.3^{a}
Malvasia	$10.2 \pm 0.3^{a,b}$	5.5 ± 0.5^{a}
Sercial	11.5 ± 0.5^{a}	7.5 ± 0.5^{a}
Verdelho	$9.5 \pm 0.5^{a,b}$	5.8 ± 0.2^{a}
Boal	$9.5 \pm 0.5^{a,b}$	5.6 ± 0.5^{a}
Terrantez	8.8 ± 0.2^{b}	5.5 ± 0.5^{a}

The results are expressed as the mean of zone size (mm) ± standard deviation

Values followed by the same letters on the same column did not differ by Tukey's HSD test ($p > 0.001$)

4 Conclusions

The µQuEChERS and SLE extractions combined with UHPLC-PDA analysis proved to be a robust and efficient method for identifying and quantifying nine phenolic compounds and vitamin C across eight diverse GP varieties, demonstrating excellent analytical performance The methods show excellent linearity ($R^2 > 0.990$), precision (%RSD < 16%), accuracy (81.0 – 116%), low LODs (0.008 – 0.140 mg/L), and LOQs (0.023 – 0.424 mg/L) values. Among the phenolic compounds examined, gallic acid, catechin, quercetin, and *trans*-resveratrol were the most prevalent and quantified in the GP extracts analysed. On average, the concentration of gallic acid was more abundant in GP from white grape varieties, while catechin, *trans*-resveratrol and quercetin were dominant in GP from red grape varieties. Contrarily, the concentration of vitamin C ranged from 17.7 mg/100 g (Malvasia Roxa) to 21.6 mg/100 g (Boal). There was no significant difference ($p > 0.001$) in the vitamin C content among the GP extracts of Boal, Terrantez, and Sercial.

The GP extracts obtained by µQuEChERS were assessed in terms of antioxidant, anti-inflammatory, and antibacterial activities. On average, the highest antioxidant activity was determined in GP from red grape varieties (6704 ± 39.2 mgTE/100 g), whereas the lowest antioxidant activity was found in GP from white grape varieties (4099 ± 25.2 mgTE/100 g). A strong positive and significant correlation ($p < 0.001$) was observed between phenolic compounds and antioxidant activities ($r = 0.98$). In addition, the investigated GP extracts showed promising inhibition of albumin protein denaturation compared to aspirin (reference standard). GP inhibited albumin denaturation in a concentration-dependent manner, reaching

over 41% inhibition at 100 µg/mL. GP extracts exhibit bacterial activities against both Gram-negative and Gram-positive bacteria, being more efficient against *S. aureus*.

This groundbreaking study reveals that GP, a wine industry by-product, is a treasure trove of bioactive compounds with potent antioxidant, anti-inflammatory, and antibacterial properties, positioning it as a sustainable and versatile resource for developing innovative health-promoting products in the food, supplement, and cosmetic industries. However, further studies should be carried out to examine the scalability of the existing approaches for industrial applications, specifically designing pilot processes, method automatisation, sustainability (e.g., reduced waste generation, eco-friendly solvents), evaluating the functional characteristics and bioavailability of the extracted phenolics in product formulations and determine their market suitability in industries such as nutraceuticals, cosmetics, or foods.

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Author contributions Teresa Abreu contributed to formal analysis, investigation, and the preparation of the original draft. Catarina Luís was involved in investigation, formal analysis, and writing the original draft. José Câmara focused on conceptualization, formal analysis, and the review and editing of the manuscript. Juan Teixeira worked on conceptualization and the review and editing process. Rosa Perestrelo handled conceptualization, formal analysis, supervision, and both the preparation and review of the original draft. All authors have read and approved the final version of the manuscript.

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Data availability All data generated or analysed during this study are included in this article.

Declarations

Consent for publication All authors have read and agreed to the published version of the manuscript.

Consent to participate All the authors agreed to participate in this work.

Competing interests 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.

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