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Grape pomace as a source of phenolic compounds and diverse bioactive properties

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ABSTRACT

The bio-residues resulting from the wine industry (grape pomace made up of skins, seeds and stems) are often undervalued but constitute a potential source of bioactive phenolic compounds that can be applied in several industries. In this context, the aim of the present study was to evaluate the phenolic profile of *Vitis vinifera* L. grape pomace (skins, seeds and their mixture), and correlate them with its antioxidant, cytotoxic and antibacterial activities. The seeds showed the highest amount of phenolic compounds and also the highest antioxidant, cytotoxic and antibacterial activities. The skins revealed the highest levels of anthocyanins and *p*-coumaric acid hexoside. Strong correlations were observed between the presence of phenolic compounds and all the bioactivities studied. These by-products are good sources of phenolic compounds with high antioxidant and antibacterial activity, and also presenting a moderate cytotoxicity activity. These added-value by-products have great applicability in food, pharmaceutical and cosmetic industries.

1. Introduction

Vitis vinifera L. is a grapevine species from the Vitaceae family, which comprises a huge variety of white (Chardonnay, Pinot blanc, Gewürztraminer, Comtessa, Noblessa) and red (Pinot noir, Cabernet Sauvignon, Cabernet franc, Merlot, Petit Verdot) grapes (Rodríguez Montealegre, Romero Peces, Chacón Vozmediano, Martínez Gascueña, & García Romero, 2006; Santos-Buelga, Francia-Aricha, & Escribano-Bailón, 1995; Terral et al., 2010). In recent years, the by-products of wine making and the agricultural residues of plant origin have attracted considerable attention as sources of bioactive phenolic compounds, that are used for various purposes in cosmetic, pharmaceutical and food industries (Makris, Boskou, & Andrikopoulos, 2007). During wine production tons of grape pomace are obtained, which is essentially made up of skins, seeds and stems. These components, particularly the seeds, are rich in phenolic compounds known as antioxidant, anti-tumor, anti-aging, anti-microbial and anti-inflammatory agents (Xia et al., 2010; Yu & Ahmedna, 2013).

In general, phenolic compounds found in wine and grapes can be classified in three main groups, phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids (catechins, flavonols and anthocyanins) and proanthocyanidins. Genetic factors, environmental conditions and the degree of plant maturation widely influence the content in these compounds (Melo et al., 2006; Yu & Ahmedna, 2013). Previous studies also showed the potential of grape pomace phenolic compounds to be used as preservatives, they prevent lipid oxidation and suppress the growth of some bacterial strains, such as *Staphylococcus aureus*, *Streptococcus mutans* and *Escherichia coli* (Yu & Ahmedna, 2013). The antitumor activity of grape pomace polyphenols has also been reported within their preventive effects in several diseases, which led to the commercialization of different dietary food supplements rich in polyphenols (Caleja, Ribeiro, Barreiro, & Ferreira, 2017; Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005).

Previous studies have established a relation between the presence of phenolic compounds and some bioactivities exhibited by red grapes varieties. For instance, Bartolomé, Nuñez, Monagas, and Gómez-Cordovés (2004) and Murthy, Singh, and Jayaprakasha (2002) described the antioxidant activity of red grapes skins from *Vitis vinifera* var. *Cabernet Sauvignon*, *Graciano* and *Tempranillo* from Spain and var. *Bangalore blue* from Indian states, respectively. Scalbert et al. (2005) related the presence of polyphenols with tumor cells' apoptosis. On the other hand, Jayaprakasha et al. (2003) and Anastasiadi, Chorianopoulos, Nychas, and Karoutounian (2009), reported the antimicrobial activity of grape extracts (var. *Bangalore blue* and var.

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Mandilaria, *Voidomato*, *Asyrtiko* and *Aidani*, respectively) against gram positive and negative bacteria.

In this context, the aim of the present study was to characterize *Vitis vinifera* grape pomace (skins, seeds and their mixture) in terms of the phenolic profile and correlation with biological properties namely antioxidant, cytotoxic and antibacterial activities.

2. Materials and methods

2.1. Standards and reagents

Acetonitrile (99.9%) was of HPLC grade from Fisher Scientific (Lisbon, Portugal). Phenolic standards ((+)-catechin, delphinidin, (−)-epicatechin, gallic acid, malvidin, peonidin-3-*O*-glucoside, *p*-coumaric acid, quercetin-3-*O*-glucoside) were from Extrasynthèse (Genay, France). Sulforhodamine B, trypan blue, trichloroacetic acid (TCA), tris (hydroxymethyl)aminomethane (Tris), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), hank's balanced salt solution (HBSS), foetal bovine serum (FBS), L-glutamine, trypsin-EDTA, penicillin/streptomycin solution (100 U/ml and 100 mg/ml, respectively) were purchased from Hyclone (Logan, Utah, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other general laboratory reagents were purchased from Panreac Química S.L.U. (Barcelona, Spain). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Preparation of the samples

Fermented grape pomace, a by-product of the winery industry, was provided by Alijó Cooperative Winery (Vila Real, Portugal). The skins and seeds of the grape pomace were manually separated in order to obtain three different samples: i) mixture; ii) skins and iii) seeds (Fig. 1). These fractions were uniformly distributed on trays and dehydrated in a forced-air-drying oven (Imperial IV Microprocessor Oven, Lab-Line Instruments, Inc., Melrose Park, III) at 50 °C until 7.0% of moisture was reached.

2.3. Analysis of the phenolic compounds

The phenolic profile was determined by LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA).

2.3.1. Non-anthocyanin compounds

To prepare the hydromethanolic extracts, 1 g of each sample was submitted to extraction with a methanol/water mixture (80:20, *v/v*; 30 ml) at 25 °C and 150 rpm during 1 h, followed by filtration through a Whatman filter paper No. 4. Afterwards, the residue was extracted with one additional portion of the hydromethanolic mixture and the combined extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland).

These compounds were separated and identified as previously described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). The obtained extracts were re-dissolved at a concentration of 5 mg/ml with a methanol/water (80:20, *v/v*) mixture. For the double online detection, 280, 330 and 370 nm were used as preferred wavelengths for the diode array detector (DAD) and in a mass spectrometer (MS) connected to HPLC system via the DAD cell outlet. The MS detection was performed in negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source.

2.3.2. Anthocyanin compounds

Each powdered sample (1 g) was extracted with 30 ml of methanol containing 0.5% trifluoroacetic acid (TFA), and filtered through a Whatman No. 4 paper. The residue was then re-extracted twice with additional 30 ml portions of 0.5% TFA in methanol. The combined extracts were evaporated at 35 °C to remove the methanol, and re-dissolved at a concentration of 5 mg/ml in 80% acidified methanol with TFA (0.01%). These compounds were separated and identified as previously described by Gonçalves et al. (2017). For the double online detection, 520 nm was used as preferred wavelengths for DAD and in a MS connected to HPLC system via the DAD cell outlet. The MS detection was performed in positive mode, using a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source.

The identification of the phenolic compounds (non-anthocyanins and anthocyanins) was performed based on their chromatographic behaviour and UV–vis and mass spectra by comparison with standard compounds, when available, and data reported in the literature giving a tentative identification. Data acquisition was carried out with Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA). For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of the most similar available standard: catechin ($y = 84539x + 269612$, $R^2 = 0.991$); delphinidin ($y = 557274\ x + 126.24$, $R^2 = 0.999$); (−)-epicatechin ($y = 28512x + 2000000$, $R^2 = 0.999$); gallic acid ($y = 280379x + 119556$, $R^2 = 0.998$); malvidin ($y = 477014.9x + 38.38$, $R^2 = 0.999$); *p*-coumaric acid ($y = 301950x + 6967$, $R^2 = 0.999$); peonidin-3-*O*-glucoside ($y = 537017x - 71.47$, $R^2 = 0.999$) and quercetin-3-*O*-glucoside ($y = 23853x + 343376$, $R^2 = 0.999$). The results were expressed as μg/g extract.

Fig. 1. Grape pomace mixtures (A), seeds (B) and skins (C).