Science of the Total Environment 634 (2018) 831–842

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Potential of Portuguese vine shoot wastes as natural resources of bioactive compounds

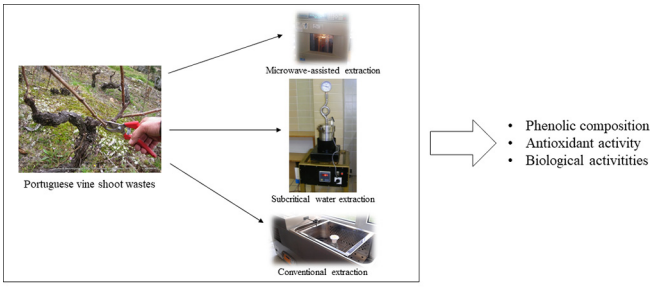
Manuela M. Moreira [a,*], M. Fátima Barroso [a], João Vasconcellos Porto [b], M.J. Ramalhosa [a], Jaroslava Švarc-Gajić [c], Letícia Estevinho [d,e], Simone Morais [a], Cristina Delerue-Matos [a]

[a] REQUIMTE/LAQV, Instituto Superior de Engenharia do Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4249-015 Porto, Portugal
[b] Sogrape Vinhos, S.A., Aldeia Nova, 4430-809 Avintes, Portugal
[c] Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[d] Agricultural College of Bragança Polytechnic Institute of Bragança, Bragança, Portugal
[e] IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Braga, Portugal

HIGHLIGHTS

- Phenolic, antioxidant and biological activities of vine shoots were examined.
- Subcritical-water, microwave-assisted and conventional extractions were employed.
- *Tinta Roriz* has higher phenolic and antioxidant activity than *Touriga Nacional*.
- The tested vine shoot extracts inhibited α-amylase and acetylcholinesterase enzymes.
- Vine shoot extracts had activity against *S. mitis*, *E. coli* and *C. albicans*.

GRAPHICAL ABSTRACT



Valorization of vine shoots through the extraction of bioactive compounds to obtain high added value products

ARTICLE INFO

Article history:
Received 6 February 2018
Received in revised form 3 April 2018
Accepted 4 April 2018
Available online xxxx

Editor: Frederic Coulon

Keywords:
Vine shoots
Antioxidant activity
HPLC-PDA analysis
Polyphenols

ABSTRACT

Since annually a high amount of wastes is produced in vine pruning, the aim of this study was to evaluate the potential of vine shoots from two Portuguese grape varieties (*Touriga Nacional* - TN and *Tinta Roriz* - TR) to be used as a natural source of phenolic compounds. To reach this goal, three techniques were explored, namely microwave-assisted extraction (MAE), subcritical water extraction (SWE) and conventional extraction (CE). The phenolic composition of the extracts, antioxidant and biological activities were evaluated by spectrophotometry and chromatography. MAE and SWE produced the highest concentrated extracts. TR vine shoot variety had the highest antioxidant activity and total phenolic (32.1 ± 0.9 mg gallic acid equivalents/g dry sample), as well as flavonoid content (18.7 ± 1.2 mg epicatechin equivalents/g dry sample). For the first time, the biological activity of the vine shoot extracts was tested. Results demonstrated that all of them had antimicrobial potential against different bacteria and yeasts, and the ability of inhibiting α-amylase and acetylcholinesterase enzymes, with MAE TR extracts being the most efficient. HPLC analysis enabled the identification of different phenolic

Abbreviations: AA, ascorbic acid; AAPH, 2,2′-azobis-2-amidinopropane; ATCC, American Type Culture Collection; CE, conventional extraction; CFU, colony forming units; DPPH-RSA, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; DW, dry weight; ESA, Escola Superior Agrária; FRAP, ferric reduction activity power; GA, gallic acid; GAE, gallic acid equivalents; HPLC-PDA, high performance liquid chromatography with photodiode array detection; IC_{50}, half maximal inhibitory concentration; MAE, microwave assisted extraction; MIC, minimum inhibitory concentration; MLC, minimum lethal concentration; PBS, phosphate buffered saline; SD, standard deviation; SWE, subcritical water extraction; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid; TE, trolox equivalents; TPC, total phenolic content; TPTZ, 2,4,6-tris(2-pyridyl)-s-triazine; UAE, ultrasound assisted extraction.

* Corresponding author at: REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal.
E-mail address: manuela.moreira@graq.isep.ipp.pt. (M.M. Moreira).

https://doi.org/10.1016/j.scitotenv.2018.04.035

Bioactive compounds
Antimicrobial activity

compounds, with gallic acid, catechin, myricetin and kaempferol-3-*O*-rutinoside being the main contributors to the phenolic composition. Portuguese vine shoot wastes could serve as easily accessible source of natural antioxidants for the food or pharmaceutical industries.

1. Introduction

One of the most important economic activities in Portugal is the cultivation of *Vitis* (*Vitaceae*) grape varieties. According to the International Organization of Vine and Wine, in 2014 the Portuguese vitiviniculture area cultivated was 221,448 ha and the grape production was 8,185,120 tons (Wine, 2017). During the harvest season, this wine-producing results in a huge amount of vineyard wastes, especially vine shoots, which could be re-used in valued added applications, as their present economic value is nearly nule (Delgado de la Torre et al., 2012; Gullón et al., 2018; Hagemann et al., 2018; Sánchez-Gómez et al., 2016). Currently, they are incorporated in the soil, which enables the biodegradation of the vegetal matter reducing the need for application of organic correctives and/or fertilizers (Hagemann et al., 2018). However, using this waste as a natural resource of bioactive compounds could increase its economic value (Delgado-Torre et al., 2012; Karacabey et al., 2012; Sánchez-Gómez et al., 2014; Teixeira et al., 2014).

Vine shoots have been shown to be a rich source of bioactive compounds, including phenolic compounds, which content depends on several factors such as grapevine variety, age, growth conditions, among others (Figueiredo-González et al., 2012a; Gullón et al., 2018; Luque-Rodríguez et al., 2006; Pérez-Lamela et al., 2007; Rajha et al., 2014c). Additionally, experimental conditions, such as temperature, time, and solvent composition, also affect the yield of phenolic compounds recovered (Figueiredo-González et al., 2013; Figueiredo-González et al., 2012b; Figueiredo-González et al., 2012c; Karacabey and Mazza, 2010; Karacabey et al., 2012).

Luque-Rodríguez et al. (2006) were the pioneers to investigate the extraction of polyphenols from vine shoots of *Vitis vinifera* by superheated ethanol-water mixtures. Although, as can be seen from the analysis of Table 1, which summarizes the collected data from the literature regarding the phenolic compounds extraction from vine shoots, only in the last 7 years higher efforts have been made in the exploitation of this vineyard waste as a potential source of phenolic compounds. The majority of the reported studies uses aqueous ethanol for phenolics extraction from vine byproducts (Alexandru et al., 2014; Çetin et al., 2011; Delgado-Torre et al., 2012; Farhadi et al., 2016; Ju et al., 2016; Karacabey et al., 2012; Luque-Rodríguez et al., 2006). In some cases (Rajha et al., 2015a; Sánchez-Gómez et al., 2014; Sánchez-Gómez et al., 2016), water was employed as extracting solvent, but the total phenolic content (TPC) reported was lower than the obtained employing mixtures with organic solvents (Delgado-Torre et al., 2012; Rajha et al., 2015a; Sánchez-Gómez et al., 2014).

Regarding the extraction techniques employed, conventional extraction (CE) is the preferred method for the recovery of polyphenols from vine wastes (Alexandru et al., 2014; Çetin et al., 2011; Gullón et al., 2017; Ju et al., 2016; Karacabey and Mazza, 2010; Rajha et al., 2015b; Sánchez-Gómez et al., 2014). Recently, the use of auxiliary energies, such as microwaves and ultrasound, has enhanced the extraction process, as well as the yield of recovered compounds (Alexandru et al., 2014; Delgado-Torre et al., 2012; Farhadi et al., 2016; Sánchez-Gómez et al., 2014). Despite of being under exploitation, another environmental-friendly extraction technique that is gaining considerable attention for polyphenols recovery is subcritical water extraction (SWE) (Aliakbarian et al., 2012; Plaza et al., 2010). As far as we know, only one study has been conducted using a pressurized low-polarity water extractor to recover *trans*-resveratrol and *trans*-ε-viniferin compounds from vine shoot wastes (Karacabey et al., 2012).

This study represents the first attempt to characterize the phenolic profile and antioxidant activity, as well as the biological activities from two important Portuguese vine shoot varieties (*Touriga Nacional*, TN and *Tinta Roriz*, TR) from Dão region (North Center of Portugal) for the potential utilization of these wastes as a source of natural bioactive compounds. For that, the efficiency of three extraction techniques (SWE, microwave-assisted extraction (MAE), and CE) for the recovery of bioactive compounds from vine shoots was evaluated. The obtained vine shoot extracts were characterized in terms of total phenolic and flavonoid content, and antioxidant activity by Ferric Reducing Activity Power (FRAP), 2,2-diphenyl-picrylhydrazyl radical scavenging activity (DPPH-RSA) and anti-hemolytic assays. The α-amylase and acetylcholinesterase inhibition and the antimicrobial activities, as well as the phenolic profile by high-performance liquid chromatography (HPLC) analysis were also determined.

2. Materials and methods

2.1. Chemicals

Sodium carbonate (≥99%), Folin's phenol reagent, and gallic acid (GA, ≥98%) were used in the Folin-Ciocalteu assay and were obtained from Sigma-Aldrich (Madrid, Spain). DPPH radical, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine, 99%), aluminium chloride hexahydrate (99%) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 98%) were also from Sigma. Absolute anhydrous ethanol (p.a.), sodium nitrite (≥97%) and ascorbic acid (AA, 99.7%) were acquired from Carlo Erba (Peypin, France), Merck and Riedel-de Haën, respectively. Methanol and formic acid for HPLC analysis were gradient grade and got from Merck (Darmstadt, Germany). Phenolic compound standards were bought to Sigma-Aldrich (Madrid, Spain). Other chemicals were from Sigma (Madrid, Spain) and analytical grade.

2.2. Samples collection and preparation

Vine shoots from two different *V. vinifera* varieties (TN and TR) were kindly provided by Sogrape Vinhos, S. A. (Portugal). Vine shoots were sampled in Quinta dos Carvalhais, located in Mangualde (North of Portugal), in November of 2015 by randomized selection. The moisture content of each vine shoot variety was determined using a Moisture Analyser (Kern MLS 50-3IR160) and was 13.4 ± 0.7 and 12.8 ± 0.9% for TN and TR, respectively. After oven-drying (Model no. 2000208, J.P. Selecta, Barcelona, Spain) the vine shoots at 50 °C for 24 h, they were milled (Retsch ZM200) to a particle size smaller than 1 mm and stored in sealed bags at room temperature until use.

2.3. Extraction of phenolic compounds

2.3.1. Microwave-assisted extraction

MAE was carried out in a MARS-X 1500 W (Microwave Accelerated Reaction System for Extraction and Digestion, CEM, Mathews, NC, USA) using the conditions previously optimized by Moreira et al. (2017). Milled dried vine shoots (0.1 g) were extracted with 20 mL ethanol: water 60:40 v/v during 20 min at 100 °C. The obtained extract was centrifugated (Heraeus™ Megafuge™ 16R Centrifuge, Thermo Scientific) for 10 min at 4000 rpm, and the supernatant was stored at −20 °C until further analysis.