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A potential use of vine-shoot wastes: The antioxidant, antifeedant and phytotoxic activities of their aqueous extracts

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ABSTRACT

One of the biggest challenges for wine producing regions is to create alternatives to increase the added value of the large amount of vine-shoot wastes generated. The antioxidant, antifeedant and phytotoxic activities of vine-shoot aqueous extracts obtained by different extraction methods (Conventional Solid-Liquid Extraction, CSLE; Solid-Liquid Dynamic Extraction, SLDE-Naviglio; Microwave Extraction, ME) have been studied. Because of their higher content of phenolic compounds, CSLE and ME extracts showed a higher DPPH radical-scavenging activities compared with SLDE-Naviglio extracts. Significant antifeedant effects against *Leptinotarsa decemlineata* were observed for CSLE and ME. None of the extracts had phytotoxic effect against *Lactuca sativa* germination and radicle growth, but SLDE-Naviglio extracts stimulated the radicule elongation. ME extracts were the most active inhibitors of *Lolium perenne* germination. These results suggest some potential applications of vine-shoot extracts including cosmetics, nutraceuticals or pharmaceuticals linked to their antioxidant effects, or in organic agriculture because of their antifeedant and allelopathic activities.

1. Introduction

Exploitation of certain plant residues as a source of phenols is an attractive option to increase their value due to the importance of natural antioxidants as health-promoting ingredients in functional foods, in the prevention of aging and diseases such as atherosclerosis, diabetes, and inflammatory processes (Fernández de Simon et al., 2010), or as bioactive molecules in cosmetics, pharmaceuticals and nutraceuticals (Billard et al., 2002; Çetin et al., 2011; Devesa-Rey et al., 2011; Karacabey et al., 2013; Macke et al., 2012; Romain et al., 2012; Ruiz-Moreno et al., 2015). More recently, some foliar applications of vegetable extracts rich in phenolic compounds are being assayed as grapevine biostimulants (Pardo-García et al., 2014a,b).

Another interesting aspect of phenolic compounds is their involvement in plant mechanism protection against insects and their role in the feeding behaviour of herbivores, which has been recently reviewed (Lattanzio et al., 2012). For example, specific phenols either individually or in combination, have been associated with defence against insect herbivores in *Quercus oleoides* (Moctezuma et al., 2014). As well, Fornoff and Gross (2014) reported the increase in phenols as one of the induced resistance and defensive traits of *Myriophyllum spicatum* to prevent herbivore damage. Some of the compounds reported as strong insecticides include gallic and ferulic acids (Senthilkumar et al., 2012), luteolin (Golawska and Lukasik, 2012), quercetin and kaempferol derivatives (Huang et al., 2012; Nenaah, 2013), carnosic acid (Santana-Méridas et al., 2014), rosmarinic acid (Sánchez-Vioque et al., 2015) and (+)-catechin (Silva et al., 2013), among others. There are a few studies on the recovery of bioactive compounds with biopecticidal activity from agriculture residues, as for example citrus peels (Did et al., 2011), rice straw (El-Maghraby et al., 2012) or from residues from essential oil extraction (Sánchez-Vioque et al., 2013).

Optimization of food processing based on the reduction of wastes has become a mandatory standard within the most developed countries. The European Union in Directive 2008/98/EC stated that "*waste prevention should be the first priority of waste management, and that re-use and material recycling should be preferred to*

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energy recovery from waste". Accordingly, one of the biggest challenges for wine producing regions is to create alternatives for processing the vast amount of grape wastes generated during the harvest season, among which stands out vine-shoots. Although they are not considered a winery waste, vineyards generate an important vine-shoots quantity: annually yield 1.4–2.0 tons per hectare that are usually left in the field as organic fertilizer (Jiménez Gómez et al., 1993) or burnt (Peralbo-Molina and Luque de Castro, 2013).

The chemical composition of vine-shoots has been widely studied in terms of their phenolic content (Sánchez-Gómez et al., 2014; Vergara et al., 2012), volatiles (Delgado de la Torre et al., 2014; Sánchez-Gómez et al., 2014) or their mineral composition (Çetin et al., 2011; Sánchez-Gómez et al., 2014). All these potential active compounds need to be properly extracted in order to exploit them in new applications. Recently, Sánchez-Gómez et al. (2014) suggested the possibility of using vine-shoot aqueous extracts for other agricultural purposes on the basis of the recovery of bioactive compounds by conventional solid-liquid extraction techniques, as well as assisted extraction by Naviglio principle or microwave. These extraction procedures encourage the use of water as extraction solvent, reducing the energy input in order to meet the requirements of the so-called "green chemistry" and consequently, of sustainable agriculture. Obviously, the method selected for the extraction affects both the yield and the chemical composition of the extract, and hence its bioactivity.

As the vine-shoot extracts biostimulant capacity has been recently investigated (Sánchez-Gómez et al., 2016), it is important to consider their effect on plants growth and their possible effect as biopesticides. For this reason, it is proposed for first time, the study of the antioxidant, antifeedant and phytotoxic effects of different vine-shoot waste aqueous extracts that may be used as future plant biostimulant. Relationships between vine-shoot extracts phenolic composition and the studied effects will be discussed.

2. Materials and methods

2.1. Vine-shoots samples

One hundred kilograms of Airén white *Vitis vinifera* L. vine-shoot wastes from Castilla-La Mancha Spanish region (O.D. La Mancha) were randomly sampled 4 months after the harvest of 2013. Samples were dried at room temperature for other three months until a final humidity of 6.5% ($_{gofwater}$/100 g $_{ofsample}$). Dry vine-shoot wastes were ground by a hammer miller (LARUS Impianti, Skid Sinte 1000, Zamora, Spain) to get a homogenous 40-mesh sieve sampling. Samples were kept at room temperature under vacuum until their use.

2.2. Vine-shoots extraction procedures

2.2.1. Conventional solid-liquid extraction (CSLE)

Fifty grams of ground Airén vine-shoot wastes were extracted with 250 mL of boiling water for 15, 30 and 60 min (CSLE-15, CSLE-30 and CSLE-60) according to Sánchez-Gómez et al. (2014).

2.2.2. Solid-liquid dynamic extraction (SLDE-Naviglio)

A dynamic solid-liquid extraction was carried out using a NAVIGLIO Extractor (FT 110, Armfield, UK) following the Naviglio methodology (Naviglio, 2003). The Naviglio extraction is based on a suction effect generated by a compression of water, used as extracting solvent, on solids at room temperature and pressure of 8 bar, followed by immediate decompression at atmospheric pressure. Two hundred grams of ground vine-shoot wastes were placed inside the extraction chamber and 1.1 L of water was added. The conditions tested for compound extraction consisted of 20 extractive cycles of: 6.5 min (5 min in the static phase and 1.5 min in the dynamic phase; SLDE-Naviglio-6.5), 8 min (5 min in the static phase and 3 min in the dynamic phase; SLDE-Naviglio-8) and 12 min (9 min in the static phase and 3 min in the dynamic phase; SLDE-Naviglio-12) according to Sánchez-Gómez et al. (2014).

2.2.3. Microwave extraction (ME)

Microwave extraction was carried out with a NEOS® apparatus (Milestone, Italy). Fifty grams of ground vine-shoot wastes were placed into the reactor with 250 mL of water at 100 °C. The extraction was performed at 100 °C (600 W) for 5, 10 and 15 min (ME-5, ME-10 and ME-15) according to Sánchez-Gómez et al. (2014). A rotating microwave diffuser ensured homogeneous microwave distribution throughout the plasma-coated polytetrafluoroethylene (PTFE) cavity. The temperature was monitored by an external IR sensor.

All extracts were centrifuged at 4000 rpm for 10 min and filtered through a polyvinylidene-fluoride (PVDF) Durapore filter of 0.45 μm (Millipore, Bedford, USA). Two independent extractions were carried out for each extraction procedure and conditions, and resulting extracts were analyzed twice, n = 2. The extracts were freeze-dried and kept in a desiccator until analysis.

2.3. Chemical characterization

2.3.1. Total reducing power

The total reducing power of samples was determined by the Folin-Ciocalteu method as described by Slinkard and Singleton (1977). Gallic acid was used as standard (Sigma-Aldrich, St. Louis, MO, USA) and the total reducing power was expressed as equivalent grams of gallic acid per 100 g of dry extract. The Folin-Ciocalteu's phenol reagent was supplied by Sigma-Aldrich (St. Louis, MO, USA).

2.3.2. Determination of low molecular weight phenolic compounds (LMWPC) by LC-DAD-MS

The analysis was based on Pardo-García et al. (2014a) and Sánchez-Gómez et al. (2014) methods. Briefly the HPLC grade solvents used were water/formic acid/acetonitrile (97.5:1.5:1 v/v/v) as solvent A and acetonitrile/formic acid/solvent A (78.5:1.5:20 v/v/v) as solvent B. The elution gradient was set up for solvent B as: 0 min, 5%; 2 min, 10%; 4 min, 14%; 9 min, 14%; 12 min, 18.5%; 35 min, 20%; 50 min, 25%; 55 min, 50%; 60 min, 5%; 65 min, 5%. The loop volume was 20 μL.

Identification of phenolic acids, stilbenes and flavanols in the chromatogram was carried out by MS and DAD detectors by comparison with the corresponding mass fragmentation, UV–vis spectra and retention time of the pure standards (Sigma-Aldrich, Steinheim, Germany). Compounds were quantified at different wavelengths: (+)-catechin, (−)-epicatechin, gallic acid and pyrogallol at 280 nm; ellagic and ferulic acids at 256 nm; piceid (*t*-resveratrol-3-glucoside) at 308 nm. The parameters for MM-ESI–MS were: dry gas, N_2, 10 mL/min; drying temperature, 350 °C; vaporiser temperature, 200 °C; nebuliser, 55 psi; capillary, 2000 V (positive and negative ionisation mode); scan range of 100–700 *m*/*z*. Quantification was based on calibration curves of the respective standards at five different concentrations achieved by UV–vis signal (0.70–175 mg L^{-1}) ($R^2 > 0.97$).

2.4. Antioxidant activity

Antioxidant activity was carried out by means of two *in vitro* antioxidant methods.

2.4.1. DPPH radical-scavenging activity

The scavenging activity against the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca et al. (2001). The method is based on the DPPH