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Valorisation of olive agro-industrial by-products as a source of bioactive compounds

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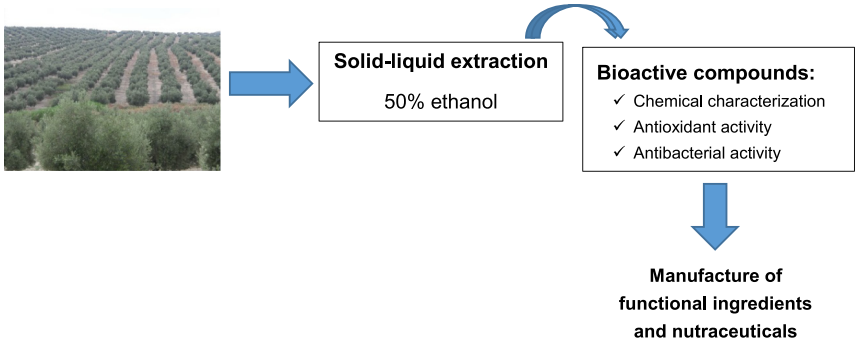
HIGHLIGHTS

- Olive-derived biomass was explored as a potential source of antioxidants and antimicrobials.
- The ethanolic extracts presented a TPC range of 23.85–27.54 mg GAE/g_{dw}.
- The ethanolic extracts presented a TFC range of 52.82–52.39 mg RE/g_{dw}.
- The ethanolic extracts were characterised by Py-GC/MS and UPLC-DAD-ESI-MS.
- The MIC varied from 20 to 40 mg/mL and the MBC ranged from 25 to 45 mg/mL.

GRAPHICAL ABSTRACT

Valorization of olive agro-industrial by-products to obtain bioactive compounds with potential applications in the food industry.



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ABSTRACT

A large amount of olive-derived biomass is generated yearly in Spain, which could be used as a potential source of bioactive compounds. The present work evaluates the recovery of natural antioxidants from olive tree pruning (OTP) and olive mill leaves (OML). For this purpose, the effect of different solvents on the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity was evaluated. The solvent was found to have a significant effect ($p < 0.05$) on the TPC, TFC, and the DPPH, ABTS, and FRAP activity, affording similar results for the extracts from the two by-products. The extracts obtained using 50% ethanol showed high TPC (23.85 and 27.54 mg GAE/g_{dw} for OTP and OML, respectively) and TFC (52.82 and 52.39 mg RE/g_{dw} for OTP and OML, respectively). Also, the OTP and OML extracts exhibited notable antioxidant activity as measured by the ABTS method (45.96 and 42.71 mg TE/g_{dw}, respectively). Using pyrolysis-gas chromatography/mass spectrometry, 30 bioactive compounds were detected in both extracts. Additionally, UPLC-DAD-ESI-MS allowed the identification of 15 compounds in the samples. Furthermore, the antioxidant extracts were found to inhibit the growth of several food pathogenic bacteria. This research demonstrates that these by-products from olive grove farming are a good source of antioxidant compounds with antibacterial properties, which have potential applications in the food and pharmaceutical industries.

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1. Introduction

The comprehensive usage of agro-food by-products to obtain added-value products and energy is an interesting approach from the economic and environmental points of view (Gullón et al., 2018a). Indeed, different biomass sources have been widely investigated for this purpose (López-Linares et al., 2014). The availability, non-food use, and low price are among the most important requirements for raw materials to achieve viable processes. Olive-derived biomass comprises different wastes and by-products from olive tree cultivation and the olive oil industry that meet these requirements (Ruiz et al., 2017a). Among them, residual biomass derived from olive trees includes two types of by-products: olive tree pruning (OTP) and olive mill leaf (OML) biomass. OTP is generated in olive groves when unproductive branches are eliminated, while OML is obtained in olive mills by separating the leaves and thin branches from the olives. These olive residues do not have industrial applications and are usually burnt or just left in the field for fertilisation purposes; however, both practices have associated environmental risks (Ruiz et al., 2017a).

More than ten million hectares of olive trees were cultivated worldwide in 2016 (FAOSTAT, 2018), a quarter of them in Spain, which is the main olive oil producer. The global availability of OTP and OML in Spain has been estimated at more than three million tons, with a strong concentration in specific areas, which is a positive factor for the sustainability of biorefineries (Ruiz et al., 2017a). Some research has been reported on the use of OTP and OML as raw materials in the biorefinery context to produce mainly sugars and bioethanol through different thermochemical pretreatments (Negro et al., 2017). The presence of antioxidant compounds in the liquid fractions issued from OTP pretreatment has also been investigated (Conde et al., 2009). Furthermore, it has been demonstrated that a previous stage of aqueous extraction improves the efficiency of the pretreatment and the fermentability of sugars (Martínez-Patiño et al., 2015). In this context, the extraction of high-value compounds with bioactive properties could improve the viability of biorefineries based on biomass derived from olive trees (Romero-García et al., 2016).

Olive leaves have been widely investigated as a source of bioactive compounds (Rahmanian et al., 2015). The antioxidant activity of phenolic compounds extracted from olive wood has also been reported (Salido et al., 2015). The major compounds identified in these extracts include hydroxytyrosol, tyrosol, caffeic acid, *p*-coumaric acid, vanillic acid, vanillin, oleuropein, luteolin, diosmetin, and rutin, among others (Ghanbari et al., 2012). All these compounds exhibit anti-hypertensive, anti-inflammatory, hypoglycaemic, and hypocholesterolemic properties (Ghanbari et al., 2012). Olive leaf extracts have also been found to exhibit antimicrobial properties against some microorganisms, such as bacteria, fungi, and mycoplasma (Ghanbari et al., 2012).

Such bioactivity drives the use of different olive oil by-products in functional and food applications. In this sense, new applications have focused in enriching the food nutritional profile, replacing or improving the technological properties/functions of food additives, and extending the food product shelf-life (Nunes et al., 2016). However, to the best of our knowledge, scarce literature exists on the extraction and characterisation of bioactive compounds from OTP and OML, the real olive-tree residual biomass available in farms and mills.

The aim of this work was to study the extraction of OTP and OML with different solvents as a first step for the valorisation of this biomass in the biorefinery context. The influence of different extraction solvents on the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of the extracts was evaluated. The correlation between the TPC and antioxidant activity (as determined by DPPH, ABTS, and FRAP methods) was analysed. The extracts exhibiting the best antioxidant characteristics were characterised by ultra-performance liquid chromatography coupled to diode-array detection and mass spectrometry (UPLC-DAD-ESI-MS). Additionally, pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) was employed for the detailed characterisation of those extracts. Finally, the antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria.

2. Materials and methods

2.1. Raw materials

OTP biomass was collected in an olive grove from the province of Jaén (Spain) in February 2016, after the fruit harvest. The OTP sample was taken from branches, which were cut from the tree and then crushed in a commercial chipper. The residue consisted of a mixture of leaves and wood from branches with different thicknesses (0–5 cm), considering that thick wood (>5 cm in diameter) is usually separated in advance for use in domestic fires. The OML sample was collected in January 2016 from the olive cleaning line at the oil mill "SCA Unión Oleícola Cambil", also located in the province of Jaén (Spain). This residue consists mainly of olive leaves mixed with small amounts of fine wood from small branches (<0.5 cm) generated upon hitting the tree during the olive harvest. Both OTP and OML samples were from olive trees of the Picual variety. Once in the laboratory, the OML biomass was washed with tap water to remove any dirt (the remains of olives and soil from the olive groves). Both OTP and OML were air-dried to a constant moisture content (~7%), milled to a particle size of 4 mm, and stored in a dry place until use. Chemical characterisation of the raw materials was performed according to the analytical methodologies of the National Renewable Energy Laboratory (NREL) of the United States. The protein content was determined from the nitrogen content obtained by elemental analysis, applying a conversion factor of 6.25.

2.2. Chemicals

All the chemicals and reagents were of analytical grade. Ethanol, methanol, acetone, gallic acid, rutin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), Folin–Ciocalteu reagent, 2,2′-azino-di(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tri(2-pyridyl)-*S*-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, sodium acetate 3-hydrate, potassium persulfate, acetic acid, hydrochloric acid, iron(III) chloride hexahydrate, and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (Barcelona, Spain). The Müeller–Hinton broth (MHB) and MH agar were purchased from Fluka (Barcelona, Spain).

2.3. Conventional extraction of phenolic compounds

In this investigation, different solvents were assessed for the extraction of antioxidant compounds from OTP and OML, including water, acidified water (acetic acid 0.5%), aqueous ethanol (20% and 50%), aqueous acetone (50%), and 1% NaOH. The antioxidant compound extraction was performed in an orbital shaker (Adolf Kühner AG, Birsfelden, Switzerland) at 55 °C for 90 min at a shaking speed of 120 rpm. These extraction conditions were selected based on preliminary experiments (data not shown) and other related research (Diaz-Reinoso et al., 2012, Mokrani and Madani, 2016; Gullón et al., 2017, Gullón et al., 2018b). In order to obtain a high concentration of antioxidant compounds, the solid/liquid ratio was fixed at 1:6 (w/v). At the end of the process, the extracts were recovered by filtration and the supernatants were filtered through a 0.22 μm membrane. The extracts were then stored at −20 °C until analysis. All extractions were conducted in triplicate.

2.4. Chemical characterisation of the extracts

2.4.1. Determination of total phenolic and flavonoid contents

The TPC was determined by the Folin–Ciocalteu assay (Singleton and Rossi, 1965) and the TFC analysis was performed according to Blasa et al.