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SECONDARY METABOLITES OF APRICOT TREES (*PRUNUS ARMENIACA* L.) PRUNING: POSSIBLE SUSTAINABLE USE IN THE INDUSTRIAL SECTORS

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Abstract:
Orchards are typical Mediterranean crops and a major feature of the heritage in the Mediterranean basin, where they play an important environmental and economic role. Orchards' biomass byproduct use, such as apricot tree wood, mainly from pruning, could improve the economic and environmental aspects of the biomass disposing. To achieve this objective, the main anti-oxidative properties and the chemical compounds of the extractives coming from apricot orchards biomass were analyzed. Furthermore, the influences of four different extraction techniques in combination with different solvents have been measured. Results demonstrated the potential antioxidant activity of the bark and wood extracts of apricot trees biomass. In addition, the chemical characterization by LC-MS showed the presence of different natural compounds. Therefore, the development of innovative applications that use the biomass derivatives, could lead to possible uses of these in the market as a commodity for the chemical, pharmaceutical or cosmetic industries, giving new added values to current use of biomass from agricultural practice. Our research was focused: i) on the recognition and mapping of the orchard cultivation of the Basilicata Region; ii) on the identification of their extractives traits; iii) on the definition of the appropriate extraction techniques.

INTRODUCTION

The EU policy after the Paris agreement, have as objective within the 2020, to reduce the waste and improve the model of *circular economy* (an economic system designed to regenerate on its own). In The EU the orchard cover an area equal to 5,994,564.87 hectares (Eurostat 2012). Of these hectares, 67,592.98 ha are cultivated with Apricot Trees (*Prunus Persica* L.). Especially in country as Italy and Spain the Apricot orchards have an agricultural area of 25% and 30% on the total area of the European orchards. In the last years, to due the price very low of the apricot fruits, many farmers have been forced, especially in Italy, to extirpate these trees. Over this, the pruning of these trees produce a huge quantitative of woody material. These wood material and the trees extirpated have an only one way to be eliminated: to be burn it. So, to avoid waste of natural resources, it was thought to analysed the woody material of apricot to investigated the secondary metabolites. Secondary metabolites are chemical compounds produced by several plant tissues (e.g. leaves, bark, roots, buds, wood) that provide different medicinal applications, including antioxidant, anticancer, anti-inflammatory, antifungal and other properties (Todaro et al. 2017). The multitude of secondary metabolite is utilised by human kind to improve their health (antibiotics, enzyme inhibitors, immunomodulators, antitumor agents, et al.) (Thirumurugan et al. 2018). On the other hand, the woody material of the orchards is poorly investigated. The only studies about orchards are mainly concentred on the evaluation of the fruits juices, part of the fruits (peel, seeds etc.) and sometimes on the leaves but, rarely on the physical and chemical proprieties from the wood. Last studies about the apricot wood have investigated the technical characteristics for use the apricot wood, rich of fibrous, noting in the apricot wood a good material for industrial paper production (Tajik et al. 2015).

OBJECTIVE

To achieve this objective, the main anti-oxidative properties and the chemical compounds of the extractives coming from biomass of apricot were analyzed. Results demonstrated the potential antioxidant activity of the extracts of bark and wood of apricot trees. In addition, the chemical characterization by LC-MS showed the presence of different natural compounds like flavonoids. Trough these studies it was understood that the derivatives of the biomass examined, contains functional chemical compounds that can be added to products for cosmetics, for feed and agriculture to raise their quality in a perspective to "zero waste".

MATERIAL AND METHODS

The analyse it was started by the choose of pruning material that came from a Mediterranean Italian region (Basilicata). Aliquots of similarly sized particles of each sample were prepared and subjected to four different solid/liquid extraction techniques: maceration extraction (ME), ultrasound assisted extraction (UAE), Accelerated Solvent Extraction (ASE) and Autoclave (AT). The solvent used for the ME, UAe and Ase was etanol/ water 70:30, while for the Autoclave the solvent utilized was water. For all the extraction procedures (ME, UAE, ASE, AT) the extraction process was repeated three times. All extracts were filtered through a paper filter. Solvent was removed with a rotary evaporator at 37°C. All the extracts was freeze-dried for 48 h. Dried extracts were kept in the dark at room temperature until their use (Fig.1). Total polyphenol (TPC) were evaluated. The antioxidant activities of all samples were investigated using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). Chemicals compounds of the extractives were analysed by means of Ultra High Performance Liquid Cromatography (U-HPLC).

Fig. 1.
From left to right, show the entire process of the extraction from the raw material of apricot branches bark and wood until to the dried extractive.

RESULTS AND DISCUSSIONS

Yield Extracts

In the Fig. 2 the extraction yields of wood and bark obtained with different extraction techniques (ME, UAE, ASE, AT) is reported. The bark ASE showed the higher extraction yields (13.5%). All bark extractives have given a greater extraction yield compared to the wood extractives. These data are in accordance with a previous study (Klarić et al. 2016) showing that the bark contains more extractives than the wood as consequence of its main biological functions to protect tree's essential living systems from extreme temperatures as well as from attacks from fungi, insects and animals explaining its high extractives contents.

Extractions performed with the ASE lead to the higher extractives yields independently of the nature of the substrate used (wood or bark). As reported by Dai and Mumper (2010), the high temperature would improve the viscosities and the surface tensions enhancing the capacity of the solvent to penetrate in the matrices increasing the extraction yield. Whereas, lower extractive yields were extracted in wood and bark using autoclave extraction (AT). Despite the high temperature involved in this extraction technique, the utilization of water alone as solvent may explain this lower extraction yield. Horvath, (2005) explained that the mixture of solvent have a mayor extractive capacity than a pure solvent alone.

Total Polyphenol Content (TPC)

The highest quantity of total polyphenols was measured in the apricot bark ASE (274.5 ± 14.2 mgGAE/g), while the lowest TPC was recorded in the apricot wood extracted with ultrasound extraction (153.2 ± 4.88 mgGAE/g) (Fig. 3). These results demonstrated that the extractions with ASE have a major quantitative of polyphenol. It has been previously demonstrated as polyphenols are more soluble in methanol and ethanol than in water, and our results are congruent with previous data (Dai and Mumper 2010; Horvath 2005). Moreover, the quantity of the phenolic compounds is influenced by the extraction time and temperature (Klarić et al. 2016), but if the solubilisation can be improved, the degradation due to the oxidation and hydrolysis can be accelerated, and *vice versa* (Robards 2003). However, as reported by Sulaiman et al. (2015), the nitrogen gas in the ASE can reduce the oxidation of the compound at high temperature. According Tuyen et al. (2017), TPC and antioxidant activity are positively correlated.

DPPH radical-scavenging assay

In all apricot tree wood and bark samples, extractives were analysed for their antioxidant capacity through three different tests as DPPH and FRAP. DPPH is a purple colour stable free radical that in presence of antioxidant compound it is decolorated. The DPPH scavenging activity (Fig. 4) showed the higher value was obtained for the bark extracted with ME (5440.2 ± 185.6 mgTE/g). The lower value is measured in the in wood ASE extracts (1597.5 ± 77.6 mgTE/g).

FRAP Ferric reducing antioxidant power

The antioxidant capacity measured by means of FRAP is valued on the base on reducing ferric ion, where antioxidants are the reducing agent. Antioxidants are molecules able to donating a single electron or hydrogen atom for reduction. For FRAP assay (Fig. 5), the highest antioxidant values were obtained for apricot bark ME 1212.6 ± 114.0 mgTE/g, while the lowest values were for apricot wood AT antioxidant values 302.8 ± 27.7 mgTE/g.

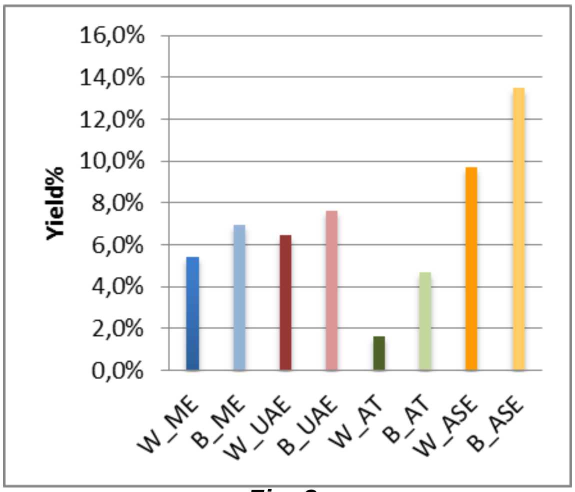

Fig. 2.
Yield (%) of wood (W) and bark (B) of apricot trees extractives obtained by using various extraction techniques. Where: maceration extraction (ME), ultrasound assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclave (AT).

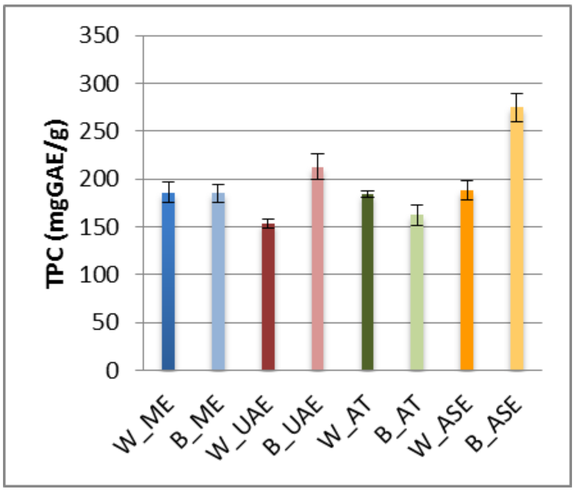

Fig. 3.
Total polyphenolic content (TPC) of wood (W) and bark (B) of apricot trees. Where: maceration extraction (ME), ultrasound assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclave.

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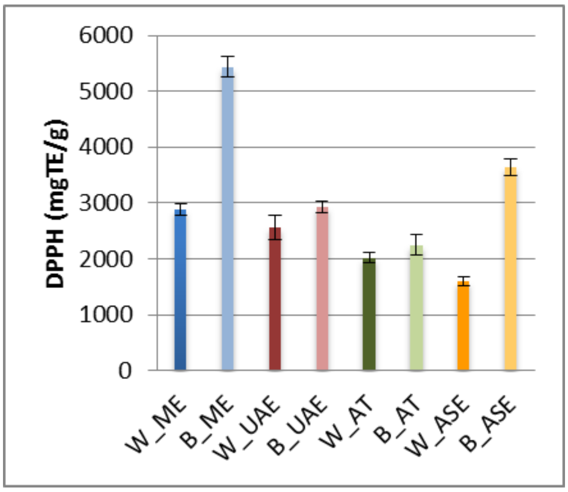

Fig. 4.
DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of wood (W) and bark (B) of apricot trees extractives obtained by using various extraction techniques. Where: maceration extraction (ME), ultrasound assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclave (AT).

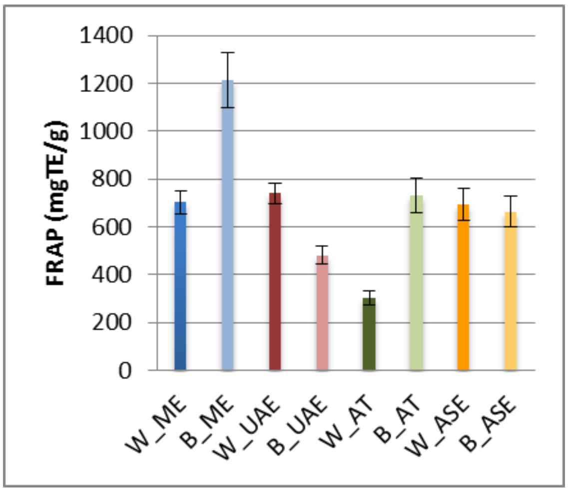

Fig. 5.
FRAP (Ferric reducing antioxidant power) of wood (W) and bark (B) of apricot trees extractives obtained by using various extraction techniques. Where: maceration extraction (ME), ultrasound assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclave (AT).

LC-MS Analysis

The LC-MS analysis is still in progress but the first datas show that especially inside the apricot bark extractives there are some flavonoids compounds such as catechine, epicatechine, naringenin and phlorentin. Flavonoids have a different functions in regulating plant development, pigmentation, and UV protection, to an array of roles in defence and signalling between plants and microorganisms (Mathesius 2018). In also, them have a widely healthy effects. One of these is the antioxidant activity that prevent the risk of age-related vascular disease development (Da Pozzo et al. 2018). In add, these compounds have applications in food stabilization due to their ability to protect against peroxidation of oxygen sensitive foods (Benavente-García et al. 1997). The identification and quantification of the compounds present in the apricot wood and bark extractives goes on due to permit understanding the possible uses of this molecules in the industrial sectors.

CONCLUSION

The aim of this study has been to analyse the wood and bark extractives of apricot orchards tree pruning to find a use sustainable of this waste material, in according with the EU waste policy. From data is emerged that the bark have a higher quantitative of total phenolic compounds and antioxidant activity than the wood. This is confirmed in all extraction techniques (ME, UAE, ASE, AT), at the same extraction condition. Between all samples, bark ASE has the major quantity of phenolic compounds due to probably, the mixture of solvent MeOH/H2O (70:30 v/v) and temperature (100°C). The datas coming from LC-MS analysis demonstrate that the extractivescontains interesting flavonoids. Flavonoids are secondary metabolites product from plant that have several properties, one on all a great antioxidant activity. The analyses and test affected on the pruning residue from apricot orchards have demonstrated that there is the possibility to use a waste product in the industrial sectors.

The results obtained are encouraging and lead us to continue the study of these materials, to better understand the compounds present inside the pruning biomass of apricot orchards and their possibly uses.

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