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Chemical analysis and antioxidant properties of orange tree (*Citrus sinensis* L.) biomass extracts obtained via different extraction techniques

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Keywords: Orange tree; bark; wood; extractives; maceration; ultrasound-assisted extraction; accelerated solvent extraction; autoclaving.

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. The high availability, low price and potential industrial application make the development of new and valuable uses of the orchards' biomass of high interest. The research was focused: *i*) on recognition and mapping of the orchard cultivation in the Basilicata Region; *ii*) on evaluation of the antioxidant capacity of extracts; *iii*) on identification of extractives' traits. To achieve these objectives, the anti-oxidative properties and chemical compounds of the extractives from orange orchard biomass were analysed. Different extraction techniques were applied, including maceration, ultrasound-assisted extraction, accelerated solvent extraction and autoclaving.

Results demonstrated the potential antioxidant activity of the bark and wood of orange tree biomass, not investigated before. Relative Antioxidant Capacity Index showed that bark extracts were more effective than wood ones, and accelerated solvent extraction was the most effective process for recovering antioxidants.

The chemical characterization by LC–MS showed the presence of different natural compounds, including caffeic acid, alkaloids and flavonoids. Therefore, the development of innovative applications that use the biomass derivatives could lead to their possible use in the market as a commodity for the chemical or cosmetic industries, giving new added value to the current use of biomass from agricultural practice. Through multi-criteria analysis, it was possible to recognize the sustainability of these cropping models and their ecological function, turning into preservation of environmental resources, environmental quality and quality of life.

1 INTRODUCTION

In the European Union (EU-27), 5,994,564.87 ha of orchards are cultivated.[1]Italy has the second largest area of orchard cultivation after Spain.[1]Among Italian orchards, the orange tree (*Citrus sinensis*L.) is one of the most representative crops, with 76,042.20ha.

Basilicata Region, located in Southern Italy, has 833,847.00 ha of agricultural cultivation. Orchards occupy 50,281.00 ha and 12% of this area is occupied by orange orchards.[2]

This cultivation needs accurate management of trees, including pruning at least once per year. The life cycle of these orchards ranges from 16 years for intensive cultivations to more than 40 years for extensive cultivations, but when trees became unproductive the farmers pull them out. The pruning and explants produce a huge quantity of biomass. The pruning biomass of orange trees is estimated to be about 1.8 $tha^{-1}year^{-1}$.[3]At the moment, the main strategy to manage the pruning biomass is to use it for fuel. Sometimes, the pruning biomass is chopped and left on the soil to improve the organic matter[4] or used for energy purposes.[5]The EU waste policy for the next few years has an objective to prevent waste and, when the waste cannot be avoided, it should if possible be re-used to obtain a green and circular economy. Thus, in agreement with the EU waste policy, it is necessary to find another way to manage the pruning biomass with a low environmental impact. Recently, more studies have been carried out on use of the wood and bark mainly coming from forests, in order to use the woody biomass and fibre for raw materials and buildings.[6–8]Furthermore, other studies on woody forest material have analysed the secondary metabolites present in the wood and bark, their extraction using different techniques[9]and their possible use in different industrial sectors, especially in chemistry.[10,11]Secondary metabolites are chemical compounds present in plant tissues (e.g. leaves, bark, roots, buds, wood) that provide different medicinal applications, including antioxidant, anticancer, anti-inflammatory, antifungal and other properties.[9]

Different methods exist to separate these fractions. Some of them are patented: US3032188A – Method of separating wood chips from bark chips – and US3826433A – Process for removing bark from wood chips. Others are available as patented but free: United StatesPatent 5577671 – Method for manufacturing low bark content wood chips from whole-tree chips.

Tree bark is a still largely underutilized side stream of the pulp and paper industries and wood works. In the production of wood pulp, for example, the felled timber is trimmed and cut to specific lengths, and its bark is then removed, either mechanically (depending on the size and shape of the

trunk) or by use of water jets. The separated bark is then dewatered and used on site as a fuel while some of it goes into horticultural use. In the case of pulp production, as bark contains cellulose (less than wood) and a large variety of phenolics and sometimes sands, it requires close-to-complete removal prior to pulping and/or extraction. Obviously, bark could be also chemically utilized at least to some extent. However, the combustion of bark represents typical integrated production of bioenergy since it is normally burned in the on-site bark-burning furnace. On the other hand, the high share of extractives in bark would constitute a potential source of interesting and valuable compounds to be used in segments such as pharmacy and cosmetics, with a higher added value. However, such utilization is impeded by the need to extract these compounds from the bark matrix, which requires sometimes the use of organic solvents. Both extraction and separation are anyway technically possible.

In this respect, our study had the scope to deepen and increase the knowledge of the chemical difference between bark and wood in the case of orange trees. One of the main scopes of our research is to demonstrate also the relevance of bark as a source of secondary metabolites.

On the other hand, the woody material from orchards is poorly investigated. The only studies about orchards are mainly concentrated on evaluation of the fruit juices, parts of the fruits (peel, seeds, etc.) and sometimes on the leaves but rarely on the physical and chemical proprieties of the wood. Recent studies about orange wood have investigated its technical characteristics for use as wood flooring,[12]assessingit as a good material for manufacturing a high-quality product.

The few available studies on extractives from orange woody sources analysed just some aspects: the possible use of orange wood extractives in the medical sector, especially for diabetic treatment,[13]the potentialities of essential oils extracted from orange tree branches and their antibacterial activity[14], and their analysis by GC–MS technique.[15]

The aim of this study is to deepen and increase the knowledge of the secondary metabolites from pruning biomass of orange trees, from both the bark and the wood particularly to determine: *i*) the yield of extractives obtained with different extraction techniques, *ii*) their phenolic content, *iii*) their

antioxidant capacity through different assay sand, finally, *iv*) their chemical composition through chromatographic analysis and liquid chromatography.

2 MATERIAL AND METHODS

2.1 Wood and bark extractives

Orange tree (*Citrus sinensis* L. var. Navelina) pruning was done in May 2018 in an orchard in the Metaponto area (Basilicata Region).Orange wood samples were collected and randomly selected then separated into bark and wood and milled to powder through a 40-mesh sieve in amilling machine (Retsch GmbH, Germany). Drive power of 1.5 kW and rotor speed of 1500 min^{-1} guaranteed a rapid size reduction. The milled wood for both types of sample, bark and wood, was used for four different extraction techniques: maceration extraction (ME), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclaving (AT). As previously reported[7]for all extraction techniques, 10 g of small pieces of orange tree bark and wood was extracted using an ethanol:water mixture (70:30 v/v) as solvent, except for AT for which only water was used, because it is not possible to use other types of solvent. Three replicates were done for each extraction.

ME was carried out at room temperature by stirring the sample for 1 h in solvent at a sample-to-solvent ratio of 1:5 (w/v), whereas UAE was carried out using an ultrasonic bath (Branson 1800) under the same conditions used fo rME. Extraction using an ASE system (ASE 150,DionexCorporation, Sunnyvale, CA)was carried out at 100°C at 1500 psi for three cycles of 5 min each. AT extracts of bark and wood were obtained using a VaporMatic 770 sterilization autoclave, following the autoclave cycle:121 °C, 1 atm, 20 min. After the extractions, all extractive solutions were filtered and ethanol was removed under vacuum with a rotary evaporator at 37 °C. After removal of ethanol, samples were freeze-dried(HetoDrywinnerDW3/RV12, Edwards High Vacuum International, Crawley, UK) for 56 h, at −48°C and 0.580 Pa, and then kept in the dark at room temperature. Extraction yields were calculated according to the following formula:

$$\% = \frac{\text{dried extracts (g)}}{\text{milled wood (g)}} \times 100$$

2.2 Total polyphenolic content (TPC)

The TPC was determined by the Folin–Ciocalteu reagent method.[16,17] An aliquot of extract (75 μL) was mixed with 500 μL of Folin–Ciocalteu reagent and 500 μL of Na_2CO_3 solution (10×) and, finally, water was added to reach a final volume of 1.5 mL. After incubation for 1 h in the dark at room temperature, the absorbance of the mixture was read at 723 nm. The TPC of the extracts was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract ± standard deviation (SD).

2.3 Antioxidant activity

2.3.1 Radical scavenging activity

Radical scavenging ability was measured using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH);200 μL of 100 μM DPPH methanol solution was added to 50 μL of extract at different concentrations in 96-well plates and kept in the dark for 30 min. Absorbance at 515 nm was recorded. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity.[18,19] The radical scavenging activity of each sample was expressed as milligrams of Trolox equivalents (TE) per gram of dried sample ± SD.

2.3.2 Ferric reducing antioxidant power (FRAP)

The FRAP method is based on the reduction of Fe^{3+} to Fe^{2+} by the action of electron-donating antioxidants. At low pH, in the presence of TPTZ, ferric–tripyridyltriazine (Fe^{3+}–TPTZ) complex is reduced to the ferrous form (Fe^{2+}–TPTZ). The reduction is monitored by measuring the change of absorbance at 593 nm. Increased absorbance of the reaction mixture indicates an increase of

reduction capability.[18,20,21]As reported by Todaro *et al.*,[7] FRAP reagent was prepared daily with 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride and 10 mM TPTZ (in 40 mM hydrochloric acid) at a ratio of 10:1:1. FRAP reagent (225 µL) was added to 25 µL of extract or methanol (for the blank) in a 96-well plate and incubated at 37 °C for 40 min. Trolox was used as standard. Results were expressed as milligrams of TE per gram of dried extract ± SD.

2.3.3 β-Carotene bleaching assay (BCB)

The inhibition of lipid peroxidation of wood extracts was assayed by the BCB method.[22]Butylated hydroxytoluene (BHT) was used as a positive control of the reaction. *β*-Carotene (0.20 mg in 0.20 mL chloroform), linoleic acid (20 mg) and Tween 20 (200 mg) were transferred into a round flask. Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary evaporator, and 50 mL of distilled water was added. Extract (50 µL) at an initial concentration of2 $mgmL^{-1}$ was added to 950 µL of *β*-carotene emulsion, and then the solution (250 µL) was transferred into a 96-well plate. The absorbance was read every 30 min (0, 30, 60, 90, 120, 150 and 180 min) at 470 nm. Inhibition of lipid peroxidation was expressed as a percentage of antioxidant activity (% AA) ± SD using the following formula:

$$\%AA = \left[1 - \left(\frac{\text{A sample T0}' - \text{A sample T180}'}{\text{A blank T0}' - \text{A blank T180}'}\right)\right] \times 100$$

2.4 Determination of Relative Antioxidant Capacity Index (RACI)

According to Sun and Tanumihardjo[23]and Russo *et al.*,[24] RACI is determined by integrating the antioxidant capacity values generated from different *in vitro* methods and allows a better comprehensive comparison. In RACI, the standard score was calculated using the following formula:

$$(x - \mu)/\sigma$$

where x is the raw data, μ is the mean, and σ is the SD. Standard scores have a mean of 0 and an SD equal to 1.[25]

2.5 LC–MS analysis

U-HPLC analysis of extracts was carried out using Shimadzu LC–MS-8030 apparatus equipped with anSPDM20A diode array detector. The separation was carried out in thermostatic conditions at 40 °C with a reversed-phase column (Phenomenex® Luna 3μm C18). Elution was carried out with a binary solvent system consisting of water with 0.1% formic acid and acetonitrile with 0.1% formic acid, running at the flow rate of 0.4 $mLmin^{-1}$. The injection volume was fixed at 1.0μL. Detection was carried out with a UV detector set at a wavelength of 280nm and under selected ion monitoring by negative- and positive-mode ESI-MS. The operating parameters for MSdetection were as follows: nebulizing gas (N_2) flow 3.0 $Lmin^{-1}$, dryinggasflow15 $Lmin^{-1}$, interface voltage 4.5 kV, gas pressure 230 kPa,DL temperature 250 °C, block heater temperature 400 °C.

2.6 Statistical analysis

Results are expressed as the mean ± SD of three independent experiments performed in triplicate. Principal component analysis (PCA), an unsupervised multivariate statistical tool that analyses data sets consisting of a large number of variables, was also used. It is able to develop a new and easier model with a smaller number of artificial variables that accounts for most of the variance in the normalized data set. To verify the correlations among antioxidant methods and chemical compounds, the Pearson correlation coefficient was determined. The relationship between compounds (present in at least three samples) and antioxidant activity results obtained from each test were considered. PCA and Pearson coefficients were computed using the R statistical software environment (http://www.r-project.org).

3 RESULTS AND DISCUSSION

3.1 Extraction yield

The extraction yield of pruning residue from *C.sinensis* bark and wood (Fig. 1) showed that extraction efficiency increased in the following order: ASE>UAE>ME>AT for wood and ASE>ME>UAE>AT for bark. Extractions performed with ASE led to the highest extraction yields (7.10% for wood and 12.50% for bark) independent of the nature of the plant matrix, whereas AT showed the lowest values, 2.80%for wood and 4.60% for bark.

As reported by Dai and Mumper,[26] high temperature improves the viscosity and surface tension, enhancing the capacity of the solvent to penetrate the matrices and increasing the extraction yield, but the solvent mixture also affects the recovery of compounds. Despite the high temperature involved in the AT technique, the utilization of water as unique solvent could explain this lower extraction yield. A previous study[9]reported that the mixture of water and ethanol is more appropriate for extracting chemical compounds from plant materials with biological activity and, according to Horvath,[27]the mixture of solvents has a higher extraction capacity than the pure solvent.

All extraction techniques showed greater yield from bark than wood, in accordance with a previous study,[28]showing that the bark contains more extractives than the wood as a consequence of its main biological functions to protect the tree's essential living systems from extreme temperatures as well as from attacks from fungi, insects and animals, explaining its high extractive content.

3.2 Total polyphenol content (TPC)

The effectiveness of different extraction techniques was evaluated in terms of TPC and antioxidant activity. As reported in Figure 2, higher TPC was found for bark extracts than for wood. TPC ranged from 79.42 ± 1.43to 57.03 ± 1.09 mgGAEg^{-1}in bark extracts and from 50.49 ± 3.45to

35.95 ± 0.41 mg GAE g^{-1}in wood. ASE was the extraction technique with the highest TPC, followed by ME, UAE and finally AT.

These results are comparable with the extraction yields, and the solvent probably affected the extraction. It has been previously demonstrated that polyphenols are more soluble in methanol and ethanol than in water, and our results are congruent with previous data.[26]Moreover,the amount of phenolic compounds is influenced by the extraction time and temperature,[28] but if the solubilization can be improved, degradation due to oxidation and hydrolysis can be accelerated and *vice versa*.[29]However, as reported by Sulaiman*et al.*,[30]the nitrogen gas in ASE can reduce oxidation of the compounds at high temperature.

3.3 Antioxidant activity

As reported by Tuyen*et al.*,[31] polyphenol compounds possess antioxidant activity. All orange tree wood and bark extractives were analysed for their antioxidant capacity using three different tests – DPPH, FRAP and BCB – to measure their radical scavenging activity, reducing power and lipid peroxidation inhibition, respectively.

According to TPC values, higher radical scavenging activity and reducing power was found for bark extracts than for wood. DPPH scavenging activity ranged from 60.51 ± 2.12 $mgTEg^{-1}$ (B_ASE) to 22.07 ± 1.93 $mgTEg^{-1}$ (W_ME), whereas reducing power varied from 181.88 ± 10.66 $mgTEg^{-1}$ (B_UAE) to 61.69 ± 5.62 $mgTEg^{-1}$ (W_ME). No significant differences were observed for bark extracts obtained by ASE, UAE and ME, which showed similar values (Figs. 3 and 4). Wood extract derived from AT extraction had higher DPPH scavenging activity (35.35 ± 3.60 $mgTEg^{-1}$) and reducing power (87.31± 6.63 $mgTEg^{-1}$) than other wood extractives(Figs. 3 and 4).

The BCB assay is a common test used for the evaluation of lipid peroxidation. It is a colorimetric method based on disappearance of the yellow colour of *β*-carotene due to its reaction with radicals generated by linoleic acid oxidation in an emulsion.[31]According to Da Pozzo *et al.*,[32] the presence of antioxidants minimizes the oxidation of *β*-carotene.

Unlike the results for reducing power and DPPH scavenging activity, similar or higher inhibition of lipid peroxidation was observed for wood extracts than for bark extracts, for all extraction techniques except for AT.

Generally, the different behaviour of extracts in the BCB assay is due to the phenomenon called the 'polar paradox'.[33–35]The polar paradox states that polar antioxidants are more effective in less polar media (bulk oil) than nonpolar ones, whereas nonpolar antioxidants are more effective in relatively more polar media (oil-in-water emulsions or liposomes) than their polar counterparts. However, the greatest inhibition of lipid peroxidation was found for bark and wood extracts obtained using the ASE technique, 70.52 ± 0.81% and 70.26 ± 1.53% AA, respectively (Fig. 5), followed by W_UAE and W_ME extracts.

It is interesting to note that B_AT also showed greater inhibition of lipid peroxidation than W_AT, contrary to that observed for the other extraction techniques. Recent evidence shows that not all antioxidants behave in a manner proposed by this hypothesis in oil and emulsion, suggesting that antioxidant effectiveness depends also on several factors not exhaustively known nor controlled.[37,38]

3.4 Relative Antioxidant Capacity Index (RACI)

The RACI is a hypothetical concept[23] to evaluate the relative antioxidant capacity of diverse extracts. In this study, results obtained from different antioxidant tests (DPPH, FRAP and BCB methods) along with TPC were used for RACI calculation. According to previous data, the RACI ranking(Fig.6)showed that bark extracts had a higher RACI than wood extracts, and the ASE technique had the highest value (1.11) followed by ME (0.80) and UAE (0.44). The lowest RACI(−0.95) was observed for W_AT.

3.5 Statistical analysis

3.5.1 Pearson coefficient

To evaluate the correlation among TPC and the antioxidant assays, Pearson values were calculated among the mean of each variable (Table 1). The outcomes show that there is a positive correlation between all methods and TPC, but the strongest correlation was found to be between TPC and FRAP (r=0.95) and radical scavenging activity (r=0.94). A low correlation between TPC and BCB could be explainedby BCB involving not only phenolic compounds but also lipophilic compounds, as mentioned previously.

3.5.2 Principal component analysis (PCA)

PCA was carried out on the data set after standardization of the antioxidant assays and TPC, and on the different extracts from the orange tree wood and bark. PCA (Fig.7a and 7b) explained 99.23% of the data set's total variance. The first component (PC1) explained 73.38%of the total variance in the data set while PC2 explained 25.85%.Figures 7a and 7b explain the relationships of antioxidant assays and TPC with the samples. In Figure7a,theresults indicate high positive antioxidant activity from B_ASE and B_ME, and negative antioxidant activity from W_AT. Furthermore, all the bark samples are located on the right while the wood samples are located on the left; this shows the significant difference between wood and bark. B_AT is situated in the middle of the PC1 zero point, quite a distance from the other samples, indicating that this sample is significantly different from the others. The location of B_ASE in the top right of PC1 can be explained by its high TPC value. In contrast, the wood samples are located in the left quadrant, demonstrating less TPC and at the same time better results in the BCB test, except for W_AT, due to the polar paradox explained in section 3.3. In Figure7b, DPPH and FRAP are overlapping, closer to TPC, and on the right side of the plot, on the opposite side to BCB. This means that DPPH and FRAP are significantly correlated with TPC as the Pearson correlation has shown, whereas BCB is not correlated with the other antioxidant tests or TPC.

3.6 LC–MS analysis

In the LC–MS analysis (Table 2), caffeic acid was detected in all samples. As reported by Magnani*et al.*,[39]caffeic acid is representative of cinnamic acid derivatives, also called phenylpropanoids. Caffeic acid (3,4-dihydroxycinnamic acid) is one of the hydroxycinnamate and phenylpropanoid metabolites more widely distributed in plant tissues. Hydroxycinnamic acid is the major subgroup of phenolic compounds.[40]The properties of this compound have been widely investigated and it is known to act as a carcinogenic inhibitor, to possess antioxidant and antibacterial activity in vitro, and to contribute to the prevention of atherosclerosis and other cardiovascular diseases.[39]

Flavonoids and alkaloids were detected, as was caffeic acid, in wood and bark samples. Flavonoids have different functions, from regulating plant development, pigmentation and UV protection, to an array of roles in defence and signalling between plants and microorganisms.[41]Flavonoids also present numerous healthy effects. One of these is the antioxidant activity that prevents the risk of developing age-related vascular disease.[32]These compounds have applications in food stabilization due to their ability to protect against peroxidation of oxygen-sensitive foods.[42]Alkaloids were present in all bark extracts, especially B_UAE, while of the wood extracts they were present only in W_UAE. The known functions of alkaloids are related to protection and to regulation of plant growth.[43]Alkaloids are used in pharmacology as analgesics, antispasmodics and bactericidals; in particular, alkaloids have effects on the nervous system.[44]Also, several potent anticancer drugs have been developed from plant alkaloids.[45]Despite careful and deep bibliographic investigations, it was difficult to identify the nature of several detected peaks. Particularly, the unknown at 1.27 min which has a very important percentage area of ESI(-) TIC, it didn't exhibit any UV absorbance and, as shown by its Retention Time, seemed to be very polar, thus its structure was probably a disaccharide with M-H =341 Da (2 hexoses 2x 180 Da - 1 equivalent of water 18 Da = 342 Da). Future researches will effort to identify these unknown peaks.

As reported in the Introduction, the importance of orchard cultivation is reflected in the number of hectares present in Basilicata Region (50,281.00). Identifying valuable alternative uses for biomass, which nowadays is mainly burned, represents for both researchers and landowners a challenge and an opportunity. As stated by Moncada and Aristizábal,[46]biorefinery processes can draw benefits from the different biomass components (extractives holocellulose and lignin) and maximize the value derived from the raw material.

The advantages can be achieved mainly by implementing and developing integrated biorefinery processes of course taking into account the size of company and the relative economic possibilities. In this respect, the creation of an association of producers might facilitate the entire process system. One of the fundamental characteristics of a producer group is integration of the enterprises in a district to maximize their business efficiency and competitive ability. Through this strategy, both greater profitability and environmental sustainability can be achieved.

The scale of a biorefinery is a crucial point because its dimension is related to the final products, from a small scale in the case of added-value products (antioxidants) to a large scale in the case of bioenergy or food products (i.e. sugar).[47]In fact, biorefineries are very sensitive to the production scale.[46,48,49]

Moncada and Aristizábal[46]reported several case studies and overall suggested that the decision of the scale dimension should be analysed for every biorefinery case during the preliminary design stages because several parameters should be taken into account: raw material, degree of development of the area, etc.

4 CONCLUSIONS

The objective of this study was to analyse the wood and bark extractives from orange orchard tree pruning residue to find a sustainable use for this waste material, in accordance with the EU waste policy. From the data, it has emerged that the bark has higher TPC and antioxidant activity than the wood. This is confirmed for all extraction techniques (ME, UAE, ASE, AT), under the same

extraction conditions. Of all samples, bark submitted to ASE has the highest TPC and antioxidant activity, probably due to the mixture of solvent (MeOH/H_2O 70:30 v/v) and temperature (100 °C). In the LC–MS analyses, important natural compounds such as caffeic acid, flavonoids and alkaloids were detected in all samples, even that with quantitative difference. Caffeic acid is present in many food sources and several medications in popular use, mainly based on propolis.[39]This confirmed the potential of caffeic acid for use in cosmetics and pharmaceuticals. While flavonoids are plant secondary metabolites that have several properties, the most important is great antioxidant activity. Alkaloids have important biological properties and practical applications in human health. The analyses and tests conducted on the pruning residue from orange orchards have demonstrated that the waste product could be used in industrial sectors such as nutraceutical, chemical, pharmacy and cosmetic sectors. The results obtained are encouraging and lead us to continue the study of these materials, to better understand the compounds present in the pruning biomass from orange orchards and their possible uses.

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Table 1. Pearson coefficient calculated between total polyphenolic content(TPC), DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric reducing antioxidant power(FRAP) and inhibition of lipid peroxidation (BCB).

	TPC
DPPH	**0.940**
FRAP	**0.948**
BCB	**0.076**

Table 2. Relative area percentage of orange wood (W) and bark (B) extractives by LC–MS in total ion chromatogram in negative mode (TIC^-).

Bark						
Ret. Time	***m/z***	**Product**	**Area (%)**			
			ME[a]	**UAE[b]**	**ASE[c]**	**AT[d]**
1.20	180	Caffeicacid	18.90	19.35	14.32	17.57
1.27	341	Unknown	23.48	21.60	18.09	18.983
3.64	264	Glycosylated compounds	1.86	1.90	2.06	0.61
4.47	594	Flavonoids	4.21	7.42	2.52	5.33
4.60	548	Glycosylated compound A	0.93	2.13	0.70	n.d.
4.71	386	Compound A without sugar	2.02	4.20	1.74	4.80
5.63	501	Alkaloid	2.91	5.04	3.30	5.33
6.30	728	Unknown	1.63	1.81	1.72	5.33
9.30	323	Alkaloid	7.04	11.43	9.16	1.36

Wood						
Ret. Time	***m/z***	**Product**	**Area (%)**			
			ME	**UAE**	**ASE**	**AT**
1.40	180	Caffeicacid	54.41	57.31	69.72	36.34
1.80	192	Glycosylated compounds	10.78	11.22	15.06	10.43
3.26	384	Lignan	n.d.[e]	1.01	n.d.	1.03
4.5-5	290	Catechin or isomer	11.56	10.31	14.35	10.45
6.44	330	Unknown	1.09	1.14	1.23	1.20
7.67	323	Alkaloid	n.d.	4.15	n.d.	n.d.

[a]Maceration extraction.
[b]Ultrasound-assisted extraction.
[c]Accelerated solvent extraction.
[d]Autoclaving (AT).
[e]Not detected.

FIGURE CAPTIONS

Figure 1. Extraction yield (%) fororange tree wood (W) and bark (B) extractives obtained using various extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE,accelerated solvent extraction; AT,autoclaving.

Figure 2. Total polyphenolic content (TPC) of orange tree wood (W) and bark (B). ME,maceration extraction; UAE,ultrasound-assisted extraction; ASE,accelerated solvent extraction;AT, autoclaving.

Figure 3.DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of orange tree wood (W) and bark (B) extractives obtained using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving.

Figure 4. Ferric reducing antioxidant power (FRAP) of orange tree wood (W) and bark (B) extractives obtained using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving.

Figure 5.*β*-Carotene bleaching (BCB) assay on orange tree wood (W) and bark (B) extractives obtained using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction;AT, autoclaving.

Figure 6. Relative Antioxidant Capacity Index (RACI) values obtained for orange tree wood (W) and bark (B) extractives using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving.

Figure 7. Principal component analysis (PCA) plots. (a) PCA scores from orange tree wood (W) and bark (B) pruning extracts using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving; (b) PCA scores for antioxidant activity (DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric reducing antioxidant power (FRAP) and inhibition of lipid peroxidation (BCB)) and total polyphenolic content (TPC).

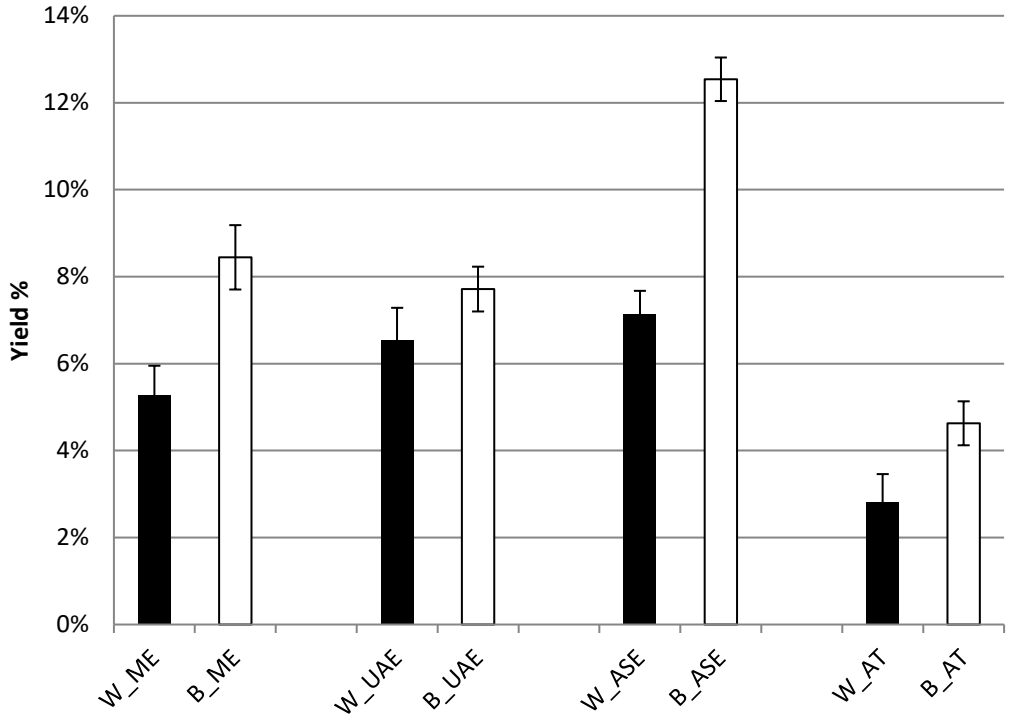

Figure 1

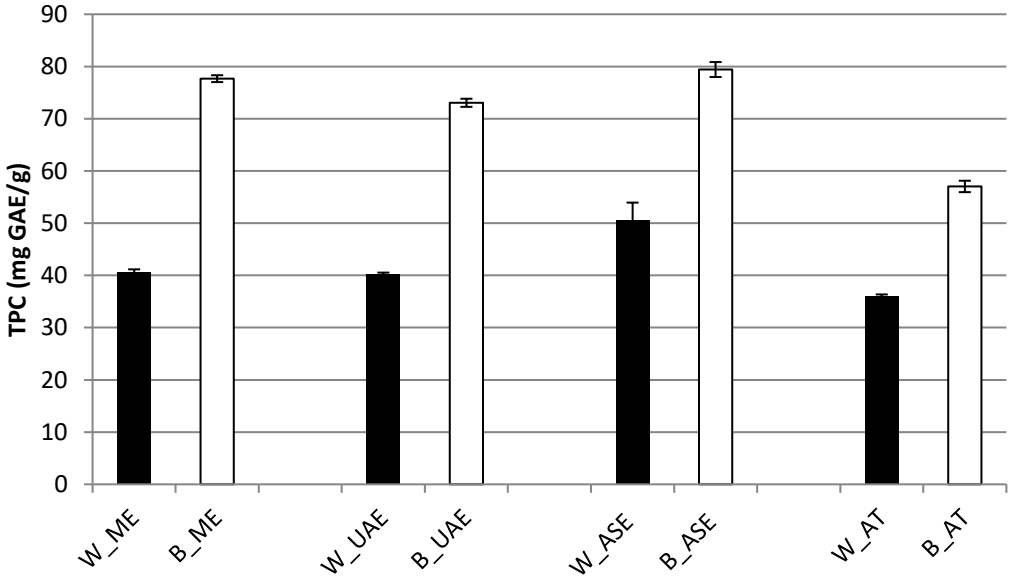

Figure 2

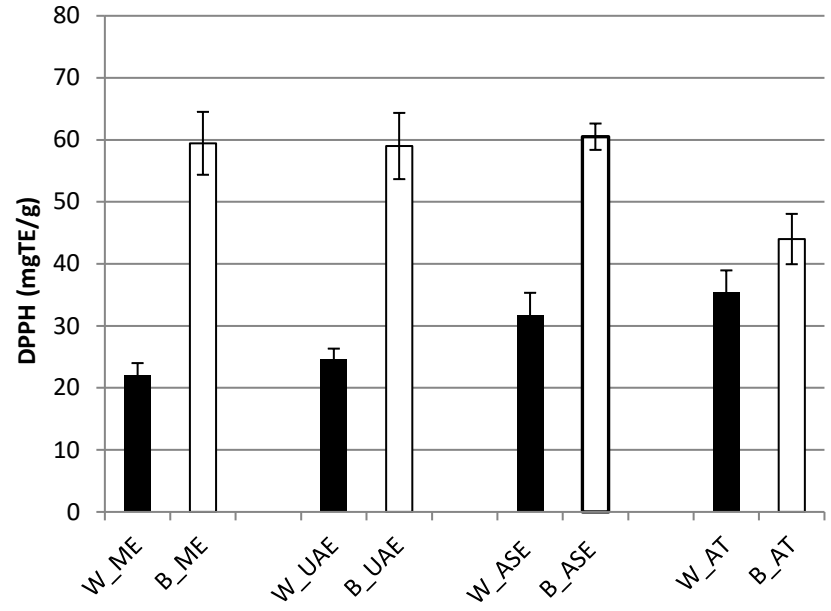

Figure 3

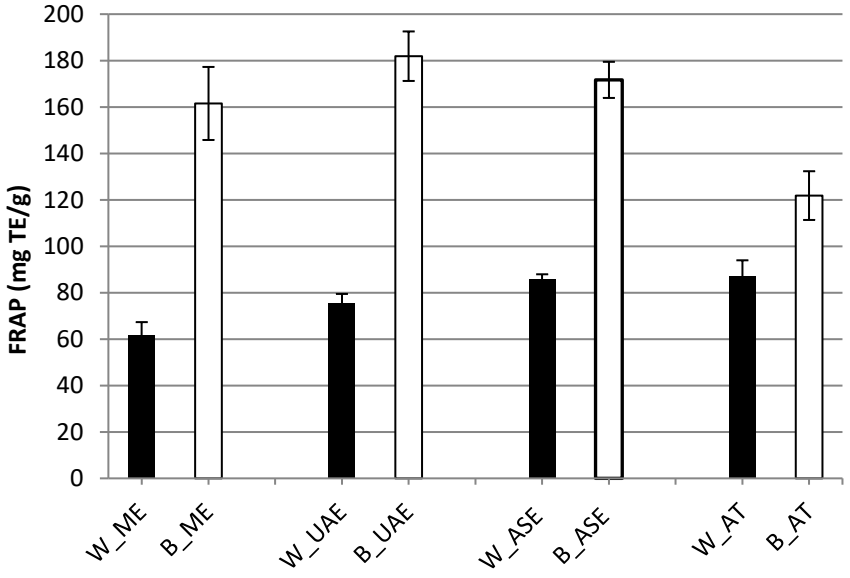

Figure 4

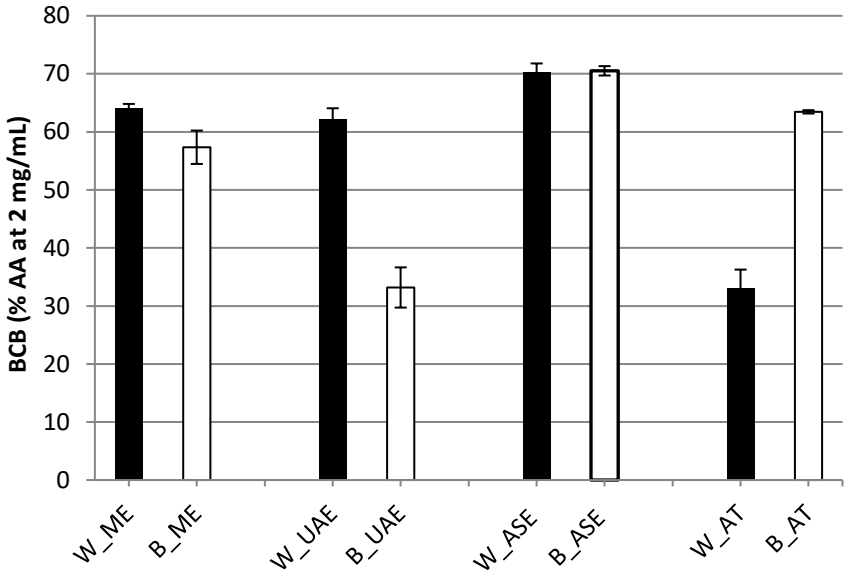

Figure 5

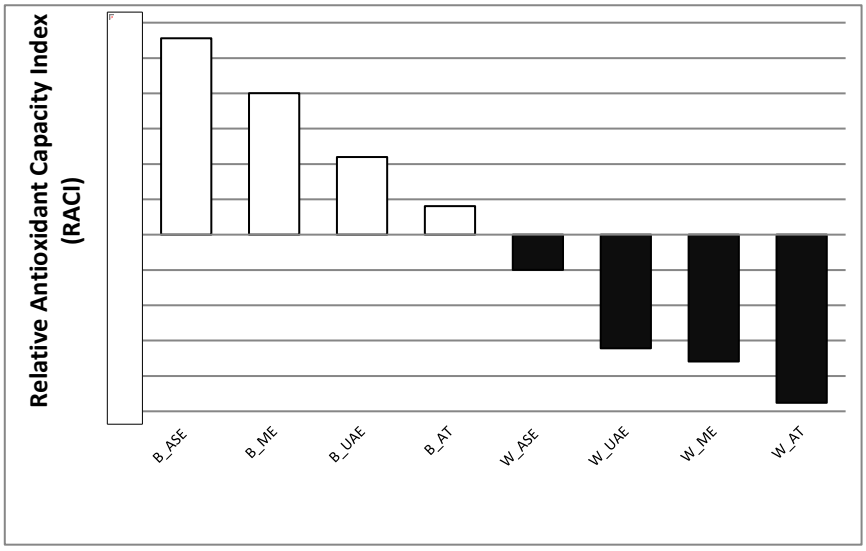

Figure 6

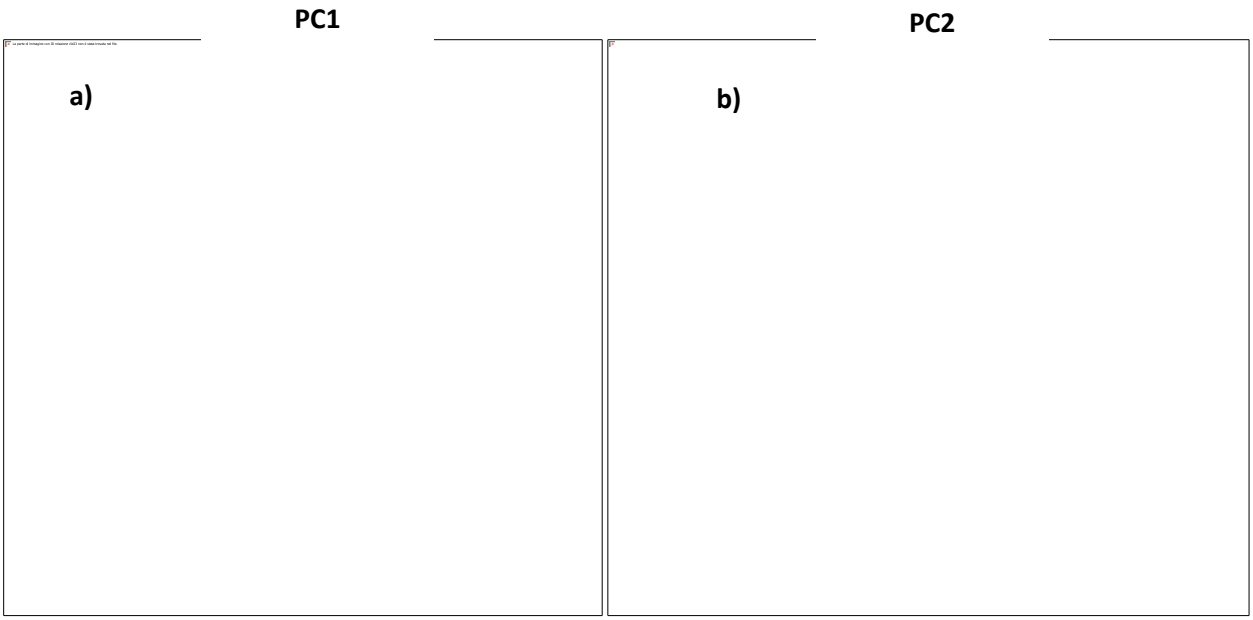

Figure 7