Original Article

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Focus on *Olea europaea* L. pruning by-products: extraction techniques, biological activity, and phytochemical profile

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Abstract: The *Olea europaea* L. tree has played a central role in Mediterranean culture since ancient times. Several studies have highlighted the health-promoting properties both of its primary products (olives) and its by-products (leaves, pomace, husk, stone, mill wastes, and wood). In this study, pruning residues from 25-year-old olive trees located in a Mediterranean region (Basilicata, Italy) were analyzed. The antioxidant activity of hydro-alcoholic extracts from wood samples were analyzed through three complementary *in vitro* assays. The molecular composition of the extracts was thoroughly evaluated using a gas chromatography apparatus coupled with a mass spectrometer (GC–MS). Our study demonstrated that all but three extracts had remarkable antioxidant activity, which was likely due to the meaningful presence of phenolic compounds, mostly derived from lignin. Moreover, the results showed that bark extracts obtained with ultrasound-assisted extraction (UAE) had the highest antioxidant activity. In this extract, several known compounds with demonstrated antioxidant activity were found, including hexylresorcinol, 1-methyl-*N*-vanillyl-2-phenethamine, and allopurinol. This research suggests that woody olive by-products are a potential natural resource of antioxidants. These compounds could be useful for functional foods and in industry, and could help to solve the problem of pruning residues, increasing their potential economic value. © 2021 Society of Chemical Industry and John Wiley & Sons, Ltd

Key words: pruning characterization; wood; bark; extractives; antioxidant activity; phytochemical composition

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Introduction

Olea europaea L. (olive tree) belongs to the Oleaceae family and is traditionally and widely cultivated in the Mediterranean region. The olive tree is a significant cultural and traditional symbol. It has been associated with joy and peace, and its leafy branches have been used historically to crown the victorious in exhibition matches and war.[1] According to Sofo *et al.*,[2] several morphological, physiological, and biochemical adaptations allow the olive tree, like many Mediterranean plant species adapted to semi-arid climates, to resist water scarcity during the summer. Olive-tree management involves annually or biennially pruning the crown with an average residual biomass of 33 kg tree^{-1}, without differences in annual or biennial pruning.[3] This means that in Mediterranean areas, the residual biomass from olive pruning reaches an average 1.31 t ha^{-1} in annual pruning and 3.02 t ha^{-1} in biennial pruning. Several studies have focused on the use of pruning residues, especially to produce heat and energy,[4–6] or to produce pulp and paper.[7–9] Furthermore, pruning waste is used for the production of bioethanol because, being a lignocellulosic waste, it is rich in sugars.[10] Some studies, instead, have investigated the radical scavenging properties, molecular compound presence,[11–13] and antifungal activity[14] of olive tree wood residues.

There are few studies on the phytochemical characterization of olive wood. Some have shown that it is rich in several specialized metabolites, also known as secondary metabolites, developed by different plant tissues (e.g., buds, wood, leaves, bark, or roots), which have biological effects, including antibiotic, antifungal, antioxidant, cytotoxic, or anti-inflammatory properties.[15]

The most representative compounds found in olive wood extract are oleuropein, ligustroside, and their derivatives. These compounds have different properties, with the most important being anti-inflammatory, antioxidant, and anti-cancer activity, together with hypolipidemic and hypoglycemic effects.[16] Several studies on olive tree bark have also demonstrated the presence of oleuropein,[17,18] known for its antioxidant activity and several other benefits for health care.

Moreover, different researchers have reported polyphenol extracts obtained from olive pomace by solid–liquid extraction, conventional, or nonconventional techniques.[19–21]

The purpose of this study was to increase the knowledge of the potential biological activity of pruning waste residues of an Italian olive tree cultivar. After separating the bark from the wood, the different parts of the branches were subjected to various extraction techniques.

In fact, the heterogeneous molecules present in *O. europaea* can be extracted in different ways, using several solvents, instruments, and parameters. The choice of the best extraction method is extremely important to recover the greatest amount of biologically active compounds.

The first goal of our research was to focus on eco-friendly solvent extraction processes. For this reason, among the solvents used, ethanol and water were selected, being greener solvents than the more commonly used methanol.[22]

Once the solvents to be used were identified, four different extraction methods were selected.

In addition to the more conventional technique, maceration extraction (ME), less conventional techniques, such as ultrasound assisted extraction (UAE), accelerated solvent extraction (ASE), and autoclaving (AT), have also been used. In particular, UAE, ASE, and AT were selected for their ability to reduce the extraction times and energy and solvent consumption.[23] Moreover, as reported by Aliakbarian *et al.*,[24] the high pressure and high temperature used during ASE and AT extractions allow recovery of most of the compounds present in the samples.

The extracts obtained were therefore tested to measure the differences between wood and bark composition and the influences of the different extraction techniques. The total polyphenolic content and different *in vitro* assays were used to identify the potential antioxidant activity of the samples. Finally, the chemical composition of the pruning samples was determined via gas chromatographic analysis (GC–MS). These analyses identified the most promising extraction method for olive wood.

Material and methods

Chemicals

Chloroform, ethanol, and glacial acetic and hydrochloric acid were purchased from Carlo Erba (Milan, Italy). Reagents and standards, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), β-carotene, butylhydroxytoluene (BHT), Folin–Ciocalteu, gallic acid, iron (III) chloride ($FeCl_3$*$6H_2O$), linoleic acid, sodium carbonate, sodium chloride, sodium hydroxide, and Tween 20, were purchased from Sigma-Aldrich (Milan, Italy). Milli-Q water was obtained from the Mill-Q purification system (Millipore, Bedford, MA, USA).

Experimental area

The research area was in Bernalda (Matera, southern Italy) at 127 m above sea level (40° 41′38″ N, 16° 69′28″ E). The trees were 25 years old. A square-shaped planting scheme was used with a distance between the plants of 5 m. Five 1000 m^2 randomized plots were used for the experiment.

Wood and bark extracts

Olive tree (*O. europaea* L.) biennial pruning was in October 2017 in an orchard localized in the Bernalda area (Basilicata region). The sample material was prepared following a previously reported procedure.[25] Each extraction technique was repeated three times on 10 g of small pieces of olive bark and wood.

All solutions were filtered and dried before using a rotary evaporator to remove ethanol. They were subsequently freeze-dried (Heto Drywinner DW3/RV12, Edwards High Vacuum International, Crawley, UK) and kept in the dark at room temperature.

The following formula was used to calculate the extraction yield of each sample:

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

The following describes the four extraction techniques. (1) The samples were subjected to *maceration extraction* (ME) at room temperature using ethanol : water (70:30 *v/v*) as a solvent. The sample to solvent ratio was 1:5 (*w/v*), and the duration of the extraction was 1 h with constant stirring. (2) The same solvent mixture and conditions of ME were used in *ultrasound-assisted extraction* (UAE) carried out using an ultrasonic bath (Branson 1800, Danbury, CT, USA); (3) an ethanol : water mixture (70:30 *v/v*) was used as a solvent, but different conditions were used in *accelerated solvent extraction* (ASE). This extraction was carried out in an ASE system (ASE 150, Dionex Corporation, Sunnyvale, CA, USA) at a temperature of 100 °C and a pressure of 1500 psi. The whole cycle lasted 5 min and was repeated for three cycles. (4) *Autoclaving extraction* (AT) was completed using a VaporMatic 770 sterilization autoclave, with water as a solvent. It was not possible to use the same mixture used for the other three extraction methods due to the impossibility of using other solvents in the autoclave. The complete extraction cycle lasted 20 min and was carried out at 121 °C with a pressure of 1 atm.

Total polyphenolic content (TPC)

Folin–Ciocalteu reagent was used to determine the total polyphenolic content (TPC) of olive samples.[25] Each sample extract was mixed with Folin–Ciocalteu reagent, Na_2CO_3 solution, and water. After 1 h at room temperature in the dark, the absorbance was read at 723 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract ± standard deviation (SD).

Antioxidant activity

Radical scavenging activity

The radical scavenging activity of the sample was investigated using DPPH, a stable and neutral radical. A DPPH methanol solution was added to different concentrations of each sample and stored in the dark for 30 min, and then the absorbance was measured at 515 nm. The radical scavenging activity of each sample was expressed as mg Trolox equivalents (TE) per g of dried sample ± SD.[26]

Ferric reducing antioxidant power (FRAP)

The reduction of the ferric complex to the ferrous form by antioxidants is the basis of the FRAP method.[15,25] This reaction is monitored by measuring the change in absorbance at 593 nm using the daily prepared FRAP reagent, which was added to the extract in a 96-well plate and incubated at 37 °C for 40 min. The results were expressed as mg of TE per g dried extract ± SD.

β-Carotene bleaching assay (BCB)

The BCB test was used to evaluate the inhibition of lipid peroxidation of samples following a previously described method.[26] The reaction was monitored at 470 nm every 30 min (0–180 min). The percentage of antioxidant activity (% AA) ± SD was calculated using the following formula:

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

Relative antioxidant capacity index (RACI) determination

According to Catronuovo *et al.*,[27] the adimensional RACI value was calculated by the integration of the data obtained from the different *in vitro* antioxidant assays, including TPC, allowing a better, comprehensive comparison of experimental results expressed in different units.

Gas chromatography–mass spectrometry (GC–MS)

The quali-quantitative organic compound determination in olive samples was assessed by GC–MS analysis as described by Lovaglio *et al.*,[28] using the NIST11 library for the identification of the compounds.

Statistical analyses

All experiments were repeated three times in three independent experiments. The results are expressed

as the mean ± SD. The Kruskal–Wallis rank was used to analyze the significant differences for multiple distributions. Principal component analysis (PCA) was also used. The Pearson correlation coefficient was calculated to verify the correlations among total polyphenolic content present in the samples and their antioxidant effects. The correlation between antioxidant activity and total phenolic content from each test was considered. To measure the significance of differences, the agricolae package was used. Statistical analyses were computed using R statistical software (http://www.r-project.org).

Results and discussions

By analyzing the data from the FAO (Food and Agriculture Organization) (Fig. 1) for the total area of olive trees harvested in the world from 2008–2018, an increase in the cultivation of olive trees (+6%) was observed. A more consistent increase was registered in Africa, Asia, and America, although the latter two do not traditionally cultivate olives. Of all the olive trees, 48% were cultivated in Europe.

In Europe, the countries with the highest olive tree harvest were Spain (about 50% of the total area in the EU-28), followed by Italy (22%), and Greece (19%; Fig. 2).

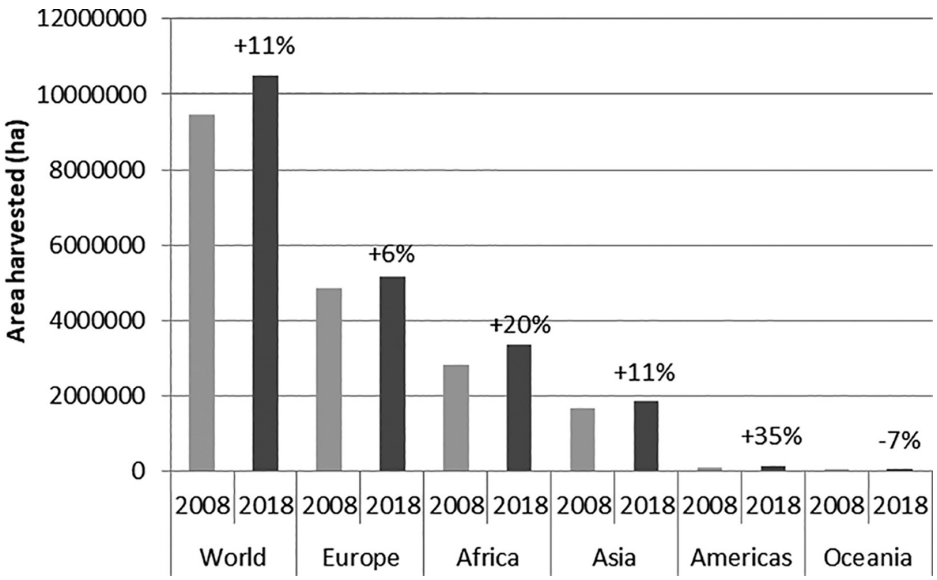

Figure 1. Comparison of olive tree area harvested in hectares (ha) in the years 2008 and 2018 in the world and by continent.

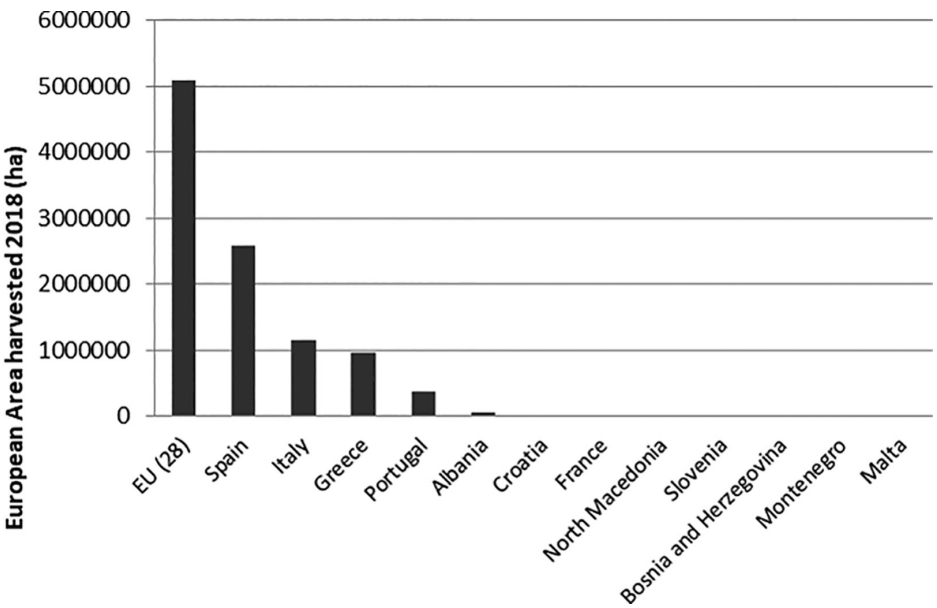

Figure 2. Total olive tree area tilled in hectares (ha) in 2018 in Europe (EU-28) and in different European countries.

These data clearly underline the importance of giving a new life to the many tons of pruning residues that are produced every year. Extraction optimization of the specialized metabolites from pruning waste and the analysis of their antioxidant activity *in vitro* is therefore essential.

Yield extracts

Figure 3 reports the extraction yield obtained using the four different selected extraction techniques. The extraction yield for wood (W) showed that ASE and UAE had the same yields, followed by ME and AT. The extraction yields for the bark (B) increased in the following order: ASE > UAE > AT > ME. The highest extraction yield was found using ASE with B (21 ± 1.0%) and W (9 ± 1.0%). The lowest yield for W was in AT (3 ± 1.0%), and for B, it was in ME (8 ± 0.8%). These results are consistent with previously published studies, in which the highest extract yield was obtained from bark rather than from wood.[25,29–31] This result can be explained because the bark is more subjected to biotic and abiotic stress, causing plants to defend themselves and producing a large amount of specialized metabolites.

According to Vázquez *et al.*,[32] the extraction yield was directly proportional to the lipophilicity of the solvent used compared with extractions with the organic solvents alone. Moreover, the highest extraction yield was obtained using ASE techniques and might be due to the higher solubility of analytes in the solvent and higher diffusion rate because of the higher temperature. Moreover, the high temperature increased the interaction between the solvent and sample breaking the bonds between molecules.[24,33]

Total polyphenolic content (TPC)

The total polyphenolic content of olive tree pruning samples is shown in Fig. 4. The highest value of TPC was found in the W_UAE group (156.04 ± 4.42 mg GAE g^{-1}), followed by B_ASE (144.63 ± 1.76 mg GAE g^{-1}) and B_AT (143.00 ± 1.66 mg GAE

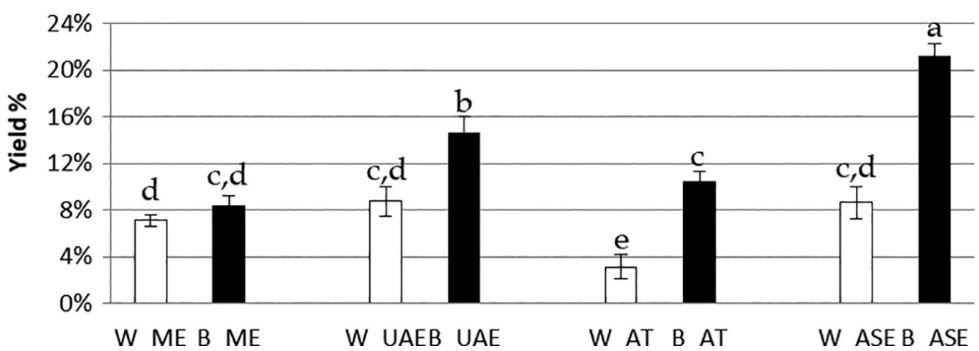

Figure 3. Yield (%) of wood (W) and bark (B) of olive tree extracts obtained using various extraction techniques. ME is maceration extraction, UAE is ultrasound-assisted extraction, ASE is accelerated solvent extraction, and AT is autoclave. Different letters (a–e) indicate a significant difference (*P* value <0.001).

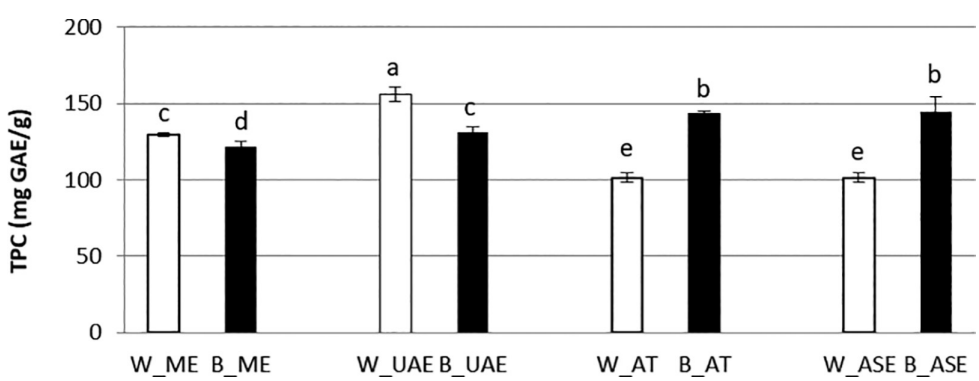

Figure 4. Total polyphenolic content (TPC) of wood (W) and bark (B) of olive trees. ME is maceration extraction, UAE is ultrasound-assisted extraction, ASE is accelerated solvent extraction, and AT is autoclave. Data are expressed as the means ± standard deviations from three experiments in mg gallic acid equivalents per gram of dried sample (mg GAE g^{-1}). Different letters (a–e) indicate a significant difference (*P* value <0.001).

g^{-1}) according to previous results reported by Bouras *et al.*[34] The bark sample extract obtained using AT, which was indicated as hydrothermal processing, caused a break in cell membranes and cell walls and made phenolic compounds more available by hydrolyzing the components of the cell wall.[35] Specialized metabolites, such as phenolics and tannins, are abundant in bark. They protect the living tissues against biotic and abiotic disease.[36] The lowest quantity of TPC was elevated in W_AT (101.45 ± 2.85 mg GAE g^{-1}) and W_ASE (101.60 ± 2.90 mg GAE g^{-1}). According to Gao *et al.*,[36] generally, sapwood and the inner bark have abundant nutrient content, such as glycosides and sucrose, and possess a lower amount of phenolic compounds.

Antioxidant activity

The antioxidant capacity of samples was analyzed utilizing three complementary *in vitro* assays: DPPH, FRAP, and BCB.

The DPPH test (Fig. 5) showed that the highest value was exhibited in the W_ME sample (188.84 ± 20.60 mg TE g^{-1}), followed by W_UAE (164.17 ± 13.80 mg TE g^{-1}), and the lowest value was determined in the W_AT (26.40 ± 0.64 mg TE g^{-1}). This latter result is in accordance with the value obtained in TPC.

The data obtained from FRAP (Fig. 6) showed the highest values in W_UAE (408.80 ± 38.23 mg TE g^{-1}) and B_ASE (400.31 ± 26.00 mg TE g^{-1}), similar to the results obtained by TPC; in this case, the lowest data were obtained in W_AT (74.87 ± 6.00 mg TE g^{-1}). According to Roby *et al.*,[37] extracts with a higher polyphenol content also showed higher antioxidant capacity, indicating that extracts derived from higher polarity solvents were more efficient as antioxidants than those obtained using solvents with less polarity. In olive pruning samples, data from TPC were not always in accordance with those obtained from DPPH and FRAP

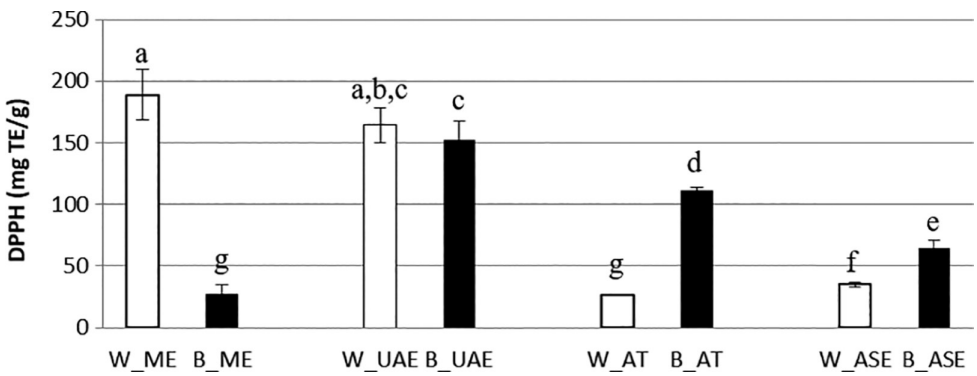

Figure 5. 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of wood (W) and bark (B) of olive tree extracts obtained using various extraction techniques. ME is maceration extraction, UAE is ultrasound-assisted extraction, ASE is accelerated solvent extraction, and AT is autoclave. Data are expressed as the means ± standard deviations from three experiments in mg Trolox equivalents per gram of dried sample (mg TE g^{-1}). Different letters (a–g) indicate a significant difference (*P* value <0.001).

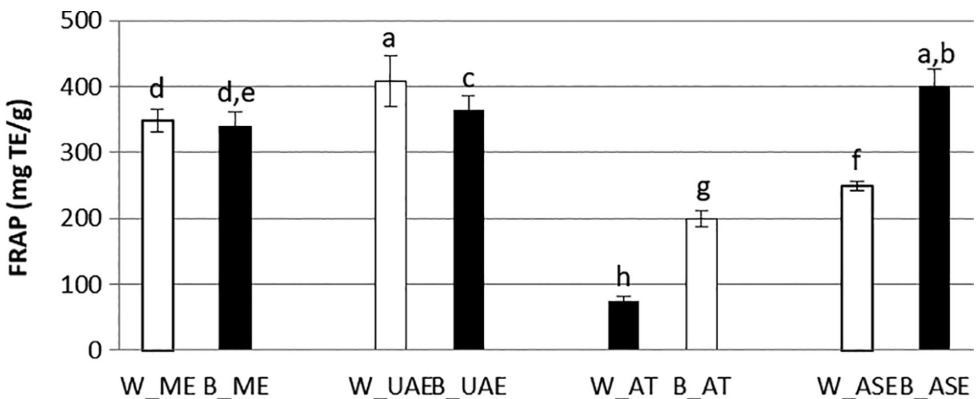

Figure 6. Ferric reducing antioxidant power (FRAP) of olive tree wood (W) and bark (B) extracts obtained by using various extraction techniques. ME is maceration extraction, UAE is ultrasound-assisted extraction, ASE is accelerated solvent extraction, and AT is autoclave. Data are expressed as the means ± standard deviations from three experiments in mg Trolox equivalents per gram of dried sample (mg TE g^{-1}). Different letters (a–h) indicate a significant difference (*P* value <0.001).

assays. Ku *et al.*[38] demonstrated a linear correlation between phenolic compound content and antioxidant activity in plant extracts due to the sensitivity of the Folin–Ciocalteu reagent against a wide range of substrates, which are easily oxidized. In contrast, the free radical DPPH shows various sensitivity to different antioxidants depending on their kinetic reactions with the radical.

The BCB assay (Fig. 7) indicates degradation during the oxidation process when linoleic acid turns to hydroperoxide at an incubation temperature of 50 °C.[39] In the BCB assay, the highest values were obtained in B_UAE and W_AT (62.74 ± 5.56 and 62.53 ± 1.00% AA, respectively), while the lowest BCB assay results were bark samples extracted through ASE and AT (17.39 ± 1.03 and 33.21 ± 2.62% AA, respectively). These data appear to contrast with those obtained from DPPH and FRAP assays, especially W_AT. Diouf *et al.*[40] explained the behavior of BCB compared with other antioxidant assays; the extraction will solubilize several other classes of molecules in addition to phenolics, like sugars and mineral constituents, which might contain transition metal cations. The latter might enhance the rate of oxidation of edible oils by increasing the rate of generation of free radicals from fatty acids or hydroperoxides. Another explanation about the difference in the data obtained from BCB compared with the other antioxidant assays might be the 'polar paradox' – lipophilic antioxidants show greater activity in emulsions in bulk oil, and hydrophilic antioxidants are more efficient than lipophilic antioxidants.[41]

Relative antioxidant capacity index (RACI)

The RACI is an index generated from an easy and rapid statistical tool that allows the evaluation of the results obtained from different antioxidant tests that are measured in different units. It provides a more comprehensive evaluation of the results that have been measured using

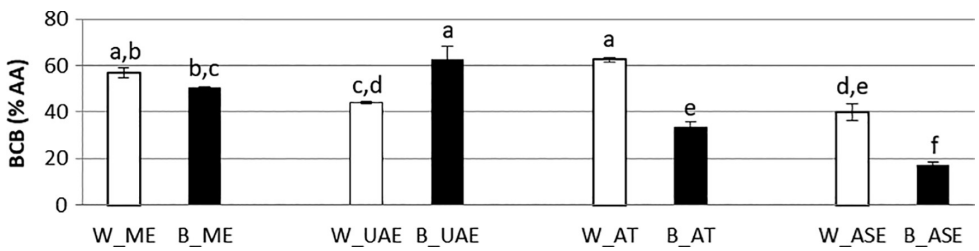

Figure 7. β-Carotene bleaching (BCB) assay of olive tree wood (W) and bark (B). ME is maceration extraction, UAE is ultrasound-assisted extraction, ASE is accelerated solvent extraction, and AT is autoclave. Data are expressed as the means ± standard deviations from three experiments in the percentage of antioxidant activity (% AA) at an initial concentration of at 2 mg mL^{-1}. Different letters (a–f) indicate a significant difference (*P* value <0.001).

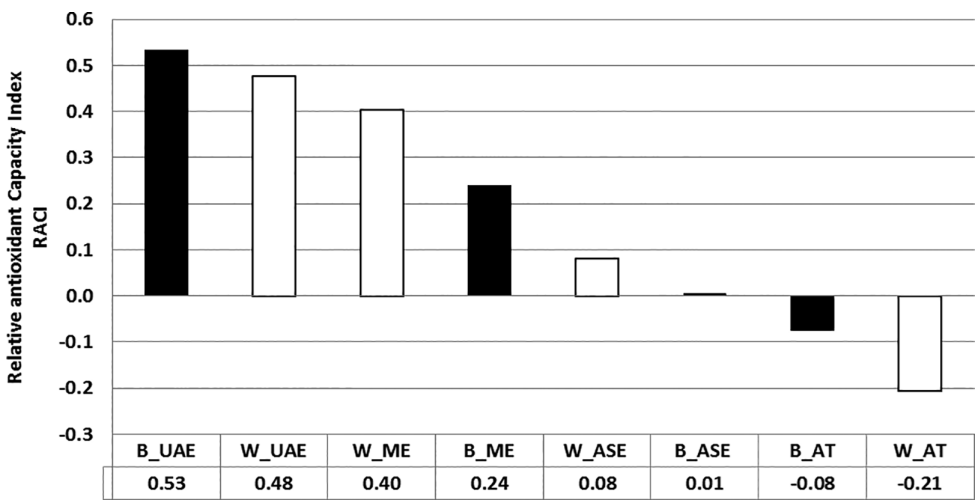

Figure 8. Relative antioxidant capacity index (RACI) values obtained for olive tree wood (W) and bark (B) extracts using various extraction techniques. ME is maceration extraction, UAE is ultrasound-assisted extraction, ASE is accelerated solvent extraction, and AT is autoclave.

the DPPH, FRAP, and BCB methods, which are often not in complete agreement.[42] Total polyphenolic content was also included because recent studies have shown that the Folin–Ciocalteu procedure could also be interpreted as an alternative way to measure the entire reducing capacity of the extracts. As seen in Fig. 8, the highest RACI was found for B_UAE, while the lowest was found for W_AT. For all samples, except those extracted by autoclaving, there were positive RACI values.

Among the four extraction methods used, UAE yielded samples with the best antioxidant activity, in accordance with the results obtained from previous works.[43,44]

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

	TPC
DPPH	0.633
FRAP	0.661
BCB	−0.440

According to Chirinos *et al.*,[45] the lowest antioxidant activity in the AT extraction could be due to solvents in different combinations that improve the phenolic glycosides' extraction. It has been demonstrated previously that polyphenols are more soluble in methanol and ethanol than water, which agrees with previously published data.[46]

Statistical analyses

Pearson coefficient

Pearson coefficient was calculated to measure the correlations between the measured variables (Table 1). It showed a moderate linear correlation between TPC and DPPH ($r = 0.633$) and TPC and FRAP ($r = 0.661$). These results suggest that the antioxidant activity of the extract might be derived from the presence of nonphenolic compounds.[47] According to Mamat *et al.*,[48] the moderate relationships might suggest that phenolic compounds were not the only components responsible for the measured antioxidant activity. For the correlation between TPC and BCB, the coefficient was negative ($r = -0.440$) due to the contribution of other substances, in addition to phenolics, to the measured

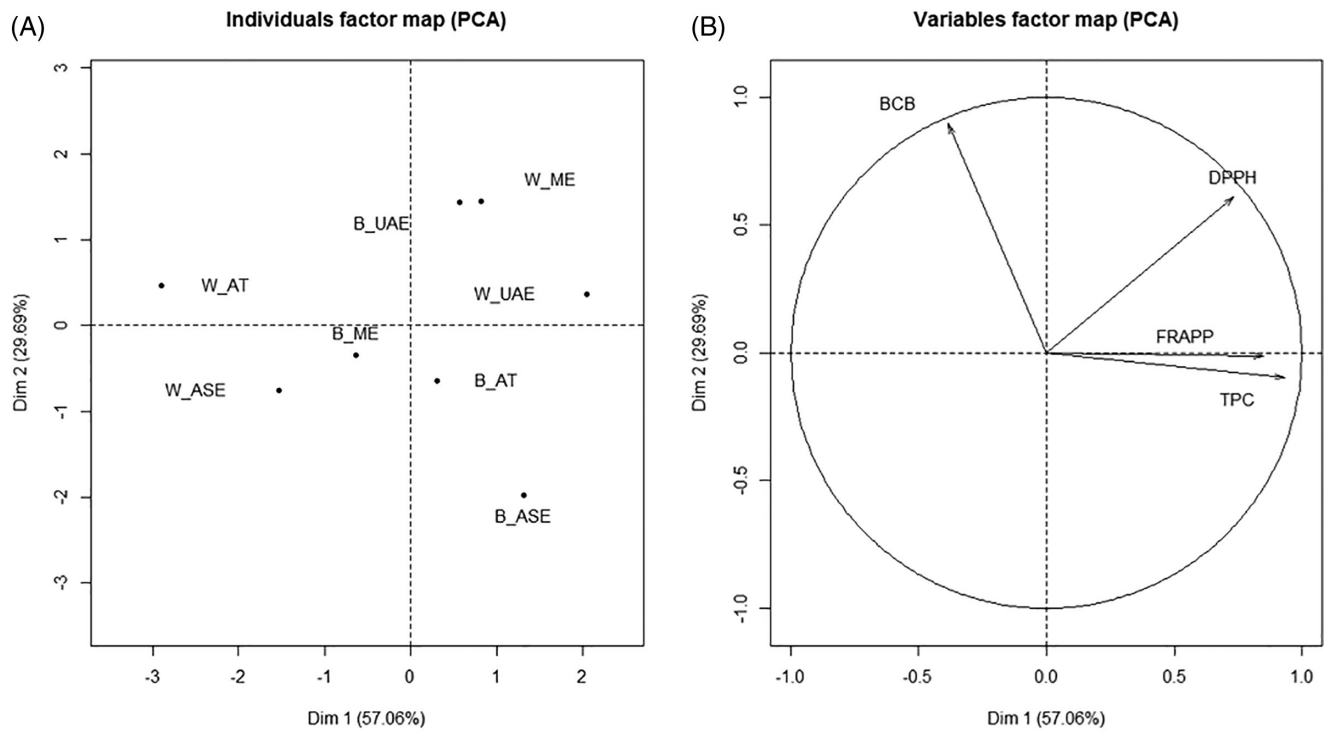

Figure 9. Principal component analysis (PCA) plots. (A) Principal component analysis scores from olive tree wood (W) and bark (B) pruning extracts using various extraction techniques. ME is maceration extraction, UAE is ultrasound-assisted extraction, ASE is accelerated solvent extraction, and AT is autoclave. (B) Principal component analysis 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).

Table 2. GC–MS analysis of olive residues extracts. ME is maceration extraction, UAE is ultrasound-assisted extraction, ASE is accelerated solvent extraction, and AT is autoclave. The results are presented as relative area percentage, of the samples peaks analyzed through the GC-MS.

Compound	r.t. (min.)	Wood				Bark			
		ME	UAE	AT	ASE	ME	UAE	AT	ASE
		Area % (±0.03)							
Benzaldehyde	3.34	0.69	0.06		0.37	1.03	0.50		0.06
2-Methoxyphenol	3.37			0.10	0.55				
(*E*)-2-Heptenal	4.24		0.22						
Catechol	4.28			0.51					
Glycerin	5.81								1.04
Dihydro-4-hydroxy-2(3*H*)-furanone	5.90							1.18	
3-(2-furanyl)-2-propenal	6.06								
2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	6.47	0.57	0.34	0.80			0.75	1.35	0.79
3-Hydroxy-butanoic acid	6.59		0.04						
5-Hydroxymethylfurfural	6.91	0.78	0.90	0.34		0.88	1.40	3.60	1.05
1,6-Anhydro-β-d-glucopyranose	7.03			1.85				1.32	
1,2,3-Propanetriol-1-acetate	7.17								
Dianhydromannitol	7.44						0.23		
Methyl 2-oxo-2*H*-pyran-5-carboxylate	7.78	0.14		0.63	0.12	0.33	0.26		
2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one	7.87			2.90					
2-Methoxy-4-vinylphenol	8.20			0.82	0.40	0.22	0.12	0.33	0.13
5-Formylsalicylaldehyde	8.51	0.27	0.23	0.28	0.19	0.69	0.42		0.56
4-Hydroxy-2-methylacetophenone	8.60				0.26				
Methyl 4-hydroxy-3-methoxybenzeneacetate	8.61			1.81					
2,6-Dimethoxyphenol	8.74		0.16	0.65	0.75	0.28	0.27		
Methyl 4-formylbenzoate	8.96	0.68	0.36	2.45			1.35	1.45	0.28
Methyl 2-formylbenzoate		0.79	0.46		1.78	2.53			0.56
2-Methylene-4-pentenal	9.29					0.64			
Vanillin	9.44		0.22	0.61	0.63	0.55	0.61		0.49
Homovanillinic alcohol	9.52	1.66		1.87	1.74				
4-(1-Methylethyl)benzoic acid	9.65					0.39	0.24	0.25	
4-Hydroxybenzeneethanol	9.89	3.40	3.74	1.82	1.91	1.24			
2-Ethylphenol	9.92							1.54	
2-Hydroxy-5-methylbenzaldehyde	9.96								2.51
3-Methoxybenzaldehyde	9.98						2.67		
4-Ethoxy-2,5-dimethoxybenzaldehyde	10.03			5.72					
4-(4-Hydroxyphenyl)-2-butanone	10.07				1.93	1.45	0.72	1.21	
Eugenol	10.09		0.42	0.63	2.55				
Methyl mandelate	10.10					0.51	0.31	0.55	1.29
2-Methyl-6-methylene-7-octen-2-ol	10.11	3.42							
3-Formylbenzoic acid	10.27		1.63		0.97	2.01	2.40		2.77
1,4-Anhydro-d-mannitol	10.31	3.08							
3,4-Dihydroxy-benzeneacetic acid	10.48	5.61		5.12					
3,4-Dihydroxyphenyl-2-propanone	10.54	3.96							3.09
Hexylresorcinol	10.58	6.68	7.64						

Table 2. (Continued)

Compound	r.t. (min.)	Wood				Bark			
		ME	UAE	AT	ASE	ME	UAE	AT	ASE
		Area % (±0.03)							
2,6-Dimethyl-4-pyrimidinamine	10.61		7.87						
1-Methyl-*N*-vanillyl-2-phenethanamine	10.75		10.27						
4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	10.78	9.47		5.57					
1-(2,4,6-Trihydroxy-3-methylphenyl)-1-butanone	10.92	1.68	1.81						
d-Allose	10.99				0.32				
Allopurinol	11.02	8.06	11.36						
1,3,5-Benzenetriol	11.19						1.54	4.35	2.58
4-Acetyl-1-methylcyclohexene	11.27							7.35	
4-(Hydroxyacetyl)-1,1′-biphenyl	11.38		2.08						
N-Formyltyramine	11.53		1.67		1.68				2.05
4-Ethoxy-2,5-dimethoxybenzaldehyde	11.69	0.57	0.61						
3-Hydroxy-4-methoxybenzoic acid	11.73		1.22			0.91	0.39	0.83	
Apocynin	11.85					0.61	1.10	0.66	
4-Propyl-1,3-benzenediol	12.27					2.62			
2-Methyl-6-methylene-7-octen-2-ol	12.42				3.88		9.06		3.28
4-Hydroxy-3,5-dimethoxybenzaldehyde	12.73				0.84				
3,4-Dihydroxybenzeneacetic acid	13.15				10.04		8.36	6.82	13.64
3,4-Dihydroxyphenyl-2-propanone	13.18					9.70		4.42	
Mannitol	13.20	1.75	5.58	0.94			1.02		
3,5-Dimethoxy-4-hydroxyphenylacetic acid	13.22				6.74	7.88			
Sorbitol	13.41	1.71					2.94		
β-(4-Hydroxy-3-methoxyphenyl)propionic acid	13.64					3.39	3.32		3.37
4-Methoxy-4′,5′-methylenedioxybiphenyl-2-carboxylic acid	13.67			1.02					
4-((1*E*)-3-hydroxy-1-propenyl)-2-methoxyphenol	13.71				17.40	3.28			2.22
Galactilol	13.94	3.01		8.43					
1-(2,4,6-Trihydroxyphenyl)-2-pentanone	14.02				1.76				
Desaspidinol	14.04						3.15	0.48	
Methyl 3-formyl-4,6-dihydroxy-2,5-dimethylbenzoate	14.14					1.67	1.63	8.30	6.49
2,4,5-trimethoxybenzaldehyde	14.75							2.51	
4-(Hydroxyacetyl)-1,1′-biphenyl	14.88								1.22
Methyl-α-(acetyloxy)-2-methoxybenzeneacetate	15.11								0.28
Hexadecanoic acid	15.77	0.75	0.90	1.03	1.18	0.87	0.41		0.74
Scopoletin	16.12	3.83	6.02		2.85	0.98	1.38	0.40	
3,5-Dimethoxy-4-hydroxycinnamaldehyde	16.18					1.52		0.44	
2′-Formyl-2,3,4,4′-trimethoxy-1,1′-biphenyl	16.20			0.72					
Glycerol 1-palmitate	16.54	0.70	0.97	4.92	1.24		1.08	1.04	
Heptadecanoic acid	16.70					0.63			
(*Z*,*Z*)-9,12-Octadecadienoic acid	17.39					0.22			0.19
Oleic acid	17.43	1.02	2.02		1.17	1.63			0.42
Xylitol	17.47						0.16		
Octadecanoic acid	17.62	0.12		2.55	0.28	0.76			0.27
Glucitol	17.84						1.33		

Table 2. (Continued)

Compound	r.t. (min.)	Wood ME	Wood UAE	Wood AT	Wood ASE	Bark ME	Bark UAE	Bark AT	Bark ASE
		Area % (±0.03)							
Hexadecanamide	17.87								0.21
Arabitinol	18.48						2.06		
Diaveridine	18.74								0.17
Eicosanoic acid	19.35		0.54		0.28	0.18			0.19
(*Z*)-9-Octadecenamide,	19.43								0.14
2-Hydroxy-1-(hydroxymethyl)ethyl hexadecanoate	20.60								1.42
Docosanoic acid	20.96		0.22		0.04	0.26			0.33
Tricosanoic acid	21.53		0.05		0.06				
4-Methoxy-4′,5′-methylenedioxybiphenyl-2-carboxylic acid	21.94				0.97	0.33			
Stigmast-4-en-3-one	22.50		0.23	0.72					0.86
4,4′-Methylenebis[2,6-dimethoxyphenol	22.57				0.19				
7,9-Diethylbenz[a]anthracene	23.88				0.13				
4-(1,1-Dimethylallyl)-9-methoxy-7*H*-Furo[3,2-g][1] benzopyran-7-one	24.10		0.07						
Methyl 3,4-dimethoxymandelate	24.36								3.08
4-[[4-(Acetyloxy)-3,5-dimethoxyphenyl]methoxy]-3-methoxybenzaldehyde	24.41					3.05			
3-Methyl-1-(2,4,6-trihydroxy-3-methylphenyl)-1-butanone	25.98		2.90						
2-Phenylnaphthalene	31.46					1.86			
γ-Sitosterol	32.60			0.22	2.30	0.40	0.15		0.64
20-Hydroxvoaluteine	39.25				0.13				

antioxidant activity. The correlation suggested a need to use more than one antioxidant assay based on several chemical reactions to consider the different mechanisms of action, overcoming the limitations of a single test.[49]

Principal component analysis (PCA)

Principal component analysis is a multivariate statistical approach used for transforming the variables obtained into new variables called principal components, and it is used to reduce the dimensions of the dataset. The complexity reduction occurs by simply analyzing the main variables (by variance) among the all variables.[50,51] The values obtained from the antioxidant assays and TPC from the different extracts of olive tree wood and bark were standardized and used for PCA. Principal component analysis (Fig. 9(A) and (B)) described 86.75% of the dataset's total variance. The first component (Dim 1) described 57.06% of the total variance in the dataset, and Dim 2 described 29.69%. Figure 9(A) and (B) explains the connection between antioxidant assays and TPC with the samples. As shown in Fig. 9(A), samples W_ME, W_UAE, and B_UAE had high DPPH, FRAP, and TPC values. In Fig. 9(B), the same position of these samples is also found. B_AT and B_ASE are reported in Fig. 9(A) and are in the same position as the TPC (Fig. 9(B)), to indicate that these samples had a high quantity of phenolic compounds. In contrast with B_AT, W_AT demonstrated the highest BCB activity (Fig. 9(B)). B_ASE was distant from the other samples, indicating that it was significantly different from the others. W_ASE demonstrated a low quantity in TPC and antioxidant tests. In Fig. 9(B), FRAP and TPC almost overlapped, and were far from DPPH and on the opposite side compared with BCB. Based on this, FRAP and TPC were correlated with each other more than with DPPH, and BCB was not linearly correlated with the remaining antioxidant tests, as shown in the Pearson correlation.

GC–MS analysis

The GC–MS analysis demonstrated 102 compounds in the olive tree extracts (Table 2). The extraction of olive residues, obtained using maceration, showed the presence of 4-hydroxybenzeneethanol, 3,4-dihydroxybenzeneacetic acid, hexylresorcinol, 4-(3-hydroxy-1-propenyl)-2-methoxyphenol, and 1,3,5-benzenetriol as the main

components. When the extraction was performed though sonication, the main components were hexylresorcinol, 1-methyl-*N*-vanillyl-2-phenethamine, and allopurinol. The use of water in the autoclave to perform the extraction yielded 4-ethoxy-2,5-dimethoxybenzaldehyde, 3,4-dihydroxybenzeneacetic acid, and 4-(3-hydroxy-1-propenyl)-2-methoxyphenol. ASE extraction revealed the presence of 3,4-dihydroxybenzeneacetic acid, 3,5-dimethoxy-4-hydroxyphenylacetic acid, and 4-(3-hydroxy-1-propenyl)-2-methoxyphenol. These compounds are phenolic compounds derived from lignin. Scopoletin, a coumarin derivative with interesting biological effects, including antibacterial, antimicrobial, and antifungal properties, was also present.[52,53] Extraction from the bark gave similar results, where mainly phenolic compounds were extracted.

Conclusion

Olive woody pruning biomass, separated into wood and bark, was subjected to four different extraction techniques. In all extracts obtained, the total polyphenol content and antioxidant activities were measured by three complementary *in vitro* assays, and the present compounds were determined by GC–MS analysis. The best extraction yield in olive tree samples was obtained through accelerated solvent extraction; furthermore, the high temperature had a positive effect on yield. This method is an environmentally friendly and green process capable of extracting bioactive compounds from different natural sources.

The extracts obtained by UAE showed better antioxidant activity, making this the most promising extraction technique.

Ultrasound-assisted extraction extracts phenolic compounds in a better way, albeit with a lower yield based on extracted material, whereas ASE offers a higher yield but a lower concentration of phenolic compound content compared with UAE.

Regarding the analyses of molecular compounds, GC–MS showed the following 15 phenolic compounds in the olive tree sample with several biological effects: 4-hydroxybenzeneticethanol, 3,4-dihydroxybenzeneacetic acid, hexylresorcinol, 4-(3-hydroxy-1-propenyl)-2-methoxyphenol, 1,3,5-benzenetriol, hexylresorcinol, 1-methyl-*N*-vanillyl-2-phenethamine, allopurinol, 4-ethoxy-2,5-dimethoxybenzaldehyde, 3,4-dihydroxybenzeneacetic acid, 4-(3-hydroxy-1-propenyl)-2-methoxyphenol, 3,4-dihydroxybenzeneacetic acid, 3,5-dimethoxy-4-hydroxyphenylacetic acid, 4-(3-hydroxy-1-propenyl)-2-methoxyphenol, and scopoletin.

In Annex 1 of the Ministerial Decree on 10 August 2018 of the Italian Ministry of Health, the bark of the surculi of *Olive europea* L, was admitted as a food supplement, together with the bud, flower, fruit, and oil. The present work adds to previous studies on the possibility of using a waste material, such as olive tree pruning residues, in different industrial sectors, such as pharmacological, cosmetic, and agriculture fields, thanks to the interesting biological activity demonstrated *in vitro*. In legislation, it is already possible to use different parts of the olive tree as a food supplement; similarly, bark should also be used because this part of the plant has demonstrated several biological proprieties.

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Author contributions

L.T., M.D.A., and L.M. were responsible for conceptualization, methodology, and software. D.R., I.F., and M.R.B. were responsible for data curation and preparation of the original draft. D.R., I.F., and M.R.B. were responsible for visualization and investigation. L.T., M.D.A., and L.M. were responsible for supervision. D.R., I.F., and M.R.B. were responsible for software and validation. D.R., I.F., M.R.B., L.T., M.D.A., and L.M. were responsible for writing, reviewing, and editing.

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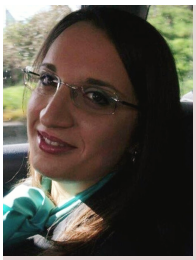

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