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A B S T R A C T

Distillation wastewaters (DWWs) are generated during the essential oil steam distillation from aromatic herbs. Despite of growing interest on novel source of natural antioxidant compounds as food additives, studies on DWWs are scarse. Herein, the potential of DWWs produced by the distillation of packaged fresh basil, rosemary and sage wastes was evaluated by chemical and antioxidant characterization.

HPLC-DAD-HRMS profiling revealed that DWWs contain water-soluble phenolic compounds, mainly caffeic acid derivatives and flavonoid glycosides, with rosmarinic acid (RA) as predominant components (29–135 mg/100 mL). DWWs demonstrated high levels of total phenolic compounds (TPC, 152–443 mg GAE/100 mL) and strong antioxidant capacities, in ORAC, DPPH and ABTS assays (1101–4720, 635–4244 and 571–3145 μmol TE/100 mL, respectively). Highly significant correlations of TEAC values with TPC and RA contents revealed that phenolic compounds and high RA content were responsible of DWWs antioxidant properties.Thus, DWWs are proposed as a new promising source of natural food additives and/or functional ingredients for cosmetic, nutraceutical and food applications.

1. Introduction

Aromatic herbs are common food adjuncts used as flavoring, seasoning, and coloring agents and sometimes as preservatives. Those mainly belonging to the Lamiaceae family are also a source of secondary metabolites with well recognized pharmacologically activities.

Recently they have been exploited as promising ingredients to develop novel products in sectors like pharmaceutical, cosmetic, food and pesticide ndustries (Trivellini et al., 2016). Particularly, there is a growing interest in the food industry to replace synthetic antioxidants and additives with compounds from natural sources or plant products. One of the most effective approaches employs the extracts of aromatic herbs as an affordable and valuable alternative to the synthetic additives. In fact, numerous studies demonstrate that herbs of Lamiaceae family (mainly rosemary, oregano, sage, basil, mint, and thyme) have food-preserving properties related to the presence of antioxidant and antimicrobial phenolic constituents (Embuscado, 2015; Trivellini et al., 2016). In addition, the consumption of aromatic herbs is growing due to their value as functional foods able to reduce the need for salt and fatty condiments (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Alvarez, 2010).

Thus, the use of natural bioactive compounds of aromatic herbs represents an attractive novelty for food sector in order to increase the shelf-life and improve the nutraceutical value of the food products. The improved interest of researchers, consumers and the food industry broadened the request and the typology of products based on aromatic plants. For instance, the production of aromatic herbs destined to packaged fresh products for Mass Market Retailers

(MMRs) had a sharp increase in recent years. The wastes resulting from processing, packaging and cultivation of packaged fresh herbs may be useful biomasses for the recovery of high-value products, in line with the concept of biorefinery and green extraction (Lin et al., 2014).
A feasible use can be the production of essential oils and aromatic waters, by steam distillation processes, to obtain quality products in a traceable supply chain. However, the essential oil steam distillation generates two main by-products: the residual plant materials and the wastewaters of the oil distillation process (distillation wastewaters, DWWs). The latter are generated by the partial condensation of hot water that passes through the vegetable matrix and is collected in the distillation chamber (Wollinger et al., 2016). After the distillation, the non-volatile compounds of aromatic herbs remain in the distillation byproducts and the hydrophilic water-soluble fraction can be dissolved in the DWWs followed to the extraction of plant material with condensed hot water. Valorization of the by-products (vegetal wastes and DWWs) generated from whole chain of production of packaged fresh aromatic herbs, via integrated biorefinery schemes, should target the production of high-value products such as essential oils, aromatic waters and natural food additives and/or functional ingredients for cosmetic, nutraceutical and food applications. In this context, the potential of aromatic herb DWWs as source of compounds with antioxidant and antimicrobial activity should be evaluated. DWWs are an unexplored by-product and very limited data are available on their chemical characterization. DWWs from some essential-oil crops have proposed as growth promoter and modifier of the essential oil composition of spearmint (Zheljazkov & Astatkie, 2012). Recently, a chemical study revealed that the rose oil DWW is a rich source of flavonoids with strong anti-tyrosinase activities (Solimine et al., 2016). Also, DWWs of rosemary have been identified as a possible source of the natural antioxidant rosmarinic acid (Wollinger et al., 2016).
Thus, the aim of the present study was to determine the qualitative and quantitative chemical profiles and antioxidant activity of DWWs obtained from the steam distillation of the waste materials generated from chain production of packaged fresh herbs. Particularly, the research was conducted by taking into account three of the most common Lamiaceae species cultivated in Southern Italy, basil (Ocimum basilicum L.) type "Genovese", rosemary (Rosmarinus officinalis L.) and sage (Salvia officinalis L.), and various waste materials produced during the processing of packaged fresh aromatic herbs. The latter were the fresh leaves of basil (BL), rosemary (RL) and sage (SL) at the vegetative stage, discarded during the packaging, and the plant materials produced by cultivation procedures, as the elimination of basil (BP) plants that have reached the flowering stage, and the pruning of sage (SP) to encourage the rejuvenation of this perennial crop. DWW samples were obtained by the steam distillation applied to these wastes to recovery essential oils and aromatic waters.
To evaluate the potential of aromatic herb DWWs as source of functional compounds, an accurate chemical characterization of DWWs, by HPLC-DAD-HRMS analysis, was firstly carried out. Later, the levels of main constituents (RA and TPC) in DWWs were determined to assess their contribution to DWW antioxidant properties, determined by three in vitro assays (DPPH, TEAC and ORAC).

2. Materials and methods

2.1. Chemicals and standards

MS-grade acetonitrile (MeCN) and water were supplied by Romil (Cambridge, UK). Ultrapure water (18 MΩ) was prepared by a Milli-Q purification system (Millipore, Bedford, USA). Analytical-grade methanol and ethanol, MS-grade formic acid (HCOOH), gallic acid (GA), butylhydroxyanisole (BHA), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), Folin & Ciocalteu's phenol reagent, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2′-

azobis(2-methylpropionamidine) dihydrochloride (AAPH), fluorescein sodium salt, potassium persulfate (K2S2O8), caffeic acid (≥98% HPLC), hesperidin (≥97% HPLC), luteolin-7-O-glucoside (≥98% HPLC) and rosmarinic acid (RA) (≥98% HPLC) were obtained from Sigma-Aldrich (Milan, Italy).

2.2. Samples

The fresh aromatic herb wastes of O. basilicum L. type "Genovese", R. officinalis L. and S. officinalis L. were collected at Azienda Agricola Nicola Palma (Capaccio, Salerno, Italy), a farm specialized in the production of packaged fresh herbs for retail chains.

Steam distillations of waste materials were performed with industrial scale essential oil extractor (Tred Technology, Campobasso, Italy) operating to low processing temperatures (75 °C) obtained with an integrated vacuum system. The extractor system was loaded with 6 L of water and 5 kg of each waste material (three independent extractions), homogeneously distributed and compacted on perforated grids to ensure the spreading of steam over the entire load. The steam distillations were carried out for 1 h from the appearance of the first drops of the distillate. The cooled DWW samples (3.4–4.5 L, clear aqueous soltions) were filtrated immediately through 1.0 μm glass fiber filters (circles size 4.7 cm, Millipore, Bedford, USA) to remove residual plant materials, added with ethanol (1%, v/v) and stored at 4 °C until analyses.

2.3. HPLC-DAD-HRMS analysis

Chromatographic analyses were performed using a Platin Blue UHPLC system (Knauer, Labservice Analytica, Bologna, Italy), consisting of two Ultra High-Pressure Pumps, an autosampler, a column temperature manager and a diode array detector, coupled to a LTQ OrbiTrap XL mass spectrometer (ThermoFisher Scientific, Milan, Italy).

A Hibar Purospher® STAR, RP-18 endcapped (3 mm×150 mm, 3 μm; Merck) column was used at a flow rate of 300 μL·min−1at 25 °C.

Volume of the injection was 5 μL. The mobile phase was a binary gradient of water (A) and MeCN (B), both containing 0.1%, v/v, formic acid. The gradient elution program is as follows: 0–1 min, 5% B; 1–5 min, 5–20% B; 5–6.5 min, 20% B; 6.5–15, 20–24% B; 15–19 min, 24% B; 19–23 min, 24–30% B; 23–26 min, 30% B; 26–38 min, 30–95% B; 38–39 min, 95–98% B; 39–45 min, 98% B; 45–46 min, 98–5% B; 46–52 min, 5% B. UV spectra were acquired in the range of 200–600 nm, and the wavelengths 245, 280, 325 and 350 nm were employed for the detection. The mass spectrometer, equipped with ESI source, was operated in negative mode. High purity nitrogen (N2) was used as sheath gas (30 arbitrary units) and auxiliary gas (10 arbitrary units). High purity helium (He) was used as collision gas. Mass spectrometer parameters were as follows: source voltage 4.0 kV, capillary voltage −33 V, tube lens voltage −41.5 V, capillary temperature 300 °C. MS spectra were acquired by full range acquisition covering 140–1500 m/z. For fragmentation study, a data dependent scan was performed and the normalized collision energy of the collision-induced dissociation (CID) cell was set at 30 eV and the isolation width of precursor ions was set at m/z 2.0. The resolution was 60,000 and 7500 for the full mass and the data dependant MS scan, respectively. Phenolic compounds were characterized according to the corresponding spectral characteristics: UV and mass spectra, accurate mass, characteristic fragmentation, and retention time. Xcalibur software (version 2.2) was used for instrument control, data acquisition and data analysis.

2.4. Determination of RA by HPLC-UV

The quantitative analyses were performed using a Dionex Ultimate 3000 UHPLC system (ThermoFisher Scientific) constituted of an Ultimate 3000 RS Pump, an Ultimate 3000 RS autosampler, an Ultimate 3000 RS column compartment and Ultimate 3000 RS variable

wavelength detector. The chromatographic conditions were the same as those used for HPLC‑DAD-HRMSn analysis. The UV chromatograms were recorded at 325 nm and calibration external standard method was used to quantify RA in DWWs. Seven different RA concentrations were prepared diluting with water appropriate volumes of RA stock solution (4 mg·mL−1, MeOH). Linearity of calibration curves were evaluated in the concentration range of 2‑300 μg·mL−1 and triplicate injections for each level. UV peak areas of the external standards were plotted against the corresponding standard concentrations (μg·mL−1). The regression curves were tested with the analysis of variance (ANOVA) and linear model was found appropriate over the tested concentration range (y = 0.9072 × −5.0669; R2 =0.9989). DWWs were diluted with water for quantification analyses, and the RA amount was finally expressed as mg/100 mL.

2.5. Determination of total phenolic content (TPC) by Folin-Ciocalteu assay TPC of DWWs was determined using the Folin-Ciocalteu (FC) colorimetric method according to the literature (Singleton, Orthofer, & Lamuela-Raventós, 1999). Briefly, 20 μL of diluted DWW samples and 5 μL of FC reagent were added to 145 μL of ultrapure water in a 96-well microplate. Then 30 μL of Na2CO3 (20%, w/v) were added at each well and the reaction mixtures were incubated at 25 °C for 45 min and then absorbances were read at 725 nm with a Multiskan Go microplate spectrophotometer (Thermo Fisher Scientific). Gallic acid (GA) was used as reference standard and TPC was estimated from the calibration curve (range 5‑200 μg/mL−1, seven levels; Abs = 0.0037 × μg·GA/mL + 0.0014; R2= 0.9992). Data were expressed as GA equivalents (mg GAE) per 100 mL of DWW.

2.6. Antioxidant capacity assays

DPPH% scavenging assay (Brand-Williams, Cuvelier, & Berset, 1995), ABTS%+ scavenging capacity assay (Re et al., 1999; Sánchez-Camargo et al., 2016) and oxygen radical absorbance capacity (ORAC) assay (Ou, Chang, Huang, & Prior, 2013) were carried out according to the literature and adapted for use in 96-well plates. Detailed experimental conditions are reported in Supplementary data.

2.7. Trolox equivalent antioxidant capacity (TEAC) calculation DPPH, ABTS and ORAC assay results were expressed as TEAC per 100 mL of DWW (μmol TE/100 mL) or per μmol of pure compound (μmol TE/μmol).

For DPPH and ABTS assays, the Trolox, standard and DWW sample curves were obtained by plotting concentration (mL/L for DWWs and μM for Trolox and standards) against the average %Inhibition of radical absorbances ((Abscontrol−Abssample) / Abscontrol×100). Concentrations corresponding to %Inhibition of 50 (50%I) were extrapolated from curves and TEAC was calculated as:

Trolox concentration50%I / Sample concentration50%I × 100 mL. In ORAC assay, the net AUC vs concentration curves were considered,and TEAC values were calculated as: Trolox concentrationnet AUC / Sample concentration net AUC ×100 mL.

2.8. Statistical analysis

The results are expressed as mean ± standard deviation (SD) of three independent steam distillations. All data were analyzed using the software Statgraphic Centurion XVI Version 16.1 from Statistical Graphics (Rockville, MD, USA). Shapiro-Wilk and Cochran tests were applied to check the normality and the homoscedasticity of variances at a significance level 0.05. The results obtained in the DWW samples characterization (TPC, RA levels, TEAC of ORAC, DPPH and ABTS assays) were submitted to a multiple sample comparison procedure (ANOVA, Multiple Range Tests) to evaluated statistical differences, and Tukey method was used to discriminate among the groups. Correlations between variables (TPC and RA contents and

TEAC values) were assessedusing Pearson's correlation coefficients (Multiple-Variable Analysis procedure).

3. Results and discussion

3.1. HPLC-DAD-HRMS analysis of DWWs

The qualitative profiles of DWWs were determined by HPLC-DADHRMS.

The HPLC conditions were optimized to obtain maximal chromatographic resolution and MS signal. Fig. 1 shows representative chromatograms of BP-, RL- and SL-DWWs under optimal conditions. In the MS analysis, both negative and positive ion modes were tested, and the results showed that the most of DWW compounds exhibited higher responses in negative ion mode. Anyway, both ESI modes were employed to establish the molecular formulas.

Metabolite assignments were made comparing retention time, UV/Vis and MS data of detected compounds with standard compounds, whenever available, or interpreting MS data (accurate masses and MS/MS fragment ions) combined with chemo-taxonomic data reported in the literature and databases.

HPLC-DAD-HRMS analysis of DWWs allowed to identify 76 secondary metabolites (36 in B-DDW, 52 in R-DWW and 45 in S-DWW), belonging to three main classes: phenolic acids, flavonoids and phenolic diterpenes (Table 1).

3.1.1. Phenolic acids

Phenolic acids, and particularly caffeic acid (CA) derivatives, resulted the predominant group of DWWs (Table 1). CA derivatives constitute the major part of the water-soluble compounds of Lamiaceae (Embuscado, 2015; Shan, Cai, Sun, & Corke, 2005; Trivellini et al., 2016; Zheng & Wang, 2001). In this plant family, CA is the building block of a wide variety of metabolites, from simple monomers to multiple oligomers. The latter derived by the condensation of CA and dihydroxyphenyl-lactic acid (danshensu, DSS) or by Diels Alder reaction between the double bond of CA and phenolic rings (Lu & Foo, 2002). CA derivatives identified in DWWs were grouped in monomers and oligomers (2–8 units), based on the number of C6-C3 units (Table 1).

In addition to CA (20) and DSS (1), other CA monomer derivatives detected in DWWs were two caffeoyl quinic acids (4 and 11), two caffeoyl glycosides (8 and 10), and two tartaric acid esters (6 and 17).

CA dimers and oligomers were characterized mainly by HRMS and MS/MS data that provided useful structural information. The predominant fragmentation patterns of $[M-H]^-$ ions are i) the loss of CO_2 and ii) cleavage of ester bond, characterized by the product ions at m/z 197.0444 (DSS) and 179.0339 (CA), and the neutral losses of 198 (DSS, $C_9H_{10}O_5$), 180 (CA, $C_9H_8O_4$) and 162 Da (CA − H_2O, $C_9H_6O_3$) (Chen et al., 2011; Liu et al., 2007). Particularly, the product ions produced from cleavage of ester bond allowed to establish the sequence of CA and DSS unit in oligomers, while fragment relative abundances were useful to distinguish the oligomers according to the presence and position of cyclic ether structures (Chen et al., 2011; Liu et al., 2007).

RA (47) was the main dimer of DWWs. Other RA analogues were 13, danshensuan C (18) and prolithospermic acid (25). In addition, RAhexose (33) and chicoric acid (32) were identified as CA dimer derivatives of DWWs.

Eleven trimers and seven tetramers were also detected in DWWs (Table 1). In the case of trimers, six compounds derived from the condensation of RA and CA were tentatively identified as four $C_{27}H_{22}O_{12}$ isomers (salvianolic acid H or I, 26; salvianolic acid J or clinopodic acid E isomers, 24 and 29; lithospermic acid, 48) and two their decarboxylated derivatives (salvianolic acid C, 64; isosalvianolic acid C, 66). Again, two salvianolic acid K isomers (27 and 46), generated from the condensation of RA and DSS, and yunnaneic acid F (30) were characterized. Tetramers detected in DWWs were all RA dimers ($C_{36}H_{30}O_{16}$, 37, 40, 52, 67 and 69,

salvianolic acid B or E isomers, and 42, clinopodic acid I), with the exception of sagerinic acid ($C_{36}H_{32}O_{16}$, 38), a RA dimer derived by a [2 + 2] union of olefinic moieties (Ribeiro et al., 2016).

Finally, some higher oligomers of CA (2 hexamers, 50 and 63; 1 heptamer, 65; and 1 octamer, 68) were detected only in B-DWW, and they were tentatively identified as clinopodic acids previously reported in Lamiaceae (Aoshima, Miyase, & Warashina, 2012; Moghadam et al., 2015).

As an example for the illustration of the fragmentation patterns of CA oligomers, the structural characterization of CA octamer 68 is described.

Based on the ions at m/z 716.1391 ([M − 2H]2−) and 1433.2834 ([M − H]−) in (−)-HRMS spectrum (Supplemental Fig. 1A), the molecular formula of $C_{72}H_{58}O_{32}$ was assigned to compound 68, which corresponds to clinopodic acids L and P in the databases (Aoshima et al., 2012; Moghadam et al., 2015). The analysis of fragmentation pattern allowed to discriminate between the two isomers and to assign the structure of clinopodic acid P to the peak 68. In fact, its MS/MS spectrum (Supplemental Fig. 1B) was characterized by the sequential loss of two terminal units of DSS: [M-H-DSS]− at 1235.2351 ($C_{63}H_{47}O_{27}$, 4.2 ppm), [M-2H-DSS]2− at 617.1117 ($C_{63}H_{46}O_{27}$, 1.5 ppm); ([M-H-2DSS]− at 1037.1794 ($C_{54}H_{37}O_{22}$, 2.2 ppm), [M-2H-2DSS]2− at 518.0855 ($C_{54}H_{36}O_{22}$, 2.2 ppm). The product ions related to the loss of a terminal CA unit ([M-H-CA]−, ([M-2H-CA]2−or ([M-H-CA-H2O]−), present in clinopodic acid L (Aoshima et al., 2012), instead were absent. Fig. 2 display the proposed fragmentation pathway of clinopodic acid P (68).

Likewise, the trimer isomers 24, 26, 29 and 48 ($C_{27}H_{22}O_{12}$) were differentiated by the analysis of MS/MS spectra (Supplemental Fig. 2).

They showed different relative intensities of the product ion [M-H-CO2]− (m/z 493.1129, $C_{26}H_{21}O_{10}$) due to the position of carboxyl group in the molecule. In the case of 24, 29 and 48, the presence of the carboxyl group on a heterocycle strongly favours the elimination of CO2 for electron-withdrawing effect of the oxygen atom (Chen et al., 2011). Thus, [M-H-CO2]− ion was the base peak in the MS/MS spectra of 24 and 29 (Supplemental Fig. 2), and it was already present in the HRMS spectrum of 48. In contrast, for trimer 26 the CO2 loss was not so favoured, suggesting the absence of the heterocycle in the structure.

The base peak in its MS/MS spectra (Supplemental Fig. 2) corresponded to the direct loss of DSS ([M-H-DSS]− at m/z 339.0502, $C_{18}H_{11}O_7$, 0.8 ppm), differently from 48, 24 and 29 that showed exclusively the product ion [M-H-CO2-DSS]− at m/z 295.0603 ($C_{17}H_{11}O_5$, 1.3 ppm) (Supplemental Fig. 2). Based on these evidences, the trimer isomers were tentatively identified as lithospermic acid (48), salvianolic acid J or clinopodic acid E isomers (24 and 29) and salvianolic acid H or I (26).

3.1.2. Flavonoids

Flavonoids constitute the second most representative class of metabolite secondary of DWWs. Except cirsimaritin (70), all detected flavonoids were glycosilated derivatives (Table 1). Flavones were the main components (16 of 20 flavonoids). Particularly, seven glycosylated derivatives of luteolin (28, 31, 34, 43, 49, 53and59) were identified, including two acetyl (53 and 59) and one malonyl (43) derivatives.

Other characteristic flavone glycosides of Lamiaceae, detected in R- and S-DWWs, were nepitrin (35), diosmin (39), homoplantaginin (45) and cirisimarin (58). Finally, a caffeoyl flavone hexoside (55) was characterized in R-DWW, and the structure of gnaphaloside A (Olennikov, Chirikova, & Kashchenko, 2015) was hypothesized by analysis of HRMS/MS data. The MS/MS spectrum (Supplemental Fig. 3) of [M-H]− ion at m/z 653.1515 ($C_{32}H_{29}O_{15}$) showed fragments at m/z 329.0660 ($C_{17}H_{13}O_7$, 0.6 ppm) and 323.0767 ($C_{15}H_{15}O_8$, 0.5 ppm) produced by glycosidic bond cleavage and corresponding to [Aglycone-H]− and [Caffeoyl-

hexose−H2O−H]− ions, respectively. Gnaphaloside A (55) is reported for the first time in R. officinalis.
Other flavonoids present in DWWs were the flavanone hesperidin (41) and three quercetin derivatives (21, 22 and 36).
3.1.3. Phenolic diterpenes
Six phenolic diterpenes (71−76) were detected as minor components of DWWs (Table 1). Their proposed structures (epirosmanol/rosmanol for 71 and 72, rosmadial for 73, carnosol for 74, carnosic acid for 75 and methyl carnosate for 76) (Borrás-Linares et al., 2014) were assumed by molecular formulas, diagnostic product ions [M−H−CO2]−and [M−H−H2O]−, and occurrence data of Lamiaceae (mainly Rosmarinus e Salvia spp).
3.2. DWWs profiling
The distribution of identified metabolites in basil (BL and BP), rosemary (RL) and sage (SL and SP) DWWs is reported in Table 1. As shown in HPLC-UV profiles of DWWs (Fig. 1), the water soluble compounds (phenolic acids and flavonoid glycosides) constitute the major part of these aqueous matrices, although less polar compounds, like phenolic diterpenes, were also found to low levels.
In detail, RA (47) was the most abundant components of all DWWs, with DSS (1) as further main compounds, according to literature data on water soluble components of Lamiaceae family (Cvetkovikj et al., 2013; Kwee & Niemeyer, 2011; Ribeiro et al., 2016; Shan et al., 2005; Zheng & Wang, 2001; Zimmermann, Walch, Tinzoh, Stühlinger, & Lachenmeier, 2011). Other constituents common to all samples were minor compounds, mainly CA derivatives (18, 20, 25, 30, 37 and 38) and phenolic diterpenes (71−74 and 76) (Table 1).
Basil DWWs showed the most distinctive chemical profile (Fig. 1).
Unlike from R- and S-DWWs, it consisted almost entirely of CA derivatives (Table 1). Flavonoids were not detected in B-DWWs, with the exception of vicenin 2 (12), that was present only in traces. Along with RA (47) and DSS (1), caftaric (6) and chicoric (32) acids were the main components of B-DWWs. Chicoric acid (32) has been reported as the second most prevalent basil phenolic compound (Lee & Scagel, 2009) at concentrations below 0.3% dry weight (Kwee & Niemeyer, 2011). The caffeoyl-tartaric acids 6 and 32 were not detected in R- and S-DWWs, therefore they could be considered as quantitative markers of B-DWWs.
Others characteristic compounds of B-DWWs were clinopodic acid I (42) and CA oligomers with n > 5(50, 63, 65 and 68). The latter are rare CA derivatives, recently isolated from two species of Lamiaceae (Aoshima et al., 2012; Moghadam et al., 2015), but reported here for the first time in O. basilicum L..
HPLC profiles of R- and S-DWWs (Fig. 1) disclosed more complex compositions than to B-DWW (Table 1). The flavonoid fraction was well represented in these DWW samples, and lipophilic components, mainly phenolic diterpenes, were also appreciable.
Besides RA (47), the most abundant and representative components of RL-DWW were the flavonoids luteolin-3′-O-glucuronide (49), nepitrin (35), hesperidin (41) and homoplantaginin (45), typically found in rosemary (Borrás-Linares et al., 2014), and the phenolic compounds salvianolic acid K isomer (27), yunnaneic acid F (30) and RA-glucoside (33). Relevant levels of 30 and 33 were also observed in R. officinalis aqueous extract (Ribeiro et al., 2016). Instead, salvianolic acid K isomer (27) has never been reported in rosemary.
On the other hand, quercetin-O-glucuronide (21), luteolin-7-O-glucoside (31), luteolin-7-O-glucuronide (34) and salvianolic acid K (46) constituted the more distinctive constituents of S-DWWs. These compounds and RA were the most frequently detected components in aqueous infusions of S. officinalis (Zimmermann et al., 2011) and culinary Salvia species (Cvetkovikj et al., 2013).
The results of DWW profiling showed a chemical composition very similar to the polar extracts of basil, rosemary and sage (Cvetkovikj et al., 2013; Kwee & Niemeyer, 2011; Ribeiro et al.,

2016; Shan et al., 2005; Zheng & Wang, 2001; Zimmermann et al., 2011), indicating that their hydrophilic compounds (extracted by the condensed water during steam distillation of essential oils) are contained in these wastewaters.

3.3. Quantitative analysis of DWWs

HPLC profiling revealed that RA (47) was the dominant phenolic compound of DWWs, according to the literature data (Cvetkovikj et al., 2013; Kwee & Niemeyer, 2011; Ribeiro et al., 2016; Shan et al., 2005; Zheng & Wang, 2001; Zimmermann et al., 2011). In fact, O. basilicum, R.
officinalis and S. officinalis are among the Lamiaceae species containing RA as the main phenolic compound and at concentration above 0.5% dry weight (Trivellini et al., 2016). Thus, RA amount represents a useful quantitative marker for the characterization of DWWs.

Moreover, based on overall chemical composition and the complexity of investigated DWWs, also the total phenolic content (TPC) is a suitable index for the standardization of DWWs. In fact, TPC value is widely used in the routine quality control and measurement of antioxidant capacity of food products, plants and dietary supplements.

Hence, in order to evaluate the phenolic content of DWWs, RA amount and TPC were determined by HPLC-UV analysis and Folin-Ciocalteu assay (Singleton et al., 1999), respectively. Quantitative data are reported in Table 2.

Consistent differences ($p < 0.05$) in RA levels were observed among the different DWWs, with the highest level in SL-DWW, followed by BP-DWW, R-DWW/SP-DWW and BL-DWW (Table 2). Zimmermann et al. reported for aqueous infusion of dried sage leaves RA content (1.2–29.6 mg/100 mL) much lower compared to aromatic herb DWWs (29–135 mg/100 mL) (Zimmermann et al., 2011). The same trend was observed for TPC values that decreased in the following order: SLDWW > BP-DWW = R-DWW > SP-DWW > BL-DWW ($p < 0.05$) (Table 2). TPC values of studied DWWs (152–443 mg GAE/100 mL) were higher than of O. basilicum (15.5 mgGAE/100 mL), R. officinalis (19 and 36 mg GAE/100 mL) and S. officinalis (14–73 and 43 mgGAE/100 mL) infusions (Gião et al., 2007; Tahirović et al., 2014).

These results show clearly that a large amount of phenolic compounds, and particularly RA, is contained in DWWs. Therefore, they are a rich source of RA and/or hydrophilic phenolic compounds of aromatic herbs.

3.4. Antioxidant capacity of DWWs

Extensive studies demonstrated that the Lamiaceae herbs, such as rosemary, sage, oregano, mint, and thyme, have very strong antioxidant activities (Embuscado, 2015; Trivellini et al., 2016), and their use has been reported to improve the shelf life of meat- and fish-products and lipid containing foods (Trivellini et al., 2016; Yanishlieva, Marinova, & Pokorný, 2006). These properties offer a possibility to use phenolic compounds of aromatic herbs, or extracts rich in them, as food additives to increase the shelf life of food products.

In this context, the in vitro antioxidant capacity (AOC) of aromatic herb DWWs was investigated as preliminary assessment of their potential as source of antioxidant compounds. The majority of the AOC assays are based on single electron/hydrogen atom transfer reactions (SET/HAT). ORAC (HAT based assay), TPC (SET based assay), and one of the SET/HAT based assays (DPPH and ABTS assays) are recommended for the representative evaluation of antioxidant properties (Prior, Wu, & Schaich, 2005).

AOC of DWWs was evaluated using ORAC, DPPH and ABTS assays.

The results, expressed in term of TEAC (μmol TE/100 mL of DWWs), are reported in Fig. 3. AOCs of RA and BHA, an authorized food antioxidant, were also determined by these three AOC assays. Their TEAC values (RA: 5.9, 1.8 and 2.2 μmol TE/μmol; BHA: 2.1, 1.1 and 0.7 μmol TE/μmol, in ORAC, DPPH and ABTS assay, respectively) agreed with literature data (Brand-Williams et al., 1995), showing the accuracy of utilized AOC procedures.

Results of AOC assays indicated that SL-DWW (4720, 4244 and 3145 μmol TE/100 mL in ORAC, DPPH and ABTS assay, respectively) exhibited the most powerful AOC ($p > 0.05$), over 4‑7 fold greater than BL-DWW (1101, 635 and 571 μmol TE/100 mL in ORAC, DPPH and ABTS assay, respectively). BP-DWW(2213, 2250 and 1445 μmol TE/100 mL in ORAC, DPPH and ABTS assay, respectively) and RLDWW (3175, 2307 and 1441 μmol TE/100 mL in ORAC, DPPH and ABTS assay, respectively) showed comparable AOCs in DPPH and ABTS assays ($p > 0.05$), whereas RL-DWW was significantly ($p > 0.05$) more potent than BP-DWW in ORAC assay (Fig. 3). It could be due to the differences observed in their chemical composition. In RL-DWW, the relative level of flavonoids respect to RA (Fig. 1) could contributed significantly to its antioxidant activity in the ORAC assay, in addition to RA.
Overall, DWWs demonstrated strong antioxidant capacities in comparison with aqueous matrices with well-known antioxidant properties: in DPPH assay SL-, BP- and R-DWWs showed higher AOC than to green tea (644 μmol TE/100 mL) and red wine (1935 μmol TE/100 mL), and comparable to pomegranate juice (3901 μmol TE/100 mL) (Plank et al., 2012). Also ORAC values indicated AOC higher than to Lamiaceae herbal infusions (303‑1322 μmol TE/100 mL) and comparable to bearberry infusion (4076 μmol TE/100 mL) (Tahirović et al., 2014).
Basically AOC data showed the same trend observed for TPC and RA levels of DWWs (Table 2), indicating that the samples with higher TPC and RA levels were generally the most active in terms of antioxidant capacity. Phenolic compounds and RA are closely associated with antioxidant activity of Lamiaceae species (Embuscado, 2015; Shan et al., 2005). So, the relationship between TEAC values and quantitative data (TPC and RA contents) of DWWs was statistically examined by Pearson's correlation coefficients (Fig.4). For all antioxidant assays, highly significant correlations between TEAC and TPC were observed (Pearson's coefficients> 0.935 and $p < 0.001$). Also RA levels showed high significant correlation (Pearson's coefficients> 0.875 and $p < 0.001$) with AOC data (Fig. 4). These correlations confirmed that phenolic compounds in DWWs were responsible for their antioxidant activity, according to literature data (Embuscado, 2015; Shan et al., 2005). In addition, the results highlighted the importance of RA in the antioxidant activity of DWWs and, generally, in aromatic herbs.

4. Conclusions

Basil, rosemary and sage DWWs, generated by the essential oil steam distillation of materials discarded from chain production of packaged fresh herbs, represent an exceptionally rich source of antioxidant phenolic compounds.
The water-soluble components of Lamiaceae constitute the major components of DWWs, with caffeic acid derivatives and flavones glycosides as the most representative classes of secondary metabolites.
Overall, DWWs contained large amounts of rosmarinic acid and phenolic compounds, closely associated with the strong antioxidant capacities demonstrated in three in vitro AOC methods. These results confirm the role of the hydrophilic phenolic compounds in the antioxidant activity of aromatic herbs, and highlight the importance of RA in the DWW properties.
Thus, these residual wastewaters should be considered as essential oil distillation coproducts rich of natural antioxidants, especially rosmarinic acid. A multistep biorefining scheme to recovery coproducts with high added-value from packaged fresh herbs production may substantially increase process effectiveness and reduce the amount of wastes.
DWWs, or their products derived from concentration or purification processes, could be employed as additives to prevent the oxidation and/ or microbiological degradation of foodstuffs, but also as functional ingredients for cosmetic, nutraceutical and food applications.

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Table 1
UHPLC-HRMS data of compounds detected in basil (B), rosemary (R) and sage (S) DWWs.

N[a]	RT (min)	$[M-H]^-$ (*m/z*)	Molecular formula	Error (ppm)	Diagnostic product ions (*m/z*)[b]	Compound[c]	DWW	Ref.
Hydroxycinnamic acids and derivates								
Caffeic acid monomers								
1	8.3	197.0449	$C_9H_{10}O_5$	2.7	**179** $[M-H-H_2O]^-$, 135 $[M-H-H_2O-CO_2]^-$	Dihydroxyphenyllactic acid (DSS)	B, R, S	1
4	9.0	353.0868	$C_{16}H_{18}O_9$	0.3	**191** $[QA-H]^-$, 179 $[CA-H]^-$, 135 $[CA-H-CO_2]^-$	5-caffeoyl quinic acid[c]	R, S	2
6	9.4	311.0399	$C_{13}H_{12}O_9$	0.4	179 $[CA-H]^-$, **149** $[M-H-CA]^-$	Caftaric acid	B	3
8	9.5	503.1385	$C_{21}H_{28}O_{14}$	− 2.1	**341** [M-H-Hex], 179 [CA-H], 161 $[CA-H-H_2O]$	Caffeoyl-hexosyl-hexose	R, S	4
10	9.8	341.0862	$C_{15}H_{18}O_9$	− 1.3	323 $[M-H-H_2O]^-$, 281 $[M-H-C_2H_4O_2]^-$, 251 $[M-H-C_3H_6O_3]^-$, 233 $[M-H-C_3H_8O_4]^-$, **203** $[M-H-C_4H_{10}O_5]^-$, 179 $[CA-H]^-$	4-caffeoyl-hexose	R, S	5
11	10.0	353.0867	$C_{16}H_{18}O_9$	− 0.1	191 $[QA-H]^-$, 179 $[CA-H]^-$, **173** $[QA-H-H_2O]^-$, 135 $[CA-H-CO_2]^-$	4-caffeoyl quinic acid	R, S	2
17	11.0	325.0554	$C_{14}H_{14}O_9$	0.0	**193** $[M-H-C_4H_4O_5]^-$	Fertaric acid	B, R	6
20	11.4	179.0344	$C_9H_8O_4$	2.8	**135** $[M-H-CO_2]^-$	Caffeic acid (CA)[c]	B, R, S	
Caffeic acid dimers								
13	10.5	355.0446	$C_{18}H_{12}O_8$	− 0.7	**311** $[M-H-CO_2]^-$, 287 $[M-H-CO_2-H_2O]^-$, 267 $[M-H-2CO_2]^-$	Dimer	B	
18	11.2	377.0866	$C_{18}H_{18}O_9$	− 0.2	**359** $[M-H_2O]^-$, 197 $[DSS-H]^-$	Danshensuan C	B, R, S	6
25	12.6	357.0601	$C_{18}H_{14}O_8$	− 1.0	313 $[M-H-CO_2]^-$, **269** $[M-H-2CO_2]^-$	Prolithospermic acid isomer	B, R, S	7
32	14.7	473.0722	$C_{22}H_{18}O_{12}$	1.7	**311** $[M-H-C_9H_6O_3]^-$, 293 $[M-H-CA]^-$, 179 $[CA-H]^-$	Chicoric acid	B	3
33	14.8	521.1283	$C_{24}H_{26}O_{13}$	− 1.3	503 $[M-H-H_2O]^-$, **359** $[RA-H]^-$	Rosmarinic acid-glucoside	R	8
47	19.7	359.0761	$C_{18}H_{16}O_8$	− 0.2	197 $[DSS-H]^-$, 179 $[CA-H]^-$, **161** $[M-H-DSS]^-$	Rosmarinic acid (RA)[c]	B, R, S	
Caffeic acid trimers								
19	11.4	537.1014	$C_{27}H_{22}O_{12}$	− 2.4	519 $[M-H-H_2O]^-$	Trimer	S	
24	12.3	537.1015	$C_{27}H_{22}O_{12}$	− 2.4	**493** $[M-H-CO_2]^-$, 359 $[RA-H]^-$, 357 $[M-H-CA]^-$, 313 $[M-H-CA-CO_2]^-$, 295 $[M-H-DSS-CO_2]^-$	Salvianolic acid J/Clinopodic acid E	S	1, 9
26	13.0	537.1017	$C_{27}H_{22}O_{12}$	− 1.9	493 $[M-H-CO_2]^-$, **339** $[M-H-DSS]^-$, 295 $[M-H-DSS-CO_2]^-$	Salvianolic acid H/I	B, R	1
27	13.2	555.1124	$C_{27}H_{24}O_{13}$	− 1.7	537 $[M-H-H_2O]^-$, **493** $[M-H-H_2O-CO_2]^-$, 359 $[RA-H]^-$	Salvianolic acid K isomer	B, R	10
29	14.0	537.1015	$C_{27}H_{22}O_{12}$	− 2.2	**493** $[M-H-CO_2]^-$, 313 $[M-H-CA-CO_2]^-$, 295 $[M-H-DSS-CO_2]^-$	Salvianolic acid J/Clinopodic acid E	B, R	1, 9
30	14.2	597.1224	$C_{29}H_{26}O_{14}$	− 2.4	329 $[M-H-C_{15}H_8O_5]^-$; **311** $[M-H-C_{15}H_{10}O_6]^-$; 197 $[DSS-H]^-$; 179 $[CA-H]^-$	Yunnaneic acid F	B, R, S	11
44	18.6	537.1023	$C_{27}H_{22}O_{12}$	− 0.9	**519** $[M-H-H_2O]^-$, 493 $[M-H-CO_2]^-$, 359 $[RA-H]^-$, 357 $[M-H-CA]^-$, 339 $[M-H-DSS]^-$	Trimer	S	
46	19.2	555.1124	$C_{27}H_{24}O_{13}$	− 1.5	**537** $[M-H-H_2O]^-$, 493 $[M-H-H_2O-CO_2]^-$, 359 $[RA-H]^-$	Salvianolic acid K	S	10
48	20.1	537.1017	$C_{27}H_{22}O12$	− 1.9	**493** $[M-H-CO_2]^-$, MS^3 (493): 313 $[M-H-CA]^-$, **295** $[M-H-DSS-CO_2]^-$	Lithospermic acid	B, R	1
64	29.9	491.0980	$C_{26}H_{20}O_{10}$	1.5	311$[M-H-CA]^-$, **293** $[M-H-DSS]^-$	Iso/Salvianolic acid C	R	6
66	31.5	491.0983	$C_{26}H_{20}O_{10}$	2.3	311$[M-H-CA]^-$, **293** $[M-H-DSS]^-$	Iso/Salvianolic acid C	R	6
Caffeic acid tetramers								
37	16.4	717.1424	$C_{36}H_{30}O_{16}$	1.3	537 $[M-H-CA]^-$, **519** $[M-H-DSS]^-$, 475 $[M-H-DSS-CO_2]^-$, 339 $[M-H-DSS-CA]^-$	Salvianolic acid E/B isomer	B, R, S	6
38	17.0	719.1589	$C_{36}H_{32}O_{16}$	− 2.4	539 $[M-H-CA]^-$, 521 $[M-H-DSS]^-$, **359** $[RA-H]^-$, 341 $[M-H-DSS-CA]^-$	Sagerinic acid	B, R, S	11
40	17.8	717.1436	$C_{36}H_{30}O_{16}$	− 1.9	673 $[M-H-CO_2]^-$, 537 $[M-H-CA]^-$, **519** $[M-H-DSS]^-$, 493 $[M-H-CA-CO_2]^-$, 475 $[M-H-DSS-CO_2]^-$, 339 $[M-H-DSS-CA]^-$, 321 $[M-H-2DSS]^-$	Salvianolic acid E/B isomer	B, S	6
42	18.5	717.1436	$C_{36}H_{30}O_{16}$	− 1.9	**555** $[M-H-C_9H_6O_3]^-$, 537 $[M-H-CA]^-$, 519 $[M-H-DSS]^-$, 475 $[M-H-DSS-CO_2]^-$, 357 $[M-H-C_9H_6O_3$-DSS$]^-$	Clinopodic acid I	B	12
52	23.9	717.1436	$C_{36}H_{30}O_{16}$	− 1.9	**519** $[M-H-DSS]^-$, 321 $[M-H-2DSS]^-$	Salvianolic acid E/B isomer	B	6
67	32.2	717.1437	$C_{36}H_{30}O_{16}$	− 1.9	**519** $[M-H-DSS]^-$, 339 $[M-H-DSS-CA]^-$	Salvianolic acid E/B isomer	S	6
69	32.6	717.1436	$C_{36}H_{30}O_{16}$	− 1.9	**519** $[M-H-DSS]^-$, 357 $[M-H-DSS-C_9H_6O_3]^-$	Salvianolic acid E/B isomer	B	6
Caffeic acid hexamers								
50	21.1	1075.2151 537.1036[d]	$C_{54}H_{44}O_{24}$	1.6	**877** $[M-H-DSS]^-$, 679 $[M-H-2DSS]^-$, 438 $[M-2H-DSS]^{-2}$, 197 $[DSS-H]^-$	Clinopodic acid O isomer	B	12
63	28.7	1075.2151 537.1036[d]	$C_{54}H_{44}O_{24}$	1.6	**877** $[M-H-DSS]^-$, 679 $[M-H-2DSS]^-$, 438 $[M-2H-DSS]^{-2}$, 197 $[DSS-H]^-$	Clinopodic acid O isomer	B	12
Caffeic acid heptamers								
65	31.3	1253.2408 626.1172[d]	$C_{63}H_{50}O_{28}$			Heptamer	B	
Caffeic acid octamers								
68	32.3	1433.2844 716.1339[d]	$C_{72}H_{58}O_{32}$	3.5	1235 $[M-H-DSS]^-$, 1037 $[M-H-2DSS]^-$, 877 $[M-H-2DSS-C_9H_4O_3]^-$, 679 $[M-H-3DSS-C_9H_4O_3]^-$, **617** $[M-H-2H-DSS]^{-2}$, 518 $[M-H-2H-2DSS]^{-2}$, 357 ($C_{18}H_{13}O_8$, 2.0 ppm)	Clinopodic acid P	B	12
Flavonoids								
Flavanones derivates								
41	17.9	609.1802	$C_{28}H_{34}O_{15}$	− 1.9	**301** ($C_{16}H_{13}O_6$, 2.7 ppm)	Hesperidin[c]	R, S	
Flavones derivates								
12	10.2	593.1495	$C_{27}H_{30}O_{15}$	− 0.9	575 $[M-H-H_2O]^-$, 503 $[M-H-C_3H_6O_3]^-$, **473** $[M-H-C_4H_8O_4]^-$, 383	Vicenin II	B, R, S	13

(continued on next page)

Table 1 *(continued)*

N[a]	RT (min)	$[M-H]^-$ (*m/z*)	Molecular formula	Error (ppm)	Diagnostic product ions (*m/z*)[b]	Compound[c]	DWW	Ref.
28	13.4	593.1491	$C_{27}H_{30}O_{15}$	− 1.6	**285** ($C_{15}H_9O_6$, 2.1 ppm)	Luteolin-7-O-rutinoside	R, S	14
31	14.6	447.0913	$C_{21}H_{20}O_{11}$	− 1.8	**285** ($C_{15}H_9O_6$, 1.6 ppm)	Luteolin-7-O-glucoside[c]	R, S	14
34	14.9	461.0707	$C_{21}H_{18}O_{12}$	− 1.6	**285** ($C_{15}H_9O_6$, 1.2 ppm)	Luteolin-7-O-glucuronide	R, S	14
35	15.4	477.1020	$C_{22}H_{22}O_{12}$	− 1.6	462 $[M-H-CH_3]^-$, **315** ($C_{16}H_{11}O_7$. 1.2 ppm), 300 [$C_{16}H_{11}O_7$-CH_3]$^-$	Nepitrin	R, S	8
39	17.6	607.1646	$C_{28}H_{32}O_{15}$	− 1.9	**299** ($C_{16}H_{11}O_6$, 1.1 ppm), 284 [$C_{15}H_8O_6$-CH_3]$^-$	Diosmin	R, S	14
43	18.6	533.0917	$C_{24}H_{22}O_{14}$	− 1.6	515 $[M-H-H_2O]^-$, **489** $[M-H-CO_2]^-$, 285 ($C_{15}H_9O_6$, 2.5 ppm)	Luteolin-O-malonylhexoside	S	15
45	19.1	461.1071	$C_{22}H_{22}O_{11}$	− 1.4	446 $[M-H-CH_3]^-$, **299** ($C_{16}H_{11}O_6$, 1.3 ppm), 284 [$C_{16}H_{11}O_6$-CH_3]$^-$	Homoplantaginin	R, S	8
49	20.4	461.0707	$C_{21}H_{18}O_{12}$	− 1.5	**285** ($C_{15}H_9O_6$, 2.1 ppm)	Luteolin-3′-O-glucuronide	R	8
53	25.3	503.0832	$C_{23}H_{20}O_{13}$	2.5	443 $[M-H-CH_3CO_2H]^-$, 399 $[M-H-CH_3CO_2H-CO_2]^-$, **285** ($C_{15}H_9O_6$, 2.5 ppm)	Luteolin-*O*-acetylglucuronide	R	8
54	25.7	491.1199	$C_{23}H_{24}O_{12}$	3.1	**329** ($C_{17}H_{13}O_7$, 0.7 ppm)	Trihydroxy-dimethoxyflavone-hexoside	R	16
55	25.7	653.1515	$C_{32}H_{30}O_{15}$	2.2	329 [caffeoyl hexose-H-H_2O]$^-$, **323** ($C_{15}H_{15}O_8$, 0.5 ppm), 300 [$C_{15}H_{15}O_8$-$2CH_3$]$^-$	Gnaphaloside A	R	17
57	25.9	491.1182	$C_{23}H_{24}O_{12}$	− 0.3	**329** ($C_{17}H_{13}O_7$, 1.1 ppm)	Trihydroxy-dimethoxyflavone-hexoside	R	16
58	26.3	475.1244	$C_{23}H_{24}O_{11}$	1.9	313 ($C_{17}H_{14}O_6$, 2.0 ppm)	Cirsimarin	R	8
59	26.4	503.0824	$C_{23}H_{20}O_{13}$	0.1	443 $[M-H-CH_3CO_2H]^-$, 399 $[M-H-CH_3CO_2H-CO_2]^-$, **285** ($C_{15}H_9O_6$, 2.5 ppm)	Luteolin-*O*-acetylglucuronide	R	8
70	35.6	313.0712	$C_{17}H_{14}O_6$	1.6	**298** $[M-H-CH_3]^-$, 283 $[M-H-2CH_3]^-$	Cirsimaritin	R, S	8
Flavonol derivates								
21	12.0	477.0656	$C_{21}H_{18}O_{13}$	− 1.7	301 ($C_{15}H_9O_7$, 2.3 ppm)	Quercetin-O-glucuronide	R, S	14
22	12.1	463.0861	$C_{21}H_{20}O_{12}$	− 2.3	301 ($C_{15}H_9O_7$, 2.5 ppm)	Quercetin-O-hexose	R, S	14
36	15.7	491.0812	$C_{22}H_{20}O_{13}$	− 1.6	315 ($C_{16}H_{11}O_7$, 2.0 ppm), 300 [$C_{16}H_{11}O_7$-CH_3]$^-$	Isorhamnetin-O-hexose	R, S	14
Diterpene phenols								
71	36.1	345.1698	$C_{20}H_{26}O_5$	0.5	**283** $[M-H-CO_2]^-$, 301 $[M-H-H_2O]^-$	Rosmanol/Epirosmanol	B, R, S	8
72	36.6	345.1722	$C_{20}H_{26}O_5$	4.5	**283** $[M-H-CO_2]^-$, 301 $[M-H-H_2O]^-$	Rosmanol/Epirosmanol	B, R, S	8
73	38.7	343.1539	$C_{20}H_{24}O_5$	− 0.1	**299** $[M-H-CO_2]$, 315 [M-H-CO]	Rosmadial	B, R, S	8
74	39.4	329.1747	$C_{20}H_{26}O_4$	0.0	**285** $[M-H-CO_2]^-$	Carnosol	B, R, S	8
75	41.1	331.1902	$C_{20}H_{28}O_4$	− 0.6	**287** $[M-H-CO_2]^-$	Carnosic acid	R, S	8
76	41.9	345.2058	$C_{21}H_{30}O_4$	− 0.7	**301** $[M-H-CO_2]^-$, 286 $[M-H-CO_2-CH_3]^-$	Methyl carnosate	B, R, S	8
Other compounds								
2	7.0	447.1489	$C_{19}H_{28}O_{12}$	− 1.9	**293** $[M-H-C_8H_{10}O_3]^-$, 233 $[M-H-C_{10}H_{14}O_6]^-$, 153 ($C_8H_9O_3$, 9.7 ppm)	Unknown	R	
3	8.7	447.1125	$C_{18}H_{24}O_{13}$	− 1.9	315 $[M-H-Pen]^-$, 297 $[M-H-Pen-H_2O]^-$, 271 $[M-H-Hexu]^-$	Unknown	B, R, S	
5	9.3	449.2006[e]	$C_{20}H_{34}O_{11}$	− 0.3	**223** $[M-H-Hex-H_2O]^-$	Unknown	R	
7	9.4	521.1493	$C_{21}H_{30}O_{15}$	− 1.5	**341** $[M-H-Hex]^-$, 323 [M-H-syringic acid]$^-$, 197 [syringic acid-H]$^-$	Syringoyl dihexoside	B	
9	9.7	417.1018	$C_{17}H_{22}O_{12}$	− 1.9	285 $[M-H-Pen]^-$, **241** $[M-H-Pen-CO_2]^-$, 152 ($C_7H_4O_4$, 10 ppm)	Dihydroxybenzoic acid-dipentoside	B	
14	10.5	387.1644	$C_{18}H_{28}O_9$	− 1.3	369 $[M-H-H_2O]^-$, **207** [tuberonic acid-H-H_2O]$^-$, 163 [tuberonic acid-H-H_2O-CO_2]$^-$	Tuberonic acid-glucoside	B, R, S	18
15	10.6	401.1436	$C_{18}H_{26}O_{10}$	− 1.5	**269** $[M-H-Pen]^-$, 161 ($C_6H_9O_5$, 7.4 ppm)	Icariside F2	R, S	19
16	10.7	385.1853	$C_{19}H_{30}O_8$	− 1.1	**223** $[M-H-Hex]^-$, 205 $[M-H-Hex-H_2O]^-$	Roseoside A	B, R, S	20
23	12.3	377.1803	$C_{17}H_{30}O_9$	− 0.8	**359** $[M-H-H_2O]^-$	Unknown	B	
51		999.1803 499.0860[d]	$C_{48}H_{40}O_{24}$	− 0.2	823 $[M-H-C_6H_8O_6]^-$, 801 $[M-H-DSS]^-$, 757 $[M-H-DSS-CO_2]^-$, **581** $[M-H-C_6H_8O_6-DSS-CO_2]^-$, **378** $[M-2H-DSS-CO_2]^-$, 411 $[M-2H-C_6H_8O_6]^-$, 197 $[DSS-H]^-$	Unknown	R	
56	25.7	593.2216	$C_{29}H_{38}O_{13}$	− 2.1	561 $[M-H-CH_3OH]^-$, 519 $[M-H-C_3H_6O_2]^-$, **387** ($C_{18}H_{27}O_9$, 1.0 ppm)	Unknown	S	
60	26.6	563.2111	$C_{28}H_{36}O_{12}$	− 2	531 $[M-H-CH_3OH]^-$, 489 $[M-H-C_3H_6O_2]^-$, **387** ($C_{18}H_{27}O_9$, 1.0 ppm)	Unknown	S	
61	27.2	651.2271	$C_{31}H_{40}O_{15}$	− 1.9	505 $[M-H-dHex]^-$, 487 $[M-H-dHex-H_2O]^-$, **475** $[M-H-C_{10}H_8O_3]^-$, 457 [M-H-ferulic acid]$^-$, 193 [ferulic acid-H]$^-$	Martynoside	S	18
62	27.9	435.0914	$C_{20}H_{20}O_{11}$	− 1.7	315 $[M-H-C_7H_6O_3]^-$, **297** [M-H-salicilyc acid]$^-$	Shimobashiraside C	S	21

Abbreviations: CA: caffeic acid; QA: quinic acid; DSS: danshensu; RA: rosmarinic acid; dHex: loss of deoxyhexose (− 146 Da); Hex: loss of hexose (− 162 Da); Pen: loss of pentose (− 132 Da); Hexu: loss of hexuronose (− 176 Da).

Reference: 1 (Liu et al., 2007); 2 (Vallverdú-Queralt et al., 2014); 3 (Lee & Scagel, 2009); 4 (Lu & Foo, 2002); 5 (Jaiswal, Matei, Glembockyte, Patras, & Kuhnert, 2014); 6 (Cao et al., 2016); 7 (Chen et al., 2011); 8 (Borrás-Linares et al., 2014); 9 (Aoshima et al., 2012); 10 (Zimmermann et al., 2011); 11 (Ribeiro et al., 2016); 12 (Moghadam et al., 2015); 13 (Piccinelli et al., 2008); 14 (Cvetkovikj et al., 2013); 15 (Schnitzler, Nolkemper, Stintzing, & Reichling, 2008); 16 (Islam, Downey, & Ng, 2013); 17 (Olennikov et al., 2015); 18 (Quirantes-Piné, Arráez-Román, Segura-Carretero, & Fernández-Gutiérrez, 2010); 19 (Wang et al., 1998); 20 (Xie et al., 2014); 21 (Zhang, Lv, Chen, Yan, & Duan, 2013).

[a] Compounds are numbered according to their elution order.

[b] In bold the base peak of MS/MS spectrum.

[c] Compared with reference standards.

[d] *m/z* values corresponding to $[M-2H]^{-2}$.

[e] *m/z* values corresponding to $[M + HCOOH-H]^-$.

Table 2
Rosmarinic acid (RA) and total phenolic content (TPC) levels of DWWs.

Sample	RA (mg/100 mL)	TPC (mg GAE/100 mL)
SL-DWW	135.3 ± 12.3[a]	443 ± 95[a]
BP-DWW	72.3 ± 9.4[b]	313 ± 32[b]
RL-DWW	46.8 ± 9.4[c]	277 ± 26[b]
SP-DWW	39.7 ± 4.6[c]	205 ± 15[c]
BL-DWW	28.8 ± 3.4[d]	152 ± 17[d]

Values are means of three replicates ± standard deviation (SD).
Different superscript letters within each column indicate significant differences among samples ($p < 0.05$).

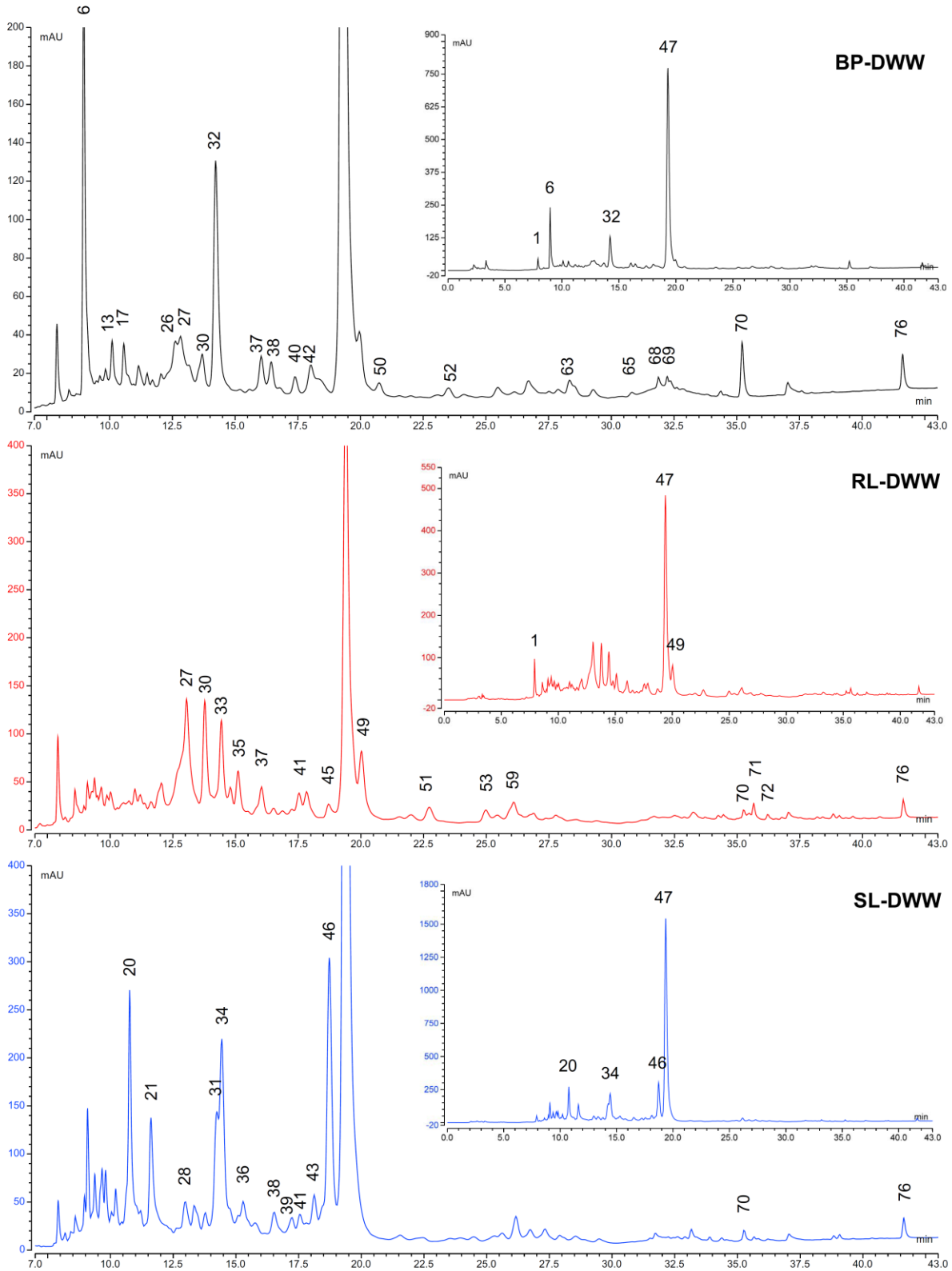

Fig. 1. HPLC-UV profiles (280 nm) of BP-DWW, RL-DWW and SL-DWW.

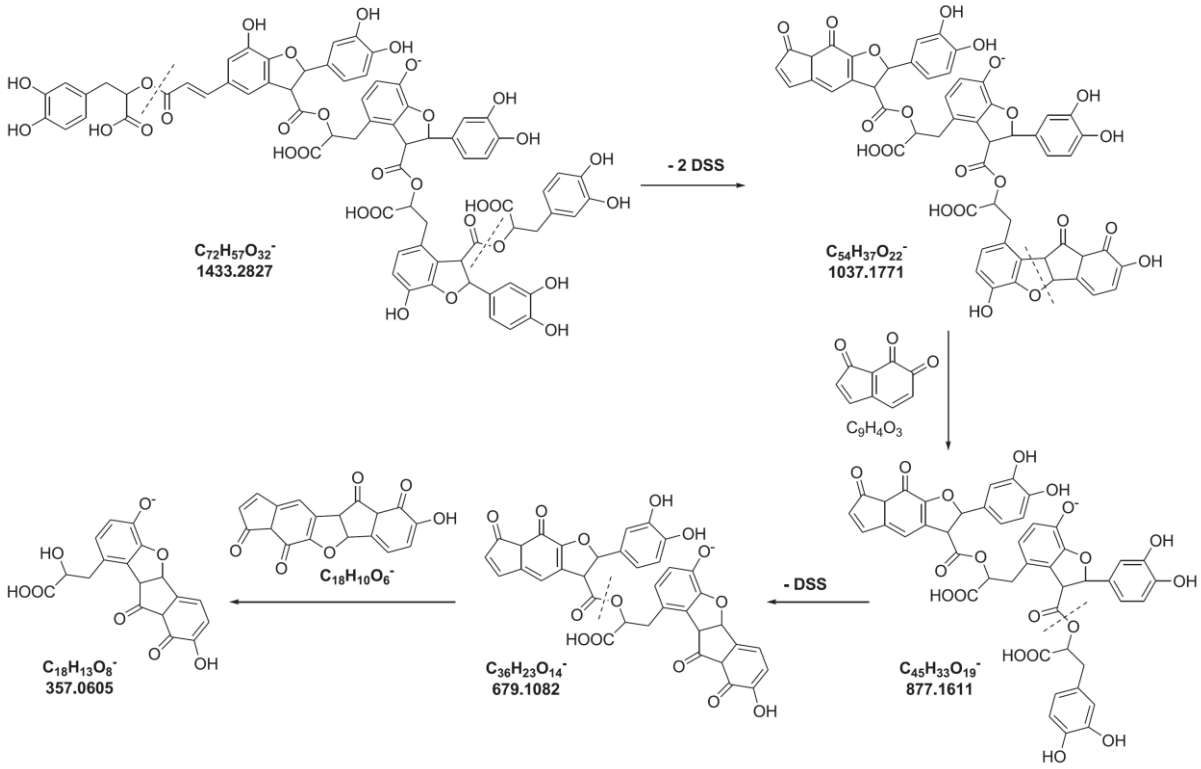

Fig. 2. Proposed fragmentation pathway of clinopodic acid P (**68**).

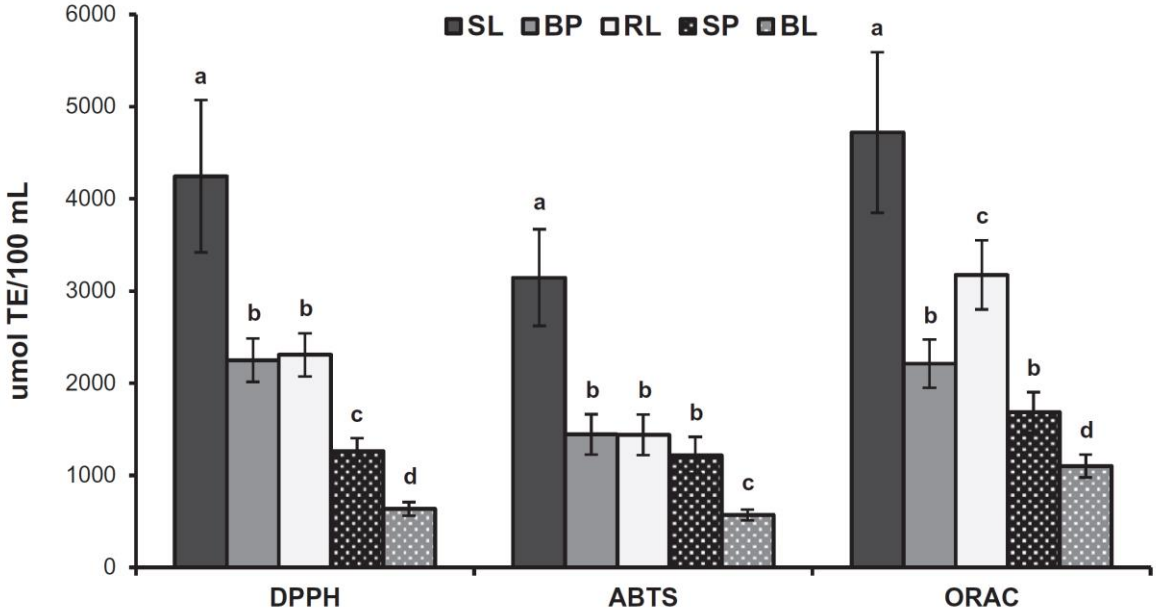

Fig. 3. Antioxidant activities of DWWs by DPPH, ABTS and ORAC assays.
Values are means of three replicates ± standard deviation (SD).
Different superscript letters within each assay indicate significant differences among samples ($p < 0.05$).

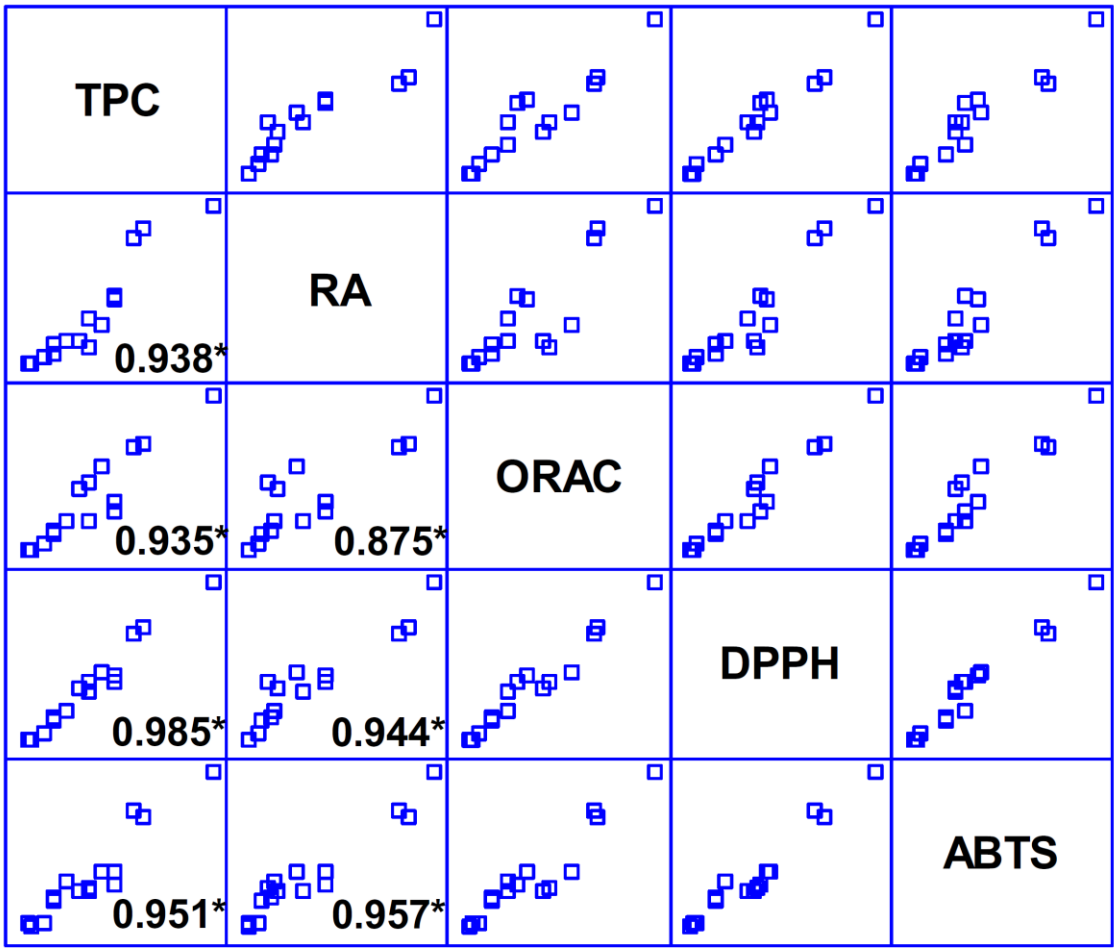

Fig. 4. Scatter plot matrix of Pearson correlations between TPC and RA levels and AOC values (ORAC, DPPH and ABTS) of DWWs.
Number of pairs of data values = 15. *denote p values < 0.001.