SPECIAL GUEST EDITOR SECTION

Antioxidant Profiles of *Vitis vinifera* L. and *Salvia triloba* L. Leaves Using High-Energy Extraction Methodologies

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The profiles of *Vitis vinifera* L. and *Salvia triloba* L. leaf extracts have been studied via photometric assays on the basis of their total phenolic and flavonoid content as well as of their radical scavenging and antioxidant activities. Ultrasound-assisted (UAE) and pressurized liquid extractions (PLE) were implemented for producing polar fractions from the plants, using different methanol–water and glycerol–water mixtures for UAE and PLE, respectively. Aqueous methanol was proved an effective solvent for the UAE of total phenolics and flavonoids as well as for increased radical scavenging and antioxidant activities. As for PLE, plain water was proved a more efficient solvent than hydroglycerolic mixtures. Overall, irrespective of the solvent(s) used, UAE extracts showed higher values compared with the PLE extracts for all the photometric determinations and for both plant species. Moreover, *Salvia* UAE and PLE extracts presented higher total phenolic and flavonoid contents, accompanied by higher radical scavenging and antioxidant activities, compared with *Vitis* extracts. The correlations among photometric results were also studied, indicating the categories of compounds that relate to the antioxidant and/or radical scavenging activities of the extracts. Mixtures of the examined extracts could be exploited as the basis of novel phytotherapeutic products in the cosmetic sector.

Consumers' changing preferences and increased demands for pharmaceutical, nutraceutical, and cosmetic products containing natural extracts prompted the development and scale-up of modern extraction techniques. Over the last years, industries have focused on developing high-energy techniques as a potent alternative to classic extraction approaches. According to Chemat et al. (1), high-energy practices are acknowledged as "green" extraction methods because of (*1*) the utilization of alternative, eco-friendly solvents [glycerol (Glyc), ionic liquids, natural deep eutectic solvents], (*2*) the shorter processing and extraction times, (*3*) the modern nonelaborated instrumentation, (*4*) the energy-saving procedures and reduced CO_2 emissions, (*5*) the improved selectivity, (*6*) the increased extraction yields, and (*7*) the high quality of the final product.

Among these practices, ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE), also known as pressure-enhanced liquid extraction or accelerated solvent extraction, emerge as ideal tools for the recovery of bioactive thermolabile compounds such as phenolic acids, polyphenols, flavonoids (2–6) and carotenoids (7–10).

The technique of PLE relies on the use of "green" solvents at controlled temperature and pressure to extract target components from various matrices (11). High-pressure systems promote enhanced analyte solubility, faster transfer, and compound diffusion between the matrix and extraction solvent as well as lower viscosity of the solvent, which is a key aspect for the convenient and successful utilization of highly viscous solvents of interest, like Glyc (12, 13). Glyc is a generally recognized as safe (GRAS) fluid of low cost because of its abundance as a byproduct of the biodiesel industry, whereas it fulfills all the criteria necessary to develop an eco-friendly extraction process with industrial prospects for cosmetics, food supplements, or pharmaceuticals. Moreover, Glyc presents a high melting point

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and relatively low dielectric constant that enables it to donate and accept protons and electrons to form hydrogen bonds, thus increasing its dissolution capacity (14). However, high-pressure techniques have been associated with low extraction yields (15).

On the other hand, UAE is a widely used and forceful method for improving extraction performance for a wide range of phytoconstituents. The use of ultrasounds presents several advantages in terms of shortening the process time, using smaller volumes of the organic solvent, and boosting the extraction rate, thus enabling process intensification and a cost-effective production of high-quality extracts (15). Additionally, UAE enhances the extraction of heat-sensitive components under conditions that would otherwise have low or unsatisfactory yields (16). Furthermore, UAE extracts are commonly dried and powdered for industrial purposes. The dried extracts are considered a most-potent preparation because of lower storage costs, reduction of shipping weights, and the higher concentration and stability of the active substances (17). They are easily standardized and could enhance the content and properties of liquiform extracts. Moreover, dried extracts are readily prepared for incorporation in health-promoting and medicinal products (17) or as functional food (18) and biopackaging ingredients (19).

Vitis vinifera L. (family Vitaceae) fruit as well as its leaves have been credited with a plethora of biological properties including hepatoprotective, spasmolytic, vasorelaxant, antibacterial, antifungal, anti-inflammatory, antiviral, and antioxidant effects (20). These therapeutic effects are due to the (poly) phenolic compounds that are abundant in leaves, particularly phenolic acids, flavonols, tannins, and anthocyanins (21–23). Annually, great amounts of residues are generated by the wine and juice industries, especially in Southwestern Europe (24), and grape leaves are one of the main vinification byproducts, causing excessive environmental problems (25). Therefore, the exploitation of *Vitis* leaves for recovering bioactive constituents presents an opportunity to solve the disposal problems arising from the large amounts of ecotoxic residues and also to generate innovative, high-value-added natural products.

Salvia triloba L. (synonym: *S. fruticosa* Mill.; common name: Greek oregano or sage) of the Lamiaceae family is one of the most important commercial species of sage. *Salvia* species has been the subject of intensive research because of its phenolic antioxidant constituents (26). The leaves of sage are well-known for their antioxidative, anticancer, and antimicrobial properties. In fact, the potent anti-inflammatory effects of sage extracts have been attributed to their antioxidant mechanisms (27). Rosmarinic acid is a major phenolic compound found in *S. triloba* and is considered responsible for the antioxidant activity observed in the methanolic and aqueous extracts of the plant (26).

Therefore, the aim of the current study is to investigate the antioxidant profiles of different extracts of *V. vinifera* L. and *S. triloba* L. leaves. Their phenolic and flavonoid content, together with the measured antioxidant/radical scavenging activities, may set the basis for their potential exploitation for novel cosmeceutical products. In order to obtain complementary phenolic/antioxidant-rich extracts, UAE and PLE have been applied with different combinations of solvent mixtures (aqueous Glyc and methanol). The overall objective is the acquisition of extracts with *(1)* high total phenolic content (TPC), (*2*) high total flavonoid yield, and (*3*) strong antiradical and antioxidant activities.

Experimental

Materials

All reagents used were of analytical grade, and they were purchased from *Fisher Scientific* (Loughborough, United Kingdom). Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) free radical, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma Chemical Co. (St. Louis, MO). Aluminum chloride anhydrous ($AlCl_3$) was supplied from Acros Organics (Geel, Belgium), and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was bought from Acros Organics (Morris Plains, NJ). Standard phenolic compounds 3,4,5-trihydroxybenzoic acid (gallic acid) and quercetin were purchased from Alfa Aesar (Karlsruhe, Germany) and Sigma-Aldrich (Steinheim, Germany). Solvents used were of HPLC grade and purchased from Sigma Aldrich Co. (Gillingham, United Kingdom) and Fisher Chemical (Loughborough, United Kingdom), while Glyc used for the extractions was >99% pure (Oleon Corporate M&S, Ertvelde, Belgium).

Plant Material and Sample Preparation

The aerial parts of *S. triloba* L. were collected at the end of July 2016 from an organic farm located in Halmyros town (regional unit of Magnesia, region of Thessaly, Greece). The stems of the plant samples were discarded, and the leaves were manually separated. Moreover, *V. vinifera* L. leaves of the ancient variety of Athiri, indigenous to Greece, were collected from conventional grapevines in October 2016 from the Greek island of Santorini in the southern Aegean Sea. The leaves of each plant were carefully washed with cold distilled water and naturally drained, followed by air drying in a dark chamber at ambient temperature for 10 days. Part of the leaves from each species was ground into powder using a high-speed grinder. Each dried plant material sample was fully homogenized, and the ground or whole leaves were stored in hermitically sealed paper bags in the dark at 7°C until further use within 3 months postcollection.

UAE and PLE Methodologies

UAE was performed identically for *V. vinifera* and *S. triloba* leaves using five different proportions of methanol and water for a comparative study. Aqueous-methanolic mixtures are considered one of the most appropriate solvent systems to recover a wide spectrum of phenolic compounds because of their different polarities (5, 14, 28, 29). In particular, H_2O, MeOH, and various ratios of MeOH–H_2O [1:4, 1:1, and 4:1 (v/v)] were used for the UAE of each plant species, one at a time. A fast methodology was used as described by Lantzouraki et al. (28) as follows: 3 g *V. vinifera* or *S. triloba* ground leaves and 30 mL each different solvent or mixture [10:1 (v/w) solvent: solid material ratio] were placed in a sealed 250 mL three-neck vessel in an ice bath (the temperature ranged from 30 to 35°C), and they were sonicated using a Vibra-Cell VCX 750 (20 kHz, 750 W) ultrasonic processor equipped with a piezoelectric converter and a 13 mm diameter probe fabricated from titanium alloy Ti–6Al–4V (Sonics & Materials, Inc., Newtown, CT). The amplitude was 80%, and the pulse sonication sequence was 10 s ON and 5 s OFF, for a total extraction time of 15 min.

After sonication, the extracts were filtered by a Buchner funnel under vacuum, and the filtrates were evaporated to dryness in a CentriVap Concentration System (Labconco Corp., Kansas City, MO) equipped with a cold trap, a concentrator, and a pump (Yellow Jacket SuperEvac Pump; Ritchie Engineering, Inc.). The dry residues were reconstituted in 30 mL methanol. The extracts were stored in sealed glass vials at –20°C until used for analysis. Each extraction was conducted in triplicate.

As for the PLE, the whole procedure took place in the controlled environment of a Timatic Micro solid–liquid extractor (capacity of 1 L; Tecnolab, Spello, Italy) to ensure the maximum yield while avoiding possible oxidations and degradations of the plant or extract ingredients.

About 30 g intact leaves (to prevent the formation of precipitates) from each plant species were placed in a filter bag (50 μm pore size; Tecnolab), and they were loaded into a stainless-steel cell of the extraction chamber. Five GRAS mixtures of aqueous Glyc were used for the PLE, as aqueous combinations of Glyc have been shown to significantly increase (poly)phenolic yield compared to plain Glyc (11, 30, 31). In detail, 1:4, 2:3, 1:1, and 3:2 (v/v) of Glyc–H_2O as well as plain H_2O were tested as solvent systems at a ratio of 40:1 (w/w) solvent(s):plant material. The solvent circulated through the filter bag throughout the extraction procedure. Each extraction procedure of 88 min included eight cycles with sequential pressure and depression phases of 5 and 6 s, respectively. A dynamic phase was obtained via a programmed pressure ranging from 4 to 9 bars, and the temperature was set at 25 ± 1°C. At the end of each cycle, the plant material was pressed to achieve the maximum extraction by applying automatic pressure control in the extraction chamber; subsequently, the solvent recirculated in the extraction chamber for another cycle. The final extracts were filtered through mixed cellulose ester filter paper of 0.45 μm pore size (Sartorius AG, Goettingen, Germany) to retain the solid plant material, and the clarified extracts remained sealed in glass vials at 10°C until further use. Each extraction was conducted in triplicate.

Determination of TPC

The TPC of the UAE and PLE extracts was determined by applying a micromethod of Folin–Ciocalteu's colorimetric assay based on the methodology by Matić et al. (32). The TPC was expressed as micrograms gallic acid equivalents (GAE) per milliliter extract using a standard curve with concentration range 0.1–1.2 mg/mL gallic acid ($y = 1.158x + 0.010$, $R^2 = 0.999$).

Determination of Total Flavonoid Content (TFC)

To estimate the TFC of the extracts, a previously published methodology of aluminum chloride was applied (33). The TFC was expressed as micrograms quercetin equivalents (QE) per volume (milliliters). The range of concentrations for methanolic standard solutions was 6.0–60 μM ($y = 0.013x + 0.027$, $R^2 = 0.998$).

Scavenging Activity on DPPH• Free Radical

The antiradical activity of each extract was assessed by monitoring its effect on the reduction of the stable DPPH• radical as previously reported (29). The antiradical activity of the ultrasound-assisted and pressurized liquid extracts was expressed as milligrams trolox equivalents (TE) per milliliter extract using a standard curve ranging from 0.10 to 1.2 mg/mL trolox methanolic solutions ($y = –0.401x + 0.508$, $R^2 = 0.995$).

Ferric Reducing/Antioxidant Power (FRAP) Assay

In the FRAP assay, the antioxidants of the studied extracts were determined as reductants of Fe(III) to Fe(II). The method was based on the reduction of a ferric-2,4,6-tripyridyl-s-triazine complex to the ferrous form, and the methodology was carried out as follows. The FRAP reagent was prepared at 37°C by mixing acetate buffer (CH_3COOH–CH_3COONa) adjusted to pH 3.6, 20 mM aqueous solution of ferric chloride hexahydrate $FeCl_3 \cdot 6H_2O$, and 10 mM TPTZ solution in hydrochloric acid 40 mM at the proportion of 10:1:1 (v/v/v). Freshly prepared FRAP reagent of 900 μL along with 500 μL acetate buffer and 2000 μL H_2O were mixed thoroughly with 7 μL each plant extract, standard solution, or blank. The absorbance of the produced blue ferrous–TPTZ complex $[Fe(II)(TPTZ)_2]^{2+}$ was recorded at 593 nm with a double-beam UV-Vis spectrophotometer (UV-1800; Shimadzu Europa, Duisburg, Germany) after 90 min (plateau of time) incubation at 37°C. The calibration curve ($y = 0.003x + 0.029$, $R^2 = 0.999$) was prepared with aqueous solutions of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in concentrations that ranged from 10 to 600 μM. The results were expressed as milligrams Fe(II) resulting from the reduction of Fe(III) per milliliter extract.

Statistical Data Analysis

All the photometric determinations were carried out in triplicate for each UAE or PLE preparation. Results were expressed as mean values ± SD. Data were analyzed on a significance level of 0.05 with one-way analysis of variance and post hoc test analysis that comprised pairwise multiple comparisons conducted with Tukey's significant difference test. Probabilities lower than 0.05 were considered statistically significant ($P < 0.05$). Moreover, Pearson correlation coefficients were calculated in order to measure the linear correlations between the different spectrophotometric assays. All statistical calculations were performed using the Origin Pro 8 SR0 (v8.0724, B724; OriginLab, Northampton, MA) statistical software package.

Results and Discussion

TPC, TFC, and Antiradical and Reducing/Antioxidant Activities of UAE and PLE Extracts from V. vinifera and S. triloba Leaves

The TPC and TFC, determined by Folin–Ciocalteu and the $AlCl_3$ assay, respectively, as well as the antiradical activity towards DPPH• radical and the reducing/antioxidant power assessed with the FRAP assay for the different UAE and PLE extracts from *V. vinifera* and *S. triloba* leaves are summarized in Table 1.

As an overall trend depicted for both plant species, the UAE extracts showed significantly ($P < 0.05$) higher values compared to the PLE ones for all the photometric determinations, irrespective of the solvent mixture system. In contrast to pure water or methanol, all aqueous methanol mixtures were proved more effective for the UAE of phenolics and flavonoids

Table 1. TPC expressed as micrograms GAE per milliliter, TFC expressed as micrograms QE per milliliter, antiradical activity expressed as milligrams TE per milliliter, and reducing/antioxidant activity expressed as milligrams Fe(II) per milliliter for the UAE and PLE extracts of *V. vinifera* and *S. triloba* leaves

		V. vinifera L.			
Method	Type of extract	μg GAE/mL	μg QE/mL	mg TE/mL	mg Fe(II)/mL
UAE, v/v	H_2O	945±31^{e}	147.7±1.0^{e}	3.128±0.033^{d}	0.559±0.041^{c}
	MeOH–H_2O 1:4	1300±21^{c}	225.0±1.0^{d}	3.947±0.049^{c}	0.723±0.011^{b}
	MeOH–H_2O 1:1	1972±10^{a}	453.8±3.0^{b}	6.025±0.023^{a}	0.798±0.010^{a}
	MeOH–H_2O 4:1	1721±27^{b}	551.3±2.4^{a}	6.054±0.051^{a}	0.735±0.033^{b}
	MeOH	1192±12^{d}	333.0±3.6^{c}	4.978±0.060^{b}	0.576±0.023^{c}
PLE, w/w	H_2O	237.4±1.9^{f}	9.89±0.23^{f}	1.134±0.017^{e}	0.179±0.013^{d}
	Glyc–H_2O 1:4	212.0±9.5^{g}	8.58±0.18^{g}	1.034±0.011^{f}	0.168±0.010^{d}
	Glyc–H_2O 2:3	197.5±5.0^{g,h*}	7.74±0.12^{h}	0.899±0.038^{h}	0.143±0.015^{e}
	Glyc–H_2O 1:1	190±5.6^{h}	7.97±0.23^{h}	0.982±0.020^{g}	0.164±0.010^{d}
	Glyc–H_2O 3:2	136±4.0^{i}	6.15±0.13^{i}	0.852±0.013^{h}	0.147±0.012^{e}
		S. triloba L.			
UAE, v/v	H_2O	1285±16^{e}	313.3±1.1^{e}	7.73±0.37^{d}	1.86±0.28^{c}
	MeOH–H_2O 1:4	3044±40^{c}	578.4±1.7^{c}	10.90±0.48^{c}	3.47±0.12^{b}
	MeOH–H_2O 1:1	4692±12^{b}	837.0±2.8^{a}	13.74±0.46^{b}	3.69±0.16^{b}
	MeOH–H_2O 4:1	5294±17^{a}	734.3±2.3^{b}	16.67±0.93^{a}	4.01±0.10^{a}
	MeOH	2760±50^{d}	347.7±4.1^{d}	9.34±0.73^{c}	1.37±0.23^{c}
PLE, w/w	H_2O	376.1±6.2^{f}	8.64±0.63^{f}	1.432±0.010^{e}	0.221±0.009^{d}
	Glyc–H_2O 1:4	279.1±1.8^{g}	6.60±0.11^{g}	1.125±0.010^{f}	0.201±0.007^{d}
	Glyc–H_2O 2:3	200.3±8.9^{h*}	4.13±0.15^{h}	1.002±0.011^{g}	0.180±0.005^{e}
	Glyc–H_2O 1:1	162.0±8.0^{i}	2.99±0.10^{i}	0.824±0.011^{h}	0.163±0.003^{f}
	Glyc–H_2O 3:2	145.9±1.2^{j}	2.30±0.41^{j}	0.705±0.012^{i}	0.142±0.001^{g*}

as well as for the enhancement of radical scavenging and antioxidant activities. A contrary outcome was observed for the binary systems of Glyc and water used in PLE, whereas plain water seemed to be a more efficient solvent according to all photometric assays for both plant species. Moreover, based on the results of Table 1 for the PLE of both species, increasing the proportion of Glyc in most cases appears to decrease the TPC, the TFC, the radical scavenging, and, to a lesser extent, the antioxidant activity of the extracts. To a step further, comparing the extracts from the different plant species, irrespective of the extraction type or the solvent system, *S. triloba* UAE and PLE extracts generally presented higher TPC and TFC accompanied by higher radical scavenging and antioxidant activities compared with the respective extracts of *V. vinifera*.

Regarding the UAE extracts of *V. vinifera*, the MeOH–H_2O 1:1 (v/v), resulted in the highest value for TPC ($P<0.05$), followed by MeOH–H_2O 4:1 (Table 1). In particular, the TPC of the aqueous extract was about half ($P<0.05$) of the corresponding value for MeOH–H_2O 1:1 (v/v). Interestingly, the TFC did not follow the same trend as for TPC, showing the MeOH–H_2O 4:1 (v/v) as the most efficient solvent, which was about 4-fold higher than the use of H_2O (Table 1). This could be justified because of the different polarities of nonglycosylated flavonoids compared with phenolic compounds. Indeed, polar phenolic acids such as 3-hydroxybenzoic, caffeic, gallic, vanillic, *p*-coumaric, *p*-caffeoyl-tartaric, and ferulic acids, resveratrol monomers and their derivatives, and astringin from stilbene groups have been previously determined in *V. vinifera* (21, 34–37). Moreover, free flavonoids and their glycosides have been identified in *Vitis* leaf extracts including (+)-catechin, (–)-epicatechin, apigenin, myricetin, quercetin, quercetin-4′-glucoside, and rutin (21).

The radical scavenging activity of the UAE extracts of *Vitis* leaves toward the DPPH$^{\bullet}$ radical ranged from 3.128±0.033 for pure water to 6.054±0.051 mg TE/mL for MeOH–H_2O 4:1 (v/v). The MeOH–H_2O 4:1 and 1:1 (v/v) extracts were found equally ($P > 0.05$) active as radical scavengers, which denotes the contribution of the high phenolic and flavonoid content to the antiradical capacity of each extract, respectively. It may be assumed that the same categories of compounds exhibiting antiradical activity were extracted by both 4:1 and 1:1 (v/v) aqueous methanol.

Based on the FRAP results as seen in Table 1, the solvent mixture MeOH–H_2O 1:1 (v/v) seems to be the system of choice for the adequate UAE of antioxidant compounds from the leaves of *V. vinifera* that resulted in 0.798±0.010 mg Fe(II)/mL extract. This is in alignment with the elevated TPC and antiradical activity observed for the denoted extract. The lowest ($P<0.05$) values were recorded for the aqueous and methanolic extracts (Table 1). It is conceivable that the

relatively low amounts of TPC and TFC recovered by plain water resulted in low antioxidant activity [0.559±0.041 mg Fe(II)/mL]. On the other hand, the weak antioxidant capacity of the methanolic extract [0.576±0.023 mg Fe(II)/mL] appears to be directly related only to the low TPC (1192±12 μg GAE/mL). From this finding, it can be deduced that the different classes of phenolics, rather than the flavonoids alone, seem to mostly account for the antioxidant power of the UAE extracts from grape leaves.

Regarding the results for the UAE extracts of *S. triloba* (Table 1), it is apparent that increasing the proportion of methanol in the binary solvent system provides extracts with higher ($P<0.05$) TPC. Nonetheless, plain methanol seemed to be rather inefficient for the extraction of total phenolics, as it was similarly observed for plain water.

With regard to the aforementioned results, methanol is widely used for UAE of natural compounds, as it has low viscosity and vapor pressure; thus, it facilitates the acoustic cavitation phenomenon and extraction efficiency (38). However, the addition of water to organic solvents has been indicated to enhance the propagation of ultrasonic waves, leading to intensification of the process. Specifically, the critical molecular distance is closely related to the production of cavitation bubbles, which are responsive to the ultrasonic effect. When methanol and water are mixed, the molecular distance changes, as do the surface tension, vapor pressure, and viscosity. Subsequently, the extraction efficiency would improve, owing to the enhanced cavitational effect (39). In addition, the appropriate ratio of hydromethanolic mixture could correspond to the relative polarity of the targeted compounds, resulting in higher solubility in the extract (40). Nevertheless, when water is added over a critical ratio, the extraction efficiency is likely to be reduced because of the decomposition of water. Acoustic cavitation can produce radical forms such as $OH^{\bullet}$ and $H^{\bullet}$ that accumulate in the surface of the cavitation bubble and can initiate the formation of degradation products, which in turn trigger radical chain reactions (41). Moreover, in aqueous media containing volatile organic gases and solutes, cavitation collapse results not only in the breakdown of water to radicals but also in the formation of organic radicals (15) that can cause degradation of the target compounds in the matrix, thus decreasing the extraction efficiency (42).

As for total flavonoid determination of the *Salvia* plant, the highest ($P<0.05$) concentration (837.0±2.8 μg QE/mL) was estimated for the 1:1 (v/v) ratio of aqueous methanol extract, followed by those for MeOH–H_2O 4:1 and 1:4, MeOH, and water. Flavonoids are the dominant phenolic group in the *S. triloba* group, including flavones like free apigenin, luteolin, and genkwanin along with their glycosides and flavonols like kaempferol glycosides, rutin, and hyperoside (43).

The values for the radical scavenging activity of the *S. triloba* UAE extracts toward the $DPPH^{\bullet}$ radical follow the same trend as the values for the TPC but not those for the TFC of the respective extracts. Based on the reaction mechanisms of each method, the Folin–Ciocalteu assay measures all the compounds with a phenolic structure [aromatic ring(s) bearing one or more hydroxyl groups], so the value of TPC comprises all the phenolic and polyphenolic groups, including simple phenols, phenolic acids, coumarins, all classes of the flavonoid family, lignins, lignans, and condensed and hydrolysable tannins. On the other hand, the assay with aluminum chloride, applied for the measurement of the TFC, includes the measurement only of the compounds containing one or more flavonoid moieties (32). Hence, it can be assumed that the radical scavenging activity of *Salvia* UAE extracts is not only attributable to flavonoids and that other (poly)phenolic compounds contribute to a greater extent in the antiradical activity measured with the $DPPH^{\bullet}$ assay. In detail, the MeOH–H_2O 4:1 (v/v) extract was the most potent ($P<0.05$) against the free radical and 2-fold higher than the H_2O extract (Table 1). The same extract also exhibited the highest ($P<0.05$) antioxidant activity as measured with the FRAP assay, while the water or methanol extracts were proved comparatively inadequate. The above findings are in contrast with those reported by Duletić-Laušević et al. (43), who showed that Salvia extracts with high concentrations of specific flavonoid glycosides exhibited strong antioxidant activity (revealed by FRAP assay), whereas extracts with high TPC or TFC possessed only weak antioxidant activity. Moreover, they indicated that extracts with significant antiradical activity ($DPPH^{\bullet}$) possessed low TPC but an average TFC.

An overall remark regarding the results obtained for both UAE plant extracts is that different solvent systems were shown to fit more appropriately to *Vitis* and to *Salvia* leaves in order to produce phenolic-rich extracts coupled with high antiradical and antioxidant activities. This outcome might bear two possible explanations as they are further developed: (*1*) The phytochemical profile of each matrix seems to set the requirements for the suitability of the solvent system to be used (44). The choice of the right solvent(s) is guided by the physicochemical characteristics of the analytes of interest but most importantly by their polarity and, consequently, by their solubility. Differences in the structures of phenolic compounds, such as the number of hydroxyl groups, conjugation or not with sugars, and acid or alkyl groups are critical for solubility, as they interfere significantly in the extraction process (45). It is therefore assumable that each plant material that has a unique phenolic/antioxidant composition requires different solvent mixtures for the efficient extraction of (poly)phenolics or other phytochemicals. (*2*) The physical features of the matrix as is are important factors to consider when selecting the solvent system or even the extraction technique. The specificities of structure, rheology, or hardness of the plant material have been related to variations in the extraction yield of natural antioxidants obtained with UAE. The physical structure of the matrix may affect the susceptibility to ultrasound waves as well as the degree of probability that cavitation bubbles will contact the plant surface (46). Therefore, differences in porosity of the dried *Salvia* and *Vitis* leaves may partially explain differences in TPC and TFC results.

Contrary to UAE, the PLE performed for *V. vinifera* leaves yielded relatively poor extracts based on TPC and TFC. Likewise, PLE with plain water achieved the highest ($P<0.05$) value of TFC from *Vitis* leaves, in contrast to Glyc–H_2O 3:2 (w/w), which presented the lowest ($P<0.05$) TFC. According to Table 1, the rest of the aqueous Glyc mixtures presented medium values and no significant ($P>0.05$) differences in the TPC and TFC of the respective extracts.

Furthermore, the radical scavenging activity of the *Vitis* PLE extracts against the $DPPH^{\bullet}$ was determined, and the aqueous extract exhibited the highest ($P<0.05$) level of activity, while the extracts of Glyc–H_2O 3:2 and 2:3 (w/w) showed the lowest ($P<0.05$) levels among all.

Concerning the FRAP assay, the results seem inconclusive on whether a specific solvent system was the most efficient for

the PLE of reducing/antioxidant compounds from *Vitis* leaves. As shown in Table 1, the different ratios of Glyc to water used in PLE did not have a significant impact on the reducing/antioxidant capacity of the *Vitis* extracts. Specifically, the H_2O extract was comparable ($P > 0.05$) to Glyc–H_2O 1:4 and 1:1 (w/w), while the 2:3 and 3:2 (w/w) proportions of Glyc–H_2O were found coequal ($P > 0.05$; Table 1).

Regarding the PLE extracts of *S. triloba*, they were found to vary within group in a similar manner to the PLE extracts of *Vitis* (as mentioned above). To be more specific, the aqueous extract was more efficient when compared to the hydroglycerolic extracts on the basis of TPC and TFC and radical scavenging and antioxidant activities. The H_2O extract presented about 2.5-fold higher ($P < 0.05$) TPC and 4-fold higher ($P < 0.05$) TFC than Glyc–H_2O 3:2 (w/w) extract, which provided the lowest ($P < 0.05$) phenolic and flavonoid yields. Similarly, based on the DPPH$^{\bullet}$ assay, the antiradical capacity of the extracts was found to decrease in the following order: H_2O > Glyc–H_2O 1:4 > Glyc–H_2O 2:3 > Glyc–H_2O 1:1 > Glyc–H_2O 3:2 (w/w). By contrast with *Vitis* PLE extracts, clear conclusions were drawn from the results of the FRAP assay for *Salvia* extracts. In particular, water and the 1:4 (w/w) proportion of the mix were identified as the most effective ($P < 0.05$) media for the production of highly antioxidant extracts.

Surveying the results for PLE of *V. vinifera* and *S. triloba*, it can be deduced that plain water was significantly more efficient ($P < 0.05$) than the studied mixtures toward the recovery of (poly)phenolics and flavonoids and providing fractions with high radical scavenging and antioxidant power. Even more, it was evident that adding increasing portions of Glyc to water resulted in proportional reduction of the extraction yield and the antiradical activity. As an interesting exception, but only for *Salvia* PLE extracts, H_2O and Glyc–H_2O 1:4 (w/w) were found equally competent ($P > 0.05$) for the recovery of compounds with high antioxidant power.

Similar to the aforementioned observations, it has been previously reported that the total phenolic yield in extracts from rice byproduct started to decrease when the concentration of Glyc was higher than 19% (47). The recovery capacity of the glycerolic solutions was significantly correlated with the corresponding viscosity, conductivity, and density of the mixtures. Similarly, according to Apostolakis et al., the optimal Glyc concentration for the efficient extraction of phenolics from olive leaves was only up to 9.3% (48). The polarity of the extraction solvent varied with changing Glyc concentrations, leading to the solubility of different phenolic groups. Additionally, a detailed study demonstrated that (poly)phenols and flavonoids of grape pomace were recovered optimally by solutions containing only 20% (w/v) Glyc in water (31).

Therefore, it can be proposed that only low amounts of Glyc in aqueous mixtures may favor the phenolics extraction rate. The increased yields obtained with solvents composed of aqueous Glyc might be ascribed to the polarity of the medium, which apparently approaches that of (poly)phenolic compounds, many of which are rather scarcely soluble in water. However, slightly higher proportions of Glyc over water seem to raise obstacles in mass transfer phenomena occurring during the extraction process because of higher viscosity of the solvent compared to plain water. Glyc density ($d = 1.261$ g/cm^3) seems to impede the penetration of the solvent into the matrix particles (48).

On the other hand, several studies revealed that increased Glyc concentration might favorably affect the extraction of phenolic compounds paired with antioxidant capacity even more efficiently than water under the same conditions, but such observations were stated for extraction procedures under high temperatures. Particularly, Karakashov et al. remarked that 10% (w/v) aqueous Glyc at 70°C provided satisfactory extraction yield in total (poly)phenols from *Hypericum triquetrifolium*, as well as extracts exhibiting strong ferric reducing/antioxidant capacity, which were significantly higher than the ones attained with water (49). The phenolic profile of the 10% (w/v) hydroglycerolic extracts was composed of polar compounds such as phenolic acids and flavonoid glycosides. Moreover, 60% (w/v) Glyc solutions at 60°C were shown to be more potent for the extraction of polyphenolics from olive leaves. The relatively low dielectric constant was underlined as the key feature for the recovery of polyphenols that are otherwise scarcely soluble in pure water (30).

The scientific results presented in this study could set the basis for the complementary usage of the UAE and PLE extracts from *V. vinifera* and *S. triloba* leaves as the basis of cosmetic products. Combining selected UAE and PLE extracts from the two plants that were proved rich in total (poly)phenolics and total flavonoids and possess high antioxidant and antiradical activities could lead to enhanced targeted formulations for the production of natural cosmeceuticals. Moreover, it is intriguing that the plant extracts could be used in combination for the development of a variety of formulations with a complemented antioxidant profile. That is feasible because the dry UAE extracts are hyperconcentrated, they are considered more stable than in liquid form, and they can be easily reconstituted with hydrophilic solvents or added in other extracts as dry matter or in the form of nanoemulsions (17). Hence, they can be used as an intermediate material.

To start with, the significant concentration of total flavonoids in *V. vinifera* UAE MeOH–H_2O 4:1 (v/v) dried extract combined with *S. triloba* PLE aqueous extract could be used for developing novel natural broad-spectrum sunscreens in lieu of synthetic ingredients. In fact, flavonoids have been associated with several photoprotection effects, including UV absorption, direct and indirect antioxidant properties, and prevention of UV-induced oxygen free radical generation (50–52). Similarly, *V. vinifera* MeOH–H_2O 1:1 and *S. triloba* MeOH–H_2O 4:1 (v/v) UAE dry extracts could be incorporated in skin anti-aging and antioxidant treatments, considering their high phenolic content as well as their antiradical and antioxidant properties. Plant phenolics have been proved effective to inhibit or even reverse the signs of aging, such as wrinkles or hyperpigmentation marks (53, 54). Furthermore, studies have shown that (poly)phenols could be effective in the treatment of skin injuries and also for the healing of wounds and burns (53). On the top of that, the glycerolic extracts possesses humectant and moisturizing properties (55). Hence, an admixture of *Salvia* flavonoid-rich UAE MeOH–H_2O 1:1 (v/v) dry extract and *V. vinifera* and *S. triloba* Glyc–H_2O 1:4 (w/w) PLE extracts, which both exhibited high phenolic content and strong antioxidant activity, could be potentially exploited for a series of pharmaceutical formulations targeting the prevention or attenuation of skin disorder symptoms and the reduction of the healing time for burns and wounds.

Statistical Correlation of Photometric Results

To assess the degree of correlation between the photometric assays employed for *V. vinifera* and *S. triloba* extracts, Pearson correlation coefficients were calculated and are presented in Table 2. All statistical calculations revealed positive and high or, in a few cases, moderate correlations among the determinations for the UAE and PLE extracts of both plant species.

Very strong to excellent Pearson correlations ($R>0.8$ and $R>0.9$) were found between TPC and TFC for the UAE and PLE extracts of *V. vinifera* and *S. triloba*. We may assume that aqueous methanol combined with UAE was able to recover not only flavonoids for the greater part but also different (poly) phenolic groups from grape leaves that are not admeasured with flavonoids by the corresponding assay. Carrera et el. investigated the significant effect of ultrasonic wave amplitudes that induce a great number of cavities, thus improving the extraction of tannins from grapes (56).

It is also apparent that as the phenolic concentration of *Salvia* extracts increases, the antiradical activity against DPPH$^{\bullet}$ radical increases, as well ($R=0.974$ for UAE and 0.919 for PLE), irrespective of the extraction type or the solvents used. This implies that the phenolic compounds in the examined extracts are those presenting the main radical scavenging properties in the DPPH$^{\bullet}$ assay. That is largely the case for *V. vinifera* extracts also, exhibiting a very strong correlation ($R=0.895$ and 0.886 for UAE and PLE, respectively) between the two estimations. A strong linear correlation between phenolic compounds and the activity against DPPH$^{\bullet}$ has been previously reported for the extracts of various *Salvia* species and for *Rosmarinus officinalis* L. from Turkey and Greece (57, 58).

Furthermore, excellent or very high correlations ($R>0.90$ and $R>0.80$) were observed between TFC and the scavenging of DPPH$^{\bullet}$ radical for all extracts of both species. Such a correlation was previously found for red wines, and it was attributed to the relevance of the mechanisms underlying the antiradical activity of certain flavonoids (59). Flavonols, in particular, such as catechin and epicatechin, have been identified as primary radical scavengers found in grape byproducts (60, 61) and olive leaf extracts, which also contain flavonol glycosides (62).

Table 2. Pearson's correlation coefficients calculated for the results of Folin–Ciocalteu (TPC), total flavonoids determination (TFC), DPPH$^{\bullet}$ (antiradical activity), and FRAP (reducing/antioxidant power) photometric assays by matrix (*V. vinifera* or *S. triloba*) and extraction type (UAE or PLE)

Correlation coefficients	*V. vinifera* L., UAE / PLE		
	TPC	TFC	DPPH$^{\bullet}$
TFC	0.856 / 0.966	1	
DPPH$^{\bullet}$	0.895 / 0.886	0.973 / 0.959	1
FRAP	0.905 / 0.746	0.640 / 0.848	0.667 / 0.961
	S. triloba L., UAE / PLE		
	TPC	TFC	DPPH$^{\bullet}$
TFC	0.905 / 0.902	1	
DPPH$^{\bullet}$	0.974 / 0.919	0.880 / 0.982	1
FRAP	0.804 / 0.843	0.918 / 0.977	0.854 / 0.983

Kaempferol and quercetin methyl ethers are widely distributed in *Salvia* species, while isorhamnetin, kaempferol, quercetin, and syringenin as well as their glycoside derivatives are also abundant in winery byproducts, including leaves (21, 63). However, the specific correlation was reported differently by Duletić-Laušević et al., who observed that the antiradical activity of methanolic and aqueous extracts of two *Salvia* species, including *S. triloba*, was weakly correlated to major flavonoids quantified in the extracts (43).

It is worth noticing the contradictory results of the correlations concerning the FRAP assay for the different extractions or plant species extracts. First, for the UAE extracts of *Vitis*, the FRAP seems to be attributed mainly to the total phenolics ($R=0.905$) but is only moderately connected ($R=0.640$) to total flavonoids. On the other hand, the antioxidant power measured with the FRAP assay in the PLE extracts from grape leaves correlates more highly with the TFC ($R=0.848$) than the TPC ($R=0.746$). It is possible that other constituents in the extracts, apart from phenolic compounds, act as Fe(III)-reducing agents (29). Therefore, it is conceivable that the application of the different extraction systems resulted in the recovery of uneven amounts of analytes and/or the production of extracts with different profiles of phytoconstituents that present antioxidant power.

The flavonoids of *Salvia* leaves extracted by either UAE or PLE were shown to be the major contributors to the reducing/antioxidant power ($R>0.90$), while TPC was correlated to a lesser degree to the FRAP assay ($R>0.80$). This contradiction might suggest that the flavonoid group included in TPC as measured by the Folin–Ciocalteu assay and extracted from *S. triloba*, regardless the extraction method, possesses the main antioxidant capacity as determined by the FRAP assay. However, a portion of the (poly)phenolic compounds contained in the *Salvia* extracts seem to be minor contributors to the reducing/antioxidant activity of either the UAE or the PLE extracts.

Moreover, the excellent correlations ($R>0.90$) estimated between the antiradical activity (DPPH$^{\bullet}$) and the antioxidant power (FRAP) of PLE extracts from *V. vinifera* and *S. triloba* imply that PLE recovered compounds bearing both antiradical and antioxidant activities. Notably, the antiradical capacities of *Vitis* and *Salvia* UAE extracts were not very strongly correlated with FRAP ($R=0.667$ and 0.854, respectively, for the two plant species). That was not unexpected, as different classes of phenolics do not always exert their antiradical and antioxidant activities under the same mechanisms, even though both DPPH$^{\bullet}$ and FRAP assays are based on electron transfer reactions (64).

Conclusions

Comparison of the antioxidant profiles of different extracts of *V. vinifera* L. and *S. triloba* L. was performed using UAE and PLE with different combinations of solvent mixtures (aqueous Glyc and methanol).

UAE achieved significantly higher values compared to PLE for all photometric determinations, regardless the solvent mixture for *V. vinifera* and *S. triloba* leaves. In particular, the aqueous methanolic mixtures 1:1 and 4:1 (v/v) generally proved more effective for the UAE of phenolics and flavonoids as well as for the improvement of antiradical and antioxidant activities. As for PLE, increasing the proportion of Glyc appeared to

attenuate the enhancement of the extracts. Subsequently, the plain aqueous extract was shown to be more competent than the hydroglycerolic for recovering total phenolics and flavonoids along with concentrating higher radical scavenging and antioxidant activities in the extracts. Additionally, comparing the fractions from the different plant species, irrespective of the extraction type or the solvent system, *S. triloba* UAE and PLE extracts presented higher TPC and TFC paired with higher antiradical and antioxidant activities compared to the corresponding extracts of *V. vinifera*.

Furthermore, very strong correlations were found between TPC and TFC of the UAE and PLE extracts for *V. vinifera* and *S. triloba*. Also, it was broadly evident that as the phenolic or flavonoid concentrations of the UAE extracts increased, the scavenging capacity against $DPPH^{\bullet}$ also increased, irrespective of the extraction type or the solvents used. Remarkably, the FRAP assay provided partly contradictory results concerning the correlations for the different extraction techniques, which supports the interpretation that the use of different solvent mixtures resulted not only in the recovery of uneven amounts of analytes but also in the production of extracts with different profiles of phytoconstituents presenting antioxidant power. Last, the flavonoid content extracted from *Salvia* leaves either by UAE or PLE were shown to be most-associated with the antioxidant power.

Overall, the present investigation has offered a framework for the exploitation of natural sources as putative alternatives to synthetic chemicals in order to align with the global trends on health consciousness and acceptance of phyto-constituents as means for health care and promotion. The phenolic and flavonoid content, together with the measured antioxidant/radical scavenging activities, may set the basis for their potential exploitation for novel cosmetic products. The complementary use of UAE and PLE may provide extracts bearing significant phenolic and flavonoid content and exerting high radical scavenging and antioxidant capacities.

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