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# Antioxidant Activity of Organic Extracts from Aqueous Infusions of Sage

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The antioxidant activity of aqueous infusions of sage emerges from specific components present in that herb. In an attempt to investigate the chemical nature and properties of these components, four organic solvent extracts from aqueous infusions of sage were examined. HPLC analyses of these extracts led to the separation of a number of components, of which four were identified and quantified through the use of standard compounds of known chromatographic HPLC profiles. These compounds are the diterpenes carnosic acid, carnosol, and rosmanol and the hydroxycinnamic acid caffeic acid. The antioxidant activity and polyphenol content were determined in the four organic solvent extracts and the left-over aqueous fraction. Both polyphenolic and nonpolyphenolic substances present in the extracts arise as significant contributors to the observed antioxidant activity of the derived extracts and thus sage itself. In this sense, they reflect the antioxidant potential of the aqueous infusions of sage toward reactive oxygen species generated through variable mechanisms of iron-promoted oxidative processes.

**KEYWORDS: Reactive oxygen species; biological damage; HPLC separation and identification; antioxidant activity in sage; *Salvia fruticosa* L.; polyphenols**

## INTRODUCTION

Reactive oxygen species (ROS) are known to exert variable oxidative damage on a number of biological sites under conditions of oxidative stress (*1*, *2*). To that end, ROS have been implicated in chronic diseases (*3*, *4*). The reactivity of ROS arises from the requisite chemistry of their generation. Hence, ROS sustain oxidative transformations on nearby substrates, based upon a number of factors such as hydrophilicity, lipophilicity, and half-life time, etc. Exacerbation of ROS-related activity may appear when metal ions, capable of promoting redox-associated reactions, give rise to such species via Fenton or Fenton-like pathways (*5*, *6*). Naturally occurring agents, known as antioxidants, are able to counteract the ROS-associated damage. These are externally ingested through the diet or biologically generated in lower as well as higher organisms for their own defense against oxidative damage (*7*−*9*).

A plethora of herbs, which contain antioxidants, are consumed in daily diets and used in traditional medicinal applications, frequently in the form of infusions (*10*−*12*). The antioxidant capacity of these herbs has been attributed to a number of components, including phenolic compounds (*13*−*15*). Nevertheless, limited information exists on the nature and potential antioxidant activity of components present in herb aqueous infusions (*16*).

Prompted by the paucity of such knowledge, we launched research efforts in our laboratory to identify components that might be responsible for the overall antioxidant activity in aqueous infusions of the Mediterranean herb sage (*Salvia fruticosa* L.). The derived knowledge is expected to enhance our understanding of the antioxidant capacity of that herb's infusions and may aid in the discovery of components with specific and distinct antioxidant activity attributes. To that end, we report herein efforts to identify such antioxidant components through organic solvent extractions from aqueous infusions of sage. Antioxidant activity assays on those extracts were coupled with polyphenol determination and HPLC separation in order to determine qualitatively and quantitatively antioxidant components present in the aqueous infusions and thus sage itself.

## MATERIALS AND METHODS

**Apparatus.** A shaking water bath was used for the incubations of the samples at 37 °C. Absorbance spectra were recorded on a Hitachi U-2001 UV−visible spectrophotometer. Components were separated on an HPLC system (HPLC, Marathon IV), which was equipped with a UV−visible detector (Fasma 525 programmable detector) and an analytical Kromasil C18, 5 $\mu$m (250 × 4.6 mm i.d.) reverse phase HPLC column. All chromatographic analyses were carried out with the EZChrom Chromatography Data System software program (version 6.7).

**Reagents.** Nanopure water (purified by double deionization and reverse osmosis) was used throughout the experiments. Aqueous infusions from leaves of the herb sage (*S. fruticosa* L.) were obtained from an herb specimen collected from specific mountainous locations, on the island of Crete, Greece. Staff members of the Department of Biology, University of Crete, Greece, facilitated herb speciation (voucher no. NHMC 9331).

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All of the organic solvents were of HPLC grade (Fluka) and were thoroughly degassed. Acrodisc 13 CR PTFE filters, with a diameter of 13 mm and a pore size of 0.2 $\mu$m, were from Pall Gelman. Phosphoric acid was from Merck. Carnosic acid, carnosol, rosmanol, and cryptotanshinone were donated by Prof. N. Okamura, Fukuyama University, Fukuyama, Hiroshima, Japan. Carnosic acid and rosmanol were also donated by Dr. Chris Wright at Hauser Inc. Boulder, CO. Catechin, 2-deoxy-D-ribose, 1,1,3,3-tetraethoxypropane, caffeic acid, and butylated hydroxytoluene (BHT) were from Sigma-Aldrich, Munich, Germany. Thiobarbituric acid (TBA), $Fe(NO_3)_3$ and citric acid were from Fluka, Buchs, Switzerland, and trichloroacetic acid (TCA) was from Merck, Darmstadt, Germany. The complex $(NH_4)_5[Fe(Cit)_2]\cdot 2H_2O$ ($Cit^{4-} = C_6H_4O_7^{4-}$) was synthesized according to the method of Matzapetakis et al. (*17*). Briefly, $Fe(NO_3)_3$ and citric acid were mixed in water with a 1:2 metal to ligand molar ratio. The reaction mixture was stirred overnight. On the following day, the pH was raised to ~8 with a solution of ammonia. Addition of ethanol resulted in the precipitation of crystalline material at 4 °C a few days later.

**Procedures.** *Preparation of Aqueous Infusions of Sage.* Aqueous infusions of sage (*S. fruticosa* L.) were extracted fresh on each experiment day by addition of the herb in boiling water, at a concentration of 4 g/100 mL. The infusion was stirred for 5 min and filtered through filter paper.

*Preparation of Extracts in Organic Solvents from Aqueous Infusions of Sage.* Aqueous infusions of sage were extracted into the following organic solvents, by shaking for 3−5 min three times or more, each with 10 mL, in the order *n*-hexane, diethyl ether, ethyl acetate, and 1-butanol, until complete decoloration of the organic phase. The aqueous layer, left after the extractions, hereafter called the "left-over aqueous fraction", was also collected. The extractions were carried out in the presence of NaCl, added in a 5 mL portion of a saturated aqueous solution, to improve phase separation. In the case of diethyl ether, the solvent was evaporated in a rotary evaporator. In the case of *n*-hexane, ethyl acetate, and 1-butanol, the solvent was removed under vacuum on a Schlenck line. The left-over aqueous fraction was also evaporated to dryness under vacuum on a Schlenck line. In all cases, the solid or oily residue was collected and stored frozen at −20 °C under an atmosphere of argon. For the antioxidant activity assays, samples were left to thaw at room temperature and redissolved in 30% (v/v) aqueous methanolic solutions to a final volume equal to that of the original aqueous infusion of sage or in water to a final volume equal to that prior to evaporation. In the case of the linolenic acid peroxidation assay in 30% (v/v) aqueous methanolic solutions, aqueous infusions of sage were also taken to dryness and redissolved in the same solvent system to a final volume equal to that prior to evaporation.

*Sample Preparation for HPLC Analysis.* Samples were left to thaw at room temperature and were dissolved in methanol or in water. All solutions were filtered through 0.2 $\mu$m Acrodisc 13 CR PTFE filters prior to use.

**Antioxidant Activity.** *Linolenic Acid Peroxidation.* Lipid peroxidation was assessed according to the procedure of Yamamoto et al. (*18*). Briefly, linolenic acid was dissolved in $CHCl_3$. The organic solvent was then removed under vacuum, leaving a thin film on the wall of the flask. The linolenic acid liposomes were then weighed into 0.30 M NaCl, pH 7.4, to a final concentration of 3 mg/mL. The resulting mixture was sonicated for 10 min under an argon-saturated atmosphere, yielding a milky solution. A 0.5 mL quantity of the redissolved organic extract or infusion was placed in screw-capped tubes, followed by the addition of 0.5 mL of the linolenic acid suspension. Assay samples were prepared by mixing solutions of 1 mL of each mixture of linolenic acid−herb organic extract or infusion with $(NH_4)_5[Fe(Cit)_2]\cdot 2H_2O$ ($Cit^{4-} = C_6H_4O_7^{4-}$), ascorbic acid, and $H_2O_2$, to a final concentrations of 50 $\mu$M in $(NH_4)_5[Fe(Cit)_2]\cdot 2H_2O$, 100 $\mu$M in ascorbic acid, and 1 mM in $H_2O_2$. The final volume of each sample was 1.2 mL. Blanks were prepared by replacing linolenic acid liposomes with 0.5 mL of 0.3 M NaCl, pH 7.4. Controls were prepared by replacing the herb extracts with water or a 30% (v/v) aqueous methanolic solution. A liposome blank, prepared in 30% aqueous methanolic solution, was also included in the experimental design. All of the samples, blanks, and controls were stirred with a vortex apparatus and incubated in a water bath at 37 °C for 2 h. At the end of the incubation period, 100 $\mu$L of 2% BHT in EtOH, 1 mL of 2.8% trichloroacetic acid (TCA) in $H_2O$, and 1% thiobarbituric acid (TBA) in 50 mM NaOH were added. Subsequently, all samples, blanks, and controls were vortexed and heated at 100 °C for 20 min. The pink chromogen, thus obtained, was extracted with 1.2 mL of 1-butanol. The samples, blanks, and controls were subsequently centrifuged for 5 min at 2500*g*, and the absorbance of the organic layer was taken at 534 nm. Malondialdehyde (MDA) equivalents were calculated using linear regression analysis of a standard curve based on 1,1,3,3-tetraethoxypropane.

*2-Deoxy-D-ribose Oxidation.* Determination of 2-deoxy-D-ribose oxidation (*19*, *20*) was modified as follows: Phosphate-buffered saline (0.5 mL in 5.4 mM PBS buffer) was placed in screw-capped tubes, followed by the addition of 0.5 mL of the organic extract redissolved in water or in the herb infusion. Assay samples were prepared by mixing solutions with 1 mL of a PBS−organic extract or PBS−herb infusion, to a final concentration of 2.8 mM in 2-deoxy-D-ribose, 100 $\mu$M in ascorbic acid, 50 $\mu$M in iron ($FeCl_3$/EDTA 1:2), and 1 mM in $H_2O_2$. The final volume of each sample was 1.2 mL. Controls were prepared by replacing the herb infusion with water. Blanks were prepared by replacing 2-deoxy-D-ribose with $H_2O$. All samples, blanks, and controls were vortexed and incubated in a water bath at 37 °C for 1 h. TBA reactive substances were detected spectrophotometrically, as described in the previous section of the lipid peroxidation assay.

*HPLC Analysis.* The extracts from *n*-hexane and diethyl ether were chromatographed under isocratic conditions, with a flow rate of 1 mL/min. The mobile phase was (a) 60% $CH_3CN$/0.1% $H_3PO_4$ for the *n*-hexane extract or (b) 40% $CH_3CN$/0.1% $H_3PO_4$ for the diethyl ether extract. Elution was monitored at 230 nm for these two extracts. The extracts from ethyl acetate and 1-butanol were chromatographed with the help of a step gradient of solvents A (10% $CH_3OH$, 2% $CH_3COOH$ in $H_2O$) and B (90% $CH_3OH$, 2% $CH_3COOH$ in $H_2O$) at 0.8 mL/min. For the ethyl acetate extract, the step gradient involved mixing of A and B to 85, 45, and 20% of A at 0, 30, and 40 min, respectively. The solvent composition %A = 50/%B = 50 was held constant for the period from $t = 30$ min to $t = 35$ min. For the 1-butanol extract, the step gradient involved mixing of A and B to 100, 70, 65, 50, and 20% of A at 0, 30, 35, 50, and 60 min, respectively. The solvent composition %A = 50/%B = 50 was held constant for the period from $t = 50$ min to $t = 55$ min. Elution was monitored at 330 nm for these two extracts.

In all cases of extracts, pure known compounds, specifically carnosic acid, carnosol, rosmanol, cryptotanshinone, catechin, and caffeic acid, were chromatographed as internal and external standards. Standard curves were produced for carnosol, carnosic acid, rosmanol, and caffeic acid, all at various concentrations in each case of extract. In particular, for the hexane extract, calibration curves of carnosol and carnosic acid were generated in the concentration ranges of 13−65 and 26−130 $\mu$g/mL, respectively. For the diethyl ether extract, a calibration curve for rosmanol was produced in the range of 0−1 mg/mL. Two calibration curves were generated for caffeic acid, one for the ethyl acetate extract and one for the 1-butanol extract, in the concentration ranges of 17−60 and 4−24 $\mu$g/mL, respectively.

*Total Polyphenol Determination.* The amount of total polyphenols in the various extracts was determined according to the Folin-Ciocalteu method (*12*, *21*).

**Statistical Analysis.** Analysis of data was carried out with Statistica, version 5.1. Differences were determined with one-way ANOVA, LSD test at 95% confidence interval.

## RESULTS

**Antioxidant Activity of Extracts.** Of all the extracts generated, only 1-butanol and the left-over aqueous fraction were fully soluble in water. Consequently, all samples and controls were reconstituted in 30% aqueous methanolic solution, which was the optimized medium ensuring complete solubility of the organic extract components. Comparison of oxidation rates of the control as well as the derived standard curve of 1,1,3,3-tetraethoxypropane in water and in 30% aqueous methanolic solutions showed that in the case of the linolenic acid assay the oxidation rates were not different, in contrast to the 2-deoxy-

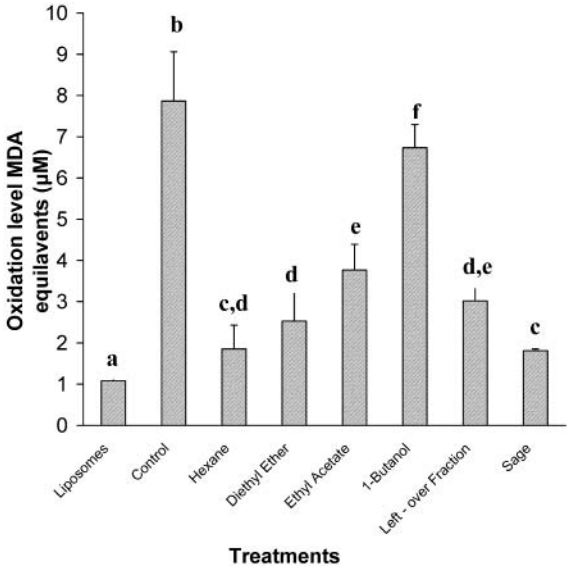


**Figure 1.** Oxidation levels (MDA equivalents in $\mu$M) in linolenic acid liposomes with iron catalyst Fe(III) + ascorbic acid + $H_2O_2$ in the presence and absence of organic extract, the left-over aqueous fraction, and sage, all reconstituted in 30% methanol. Results are the means ± standard deviation of multiple experiments ($4 < n < 8$). Samples bearing different letters (**a**–**f**) are significantly different from the remainder of the samples.

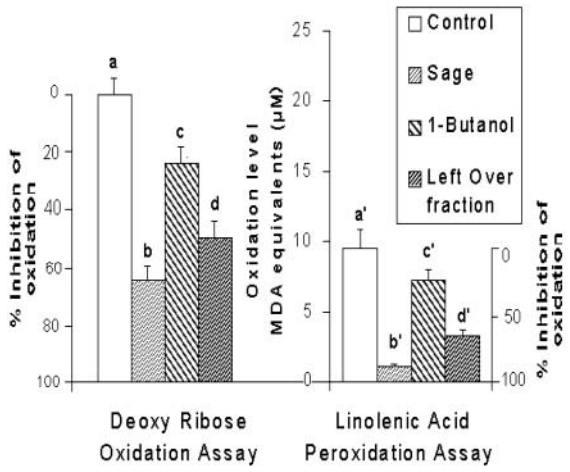


**Figure 2.** Oxidation (MDA equivalents in $\mu$M) and percent inhibition of oxidation levels in (a, left) Fe(III)-catalyzed 2-deoxy-D-ribose degradation and (b, right) linolenic acid liposomes with iron catalyst Fe(III) + ascorbic acid + $H_2O_2$ in the absence and presence of 1-butanol extract and the left-over aqueous fraction, reconstituted in water, and aqueous infusions of sage. Results are the means ± standard deviation of multiple experiments ($3 < n < 6$). All samples are significantly different from each other ($P < 0.05$), and they bear different letters (**a**–**d** for panel a), and **a′**–**d′** for panel b).

D-ribose assay where the oxidation rates were different ($P < 0.05$). Concomitantly, all samples and controls prepared in 30% aqueous methanolic solutions were tested by the linolenic acid assay (**Figure 1**). Sage, the control, the 1-butanol extract, and the left-over aqueous fraction in water were tested by both the linolenic acid peroxidation and the 2-deoxy-D-ribose assays (**Figure 2**).

The linolenic acid peroxidation assay was carried out under Fenton reaction conditions, formulated by the presence of Fe(III), ascorbic acid, and hydrogen peroxide. In this work, too, $(NH_4)_5[Fe(Cit)_2]\cdot 2H_2O$ was used as a source of Fe(III) capable of generating radicals attacking the substrates under investigation (*16*). The advantage of using such a form of Fe(III) denotes not only the hydrolytic stability and retention of Fe(III) in $(NH_4)_5[Fe(Cit)_2]\cdot 2H_2O$ at or near physiological pH values but also the availability of a well-defined form of Fe(III) being capable of promoting peroxidation under the herein examined Fenton reaction conditions. Under these conditions the results revealed the following (**Figure 1**): (a) Sage displayed antioxidant activity, reflecting 77% inhibition of oxidation in comparison to the control ($P < 0.05$). This result was in line with our previous findings on sage aqueous infusions (*16*). (b) The less polar solvent extracts of *n*-hexane, diethyl ether, and ethyl acetate exhibited high antioxidant activity, reflecting 76, 68, and 52% inhibition of oxidation, respectively, in comparison to the control ($P < 0.05$). The antioxidant activity of the *n*-hexane fraction was comparable to that of sage ($P < 0.05$). The diethyl ether and ethyl acetate extracts also displayed antioxidant activity, which was high with respect to the control ($P < 0.05$). The ether extract had an antioxidant activity comparable with that of the *n*-hexane extract ($P > 0.05$). The ethyl acetate had a lower antioxidant activity in comparison to the *n*-hexane extract and sage ($P < 0.05$). (c) The most polar solvent extract in 1-butanol exhibited low activity ($P < 0.05$), reflecting 14% inhibition of oxidation with respect to the control. Undoubtedly, the displayed activity was significantly lower than that of all of the aforementioned extracts ($P < 0.05$). (d) The left-over aqueous fraction also exhibited high antioxidant activity, reflecting 62% inhibition of oxidation with respect to the control ($P < 0.05$).

The antioxidant activities of the 1-butanol extract and the left-over aqueous fraction were also evaluated in samples reconstituted in water (**Figure 2**). In that case, the observed antioxidant activity of all samples was significant in comparison to the control ($P < 0.05$). The aqueous infusion of sage, the 1-butanol extract, and the left-over aqueous fraction inhibited oxidation by 89, 23, and 65%, respectively, in comparison to the control.

The antioxidant activity of the aqueous infusion of sage, the 1-butanol extract, and the left-over aqueous fraction was examined by the 2-deoxy-D-ribose oxidation assay (**Figure 2**). Other extracts were not tested by this method because they were not soluble in water. All three samples showed significant activity in comparison to the control ($P < 0.05$). The aqueous infusion of sage, the 1-butanol extract, and the left-over aqueous fraction inhibited oxidation by 65, 24, and 50%, respectively, in comparison to the control. The observed activities in the 1-butanol extract and the left-over aqueous fraction were clearly lower than that of the aqueous infusion of sage ($P < 0.05$).

**UV−Visible Investigation of the Organic Solvent Extracts from Aqueous Infusions of Sage (*S. fruticosa* L.).** The UV−visible spectra of the organic extracts are shown in the range from 190 to 600 nm in **Figure 3**. It appears that all of the extracts exhibit a pattern of similar absorptions that extends from 200 to 350 nm. The absorptions observed in each extract are clearly resolved in the respective spectra. Shifts in the maxima of the displayed absorptions are also distinct and may be due to the presence of chromophores capable of effecting hypsochromic or bathochromic phenomena. The corresponding spectrum of the left-over aqueous fraction displays a strong absorption at 200 nm with weak shoulders around 274 and 340 nm. The presence of absorptions in the 200−340 nm range is indicative of chromophores, such as substituted aromatic rings, carbonyl groups, and organic acids. The presence of discrete absorptions at 280 nm indicates the presence of groups containing benzene ring(s). On the basis of the above findings,

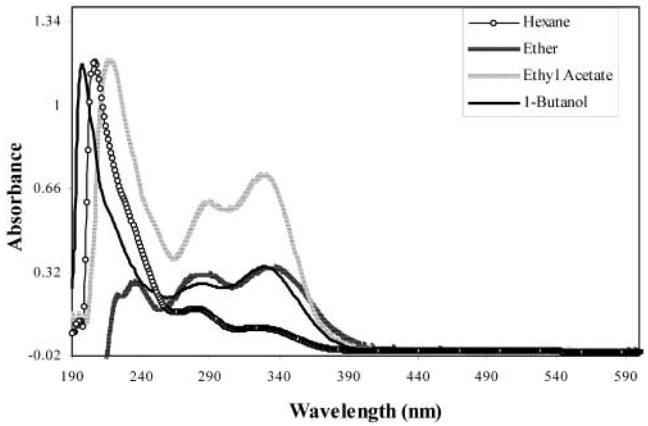


**Figure 3.** UV–visible spectra of organic extracts from aqueous infusions of sage (*S. fruticosa* L.).

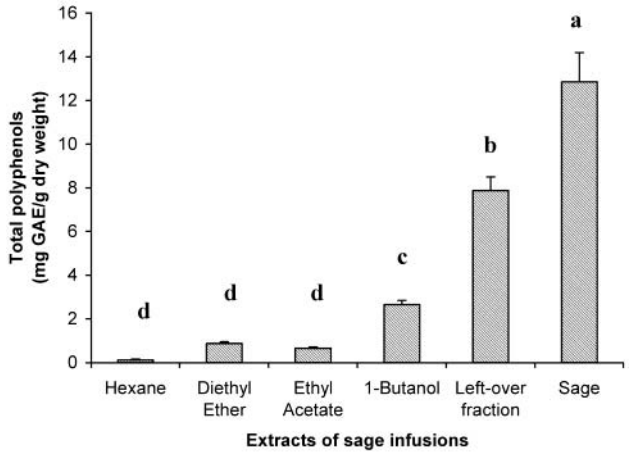


**Figure 4.** Total polyphenol content in the four organic extracts and the left-over aqueous fraction from aqueous infusions of sage (*16*). Results are means ± standard deviation of multiple experiments ($4 < n < 8$). Samples bearing different letters (**a**–**d**) are significantly different from the remainder of the samples.

the analyses of the extracts by HPLC were pursued at two different wavelengths, namely, 230 and 330 nm.

**HPLC Analysis.** In the *n*-hexane extract, the compounds identified were carnosol and carnosic acid. These compounds were quantified with the help of available standards. In the diethyl ether extract, the identified compound was rosmanol. In the ethyl acetate extract, caffeic acid was identified. Caffeic acid was also identified in the 1-butanol extract. Beyond the already mentioned compounds, cryptotanshinone and catechin were also examined but not found to be present in all employed extracts.

Carnosol, carnosic acid, rosmanol, and caffeic acid were quantified with the aid of standard curves. Their concentrations were 0.66 ± 0.19, 1.31 ± 0.33, 0.56 ± 0.12, and 0.26 ± 0.07 mg, respectively, per 100 mL of aqueous infusion of sage. The reported amount of caffeic acid is the total amount found in the ethyl acetate and 1-butanol extracts.

**Polyphenol Determination in the Extracts.** The variable polyphenol content of the extracts is shown in **Figure 4**. The *n*-hexane, diethyl ether, and ethyl acetate extracts had a low content of polyphenols compared with the more hydrophilic 1-butanol extract. The left-over aqueous fraction had the highest polyphenol content. The presence of polyphenolic compounds has been previously linked with the antioxidant activity toward metal ion promoted oxidation in a number of plant extracts (*22*, *23*).

## DISCUSSION

**Antioxidant Activity of Organic Extracts from Sage Aqueous Infusions.** In an attempt to investigate the specifics of the relationship between antioxidant activity and antioxidant compounds present in herbs, we have examined the aqueous infusions of *S. fruticosa* L., an herb habitually consumed in the Mediterranean region. Our goal was to identify constituents in aqueous infusions of sage that would be linked eventually with the exhibited antioxidant activity toward iron-promoted oxidation. To pursue such a strategy, we opted for a process of organic solvent extraction, affording isolation of groups of compounds based on chemical properties such as polarity, lipophilicity, or hydrophilicity. To this end, we adopted a sequence of extractions in the order *n*-hexane, diethyl ether, ethyl acetate, and 1-butanol. This approach afforded four extracts, each of which could be tested, as a start, for their antioxidant capacity. Subsequent HPLC separation would target specific components of potential antioxidant activity.

The antioxidant activity of herb infusions has long been associated with the polyphenol content in herbs (*22*−*24*). The polyphenol determination showed that the highest amount of polyphenols was present in 1-butanol, the most polar fraction. The lowest amount of polyphenols was present in the *n*-hexane extract, consistent with the lowest polarity and highest lipophilicity of that solvent. The determination of the total polyphenol content in the left-over aqueous fraction showed that a portion of the total polyphenols had not been extracted into the organic solvents and remained in the aqueous phase. The antioxidant activity exhibited by the 1-butanol extract and the left-over aqueous fraction, evaluated by the linolenic acid peroxidation and 2-deoxy-D-ribose oxidation assays, reflects the capacity of polyphenols to act as antioxidants, counteracting hydroxyl radicals generated under experimental conditions akin to those of Fenton or Haber−Weiss reactions. These two reactions provide the pro-oxidant species widely suspected for their destructive assault on a variety of biological tissues. To this end, polyphenols have been widely established as efficient radical scavengers through a variety of mechanisms, including that of the chelation of iron and concomitant neutralization of its promoted ROS generation (*25*). Observed differences in activity of the 1-butanol extract between the two assays may be attributed to the presence of different substrates as well as to the variable nature of products generated by the two reaction systems (*26*, *27*).

The results of the linolenic acid assay indicate that the less polar solvents exhibit the highest antioxidant activity despite the low content in polyphenols. Hence, it is likely that under the employed experimental conditions either (a) the polyphenols, albeit at low concentration, present in *n*-hexane, diethyl ether, and ethyl acetate are more potent in antioxidant activity than the corresponding polyphenols in the 1-butanol extract or the left-over aqueous fraction or (b) constituents other than polyphenols, such as carnosol, carnosic acid, and rosmanol, all of them known antioxidants, are very powerful in comparison to antioxidants in 1-butanol or the left-over aqueous fraction. Such variability in composition and associated antioxidant activity may be key attributes to the overall antioxidant capacity of various herbs that have been investigated for their potential antioxidant properties in different biological systems (*28*−*30*).

The association of the nature of antioxidants in herb infusions with their variable antioxidant activities toward iron-promoted oxidation through different mechanistic pathways has been previously observed by us and others (*16*, *31*−*33*). As a whole, therefore, it appears that polyphenols may contribute to the

overall antioxidant activity of sage aqueous infusions, but they are likely not the sole regulators of such activity (*30*). Attempts to correlate the observed antioxidant activity with the polyphenol content of the extracts did not reveal any direct association between the two parameters. Collectively, the data support the existence of both lipophilic and hydrophilic components in sage aqueous infusions. These substances are capable of exerting antioxidant activity through different pathways, reflecting variable mechanisms operating on diversely generated ROS.

**HPLC Separation and Identification of Antioxidant Substances.** The analytical investigations of herb extracts by HPLC present a scientific challenge, because the complexity in the number of components and the conditions under which they can be separated continue to be serious impediments in contemporary research in this field. Owing to the biological importance of individual components in the established antioxidant activity of herb infusions from the Mediterranean region, the qualitative and quantitative analysis of such herbs constitutes a task of formidable dimensions. Despite these difficulties, the separation of different compounds in the aqueous infusions and their subsequent identification and quantification are obviously essential in rationalizing the antioxidant activity of these herbs. There have been a number of reports in the literature describing the analytical endeavors on plant extracts, focusing on various parts of the plants, such as leaves and roots, by HPLC (*34*−*36*). In this work, attention was directed toward the analytical determination of components present in aqueous infusions of sage (*S. fruticosa* L.). In an effort to simplify and expedite the separation of these infusions into the large number of their constituents, initial trials concentrated on parting the infusions into four different organic solvent extracts, to be further separated by HPLC. The components identified, as being present in the extracts, and quantified were the following: carnosol and carnosic acid in *n*-hexane and rosmanol in diethyl ether. Caffeic acid was found in both ethyl acetate and 1-butanol extracts, testimony to the linkage between the increasing polarity of the extracting solvent and the hydrophilic nature of the compound being extracted. The above compounds are well established in the literature for their in vitro antioxidant activity in various systems (*37*−*40*). Specifically, of the four compounds, carnosol and carnosic acid have been recognized as the most potent antioxidants in herb extracts, like those from rosemary (*41*, *42*). Furthermore, of the aforementioned two molecules, carnosic acid was reported to exhibit a higher antioxidant activity than carnosol (*38*, *42*, *43*). Rosmanol and caffeic acid were equally allotted their share of antioxidant potency in counteracting oxidative processes (*44*−*46*). It should be emphasized, at this point, that (a) the literature available in the relevant field of herbs and (b) the UV−visible spectra of the extracts derived from aqueous infusions of sage were essential in the employment of such standards for the identification of compounds in the extracts.

On the basis of a number of standards, the HPLC separation and concomitant identification procedures revealed the identity of only a limited number of compounds present in the extracts. A plethora of compounds, which do not match the standards used under the specified experimental conditions, are present in our extracts from *S. fruticosa* L. Hence, further perusal of the composition of the extracts is well warranted through a multitude of characterization approaches including chromatographic methods coupled with spectroscopic (NMR, MS, etc.) and structural techniques (X-rays).

Finally, in light of the antioxidant properties of the identified compounds, it appears that the latter, as components of the organic solvent extracts, contribute to the overall antioxidant activity toward iron-promoted oxidative processes in sage. It is not, then, unreasonable to envisage that lipophilic molecules along with hydrophilic polyphenols and other as yet unidentified components in the aqueous infusions of sage modulate the antioxidant defense against oxidative processes.

**Conclusions.** Extracts in *n*-hexane, diethyl ether, ethyl acetate, and 1-butanol were generated from aqueous infusions of sage (*S. fruticosa* L.). The extracts and the left-over aqueous fraction were found to exhibit variable antioxidant activity toward Fe(III)-promoted oxidation of linolenic acid and 2-deoxy-D-ribose.

The presence of polyphenols is reflected in the overall antioxidant activity observed in the aqueous infusions of sage, yet polyphenols are not the sole determinants of that activity. Among others, lipophilic diterpenes and hydrophilic polyphenols, found in sage, both contribute to the antioxidant activity of the infusions toward oxidative processes.

There is no direct association of the observed antioxidant activity with the polyphenol contents in the organic solvent extracts (*n*-hexane, diethyl ether, ethyl acetate, and 1-butanol).

HPLC work on the four organic extracts revealed a plethora of substances, four of which were identified and determined quantitatively. These substances are carnosol, carnosic acid, rosmanol, and caffeic acid. All of them are known antioxidants against variably generated ROS linked oxidative processes.

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