

# Antioxidative Activity and Phenolic Composition of Pilot-Plant and Commercial Extracts of Sage and Rosemary

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**ABSTRACT:** Eight sage (*Salvia officinalis*) and twenty-four rosemary (*Rosmarinus officinalis*) extracts, originating from pilot-plant or commercial sources, had different antioxidative activities as measured by accelerated autoxidation of methyl linoleate. Twenty-seven compounds were characterized in the Labiatae family extracts by high-performance liquid chromatography (HPLC) coupled with mass spectrometry, equipped with an atmospheric pressure chemical ionization interface, and by HPLC coupled with a photodiode array spectrophotometer. Twenty-two compounds were identified, including phenolic acids, carnosol derivatives, and flavonoids. The extracts showed great variation in their HPLC profiles, and no correlation was apparent between their antioxidative efficiency and their composition, in twenty specific phenols. Data indicated that the most effective compounds were carnosol, rosmarinic acid, and carnosic acid, followed by caffeic acid, rosmanol, rosmadial, genkwanin, and cirsimaritin.

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**KEY WORDS:** Antioxidant, antioxidative activity, HPLC–MS, phenolic compounds, rosemary, sage.

To prevent oxidation of fats and oils, antioxidants are widely used in foods and cosmetics. Because of possible toxicity of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), together with consumers' preference for "natural" products, much natural antioxidant research has been undertaken during the past ten years. Among the plants reported to have antioxidative activity (1–4), rosemary is the most widely used and commercialized.

The main antioxidative effect of rosemary (Rom) and sage has been reported to relate to the presence of three phenolic compounds: carnosic acid, carnosol, and rosmarinic acid (5–8). Indeed, the composition of sage and Rom extracts is quite complex, and many other components have been identified, including other phenolic acids, diterpenes such as rosmanol and carnosol derivatives, and flavonoids, in particular, flavones (2,9–13). All these phenolic compounds are potent antioxidants. The antioxidative behavior and synergistic action of most of the compounds remain unknown.

We have recently published data regarding the chromatographic separation of sage phenolic compounds of various po-

larities (14). The purpose of this current work was to study the relationship between phenolic composition and antioxidative activity of eight sage and twenty-four rosemary extracts.

## EXPERIMENTAL PROCEDURES

**Materials.** Table 1 lists the characteristics of the studied extracts and their reference codes. Four sage (*Salvia officinalis*)

**TABLE 1**  
List of the Thirty-Two Extracts Analyzed for Antioxidant Activity and Phenolic Content

| Code    | Source      | Extraction mode                           | Formula <sup>a</sup>      |
|---------|-------------|---|---------------------------|
| Sage 1  | Pilot plant | Hexane extraction                         | Liquid                    |
| Rom 2   | Commercial  | n.m. <sup>b</sup>                         | Powder                    |
| Sage 3  | Pilot plant | Hexane extraction                         | Liquid                    |
| Rom 4   | Commercial  | n.m.                                      | Oil-soluble liquid        |
| Rom 5   | Commercial  | n.m.                                      | Oil-dispersible liquid    |
| Rom 6   | Commercial  | Oleoresin                                 | Liquid                    |
| Rom 7   | Commercial  | n.m.                                      | Oil-dispersible liquid    |
| Sage 8  | Commercial  | CO <sub>2</sub> Extraction                | Liquid                    |
| Sage 9  | Commercial  | n.m.                                      | Oil-dispersible liquid    |
| Rom 10  | Commercial  | n.m.                                      | Liquid in 1,2-propanediol |
| Rom 11  | Commercial  | n.m.                                      | Oil-soluble powder        |
| Sage 12 | Pilot plant | Ethanol extraction                        | Liquid                    |
| Rom 13  | Commercial  | n.m.                                      | Oil-soluble liquid        |
| Rom 14  | Commercial  | CO <sub>2</sub> Extraction                | Oil-soluble powder        |
| Sage 15 | Commercial  | n.m.                                      | Oil-soluble liquid        |
| Rom 16  | Commercial  | n.m.                                      | Water-dispersible liquid  |
| Rom 17  | Commercial  | n.m.                                      | Powder                    |
| Rom 18  | Commercial  | n.m.                                      | Oil-soluble liquid        |
| Sage 19 | Commercial  | Oleoresin                                 | Liquid                    |
| Rom 20  | Commercial  | n.m.                                      | Oil-soluble powder        |
| Rom 21  | Commercial  | n.m.                                      | Powder                    |
| Rom 22  | Commercial  | CO <sub>2</sub> Extraction + distillation | Oil-soluble liquid        |
| Rom 23  | Commercial  | CO <sub>2</sub> Extraction + distillation | Oil-soluble liquid        |
| Rom 24  | Commercial  | n.m.                                      | Oil-soluble powder        |
| Rom 25  | Commercial  | n.m.                                      | Powder                    |
| Rom 26  | Commercial  | n.m.                                      | Oil-soluble powder        |
| Sage 27 | Pilot plant | Ethanol extraction                        | Liquid                    |
| Rom 28  | Commercial  | n.m.                                      | Powder                    |
| Rom 29  | Commercial  | n.m.                                      | Poorly oil-soluble powder |
| Rom 30  | Commercial  | n.m.                                      | Oil-dispersible powder    |
| Rom 31  | Commercial  | n.m.                                      | Oil-soluble powder        |
| Rom 32  | Commercial  | n.m.                                      | Oil-dispersible powder    |

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<sup>a</sup>Set by manufacturers for commercial extracts; Rom, rosemary.

<sup>b</sup>n.m. = Not mentioned by manufacturers.

samples were specifically processed in a pilot plant by the CAL-Pfizer Company (Grasse, France). Two commercial extracts were hexane oleoresin, and four others were supercritical carbon dioxide extracts. The technology applied to the remaining twenty-two commercial extracts was unknown because it was not mentioned by manufacturers. Fourteen extracts were in powder form; the others were liquids or diluted in a vegetable oil. Most of the commercial extracts had been formulated by manufacturers to be dissolved or dispersed in oil. All extracts were dissolved in methanol and filtered before phenolic content analyses.

**High-performance liquid chromatography–mass spectrometry (HPLC–MS) analysis.** Analyses were performed with a quadrupole mass spectrometer (Trio 1000; Fisons Instruments, Manchester, United Kingdom) by using an atmospheric-pressure chemical ionization interface (APCI). The separation was carried out on an HPLC apparatus (600 ms; Waters, St. Quentin en Yvelines, France) equipped with a 25 cm × 4.6 mm Hypersil ODS column (5 mm). The mobile phase was a mixture of solvent A (acetonitrile/water/acetic acid, 15:84:0.85) and solvent B (methanol) according to a step gradient, lasting 90 min, from 0% B to 100% B, at a flow rate of 1 mL/min (14). In the APCI method, the eluted compounds were mixed with nitrogen in the heated nebulizer interface and chemically ionized by proton transfer by using a corona discharge needle. The generated positive ions were then introduced into the Trio 1000 MS. Adequate calibration of APCI parameters (needle potential 4000 V, heater 500–600°C, cone voltages SKM 1–3V and SMP 15–30V) was required to optimize the response and to obtain a high sensitivity on the molecular ion (15).

**Quantitative composition.** Before admission into the APCI interface, the absorbance of eluates was measured at 284 nm, near the maximum absorption of most phenols [Waters 486 ultraviolet (UV) detector]. Unfortunately, the lack of standards for most of the components and their unknown molecular absorption coefficient did not allow quantitation of their content. Nevertheless, peak areas of each compound can be compared from one extract to another, even for flavones where 284 nm was not the maximum absorption. The values were calculated from at least three replicates.

**UV spectrophotometry.** To collect spectral data of each separated component, sage and rosemary extracts were analyzed by HPLC with an HP 1040A photodiode array detector (Hewlett-Packard, Waldbronn, Germany) under the chromatographic conditions described above.

**Antioxidative power (AOP) measurements.** The antioxidative activity was evaluated according to the method of Cuvelier *et al.* (16). The disappearance of methyl linoleate under strong oxidizing conditions (i.e., 110°C and intensive pure oxygen bubbling at a flow rate of 7 mL/min) in a lipophilic solvent (dodecane) was followed by gas-chromatographic analyses. The half-life of methyl linoleate was measured from the kinetics of its disappearance. Experimental data were collected every 30 min until methyl linoleate was half consumed, which took less than four hours with 4% (vol/vol) initial con-

centration of methyl linoleate. Activity of antioxidants was assessed by the relative increase in the half-life reaction time of methyl linoleate. Because efficiency varies according to the nature of the antioxidant and its concentration in the medium, extract activities were defined by the quantity of each plant extract required to double the half-life reaction time of the control (EQ = efficient quantity). The AOP was compared to that of BHT (EQ<sub>BHT</sub>) in Equation 1:

$$\text{AOP} = \frac{\text{EQ}_{\text{BHT}}}{\text{EQ}_{\text{extract}}} \quad [1]$$

The greater the AOP, the stronger the antioxidative power of the extract. At least three concentrations of each extract were tested to get EQ.

To improve dissolution or dispersion in dodecane, most of the extracts had to be previously dissolved in 1-butanol. The extracts Sage 1, 3, 8, 19 and Rom 7, 22, 23 (see codes in Table 1) were directly used in dodecane.

**Statistical methods.** The peak areas of specific compounds were taken as variables related to AOP in four statistical methods: linear multiple regression, ascendant hierarchic classification, principal component analysis (17), and neuronal network (18).

## RESULTS AND DISCUSSION

**Characterization of sage and rosemary phenolic compounds.** HPLC analyses of the sage and rosemary extracts revealed at least fifty compounds. Most of these compounds are seen on the chromatogram of Figure 1, which was obtained by mixing two different sage extracts prepared in the pilot plant (14). Twenty-seven compounds were characterized by HPLC retention time, UV spectrum, and mass spectrum (Table 2). Twenty-two compounds were identified after comparison with published data or commercial standards. They can be grouped into three classes of phenolic compounds: (i) phenolic acids: vanillic, caffeic, ferulic, and rosmarinic acids; (ii) diterpenes: carnosol, rosmadial, carnosic acid, methyl carnosate, rosmanol, epirosmanol, epiisosmanol, epirosmanol methyl ether, and epiisosmanol ethyl ether; and (iii) flavonoids: hesperetin, apigenin, genkwanin, 4'-methoxytecto-chrysin, cirsimaritin, scutellarein, 4',5,7,8-tetrahydroxyflavone, homoplantaginin, and 6-hydroxyluteolin 7-glucoside. Their structures are presented in Figure 2.

Some of these compounds had been identified previously (11,14). Epiisosmanol, epirosmanol methyl ether, and epiisosmanol ethyl ether were identified from their UV spectra, which were closely related to that of carnosol derivatives and particularly to that of epirosmanol, and from their characteristic mass spectra. The mass fragmentations of epiisosmanol (MH<sup>+</sup> = 347), epirosmanol methyl ether (MH<sup>+</sup> = 361), and epiisosmanol ethyl ether (MH<sup>+</sup> = 375) were similar to that of epirosmanol (Fig. 3). A fragment at *m/e* 283 is characteristic of the HCOOH loss, which corresponds to lactone cleavage, and of the loss of H<sub>2</sub>O or CH<sub>3</sub>OH or CH<sub>3</sub>CH<sub>2</sub>OH (R<sub>1</sub>). A fragment at *m/e* 329 corresponds to MH<sup>+</sup> – H<sub>2</sub>O for

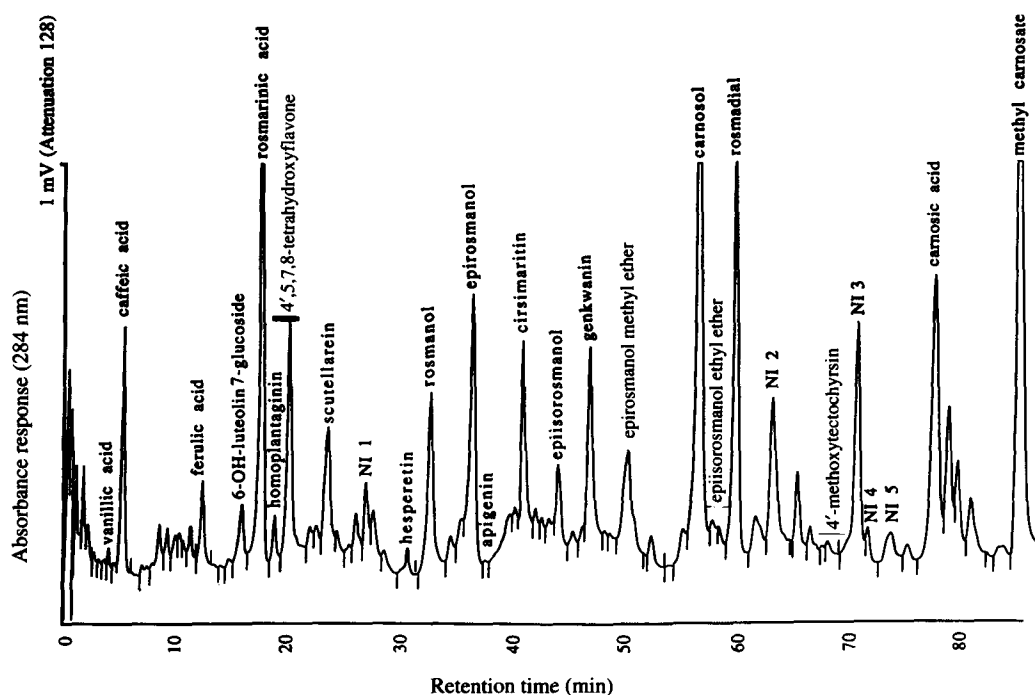


FIG. 1. High-performance liquid chromatography profile of an experimental solution obtained by mixing two pilot-plant extracts of sage. Waters (St. Quentin en Yvelines, France) 600-MS, 25 cm  $\times$  4.6 mm Hypersil ODS column (5  $\mu$ m). Mobile phase: 0–100% methanol step gradient over 90 min in acetonitrile/water/acetic acid (15:84:0.85), flow rate 1 mL/min. Detection at 284 nm; NI, not identified.

TABLE 2  
Characterization of Twenty-Seven Phenolic Compounds Separated by HPLC  
in Rosemary and Sage Extracts

| Compounds                     | Retention time (min) | UV Absorbance maximum (nm) | Mass ions (APCI) |                 |
|-------------------------------|----------------------|----------------------------|------------------|-----------------|
|                               |                      |                            | MH <sup>+</sup>  | Major fragments |
| Vanillic acid                 | 6                    | 260, 292                   | 169              |                 |
| Caffeic acid                  | 7                    | 242, 296, 324              | 181              |                 |
| Ferulic acid                  | 14                   | 244, 298, 324              | 195              |                 |
| 6-Hydroxyluteolin 7-glycoside | 17                   | 255, 272, 345              | 465              | 303             |
| Rosmarinic acid               | 19                   | (290) <sup>a</sup> , 328   | 361              | 163, 181, 195   |
| Homoplantagin                 | 20                   | 266, 334                   | 463              | 301             |
| 4',5,7,8-Tetrahydroxyflavone  | 22                   | 268, 340                   | 287              |                 |
| Scutellarein                  | 25                   | 268, 337                   | 287              |                 |
| NI 1 <sup>b</sup>             | 27                   | 258                        | 151              | 109             |
| Hesperetin                    | 31                   | 287                        | 303              |                 |
| Rosmanol                      | 33                   | (226), 284                 | 347              | 301             |
| Epirosmanol                   | 37                   | (227), 288                 | 347              | 283             |
| Apigenin                      | 38                   | 367, 340                   | 271              |                 |
| Cirsimaritin                  | 42                   | 274, 334                   | 315              |                 |
| Epiisorosmanol                | 44                   | (228), 288                 | 347              | 283, 329        |
| Genkwanin                     | 47                   | 266, 336                   | 285              |                 |
| Epirosmanol methyl ether      | 50                   | (228), 288                 | 361              | 283             |
| Carnosol                      | 56                   | 284                        | 331              | 283             |
| Epiisorosmanol ethyl ether    | 58                   | (228), 288                 | 375              | 283, 329        |
| Rosmadiol                     | 60                   | 234, 290                   | 345              |                 |
| NI 2                          | 62                   | (246), 308                 | 331              | 285, 301        |
| 4'-Methoxytecto-chrysin       | 68                   | 268, 332                   | 299              |                 |
| NI 3                          | 69                   | 278                        | 347              | 301             |
| NI 4                          | 70                   | 276                        | 281              |                 |
| NI 5                          | 72                   | 224, 272                   | 299              |                 |
| Camosic acid                  | 77                   | (228), 284                 | 333              | 287             |
| Methyl carnosate              | 84                   | (228), 282                 | 347              | 301             |

<sup>a</sup>Parentheses indicate a shoulder; HPLC, high-performance liquid chromatography; UV, ultraviolet; APCI, atmospheric pressure chemical ionization. <sup>b</sup>NI = not identified.

epiisorosmanol and  $MH^+ - CH_3CH_2OH$  for epiisorosmanol ethyl ether. An additional ion  $[(M + OH) + H]^+$  is a common occurrence in chemical positive ionization.

In the flavonoid group, one flavanone (hesperetin) and eight flavones with one to four hydroxyl groups were identified (Fig. 2). The UV spectra of these flavonoids were specific, and their mass spectra were characterized by a strong molecular ion, as shown by the example of 4',5,7,8-tetrahydroxyflavone in Figure 3. The identification of 4',5,7,8-tetrahydroxyflavone and scutellarein was confirmed by UV spectral and retention time data of closely related commercial

standards: 5,7,8-trihydroxyflavone and 5,6,7-trihydroxyflavone. Two glycosylated flavones were found in the extracts. They showed a characteristic fragmentation that led to a simple mass spectrum with only two ions,  $MH^+$  and the aglycone, as observed for 6-hydroxyluteolin 7-glucoside in Figure 3.

*AOP of sage and rosemary extracts.* Ranking of 32 extracts according to their AOP values is reported in Figure 4. Each value represents the result of three measurements with a mean range of variation of about 8%. A great variation in activity was observed between the extracts: the strongest ex-

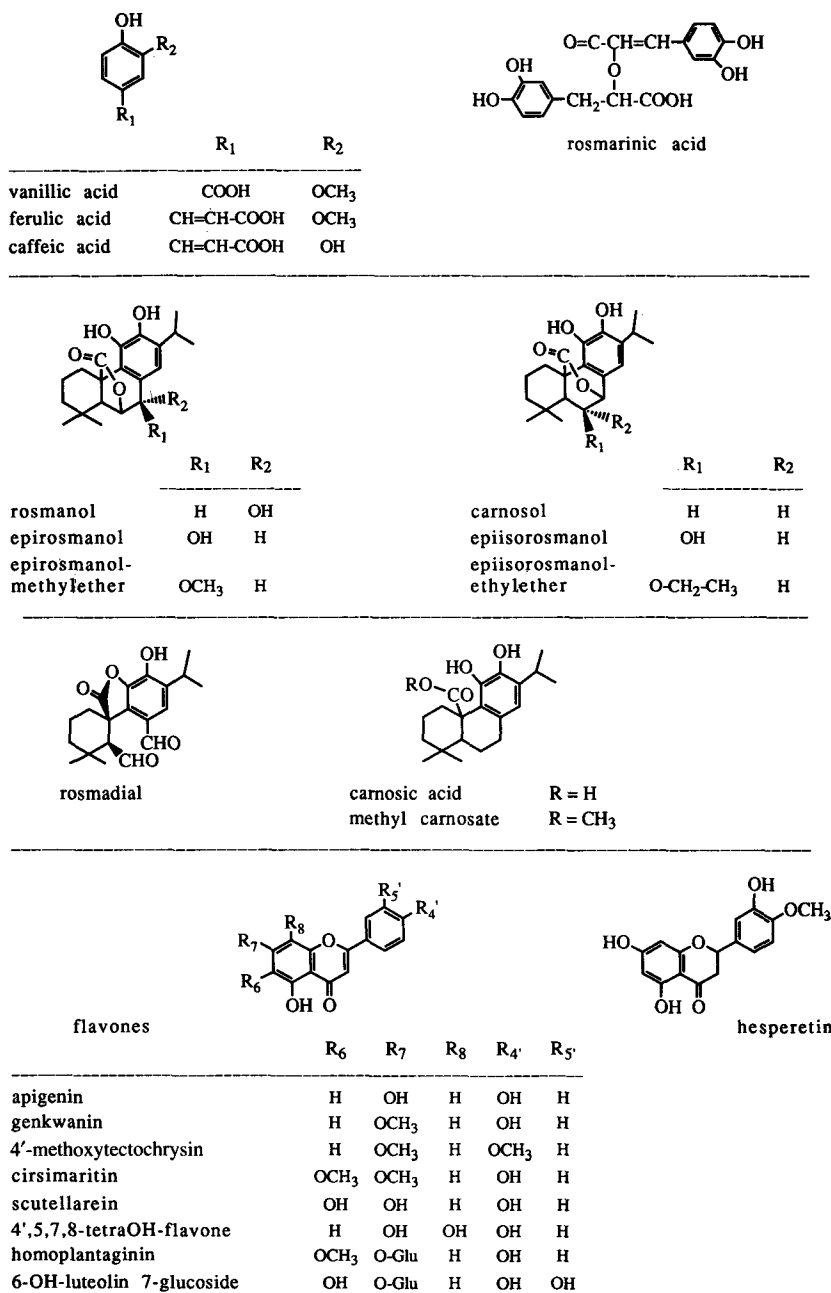


FIG. 2. Structures of the twenty-two phenolic compounds identified from rosemary and sage extracts.

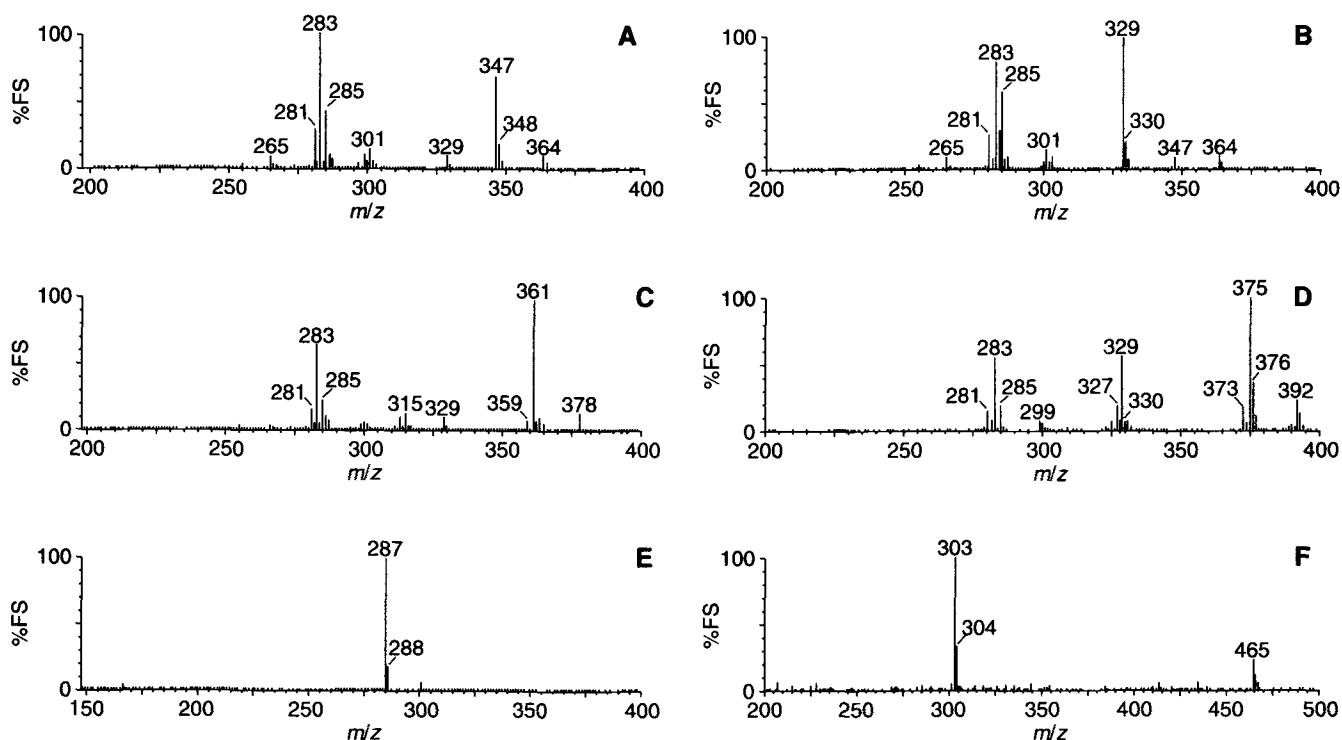


FIG. 3. Mass spectra of (A) epirosmanol, 346 amu; (B) epiisosmanol, 346 amu; (C) epirosmanol methyl ether, 360 amu; (D) epiisosmanol ethyl ether, 374 amu; (E) 4',5,7,8-tetrahydroxyflavone, 286 amu; (F) 6-hydroxyluteolin 7-glycoside, 464 amu.

tract, Rom 32, was 30 times more powerful than Sage 1 or Rom 2. Some observations can be made to explain these diverse results.

We know that the quality of natural extracts and their antioxidative performances depend first of all upon the quality of the original plant, its geographic origin, the climatic conditions, the harvesting date, its storage. This is illustrated by the difference measured between the activity of Sage 12 and Sage 27, which were two ethanol pilot plant extracts made at different harvesting dates: Sage 12, the less active, was harvested one month later than Sage 27. The same observation can be made to explain some large differences within commercial extracts. But pertinent information from manufacturers was lacking to further our interpretation.

Few of the sage extracts had good AOP values, and the commercial rosemary extracts were generally the most powerful. In the literature, the two Labiatae species are often quoted for their antioxidative activity (1–3,5,11). But, rosemary has been studied more than sage, and several new extraction processes (3,6,7,19) have been developed to bring rosemary extracts on the market.

Systematically, powdered commercial formula showed better AOP values than the liquid commercial extracts. These results can be explained first by better solubility of the powders in butanol, which was used before the AOP test, and secondly by a lower antioxidant content in the liquid formula. In fact, several liquid formula were oil dilutions of a powder extract, for specific applications as mentioned by manufacturers: i.e.,

Rom 7 was diluted from Rom 30; Rom 16 and Rom 18 from Rom 24; Rom 4 from Rom 11; and Sage 9 from Sage 15.

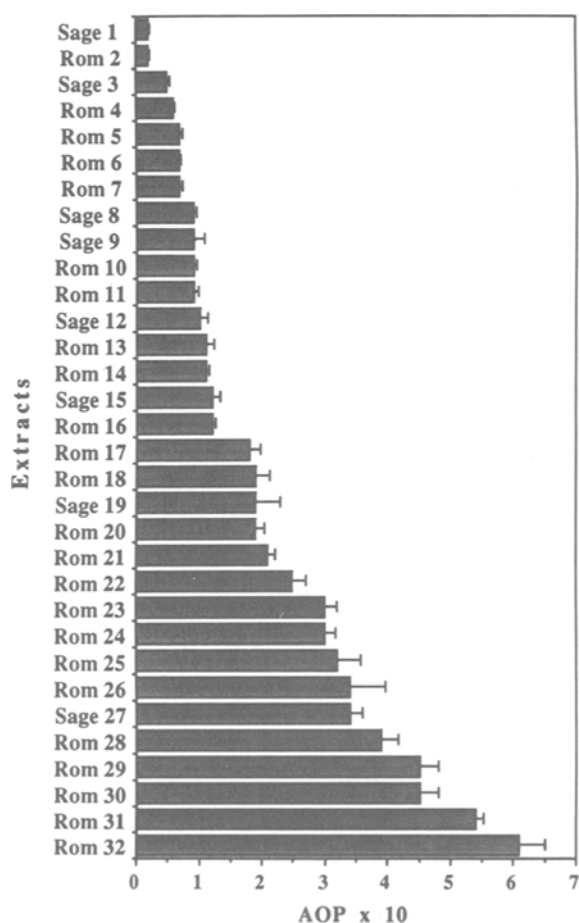
Antioxidative performance can depend also upon the extraction parameters, as mentioned by Chen *et al.* (20), who obtained better antioxidative efficacy of rosemary hexane extracts than of methanol extracts. However, our results reveal no clear solvent effect on activity between hexane, ethanol, and CO<sub>2</sub> extracts. They indicate the presence of antioxidant compounds with various polarities, ranging from polar rosmarinic acid to apolar carnosic acid.

Finally, the above parameters only partly explain the great AOP differences between extracts. Because plant origin and extraction conditions play a role in phenolic content of the extracts, it would be interesting to try to relate AOP to the content of antioxidant compounds.

*Qualitative composition and antioxidant performances.* Among the twenty-seven separated compounds, ferulic acid, epiisosmanol, homoplantagin, and hesperetin were detected only in one to three extracts, and vanillic acid, apigenin, and epiisosmanol ethyl ether were present only in trace amounts and could not be quantitated. Therefore, these seven compounds are not considered when comparing the profiles of the different extracts (Table 3).

Rosemary and sage both belong to the Labiatae family. Their chromatographic profiles appear to be close. Carnosol and rosmadial have a high frequency of appearance in extracts from both species (Table 4). Methyl carnosate and compound NI 2 have a higher frequency in sage than in rosemary, while





**FIG. 4.** Antioxidative power [AOP, with relation to butylated hydroxytoluene, was measured by accelerated autoxidation of methyl linoleate (apolar medium, 110°C, pure oxygen bubbling) (Ref. 16)] of thirty-two extracts of sage and rosemary (Rom) (see Table 1 for description of extracts).

the opposite is true for rosmarinic acid and genkwanin. Only three flavones out of nine flavonoids (6-hydroxyluteolin, 4',5,7,8-tetrahydroxyflavone, and scutellarein) and compound NI 1, present in some rosemary extracts, were not detected in the sage extracts (Table 3). The number of analyzed sage samples, however, was too small to rule out the possible presence of these compounds in this species.

**TABLE 4**  
Frequency of Appearance in Extracts from Sage and Rosemary of Seven Selected Compounds<sup>a</sup>

|                  | Frequency of appearance (%) |                   |
|------------------|-----------------------------|-------------------|
|                  | Sage (n = 8)                | Rosemary (n = 24) |
| Rosmarinic acid  | 37                          | 83                |
| Genkwanin        | 12                          | 75                |
| Carnosol         | 87                          | 100               |
| Rosmadial        | 75                          | 71                |
| Compound NI 2    | 87                          | 37                |
| Carnosic acid    | 87                          | 71                |
| Methyl carnosate | 75                          | 29                |

<sup>a</sup>See Table 2 for abbreviation.

Profiles of all extracts showed great compositional variation. Some extracts contained fewer than five compounds, whereas others contained twelve or more. There was no clear similarity between the composition of extracts with close AOP values (Table 3). The antioxidant power seems to come essentially from rosmarinic acid for some extracts (Sage 12, Sage 27), from carnosic acid for others (Rom 22, Rom 23), or from carnosol and rosmadial for Rom 21. However, the most active extracts generally contained the eight following compounds: caffeic acid, rosmarinic acid, rosmanol, cirsimaritin, genkwanin, carnosol, rosmadial, and carnosic acid. This result was of great interest, namely that caffeic and rosmarinic acids previously had been shown to be the most antioxidative among fifteen synthetic acid phenols (AOP = 3.5 and 2.15, respectively), and better than  $\gamma$ -tocopherol (AOP = 1.55) (21). In addition, synthetic carnosic acid had shown, in lard, a protection factor similar to  $\gamma$ -tocopherol (7). In our previous work (11), among the compounds isolated from sage, carnosic acid (AOP = 0.24) was approximately two times more active than rosmadial (AOP = 0.13), carnosol (AOP = 0.13), and rosmanol (AOP = 0.10). Because these three diterpenes are probably derivatives of carnosic acid (11,22), the latter is likely a main compound.

Taking into account the HPLC peak areas of the compounds in the extracts, there were no meaningful mathematical correlations between antioxidative activity and chromatographic profile, by various statistical methods. Through ascendant hierarchic classification, similar extracts were gathered into different groups; however, this analysis did not lead to well differentiated groups. By linear multiple regression, a cumbersome equation with eleven variables (compounds) was needed; in addition, this model was not validated by additional extracts. The same difficulty was raised with the neuronal network because it requires more than thirty samples. Only principal component analysis verified that rosmarinic acid, carnosol, and carnosic acid were equally the most influential compounds in contributing to antioxidative activity of the extracts. These results reinforce the importance of having rosmarinic acid and carnosic acid present. To improve the antioxidative quality of the original plants, genetic researchers might select species rich in the first or the second phenolic families.

A better knowledge of synergisms and antagonisms between phenolic acids, diterpenoids and flavones, present together in the extracts, is necessary to progress in this field. This research would be aided by having available standard compounds.

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