Antioxidant Activity in Leaves of Some Mediterranean Plants

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Hexane and methanol leaf extracts of sixteen Mediterranean plant species were obtained by percolation. Higher yields were observed for methanol (16.4–47.8%) than for hexane extracts (1.3–13%). Antioxidant activity of these extracts was determined by a rapid spectrophotometric method involving the combined oxidation of beta-carotene and linoleic acid. Leaf extracts were thus characterized by an Antioxidant Activity Coefficient (AAC) ranging from 0 to 1000. Hexane extracts gave much higher AAC values than the corresponding methanol extracts. In both cases, myrtle *(Myrtus communis)* showed the best antioxidant effectiveness.

KEY WORDS: Antioxidant activity, hexane extracts, Mediterranean plant leaves, methanol extracts.

Lipid protection against autoxidative degradation is ensured by antioxidants. Among the naturally occurring antioxidants, the liposoluble tocopherols have been studied extensively. The search for new natural antioxidants began in the fifties (1–3) and has received increasing interest recently (4–10). Most of the isolated compounds are found in plant leaves and have been characterized as polyphenols.

Food, cosmetic and pharmaceutic industries are interested in new sources of natural antioxidants. The plant kingdom is rich in phenolic compounds (11), which are particularly found in spices and aromatic plants of warm and dry regions (12,13).

This paper describes a method for obtaining plant extracts as well as determination of their antioxidant activity. The species we have examined occur frequently near the Mediterranean coast. Our results constitute the first report on these plants.

EXPERIMENTAL PROCEDURES

Air-dried leaves were ground, and 10 g of the resulting powder was introduced into a glass column (55 × 2 cm). Hexane (300 mL) was allowed to percolate through at room temperature and protected from light. Percolation was complete after about 2 h. The crude extract solution was vacuum evaporated to dryness at 40°C and stored under argon at −20°C. A second extraction process was carried out with 300 mL methanol. Flow rate was comparatively slower with this solvent. After vacuum evaporation, the dry methanol extract was stored as described above.

Hexane and methanol extracts were prepared as methanol solutions (100 mg/L), and their antioxidant activity was determined by a spectrophotometric method (14–16). The experimental procedure has been described previously by Taga *et al.* (15). Optical density measurement at 470 nm was made on a Sequoia Turner 690 visible-light spectrophotometer (Sequoia Turner Corporation, Mountain View, CA). Chemical reagents and products were obtained from Fluka (Mulhouse, France). An α-tocopherol solution (15 mg/L) was used to establish, from 20 experiments, the relative dispersion of the results (4.8%).

RESULTS AND DISCUSSION

Several preliminary experiments were performed in our laboratory concerning plant extraction with hexane and methanol (Chevolleau, M., unpublished results). Leaves were dried by various methods, such as heating (40°C) under vacuum, azeotropic distillation and lyophilization. Best results were obtained from air-dried leaves at room temperature, protected from light, humidity and dust.

Choice of solvent and extraction procedures were also considered. Among all the common solvents, hexane was preferred to alkyl chlorides, aromatic hydrocarbons and ethers, owing to their disadvantages. On the other hand, we found that methanol, in spite of its toxicity, was convenient for solubilization of the more polar compounds.

Efficiencies of some extraction methods (Soxhlet, Kumagawa, maceration, percolation) were compared, and it was observed that percolation was less time-consuming and gave rise to higher antioxidant activity. However, the yield was less than that obtained by the other methods.

Sixteen plant species were studied, and the yields resulting from hexane followed by methanol extractions of the leaves are given in part A of Table 1.

It must be noted that in all cases, low amounts of hexane extracts were obtained. The yield varied from 1.3% *(R. alaternus)* to 13% *(L. angustifolia)* and was rarely above 5%. Higher yields for some species *(E. globulus, L. angustifolia, P. halepensis)* could be explained by the presence of essential oils and oleoresins. Hexane extracts were all viscous oils with a brownish green color.

Presence of chlorophyll in the methanol extracts was responsible for the deep-green coloration. Extraction with methanol gave yields ranging from 16.4% *(F. communis)* to 47.8% *(G. alypum)*, and more than half of them were over 20%.

Determination of antioxidant activity of a pure compound or a mixture is always achieved through measurement of lipid oxidation. The methods currently utilized have been reviewed recently (17), and all of them give comparable results, although the compounds involved are different.

We used a simple and rapid method to evaluate the antioxidant activity of the plant extracts. Inhibition rate of the combined oxidation of beta-carotene and linoleic acid was monitored by optical density readings at 470 nm of an oxygenated aqueous emulsion containing these two substrates and the extracts. Antioxidant effectiveness was calculated in the following way. At time t = 0, the absorbance of beta-carotene at 470 nm is maximum and a coefficient of 1000 was thus attributed. At time t = 120 min, the minimum absorbance was observed for the control, to which we attributed a 0 coefficient. For each extract, an antioxidant activity coefficient (AAC), ranging from

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TABLE 1

Percentage of Leaf Extracts and Antioxidant Activity of Various Plant Species

Plant species	A Leaf extract (%) (dry weight basis)		B Antioxidant activity coefficient (AAC)	
	Hexane	Methanol	Hexane	Methanol
Centranthus ruber DC. (Valerianaceae)	2.5	24.0	29 (0.7)[a]	0 (0)
Cistus albidus L. (Cistaceae)	3.3	26.9	5 (0.2)	20 (5.4)
Conium maculatum L. (Apiaceae)	3.5	20.8	102 (3.6)	14 (2.9)
Coronilla juncea L. (Fabaceae)	1.8	31.6	7 (0.1)	10 (3.2)
Eucalyptus globulus Labil. (Myrtaceae)	11.1	31.2	18 (2.0)	2 (0.6)
Ferula communis L. (Apiaceae)	2.5	16.4	2 (0.1)	14 (2.3)
Globularia alypum L. (Globulariaceae)	1.7	47.8	464 (7.9)	6 (2.9)
Hedera helix L. (Araliaceae)	1.5	32.8	214 (3.2)	8 (2.6)
Lavandula angustifolia L. (Lamiaceae)	13.0	18.4	2 (0.3)	14 (2.6)
Myrtus communis L. (Myrtaceae)	1.9	19.8	641 (12.2)	260 (51.5)
Phillyrea angustifolia L. (Oleaceae)	2.0	26.2	243 (4.9)	7 (1.8)
Pinus halepensis Mill. (Pinaceae)	10.5	16.8	4 (0.4)	10 (1.7)
Quercus ilex L. (Fagaceae)	1.8	23.7	515 (9.3)	150 (35.6)
Rhamnus alaternus L. (Rhamnaceae)	1.3	22.3	585 (7.6)	13 (2.9)
Smilax aspera L. (Liliaceae)	1.7	17.3	292 (5.0)	27 (4.7)
Staehelina dubia L. (Asteraceae)	4.6	16.6	11 (0.5)	8 (1.3)

[a]Values in parentheses correspond to AAC of dry leaves.

0 to 1000, was determined by the following expression (16):

$$AAC = 1000\ [(A_{E120} - A_{C120})/(A_{C0} - A_{C120}) \quad [1]$$

where A_{E120} corresponds to absorbance of the extract at t = 120 min, A_{C0} and A_{C120} correspond to absorbance of the control at t = 0 and t = 120 min, respectively.

The calculated AAC values are given in part B of Table 1. Hexane extracts have much higher AAC (up to 641) than the corresponding methanol extracts, for which AAC ranges from 0 to 260. By comparison of the AAC values, it becomes possible to establish a classification of the extracts according to their antioxidant potencies. In decreasing order, we have: hexane extracts of *M. communis* > *R. alaternus* > *G. alypum* > *P. angustifolia* . . .; methanol extracts of *M. communis* > *Q. ilex* > *S. aspera* > *C. albidus* . . . .

Table 1 also provides AAC data relative to dry leaves and corresponding to the product of extract yield (%) × AAC. The AAC values attributed directly to leaves are much more meaningful for comparison of their antioxidant activity. On such a basis, search for highly antioxidant species would be of interest for their possible use as protective agents in various fields.

We have thus, in decreasing order, for the hexane extracts, *M. communis* > *Q. ilex* > *G. alypum* > *R. alaternus* . . ., and for the methanol extracts, *M. communis* > *Q. ilex* > *C. albidus* > *S. aspera* . . . .

M. communis (myrtle) showed the best antioxidant effectiveness whatever the solvent used. In a future paper, results concerning other plant species and structural data on some antioxidants present therein will be discussed.

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