JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Methanolic Extract of *Verbascum macrurum* as a Source of Natural Preservatives against Oxidative Rancidity

NEKTARIOS ALIGIANNIS,† SOFIA MITAKU,*,† EUGENIA TSITSA-TSARDIS,† CATHERINE HARVALA,† IOANNIS TSAKNIS,‡ STAVROS LALAS,‡ AND SERKO HAROUTOUNIAN§

Department of Pharmacy, University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece; Department of Food Technology, TEI Athens, Egaleo, Greece; and Chemistry Laboratory, Agricultural University of Athens, Iera odos 75, Athens 11855, Greece

The antioxidant properties of various fractions of a methanolic extract obtained from the aerial parts of *Verbascum macrurum* have been determined by monitoring their capacity to scavenge the stable free−radical DPPH. They were also evaluated as natural preservatives against oxidative rancidity using the accelerated Rancimat method. Their activities expressed as protection factor (PF_r) indicated that the fractions rich with phenylpropanoid glycosides were more potent compared to α−tocopherol and of the same magnitude as BHT, which were used as reference standards. Ten natural compounds were identified as components of this methanolic extract and were isolated by medium-pressure liquid chromatography (MPLC). Assessment of their antioxidant activities established that acteoside, a polyhydroxylated phenylpropanoid glycoside derivative, is the most potent free radical scavenger and showed the highest protection factor (PF_r) against sunflower-oil-induced oxidative rancidity. Its activity is comparable to the synthetic antioxidant BHT and clearly superior to natural α-tocopherol. This compound therefore represents a very interesting candidate for use in food preservation as natural protecting agent against oxidative rancidity.

KEYWORDS: ***Verbascum macrurum*; oxidative rancidity; food preservation; antioxidant**

INTRODUCTION

Lipid oxidation, which results in food rancidity, constitutes one of the major changes that can occur during processing, distribution, storage, and final preparation of foods. The prime cause of this oxidative transformation is the action of free radicals, which initiate the degradation of unsaturated fat, producing volatile compounds (*1*). Particularly susceptible to rancidity are foods that contain high concentrations of prooxidants (transition metals, heme-containing proteins, etc) and large amounts of polyunsaturated fatty acids (*2*). To control and delay the onset of rancidity, there is a growing demand for substances that can be introduced directly into the food product to inhibit this process. In this context, various synthetic antioxidants, such as ascorbyl palmitate and BHT have been approved and routinely used as food protecting agents (*3*). The growing recent public demand, however, toward the replacement of synthetic with natural antioxidants has initiated intense research activity, which resulted in the screening of a broad variety of herbal extracts in an effort to discover effective preservatives for a wide range of foods (*4*−*6*). Previous reports have established that relatively high concentrations of herb extracts (500−700 ppm) are needed to produce a marked effect (*7*), because only selective phytochemicals are responsible for their activity. Consequently, subsequent research has been directed toward a thorough study of these natural components.

As a part of our ongoing program concerning the use of various plant species of Greek flora as source of biologically interesting compounds (*8*−*9*), our research interest was focused on the antioxidant activities of herbal extracts and their components (*10*). For the assessment of their antioxidant activities, we have used as an indicator the measurement of their capacity to scavenge the stable free 2,2-diphenyl-1-picryhydrazyl (DPPH) radical. The DPPH assay constitutes a quick and low-cost method, which has frequently been used for the evaluation of the antioxidant potential of various natural products (*11*, *12*). Due to its odd electron, DPPH gives a strong absorption band at 515 nm (deep violet color). In the presence of a free radical scavenger, this electron becomes paired, resulting in the absorption loss and consecutive stoichiometric decolorization with respect to the number of electrons acquired. The absorbance change produced by this reaction is assessed to evaluate the antioxidant potential of test samples.

During explorative research concerning the antioxidant activities of various herbal extracts, we have reported that various plants of *Verbascum* genus (*13*, *14*) contain phytochemicals

* Corresponding author. Tel.: +30 2107274290. Fax: +30 2107274594. E-mail: mitakou@pharm.uoa.gr.
† University of Athens.
‡ TEI Athens.
§ Agricultural University of Athens.

10.1021/jf034528+ CCC: $25.00
Published on Web 11/01/2003

known to possess antioxidant activities (*10*, *15*, *16*). Recently, we have determined that the methanolic extract of the aerial parts of *Verbascum macrurum* also possesses significant radical scavenging capacity. This species belongs to the Scrophulariaceae family which comprises more than 300 species of wild-growing plants. It is a biennial plant of Southern Europe that grows mostly in dry and rocky places of central and southern Greece and Italy (*17*), and there are no reports on its detailed phytochemical analysis. Thus, we were interested in investigating the plant more thoroughly. The main objective of this study was to determine the phytochemical profile of the methanolic extract of *V. macrurum* and evaluate its fractions and major components as natural antioxidants and inhibitors of lipid peroxidation. The inhibitory effect against lipid peroxidation was assessed by measuring the oil stability index using a Rancimat instrument.

MATERIALS AND METHODS

Plant Material. The fresh aerial parts of *V. macrurum* were collected from Likaio mountain in the Peloponnese region (Greece). A voucher specimen (No KL 049) is deposited in the herbarium of the Laboratory of Pharmacognosy, Department of Pharmacy, University of Athens.

Extraction and Isolation The dried and pulverized leaves (cauline and basal, 750 g) were defatted by extraction with CH_2Cl_2 and subsequently extracted with MeOH (2.5 L × 3). The combined MeOH extracts were evaporated to produce a residue (40 g), which was subjected to vacuum-liquid chromatography on silica gel (0.015−0.04 mm). Elution with a CH_2Cl_2/MeOH gradient yielded eight fractions. Fraction 4 (550 mg), which was eluted with CH_2Cl_2/MeOH (85:15), was submitted to MPLC on RP-18. Elution with H_2O/MeOH (80:20) gave 6-*O*-p-coumaroylaucubin **(6)** (9 mg) and elution with H_2O/MeOH (70:30) furnished martynoside **(2)** (92 mg). The sixth fraction (2.1 g), which was eluted with CH_2Cl_2/MeOH (75:25), was rechromatographed by the same procedure. Elution with H_2O gave aucubin **(5)** (82 mg), ajugol **(8)** (63 mg), and geniposidic acid **(9)** (12 mg), and elution with H_2O/MeOH (70:30) gave acteoside **(1)** (160 mg), a 1:1 mixture of saccatoside **(10)** and acteoside (130 mg), 3′′-*O*-p-coumaroylsinuatol **(7)** (7 mg), and a mixture of 6′-*O*-α-L-arabinopyranosyl- and 6′-*O*-*β*-D-xylopyranosyl-martynoside **(3** and **4)** (23 mg).

Solvents and Standards. All solvents were purchased from Lab-Scan (Stillorgan Ind. Park, Co. Dublin, Ireland). Those used for the extractions were of analytical reagent grade, while those for chromatographic analysis were of HPLC grade. All HPLC-solvents were filtered through a 47 mm all-glass filter holder system (Waters) prior to use. Water was double distilled prior to use. Methanol-d4 (MeOD) and dimethyl sulfoxide-d6 (DMSO) were obtained from Merck (Darmstadt, Germany).

2,2-Diphenyl-1-picryhydrazyl radical (DPPH), α−tocopherol, caffeic acid, and BHT were purchased from Sigma-Aldrich (Steinheim, Germany). Pure sunflower oil was purchased locally.

Chromatography. Column chromatography was conducted using Si gel (0.04−0.06 mm (flash) and 0.015−0.04 mm) (Merck) with an applied pressure of 300 mbar. MPLC was performed with a Buchi model 688 apparatus on columns containing 0.02−0.04 mm RP-18 silica gel (Merck,). TLC analyses, with observation of developed chromatograms in UV light (254 and 366 nm) and spraying with a methanolic solution of vanillin- 5% H_2SO_4 and a ethanolic solution of DPPH (315 μM), were carried out using glass precoated silica gel 60 F_{254} and RP-18 F_{254} sheets (Merck).

Identification of Pure Compounds. Structural elucidation of the isolated compounds was achieved by means of spectroscopic data analyses. ^{1}H NMR spectra were measured on a Bruker DRX400 spectrometer (400 MHz) and ^{13}C NMR on a Bruker AC200 spectrometer (50 MHz). Chemical shifts are given in δ values compared to TMS, which was used as an internal standard. Coupling constants (*J*) are given in Hz. The signals in the ^{1}H and ^{13}C spectra were unambiguously assigned using the following 2D NMR techniques: COSY, COSY-LR, HMQC, and HMBC. All the aforementioned 2D experiments were performed using standard Bruker programs. ES-MS (Electrospray Mass Spectroscopy) was obtained on a Nermag R10−10C spectrometer. Optical rotations were measured with a Perkin-Elmer 341 polarimeter.

Radical DPPH Scavenging Activity. The free radical scavenging capacities were determined according to a previously reported procedure using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH) (*11*). In brief, 100 μL of sample solution, diluted in DMSO, was added to 1.9 mL of a 315 μM DPPH solution (in ethanol) and allowed to react for 30 min at 37 °C. Then, the optical density was measured at 515 nm, and the actual decrease of absorption induced by test compound was calculated. The IC_{50} values correspond to the required amount of each sample to scavenge 50% of the DPPH free radicals. They were calculated from regression lines, where the abscissa represents the sample concentration, and ordinate is the average percent reduction of DPPH radical. Each IC_{50} value corresponds to an average of three separate tests.

Preservation Against Sunflower-Oil-Induced Oxidative Rancidity. Although the operation of Rancimat technique is associated with problems related to all accelerated methods for the measurement of rancidity development, because they involve bubbling air through an oil sample at a constant temperature, this method has a series of advantages. It is simple to run and leads to automatic measurement of the oxidative products (*18*). The effective period that oil resists to oxidation is defined as induction time, and the results are expressed as protection factor for rancidity (PF_r) that corresponds to the ratio of the induction times with and without antioxidant (*7*). Samples were added to sunflower oil (2.5 g) and dissolved in absolute ethanol (5 mL) with the aid of a glass rod. The mixture was heated in a Rancimat apparatus (Metrohm, Herison Switzerland) at 110 °C, with an airflow rate of 20 L/hr. The induction period was determined in regard to the volatile degradation products and recorded by plotting the conductivity against time (*19*). More specifically, oil oxidation produced volatile acids that were swept by the air current into a conductivity cell containing water, where they dissolved and changed the water conductivity. The latter was recorded automatically, allowing the measurement of the induction period by a graph recorder. The electrodes, conductivity, and Rancimat reaction vessels were thoroughly cleaned according to manufacturer's procedure between two measurements.

RESULTS AND DISCUSSION

Phytochemical Analysis. Ten known phytochemicals, four phenylpropanoid glycosides, and six iridoids have been isolated and identified as components of the methanolic extract of the aerial parts of *V. macrurum* (**Figure 1**). In regard to phenylpropanoid glycosides, acteoside **(1)** (*20*) and martynoside **(2)** (*21*) constitute two of the most commonly found compounds of this class, while there is only limited information concerning the isolation of 6′-*O*-α-L-arabinopyranosyl- and 6′-*O*-*β*-D-xylopyranosylmartynoside **(3** and **4)** (*13*, *22*). More specifically, glycoside **3** has been detected only as a component of *V. spinosum* and plants belonging to the genus *Scrophularia* (Scrophulariaceae) (*13*). On the other hand, aucubin **(5)**, ajugol **(8),** and geniposidic acid **(9)** are the most common of the isolated iridoid glycosides, because they have been isolated from a broad variety of plants (*23*−*25*). Saccatoside **(10)** has been previously isolated only from plants belonging to the family Scrophulariaceae (*26*), while 3′′-*O*-p-coumaroylsinuatol **(7)** and 6-*O*-p-coumaroylaucubin **(6)** have been detected as natural components of *Verbascum* species and *Utricularia vulgaris*, respectively (*27*, *28*). The latter plant belongs to the family Lentibulariaceae, which is considered by most botanists to be a member of the Scrophulariale family (*28*).

All isolated compounds were unambiguously characterized based on their physical and spectral data as follows:

Acteoside **(1).** $[\alpha]^{25}_D$ −69.6 (c 0.1, MeOH) (Lit. $[\alpha]^{25}_D$ −73.8 (c 1.0, MeOH) *(29)*). ^{1}H NMR: data are consistent with those previously reported (*20*). ^{13}C NMR: data are consistent with those previously reported (*20*). ES-MS *m*/*z* 647 [M + Na].

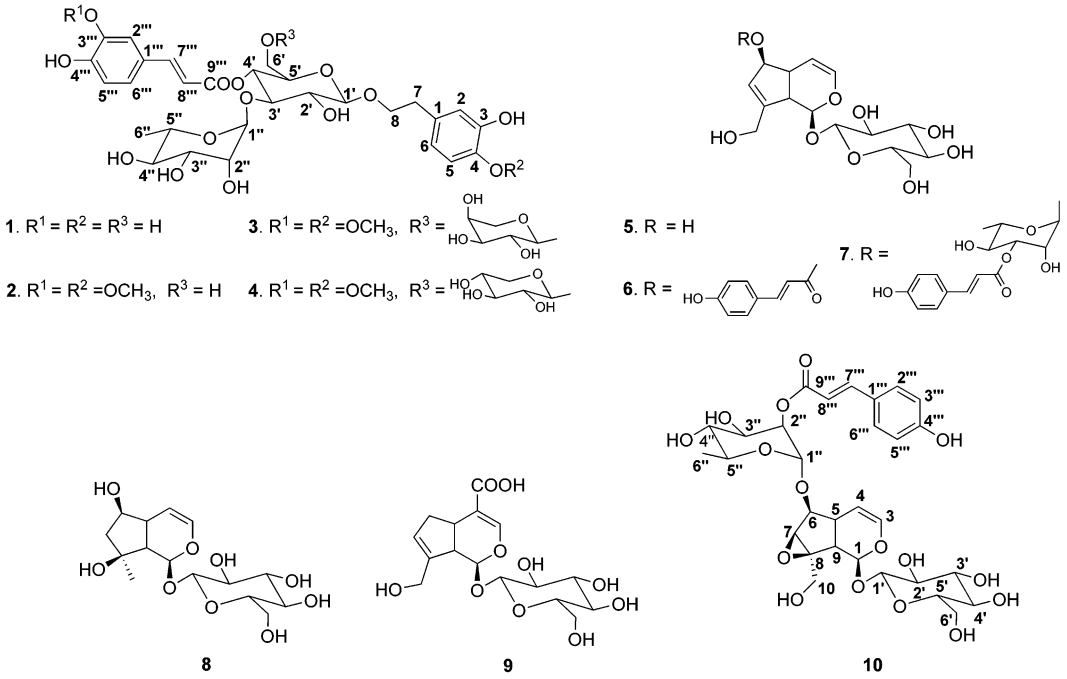

Figure 1. Structures of isolated compounds from methanolic extract of *V. macrurum*.

Martynoside **(2)**. $[\alpha]^{25}_D$ −63.2 (c 0.1, MeOH) (Lit. $[\alpha]^{20}_D$ −68.7 (c 0.69, MeOH) *(21)*). ^{1}H NMR: data are consistent with those previously reported (*21*). ^{13}C NMR: data are consistent with those previously reported (*21*). ES-MS *m/z* 675 [M + Na].

6′-*O*-α-L-Arabinopyranosylmartynoside **(3)**. ^{1}H NMR: data are consistent with those previously reported (*13*). ^{13}C NMR: data are consistent with those previously reported (*13*). ES-MS *m/z* 807 [M + Na].

6′-*O*-β-D-Xylopyranosylmartynoside **(4)**. ^{1}H NMR: data are consistent with those previously reported (*22*). ^{13}C NMR: data are consistent with those previously reported (*22*). ES-MS *m/z* 807 [M + Na].

Aucubin **(5)**. $[\alpha]^{25}_D$ −149.8 (c 0.5, H_2O) (Lit. $[\alpha]^{25}_D$ −162 (c 2.0, H_2O) *(30)*). ^{1}H NMR: data are consistent with those previously reported (*23*). ^{13}C NMR: data are consistent with those previously reported (*23*). ES-MS *m/z* 369 [M + Na].

6-*O*-p-Coumaroylaucubin **(6)**. $[\alpha]^{25}_D$ −153.2 (c 0.5, MeOH) (Lit. $[\alpha]^{21}_D$ −153 (c 0.5, MeOH) *(28)*). ^{1}H NMR: data are consistent with those previously reported (*28*). ^{13}C NMR: data are consistent with those previously reported (*28*). ES-MS *m/z* 515 [M + Na].

3″-*O*-p-Coumaroylsinuatol **(7)**. $[\alpha]^{25}_D$ −142.5 (c 0.5, MeOH)) (Lit. $[\alpha]^{23}_D$ −139.3 (c 0.5, MeOH) *(27)*). ^{1}H NMR: data are consistent with those previously reported (*27*). ^{13}C NMR: data are consistent with those previously reported (*27*). ES-MS *m/z* 661 [M + Na].

Ajugol **(8)**. $[\alpha]^{25}_D$ −165.4 (c 0.5, H_2O) (Lit. $[\alpha]^{25}_D$ −172.1 (c 0.53, MeOH) (*24*)). ^{1}H NMR: data are consistent with those previously reported (*24*). ^{13}C NMR: data are consistent with those previously reported (*24*). ES-MS *m/z* 371 [M + Na].

Geniposidic Acid **(9)**. $[\alpha]^{25}_D$ 21.5 (c 1.0, H_2O) (Lit. $[\alpha]^{24}_D$ −19.3 (c 1.0, MeOH) *(31)*). ^{1}H NMR: data are consistent with those previously reported (*25*). ^{13}C NMR: data are consistent with those previously reported (*25*). ES-MS *m/z* 397 [M + Na].

Saccatoside **(10)**. ^{1}H NMR: data are consistent with those previously reported (*26*). ^{13}C NMR: data are consistent with those previously reported (*26*).

Radical DPPH Scavenging Activity. The methanolic extract of *V. macrurum* (**Ext**), and its fractions **1**−**8** (**Fr1**−**Fr8**) were evaluated as free radical scavengers against DPPH at a final concentration of 200 μg/mL, according to a previously described method (*11*). The results of this primary test are presented in **Figure 2**, indicating that the methanolic extract and fractions **4**−**6** were active, because they inhibited the DPPH by over 70% (*12*). Fractions **3** and **7** exhibited moderate activities (51 and 63% inhibition), while fractions **1**, **2**, and **8** were characterized as inactive (inhibition less than 50%). Subsequently, the TLC analysis showed that fractions **7** and **8** contained mainly carbohydrates and only a low amount of acteoside (**1**). Thorough analyses of fractions 4 and 6 led to the isolation of the aforementioned 10 natural products. The isolated phenylethanoid glycosides (**1**, **2,** and mixture **3** + **4**) were found to possess significant free radical scavenging properties (over 70% inhibition of DPPH), while the two examined iridoids (aucubin and ajugol) were totally inactive (only 3 and 8% inhibition). On the other hand, the corresponding IC_{50} values (**Table 1**) indicated that acteoside, a molecule that possesses four phenolic hydroxy groups, is the most potent (IC_{50} = 51 μM) among the phenylpropanoid glycosides tested. Its antioxidant activity was

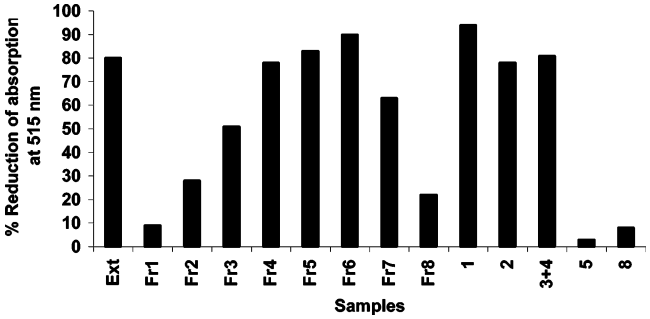

Figure 2. Radical DPPH scavenging activity of methanolic extract of *V. macrurum* (**Ext**), its eight fractions (**Fr1**–**Fr8**), its isolated components (acteoside (**1**), martynoside (**2**), aucubin (**5**), and ajugol (**8**)) and a mixture of these (6′-*O*-α-L-arabinopyranosyl- and 6′-*O*-β-D-xylopyranosyl-martynoside (**3** + **4**)). The results are expressed in % percentage of inhibition of free radical DPPH (% reduction of absorption at 515 nm) at the final concentration of 200 μg/mL. All tests were conducted in triplicate, and the mean values are used.

Table 1. IC_{50} Values of DPPH Radical Scavenging Activity of Methanolic Extract (**Ext**), Its Fractions (**Fr4**–**Fr6**), Its Isolated Components (Acteoside (**1**), Martynoside (**2**), and a Mixture of These (6′-*O*-α-L-Arabinopyranosyl- and 6′-*O*-β-D-Xylopyranosyl-Martynoside (**3** + **4**))[a]

sample	IC_{50} in μg/mL (μM)
Ext	67
Fr 4	112
Fr 5	86
Fr 6	43
Cf	12 (67)
1	31 (51)
2	94 (144)
3 + **4**	115 (146)

[a] Caffeic acid (**Cf**) was used as standard. All tests were conducted in triplicate, and the mean values are used.

Table 2. Preservation against Sunflower Oil Oxidative Rancidity of Methanolic Extract of *V. Macrurum* (**Ext**), Its Fractions (**Fr3**–**Fr8**), Its Isolated Components (Acteoside (**1**), Martynoside (**2**), Aucubin (**5**), and Ajugol (**8**)) and Two Mixtures of These (6′-*O*-α-L-Arabinopyranosyl- and 6′-*O*-β-D-Xylopyranosyl-Martynoside (**3** + **4**), Saccatoside and Acteoside (**1** + **10**))[a]

samples	induction period of sample (h)	induction period of blank (h)	protection factor (PF_r)
Ext	9.17	7.90	1.16
Fr3	9.25	7.65	1.21
Fr4	10.30	7.18	1.43
Fr5	12.15	7.58	1.60
Fr6	12.48	7.60	1.64
Fr7	9.92	7.75	1.28
Fr8	8.11	7.73	1.05
α-tocopherol	9.86	8.73	1.13
BHT	11.2	7.53	1.49
1	10.10	7.08	1.43
2	9.78	7.90	1.24
5	8.22	7.91	1.04
8	6.80	8.30	0.82
1 + **10**	10.48	8.66	1.21
3 + **4**	7.79	7.08	1.10

[a] α-Tocopherol and BHT were used as standards. The results are expressed as protection factor for rancidity (PF_r) at the final concentration of 200 ppm.

clearly superior to caffeic acid (IC_{50} = 67 μM), which was used as the reference standard. Martynoside (**2**), the corresponding phenylethanoid glycoside with two of its phenolic hydroxy groups methylated, showed relatively weak activity (IC_{50} = 144 μM) (*15*). Finally, a mixture of the more substituted phenylethanoid glycoside derivatives (**3** and **4**) was much less potent, presumably because the free phenolic hydoxy groups are essential for the free radical scavenging process. Consequently, the free radical scavenging capacity of the fraction **6** richer in acteoside (IC_{50} = 43 μg/mL) was higher compared to fractions **5** (IC_{50} = 86 μg/mL) and **4** (IC_{50} = 112 μg/mL).

Preservation Against Sunflower-Oil-Induced Oxidative Rancidity. The preservation potentials of the methanolic extract (**Ext**), its fractions **Fr3**−**Fr8**, pure compounds (**1**, **2**, **5**, **8**) and mixtures (**3** + **4** and **1** + **10**) against sunflower-oil-induced oxidative rancidity were assessed using the Rancimat method in a final concentration of 200 ppm. Their preservation indexes, expressed as protection factor (PF_r), are summarized in **Table 2**; a protection factor greater than 1 indicates that the sample inhibits the oxidative rancidity. Consequently, higher values correspond to better preservation properties (*32*). It is also evident that it is requirement for a potent antioxidant compound to be relatively stable at high temperatures to possess high safeguarding ability against oxidative rancidity. In our case, the results of the accelerated Rancimat method were in the same order with the radical scavenging activities of methanolic extract fractions and pure compounds, indicating that most of these natural products are relatively stable at high temperatures. More specifically, the methanolic extract exhibited moderate activity (PF_r = 1.16), which is comparable with the activity of natural α-tocopherol (PF_r = 1.13). Fractions **3** and **7** also exhibited similar activity (1.21 and 1.28, respectively), while fraction **8** was considered as inactive (1.05). On the other hand, fractions **4**, **5**, and **6** were far more potent, showing high preservation abilities against sunflower-oil-induced oxidative rancidity (1.43, 1.60 and 1.64, respectively). These values are comparable to values recorded for the very active synthetic preservative BHT (PF_r = 1.49) and are in full agreement with their analyses which indicated that they contain large amounts of phenylpropanoid glycosides.

In regard to the activity of each individual isolated phytochemical, it is interesting to point out that the mixture of the most substituted phenylpropanoid glycosides(**3** + **4**) was almost inactive (PF_r = 1.10), while the less substituted martynoside (**2**) showed activity (PF_r = 1.24) superior to natural α-tocopherol. On the other hand, the polyhydroxylated derivative acteoside (**1**) was the most active, exhibiting preservation capability (PF_r = 1.43) that is comparable to the synthetic preservative BHT. Finally, iridoids did not show any interesting activity.

In conclusion, various fractions of a methanolic extract obatained from the aerial parts of *Verbascum macrurum* and their phytochemical components have been evaluated as potential antioxidants using two simple methods of assessment. Two of the fractions were found to possess significant antioxidant activities and high preservation index (PF_r) against sunflower-oil-induced oxidative rancidity. Phytochemical analysis and subsequent antioxidant activity evaluation showed that acteoside (**1**), a polyhydroxylated phenylpropanoid glycoside, was the component possessing activity comparable to the synthetic antioxidant BHT and clearly superior to natural α-tocopherol. This compound is known to be nontoxic, because it has been detected as a component of several edible plants, such as olive drupes (*33*) and lemon verbena tea (*34*). It thus constitutes a very interesting candidate for use in food preservation as a natural protecting agent against oxidative rancidity.

LITERATURE CITED

(1) Wong, J. W.; Hashimoto, K.; Shibamoto, T. Antioxidant activities of rosemary and sage extracts and Vitamin E in a model meat system. *J. Agric. Food Chem.* **1995**, *43*, 2707−2712.

(2) Kanner, J. Oxidative processes in meat and meat products: Quality implications. *Meat Sci.* **1994**, *36*, 169−189.

(3) McMullen, L. M.; Hawrysh, C.; Tokarska, B. Ascorbyl palmitate efficiency in enhancing the accelerated storage stability of canola oil. *J. Food Science* **1991**, *56*, 1651−1659.

(4) Banias, C.; Oreopoulou, V.; Thomopoulos, C. D. The effect of primary antioxidants and synergists on the activity of plant extracts in lard. *J. Am. Oil Chem. Soc.* **1992**, *69*, 520−524.

(5) Gordon, M. H.; Kourimska L. Effects of antioxidants on losses of tocopherols during deep-fat frying. *Food Chem.* **1995**, *52*, 175−177.

(6) Hras, A. R.; Hadolin, M.; Knez, Z.; Bauman, D. Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate, and citric acid in sunflower oil. *Food Chem.* **1992**, *71*, 229−233.

(7) Beddows, C. G.; Jagait, C.; Kelly, M. J. Preservation of α-tocopherol in sunflower oil by herbs and spices. *Int. J. Food. Sci. Nutr.* **2000**, *51*, 327−339.

(8) Aligiannis, N.; Mitakou S.; Mitrokosta, D.; Leclerc S. Flavonids as cycline-dependent kinase inhibitors: Inhibition of cdc25 phosphatase activity by Flavonoids belonging to the quercetin and kaempferol series. *Planta Med.* **2001**, *67*, 468−470.

(9) Aligiannis, N.; Kalpoutzakis, E.; Chinou, I. B.; Mitakou, S.; Gikas, E.; Tsarbopoulos, A. Composition and antimicrobial activity of the essential oils of five taxa of *Sideritis* from Greece. *J. Agric. Food Chem.* **2001**, *49*, 811−815.

(10) Kyriakopoulou, I.; Magiatis, P.; Skaltsounis, A. L.; Aligiannis, N.; Harvala, C. Samioside, a new phenylethanoid glycoside with free-radical scavenging and antimicrobial activities from *Phlomis samia*. *J. Nat. Prod.* **2001**, *64*, 1095−1097.

(11) Cotelle, N.; Bernier, J. L.; Catteau, J. P.; Pommery, J.; Wallet, J. C.; Gaydou, E. M. Antioxidant properties of hydroxylflavones. *Free Radic. Biol. Med.* **1996**, *20*, 35−43.

(12) Lee, S. K.; Mbwambo, Z. H.; Chung, H.; Luyengi, L.; Gamez E. J. C.; Mehta, R. G.; Kinghorn, A. D.; Pezzuto, J. M. Evaluation of the antioxidant potential of natural products. *Combin. Chem. High Through. Screen.* **1998**, *1*, 35−46.

(13) Kalpoutzakis, E.; Aligiannis, N.; Mitakou, S.; Skaltsounis, A. L. Verbaspinoside, a new iridoid glycoside from *Verbascum spinosum*. *J. Nat. Prod.* **1999**, *62*, 342−344.

(14) Magiatis, P.; Mitaku, S.; Tsitsa, E.; Skaltsounis, A. L.; Harvala, C. Verbascoside derivatives and iridoid glycosides from *Verbascum undulatum*. *Nat. Prod. Lett.* **1998**, *12*, 111−115.

(15) Xiong, Q.; Kadota, S.; Tani, T.; Namba, T. Antioxidative effects of phenylethanoids from *Cistanche deserticola*. *Biol. Pharm. Bull.* **1996**, *19*, 1580−1585.

(16) He, Z. D.; Lau, K. M.; Xu, H. X.; Li, P. C.; But, P. P. H. Antioxidant activity of phenylethanoids from *Brandisia hancei*. *J. Ethnopharmacol.* **2000**, *71*, 483−486.

(17) Tutin, G. T.; Heywood, H. V.; Burges, A. N.; Moore, M. D.; Valentine, H. D.; Walters, M. S.; Webb, A. D. *Flora Europaea*; Cambridge University Press: London, 1972; Vol. 3, pp 210.

(18) Laubli, M. W.; Bruttel, P. A. Determination of the oxidative stability of fats and oils: Comparison between the active oxygen method (AOCS cd 12−57) and the Rancimat method. *J. Am. Oil Chem. Soc.* **1986**, *63*, 792−795.

(19) Hudson, B. J. F. Evaluation of oxidative rancidity techniques. In *Rancidity in foods*; Allen, J. C., Hamilton R. J., Eds.; Applied Science Publishers: London, 1983; pp 47−57.

(20) Kobayashi, H.; Oguchi, H.; Takizawa, N.; Miyase, T.; Ueno, A.; Usmanghani K.; Ahmad, M. New phenylethanoid glycosides from *Cistanche tubulosa*. *Chem. Pharm. Bull.* **1987**, *35*, 3309−3314.

(21) Calis, I.; Lahloub, M. F.; Rogenmoser, E.; Sticher, O. Isomartynoside, a phenylpropanoid glycoside from *Galeopsis pubescens*. *Phytochemistry* **1984**, *23*, 2313−2315.

(22) Warashina, T.; Miyase, T.; Ueno, A. Phenylethanoid and lignan glycosides from *Verbascum thapsus*. *Phytochemistry* **1992**, *31*, 961−965.

(23) Akdemir, Z.; Calis, I.; Junior, P. Iridoid and phenylpropanoid glycosides from *Pedicularis condensata*. *Phytochemistry* **1991**, *30*, 2401−2402.

(24) Nishimura, H.; Sasaki, H.; Morota, T.; Chin, M.; Mitsuhashi, H. Six iridoid glycosides from *Rehmania glutinosa*. *Phytochemistry* **1989**, *28*, 2705−2709.

(25) Yahara, S.; Kato, K.; Nakazawa, Y.; Toda, Y.; Nohara, T. New iridoid trimers and tetramers from seeds of *Eucommia ulmoides*. *Chem. Pharm. Bull.* **1990**, *38*, 267−269.

(26) Otsuka, H.; Sasaki, Y.; Yamasaki, K.; Takeda, Y.; Seki, T. Iridoid diglycoside monoacyl esters from the leaves of *Premna japonica*. *J. Nat. Prod.* **1990**, *53*, 107−111.

(27) Vesper, T.; Seifert, K. Iridoids from *Verbascum nigrum*. *Liebigs Ann. Chem.* **1994**, 751−753.

(28) Damtoft, S.; Jensen S. R.; Thorsen J.; Molgard, P.; Olsen, C. E. Iridoids and verbascoside in Callitrichaceae, Hippuridaceae and Lentibulariaceae. *Phytochemistry* **1994**, *36*, 927−929.

(29) Shoyama, Y.; Matsumoto, M.; Nishioka, I. Phenolic glycosides from diseased roots of *Rehmannia glutinosa* var. *purpurea*. *Phytochemistry* **1987**, *26*, 983−986.

(30) Su, B. N.; Yang, L.; Gao, K.; Jia, Z. J. Aquaticol, a bis-sesquiterpene and iridoid glucosides from *Veronica anagallis-aquatica*. *Planta Med.* **2000**, *66*, 281−283.

(31) Takeda, Y.; Nishimura, H.; Inouye, H. Two new iridoid glucosides from *Ixora chinensis*. *Phytochemistry* **1975**, *14*, 2647−2650.

(32) Elizable, B. E.; Bressa, F.; Dalla Rosa, M. Antioxidative action of maillard reaction volatiles: Influence of maillard solution browning level. *J. Am. Oil Chem. Soc.* **1992**, *69*, 331−334.

(33) Owen, R. W.; Haubner, R.; Mier, W.; Giacosa, A.; Hull, W. E.; Spiegelhalder, B.; Bartsch, H. Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food Chem. Toxicol.* **2003**, *41*, 703−717.

(34) Carnat, A.; Carnat A. P.; Fraise, D.; Lamaison, J. L. The aromatic and polyphenolic composition of lemon verbena tea. *Fitoterapia* **1999**, *70*, 44−49.

Received for review May 22, 2003. Revised manuscript received September 16, 2003. Accepted October 1, 2003.

JF034528+