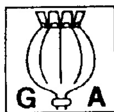

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Reprint

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Statistical analysis: All numerical data were expressed as the mean ± S. E. M. The statistical significance of the difference was determined by an unpaired Student's t-test.

References

1 Torii Y, Mutoh M, Saito H, Matsuki N. Eur. J. Pharmacol. 1993; 248: 131–135.
2 Matsuki N, Torii Y, Saito H. Eur. J. Pharmacol 1993; 248: 329–331.
3 Matsuki N. Yakugaku Zasshi 1996; 116: 710–718.
4 Kitagawa S, Sakuma T, Yonemura S. Chem. Pharm. Bull. 1995; 43 (8): 1263–1266.
5 The Japanese Phamacopoeia Thirteenth Commentary Edition. Hirokawa Publishing Co. 1996: C-88–90.
6 Yang Y, Kinoshita K, Koyama K, Takahashi K, Tai T, Nuoura Y, Watanabe K. Nat. Prod. Sci., 1999; 5 (1): 20–24.
7 Kawai T, Kinoshita K, Koyama K, Takahashi K. Planta Med. 1993; 60: 17–20.
8 Kinoshita K, Kawai T, Imaizumi T, Akita Y, Koyama K, Takahashi K. Phytomedicine 1996; 3: 51–58.

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Protective Effects of the Aerial Parts of *Salvia officinalis, Melissa officinalis* and *Lavandula angustifolia* and their Constituents against Enzyme-Dependent and Enzyme-Independent Lipid Peroxidation

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Abstract: The antioxidant effects of aqueous methanolic extracts from three medicinal Lamiaceae species were investigated in enzyme-dependent and enzyme-independent lipid peroxidation systems. All these extracts caused a considerable concentration-dependent inhibition of lipid peroxidation. Phenolic components present in the plant extracts were evaluated for antioxidant activity and were found effective in both tests. Their concentrations in each extract were determined by TLC-densitometry.

The family Lamiaceae is considered to be a promising source of natural antioxidants. 70 taxa of Lamiaceae were studied by Lamaison et al., who found that many species displayed 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity (1). The compounds responsible for this activity have not yet been investigated in detail.

The aim of this study was to evaluate the antioxidant capacity of extracts of *Salvia officinalis* (sage), *Melissa officinalis* (lemon balm) and *Lavandula angustifolia* (lavender) and their main phenolic components [rosmarinic acid (**1**), caffeic acid (**2**), luteolin (**3**), luteolin 7-*O*-glucoside (**4**) and methyl carnosoate (**5**)] in two different biological systems of lipid peroxidation (LPO), with quantification of the active compounds in order to clarify the connection between activity and chemical composition.

The aqueous MeOH extracts of all investigated plants demonstrated considerable inhibition of LPO in both enzyme-dependent and enzyme-independent test systems (Fig. **1**). All extracts exerted a somewhat more pronounced effect on the enzyme-dependent LPO, indicating a moderate direct enzyme inhibitory activity as a component of the total antioxidant effect. These *in vitro* tests were performed on the known main phenolic components of the extracts (**1–5**), with α-tocopherol acid succinate (**6**) as standard substance. Compounds **1–4** proved to be more potent inhibitors of enzyme-dependent

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than enzyme-independent LPO, but a difference in order of magnitude was found for **1** and **2**, suggesting that these compounds have substantial direct enzyme inhibitor activity. It is noteworthy that each tested compound displayed a more pronounced antioxidant activity than that of **6**. The concentrations of these compounds were determined in the extracts by TLC-densitometry (Table **1**). These data and the antioxidant IC_{50} values indicate that **1** is the only compound that contributes significantly to the antioxidant activities of the plant extracts. These data are in accord with the finding of Tagashira et al. on an aqueous hydroalcoholic extract of lemon balm (2). Compounds **2**–**5** cannot be responsible for the antioxidant effects of the extracts, since they are not present in a sufficient amount. The high efficacy of hydroxycinnamic acid derivatives accords with literature data (1, 3), but the relatively low activity of **5** is surprising, as similar diterpenes were previously reported to be the main antioxidative agents in *Salvia officinalis* and *Rosmarinus officinalis* (4–6). The total flavonoid and total hydroxycinnamic acid contents of the extracts were determined spectrophotometrically (Table **2**). A tendency was detected between the antioxidant potency (expressed as the reciprocal of the calculated IC_{50}) and the total hydroxycinnamic acid content (Fig. **2**). No similar relationship was observed for the total flavonoid content.

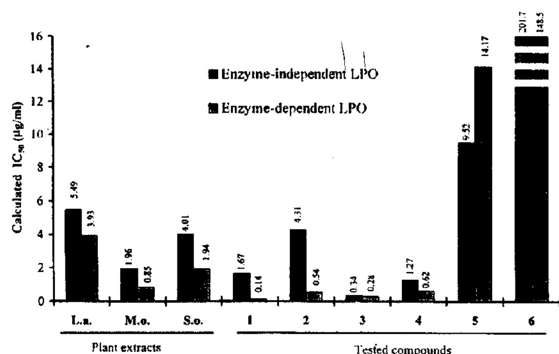

Fig. 1 Antioxidant activities of the plant extracts (L.a.: *Lavandula angustifolia*, M.o.: *Melissa officinalis*, S.o.: *Salvia officinalis*), their constituents and α-tocopherol.

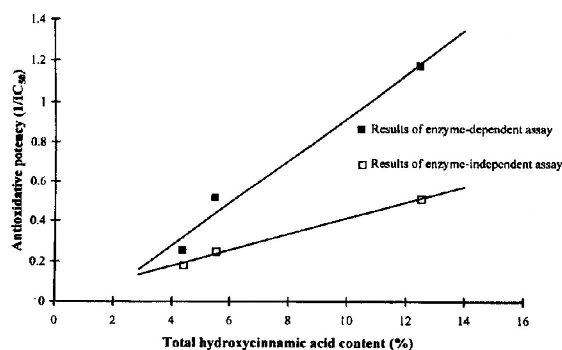

Fig. 2 Relationship between the antioxidant potencies of the investigated plant extracts and their total hydroxycinnamic acid content.

Table 1 Concentrations of some antioxidant compounds in the investigated extracts, determined by densitometry.

Extract	1	2	3	4	5
S. officinalis	3.37%	0.320%	0.059%	0.177%	0.380%
M. officinalis	2.21%	0.196%	0.027%	0.175%	–
L. angustifolia	1.16%	0.111%	0.011%	0.140%	–

Table 2 Total hydroxycinnamic acid and total flavonoid contents of the investigated plant extracts.

Plant extract	Total hydroxycinnamic acid content %	Total flavonoid content %
S. officinalis	5.519	0.485
M. officinalis	12.508	0.229
L. angustifolia	4.386	0.282

These results suggest that **1** may be responsible for part of the antioxidant effect of the plant extracts, but components **2**–**5** are present in concentrations too low to exert a significant antioxidant effect.

Materials and Methods

Materials: The aerial parts of *Lavandula angustifolia* Mill. and *Salvia officinalis* L. were gathered in July 1995 and those of *Melissa officinalis* L. in September 1995 in the Botanical Garden of the Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátót, Hungary. Voucher specimens (L987/1, 2 *L. angustifolia*, L963/A *S. officinalis*, L967/A *M. officinalis*) are deposited in the Herbarium of the Institute of Ecology and Botany. Compound **1** was purchased from ICN Pharmaceuticals, Inc. (Costa Mesa, USA), and **2** from Sigma (St. Louis, USA); **3** and **4** were isolated from the flowers of *L. angustifolia* and identified via the UV and NMR data (7). Compound **5** was obtained from the *n*-hexane fraction of a 50% aqueous MeOH extract of *S. officinalis* by repeated chromatography on a silica gel layer, using $CHCl_3$-MeOH (49:1) and *n*-hexane-diethyl ether-AcOH (79:20:4). The ^{1}H-NMR, ^{13}C-NMR, and MS data agreed with the literature values (8).

Extraction: The air-dried and powdered plant materials (5 g) were extracted with 2 × 100 ml of 50% aqueous MeOH at room temperature, using an ultrasonic bath (2 × 15 min). The filtered extracts were evaporated to dryness in vacuo to yield: 17.5% (*L. angustifolia*), 18.0% (*M. officinalis*) and 16.6% (*S. officinalis*).

Measurements of antioxidant activities: The assays suitable for independent measurements of enzyme-dependent and enzyme-independent LPO were performed as described earlier (9, 10). All *in vitro* experiments were conducted in duplicate and means were calculated. No error was computed, as the differences between the two samples were approximately 1%. Saturation curves were fitted to the measurement data and IC_{50} values (the concentration at which 50% of the maximal LPO inhibition is exerted) were calculated by means of the computer program GraphPad Prism 2.01.

TLC-densitometry: Compounds **1** and **2** in the 50% aqueous MeOH extracts were determined as described previously (11). Compound **5** was measured in the 50% aqueous MeOH extracts, and **3** and **4** in their EtOAc-soluble fractions, using silica gel 60 F_{254} (Merck 5729) plates and the solvent systems toluene-EtOAc-HCOOH (10:4:1) for **3**, EtOAc-HCOOH-AcOH-H_2O (25:2:2:4) for **4**, and *n*-hexane-diethyl ether-AcOH (79:20:4) for **5**. Compounds **3** and **4** were detected at 245 nm and **5** at 317 nm.

Determination of total hydroxycinnamic acid content: The determination was performed as in (12). Results are expressed as **1** (g) per 100 g of dry extract.

Determination of flavonoid content: The total flavonoid content in the EtOAc-soluble fraction of the 50% aqueous MeOH extracts was determined by the aluminium chloride method described in DAB 10. Each sample was analysed in duplicate, and a calibration graph with 3 datapoints for **4** was used. The amounts of flavonoids were expressed as **4** (g) per 100g of dry extract.

References

1 Lamaison JL, Petitjean-Freytet C, Duke JA, Walker J. Plant. Med. Phytother. 1993; 26: 143-148.
2 Tagashira M, Ohtake Y. Planta Med. 1998; 64: 555-558.
3 Montesinos C, Ubeda A, Ferrándiz ML, Alcaraz MJ. Planta Med. 1991; 57: Suppl., A54.
4 Inatani R, Nakatani N, Fuwa H. Agric. Biol. Chem. 1983; 47: 521-528.
5 Schwarz K, Ternes W. Z. Lebensm. Unters. Forsch. 1992; 195: 99-103.
6 Haraguchi H, Saito T, Okamura N, Yagi A. Planta Med. 1995; 61: 333-336.
7 Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids 1970; pp. 95-96 and 285-286, Springer, Berlin, Heidelbèrg, New York.
8 Al-Hazimi HMG. Phytochemistry 1986; 25: 1238-1239.
9 Stocks J, Gutteridge JMC, Sharp RJ, Dormandy TL. Clin. Sci. Mol. Med. 1974; 47: 215-222.
10 Engineer FN, Sridhar R. Biochem. Biophys. Res. Commun. 1991; 179: 1101-1106.
11 Janicsák G, Máthé I. Chromatographia 1997; 46: 322-324.
12 Lamaison JL, Petitjean-Freytet C, Carnat A. Pharm. Acta Helv. 1991; 66: 185-188.

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A Novel Flavonoid Glycoside from *Drymaria diandra*

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Abstract: **A novel flavonoid glycoside, drymariatin A, was isolated from the whole plants of *Drymaria diandra* (Caryophyllaceae). By spectroscopic analysis, its structure was elucidated as 6-*trans*-[2″-O-(α-rhamnopyranosyl)]-ethenyl-5,7,4′-trihydroxyflavone.**

Previous chemical studies of the genus *Drymaria* have not been very extensive. An alkaloid, norditerpenes and their glycosides, triterpenoids and mixtures of long chain fatty acids were isolated from this genus (1-4). *Drymaria diandra* Bl. (Caryophyllaceae) is used as a folk drug for treatment of acute hepatitis in China (5). In a search for its biologically active compounds, a chemical study on this plant was carried out and a novel flavonoid glycoside, named drymariatin A (**1**), was obtained from the *n*-butanol fraction of its ethanol extract by column chromatography.

Drymariatin A was obtained as a yellow powder. Its negative FAB-MS exhibited the molecular ion peak at *m/z* 457 ($[M-1]^-$, base peak) and one fragment peak at *m/z* 311 $[M-146-1]^-$. The molecular formula was established by HR-FABMS as $C_{23}H_{22}O_{10}$. The IR spectrum had adsorptions at 3400 and 1627 cm^{-1} corresponding to the hydroxy and hydrogen bonded unsaturated carbonyl groups. The UV spectrum showed bands at 270.5, 309.0 and 338.0 nm. This information along with the analysis of its ^1H- and ^{13}C-NMR signals indicated that **1** was a flavone glycoside.

The ^1H-NMR spectrum of this compound revealed a high field methyl doublet at δ 1.61 (J = 6.0 Hz), two olefinic doublets at δ 8.48 and 7.20, two aromatic singlets at δ 6.88 and 6.89, an AX pair of aromatic doublets at δ 7.84 (2H, J = 8.8 Hz) and 7.16 (2H, J = 8.8 Hz) characteristic of a *para*-disubstituted aromatic ring, and the signals of the sugar portion in the low-field aliphatic region. The DEPT spectrum showed one carbonyl, eight quaternary carbon, eleven methine carbon and one methyl signals. The NMR data and HMQC, HMBC spectra indicated that the sugar was rhamnose, and its anomeric proton and carbon were at δ 5.84 (s) and 102.2, respectively, suggesting the presence of a α-O-glycosidic bond.

Compared with ^{13}C-NMR signals of 5,7,4′-trihydroxyflavone (6), it had two additional olefinic carbons. The HMBC spectrum showed correlations between one olefinic proton (H-2″) and C-6, H-2″ and C_{rha}-1, and correlations between another olefinic proton (H-1″) and C-5, H-1″ and C-2″, H_{rha}-1 and C-2″ were also observed. This indicated that the olefinic carbon C-1″ was linked to C-6, and the olefinic carbon C-2″

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