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ORIGINAL PAPER

Polar constituents of *Ligustrum vulgare* L. and their effect on lipoxygenase activity

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The present work summarizes results of isolation and identification of polar constituents of the methanolic extract of *Ligustrum vulgare* L. leaves and of the evaluation of inhibiting activity of selected isolates on rat lung cytosol fraction lipoxygenase. Six different compounds were isolated from the ethylacetate and butanol portions of the methanolic extract (hydroxytyrosol and its glucoside, ligustroflavon, oleuropein, acteoside, echinacoside). The inhibitory activity of oleuropein, echinacoside and the water infusion of *Ligustrum vulgare* leaves tested on LOX was expressed as IC_{50}. Kinetic parameters (K_M, V_{max}) and type of inhibition were determined. As the most effective in competitive inhibition of LOX, oleuropein was proved.

Keywords: *Ligustrum vulgare* L., oleuropein, echinacoside, lipoxigenase activity

Introduction

Different species of the *Ligustrum* genus have been used in traditional Chinese and Japanese medicine due to its liver-protecting (Yim et al., 2001), antiviral (Ma et al., 2001), and anti-mutagenic activities (Shoemaker et al., 2005). In the folk medicine of Azerbaijan, common privet leaves (*Ligustrum vulgare*) are used in hypertension therapy (Hammermann et al., 1971), which has been supported by recent studies of hypotensive and diuretic effects of common privet since different extracts of this plant act as dual angiotensin converting enzymes and neutral endopeptidase inhibitors (Kiss et al., 2008). Common privet leaves are currently used as an oropharyngeal anti-inflammatory agent in the ethnomedicine of southern Italy and the plant is regarded as an antirheumatic agent in Cyprus. In Anatolia (Turkey), fresh plant leaves are still chewed to cure aphtae (Pieroni & Pachaly, 2000a). Most of these diseases can be connected with the reactive oxygen species balance in all human tissues. Phenolic antioxidants are recognized as the main active principles (Nagy et al., 2006, 2009); however, other classes of secondary metabolites such as essential oils or polysaccharides also exhibit antioxidant properties (Stojanovic-Radic et al., 2010; Hromádková et al., 2010, Jiménez et al., 2010).

A variety of redox active compounds have been

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identified as inhibitors of lipoxygenases. Lipoxygenases (EC 1.13.11.12) (LOXs) catalyze dioxygenation of polyunsaturated fatty acids with a *cis*, *cis*-1,4-pentadiene configuration to their corresponding hydroperoxide derivatives (Bezáková et al., 1996). LOX is the key enzyme in the biosynthesis of leukotrienes playing an important role in the pathophysiology of several inflammatory diseases since products of lipoxygenase catalyze oxygenation as hydroxyeicosatetraenoic or hydroperoxy eicosatetraenoic acid, leukotrienes, and lipoxins seem to be involved in inflammatory reactions (rheumatoid arthritis and psoriasis). There is good evidence that leukotrienes are mediators of asthmatic responses, glomerular nephritis, myocardial ischemia, and cancer (Young, 1999).

The aim of the present study was the isolation and identification of constituents of *Ligustrum vulgare* L. leaves and the evaluation of their potential anti-inflammatory activity. From the anti-inflammatory test systems, the in vitro assay of inhibitory activity on the rat lung cytosol fraction lipoxygenase was chosen.

Experimental

Leaves of *Ligustrum vulgare* L. were collected during September 2006 in Arborétum Mlyňany, Institute of Dendrobiology, Slovak Academy of Sciences, and dried at room temperature. Samples were identified by Dr. Tomaško (Arborétum Mlyňany) and a voucher specimen is deposited there.

HPLC analysis of samples was performed using a Hewlett–Packard HP 1100 (Agilent Technologies, USA) liquid chromatograph with a diode-array detector (DAD; monitoring wavelengths: 230 nm, 254 nm, 290 nm, and 334 nm). Compounds were separated on a 50 mm × 2.1 mm, 1.8 μm particle Eclipse XDB C18 column (Agilent Technologies, USA) using a gradient prepared from acetonitrile (component A) and 0.2 % of aqueous HCOOH (component B). The gradient was: 0–36 min, 10 % A + 90 % B; 36–40 min, 100 % A. The flow rate was 0.3 mL min^{-1} and the column temperature was 30 °C. Mass spectra were acquired with an Agilent 1100 LC/MSD Ion Trap VL (Agilent Technologies, USA) in the negative-ion mode. Compounds were separated as described for HPLC–DAD. The mass spectrometer settings were: MWD 254, target mass of 300, compound stability of 35 %, trap drive level of 100 %, and MS–MS. UV spectra were recorded on-line using DAD during the HPLC analysis and a Specord UV-VIS (C. Zeiss, Jena, Germany) in methanol after an addition of specific diagnostic reagents in accordance with literature (Mabry et al., 1970). NMR spectra were recorded on a Varian Inova 600 MHz spectrometer (Agilent Technologies, USA); CD_3OD was used as the solvent. Column chromatography (CC) was performed on silica gel (Silpearl, particle size of 0.40–0.063 mm; Kavalier, Votice, Czech Republic), Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden), and polyamide S6 (particle size of 0.05–0.16 mm; Riedel–De Haen, Seelze-Hannover, Germany). TLC was performed on silica gel (Silufol UV 254) and polyamide (DC-Alufolien Polyamid 11 F 254; layer thickness of 0.15 mm) plates from Merck (Darmstadt, Germany). TLC detection reagents (Naturstoffreagent A, polyethylene glycol 4000, and aniline phthalate) were prepared according to www.cob.uha/site%20cot/toolbox.php_files/TLC%20bible.pdf (Retrieved 16 December 2010).

For HPLC identification, standards of echinacoside (PhytoLab, Germany), oleuropein, apigenin, glucose, and rhamnose were purchased from C. Roth (Karlsruhe, Germany). Acteoside was isolated from *Paulownia tomentosa* fruits (Šmejkal et al., 2008) and kindly provided by the authors.

Powdered dried leaves of *Ligustrum vulgare* L. (442 g) were extracted using methanol in a Soxhlet apparatus until the extract became colorless. After filtration, the methanol extract was evaporated to dryness by a vacuum rotary evaporator. The residue (216.8 g) was then suspended in distilled water and then partitioned successively with chloroform (30.4 g, 14 %), diethyl ether (2.4 g, 1.11 %), ethyl acetate (28.7 g, 13.24 %), and butan-1-ol (54.3 g, 25 %), leaving the H_2O portion (101 g, 46.6 %). The butan-1-ol portion (54.3 g) was adsorbed on silica gel and subjected to column chromatography on a silica gel column with chloroform–methanol mixtures of increasing polarity as the eluents (0–100 %). A total of 348 fractions (each of approximately 100 mL) were collected. Fractions with similar TLC profiles were pooled to provide combined fractions (I–XV). Fraction VII was further purified on a Sephadex LH-20 column using propan-2-ol as the eluent. According to the TLC assay, twelve combined subfractions were obtained (each of approximately 50 mL). Fraction 6 from this separation was submitted for further CC on polyamide using $CHCl_3$/MeOH/butan-2-one ($\varphi_r = 12 : 2 : 1$) as the eluent. This resulted in the isolation of compound *I* (10.2 mg). Fraction XIV was purified on a Sephadex LH-20 column using methanol as the eluent. According to the TLC assay, seven combined subfractions were obtained (each of approximately 100 mL). Fraction 6 from this separation was submitted for further CC on polyamide using $CHCl_3$/MeOH/butan-2-one ($\varphi_r = 10 : 2 : 1$) as the eluent. Totally, 409 fractions were collected (approximately 1 mL of each). Fractions 40–100 gave 7.2 mg of compound *II*. Fractions 119–206 were combined and divided by SPE on column C-18 (Varian Mega Bond Elut, Agilent Technologies, USA) using a LiChrolut (Merck, Germany) equipment and an H_2O/MeOH ($\varphi_r = 9 : 1$) mixture as the eluent system with an increasing MeOH portion. This separation resulted in the isolation of compound *III* (4.5 mg). The ethyl acetate portion (28.7 g) was submitted to column chromatography on a Sephadex LH-20

with propan-2-ol as the eluent. Totally, 112 fractions were collected (about 100 mL of each). Collected fractions were combined to ten fractions according to their TLC profiles. Fraction IV was repeatedly purified on a silica gel column using $CHCl_3$/MeOH ($\varphi_r = 9 : 1$), which resulted in the separation of compound *IV* (5.5 mg). Fraction V was purified on a silica gel column $CHCl_3$/MeOH ($\varphi_r = 9 : 1$) and compound *V* was isolated (56.8 mg). Fraction IX was purified on a silica gel column with $CHCl_3$/MeOH ($\varphi_r = 9 : 1$) as the eluent. From this separation, compound *VI* (3.6 mg) was isolated. The amounts of these substances do not correspond with the real amounts in the plant.

In enzymatic hydrolysis, 2 mg of compound *I* were dissolved with 2 mg of β-glucosidase in 5 mL of a methanol/H_2O mixture ($\varphi_r = 0.5$) and left standing for 24 h at room temperature. Then, methanol was evaporated on a vacuum rotary evaporator and the water phase was used for TLC of glycoside and aglycon.

In acidic hydrolysis; compound *II* (2 mg) was dissolved in methanol (2 mL) and heated under reflux with 11 % HCl for 3 h. Aglycone was extracted with chloroform and the aqueous phase was neutralized by the anion-exchange resin Dowex 2×8, (Fluka, Germany) and submitted for TLC comparison with saccharide standards.

The activity of LOX was monitored as an increase in the absorbance at 234 nm, which reflects the formation of the hydroperoxylinoleic acid. Inhibitory effect of the tested compounds was expressed as IC_{50} (Table 1).

The cytosolic fraction from rat lungs (Wistar rat, male 180 g), as a source of LOX, was isolated according to a procedure reported by Kulkarni et al. (1992). Briefly, rat lung homogenate was centrifuged at 1000 × *g* for 5 min. The pellet obtained contained unbroken cells and the debris was discarded. The resulting supernatant was centrifuged at 10 000 × *g* for 15 min to obtain the mitochondrial fraction. The post-mitochondrial supernatant was further centrifuged at 100 000 × *g* for 60 min to obtain microsomes and cytosol. This fraction was further purified by ammonium sulfate precipitation (60 %), chromatography on Sephadex G-150 (Pharmacia, Sweden), and on Phenyl-Sepharose CL-4B (Pharmacia, Sweden). The purified enzyme was used for LOX activity determination. The protein content in the enzyme preparation was estimated by the method of Bradford (1976).

Linoleic acid (99 %, Sigma, USA) was used as a substrate prepared in solubilized state as described (Kemal et al., 1987) in the concentration of 0.2143×10^{-5}–0.7143×10^{-5} mol L^{-1}.

For the LOX activity assay, a UV/VIS Spectrometer Perkin–Elmer Lambda 35 (USA), was used. The reaction medium contained a 50 mM Tris-HCl buffer (pH 7.0), 5 μL of the enzyme protein and solubilized linoleic acid. The compounds were tested in the final concentration range of 2.5×10^{-5}–15×10^{-5} mol L^{-1}.

Results and discussion

Compound *I* was isolated as a white powder, λ_{max} = 280 nm. Mass spectra of compound *I* show a molecular ion at $m/z = 316$ with a fragment at $m/z = 136$ corresponding to aglycone after the elimination of hexose and water (316–162 = 154; 154–18 = 136). ^{13}C NMR spectra confirmed the presence of 14 carbonyl atoms. A comparison of the obtained data with data available in literature (Franzyk et al., 2004; Nagao et al., 2001) allows to identify the isolated compound as 2-(3,4-dihydroxyphenyl)-ethyl-β-D-glucopyranoside (hydroxytyrosol glucoside). Enzymatic hydrolysis of compound *I* gave aglycon and glucose, confirmed after the TLC analysis on silica gel with a glucose standard (EtOAc/HCOOH/CH_3COOH/H_2O, φ_r = 100 : 11 : 11 : 23, developed four times, aniline–phthalate detection). It is interesting that due to high polarity of aglycone, its extraction with chloroform was not successive and its TLC analysis had to be realized from the water portion.

Compound *II* was isolated as a yellow powder with two absorption maxima at 270 nm and 330 nm indicating a flavonoid structure of the isolated compound. Specific diagnostic reagents shifts (NaOMe, $AlCl_3$, NaOAc) confirmed the presence of such a structure. According to TLC, acid hydrolysis of compound *II* yielded apigenin as aglycone (benzene/ethylacetate/HCOOH, $\varphi_r = 7 : 3 : 1$, Naturstoffreagent A + polyethylene glycol) with glucose and rhamnose as the saccharide moiety (TLC as for compound *I*). This structure is supported by mass spectra which show a molecular ion peak at $m/z = 724$ and a fragment at $m/z = 270$ (aglycon). A difference of 454 atomic mass units (AMU) (724–270 = 454) indicating the presence of one molecule of hexose and two molecules of deoxyhexose in the structure of the isolated compound (162 + 146 + 146 = 454). ^1H and ^{13}C NMR are in good agreement with the spectra of ligustroflavone (Pieroni & Pachaly, 2000b), which allowed the identification of the isolated compound as ligustroflavone (apigenin-7-*O*-β-(2″,6″-di-α-D-rhamnopyranosyl)-D-glucopyranoside).

Compound *III* exhibited maxima at 220 nm, 250 nm, 290 nm, and 330 nm in UV spectrum. MS spectra in the negative mode show a molecular ion at m/z = 785 $[M—H]^-$ and fragments at $m/z = 623$ (785 – 162 = glucose – 18), $m/z = 477$ (785 – 162 = glucose – 18 – 146; rhamnose – 18), $m/z = 179$ (caffeic acid), $m/z = 135$ (hydroxytyrosol; 154 – 18). A direct TLC and HPLC comparison with the standard of echinacoside (PhytoLab, Germany) confirmed the structure of compound *III* as echinacoside.

Compound *IV*, on TLC after spraying with H_2SO_4 in ether, exhibited a characteristic purple color similar to compound *I* with λ_{max} = 280 nm. Direct

Table 1. Inhibitory activity of isolated compounds and their kinetic parameters tested on LOX

Substrate concentration/(10^{-5} mol L^{-1})	Parameter	Oleuropein	Echinacoside	*L. vulgare* L.
0.214		0.193	0.214	0.212
0.357		0.192	0.196	0.212
0.500	K_M/(mmol L^{-1})	0.191	0.213	0.211
0.643		0.189	0.215	0.207
0.714		0.189	0.186	0.192
	V_{max}/(μmol s^{-1} L^{-1})	59.9	23.3	286
	IC_{50}/(μmol L^{-1})	82.2	296	0.388[a]

a) mg L^{-1}.

TLC comparison of the isolated compound (silica gel; $CHCl_3$/MeOH, $\varphi_r = 9 : 1$) with aglycon obtained by enzymic hydrolysis of hydroxytyrosol glucoside (compound *I*) confirmed the structure of hydroxytyrosol for compound *IV*.

Compound *V* was isolated as a white powder with maxima at 230 nm and 280 nm in the UV spectrum. In mass spectra of compound *V*, a molecular ion at $m/z = 540$ is present. A direct TLC and HPLC (Rt, DAD, MS/MS fragmentation) comparison with the standard of oleuropein (C. Roth, Karlsruhe, Germany) allowed the identification of compound *V* as oleuropein.

Compound *VI* exhibited similar TLC properties as compound *III* (blue fluorescence under UV light with higher R_f compared to compound *III*, silica gel EtOAc/HCOOH/CH_3COOH/H_2O, $\varphi_r = 100 : 11 : 11 : 23$). UV maxima of compound *VI* are identical with those of compound *III*. MS spectra in the negative mode show a molecular ion at $m/z = 623$ $[M—H]^-$. According to the obtained results and the direct TLC (silica gel as mentioned above, polyamide, $CHCl_3$/MeOH/butan-2-one, $\varphi_r = 12 : 2 : 1$) and HPLC comparison with a standard, compound *III* was identified as acteoside.

It is known from literature (Tattini et al., 2004) that *Ligustrum vulgare* L. is an abundant source of iridoids and phenolics. HPLC-DAD profiles show the complex polyphenol composition of *L. vulgare* leaves which include secoiridoids and tyrosol derivatives, and both hydroxycinnamates and flavonoid glycosides. Oleuropein, ligustaloside A and ligustaloside B, and ligstroside constitute the relevant class of secoiridoids. The flavonoid composition of *L. vulgare* leaves comprises quercetin 3-*O*-rutinoside, luteolin glucosides, namely luteolin 7-*O*-glucoside and luteolin 4′-*O*-glucoside, and both apigenin 7-*O*-glucoside and apigenin 7-*O*-rutinoside. Hydroxycinnamates are represented by *p*-coumaric acid and echinacoside. Appreciable amounts of hydroxytyrosol and hydroxytyrosol glucoside were also detected in *L. vulgare* leaves. The distribution of phenylpropanoid glycoside acteoside (Scogin, 1992) is restricted to a constellation of families which may constitute a natural ordinal grouping. This distribution includes many families traditionally clustered in a Scrophulariales-Lamiales com-

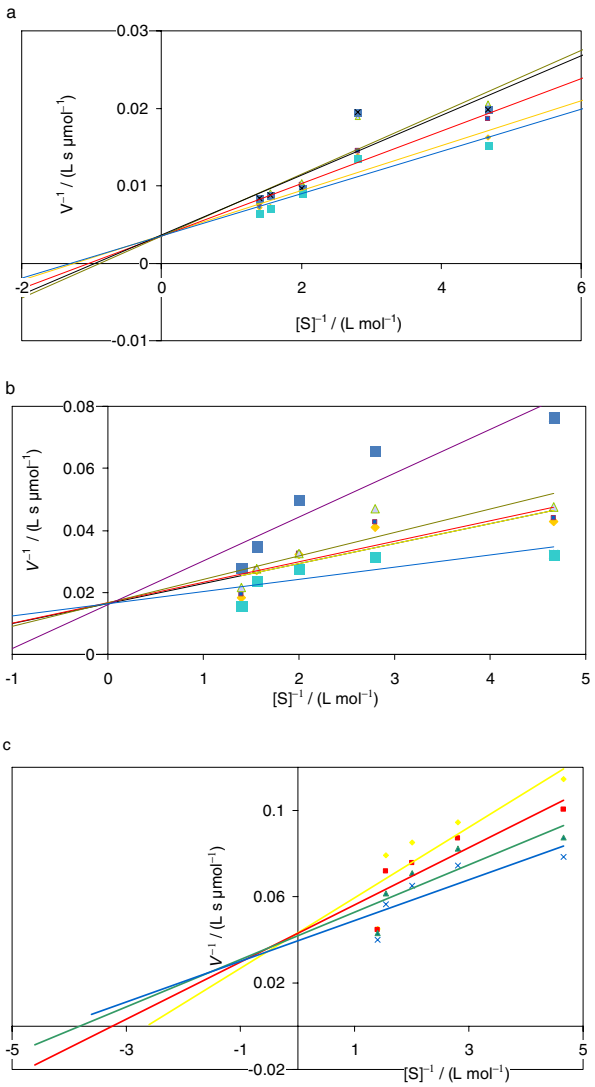

Fig. 1. Kinetic types of LOX inhibition by the tested extracted and isolated compounds: a) echinacoside, b) echinacoside, c) *L. vulgare* L.

plex containing Retziaceae, Oleaceae, Stilbaceae, and Hippuridaceae, excluding Hydrostachyaceae; the presence of this complex was confirmed in different *Ligustrum* species (Wong et al., 2001; Pan et al., 2002).

The aim of this study was also the evaluation of the

inhibitory effect of the isolated compounds and water infusion from the leaves of *L. vulgare* on the activity of lipoxygenase isolated from the cytosolic fraction of rat lungs. Data are reported as IC_{50} values (Table 1). Water infusion and two isolated compounds, oleuropein and echinacoside, exhibited remarkable inhibitory effect on LOX. An iridoid derivative, oleuropein, was proved to be the most effective. This is in good correlation with literature data. Oleuropein is one of the most active substances in virgin olive oil (de la Puerta et al., 1999). In intact rat peritoneal leukocytes stimulated with calcium ionophore, oleuropein, tyrosol, hydroxytyrosol, and caffeic acid from virgin olive oil, leukotriene B4 generation was inhibited at the 5-lipoxygenase level.

Fig. 1 shows the determination of the character of inhibition in the presence of inhibitors. Kinetic parameters K_M and V_{max} of Michaelis–Menten equation were expressed from Lineweaver–Burk equation: $V^{-1} = K_M V_{max}^{-1}[S]^{-1} + V_{max}^{-1}$; where $[S]/(\text{mol L}^{-1})$ is a substrate concentration; they demonstrate competitive inhibition of all tested inhibitors. This activity can be potentiated with the activity of flavonoids, exhibiting remarkable activity against LOX (Bezáková et al., 2007), isolated from this plant previously (Šeršeň et al., 2006; Mučaji et al., 2006) or on the classical pathway of the complement system (Pieroni & Pachaly, 2000a). These results clearly indicate that the tested samples contribute to the inhibitory activity of *L. vulgare* water infusion used for anti-inflammatory purposes in traditional medicine. However, the potential therapeutic value and the real clinical efficacy of this plant should be proved by further in vitro and in vivo experiments.

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References

Bezáková, L., Grančai, D., Obložinský, M., Vanko, M., Holková, I., Pauliková, I., Garaj, V., & Gáplovský, M. (2007). Effect of flavonoids and cynarine from *Cynara cardunculus* L. on lipoxygenase activity. *Acta Facultatis Pharmaceuticae Universitatis Comenianae*, *54*, 48–53.

Bezáková, L., Misik, V., Máleková, L., Svajdlenka, E., & Kostálová, D. (1996). Lipoxygenase inhibition and antioxidant properties of bisbenzylisoquinoline alkaloids isolated from *Mahonia aquifolium*. *Pharmazie*, *51*, 758–761.

Bradford, M. M. (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254. DOI: 10.1016/0003-2697(76)90527-3.

de la Puerta, R., Ruiz Gutierrez, V., & Hoult, J. R. S. (1999). Inhibition of leukocyte 5- lipoxygenase by phenolics from virgin olive oil. *Biochemical Pharmacology*, *57*, 445–449. DOI: 10.1016/S0006-2952(98)00320-7.

Franzyk, H., Olsen, C. E., & Jansen, S. R. (2004). Dopaol 2-keto- and 2,3-diketoglycosides from *Chelone obliqua*. *Journal of Natural Products*, *67*, 1052–1054. DOI: 10.1021/np04994 16.

Hammermann, A. F., Damirov, J. A., & Sokolov, W. S. (1971). Einige aussichtsreiche Pflanzen der Volksmedizin von Azerbajdschan. *Planta Medica*, *20*, 374–380. DOI: 10.1055/s-0028-1099719.

Hromádková, Z., Hirsch, J., & Ebringerová, A. (2010). Chemical evaluation of *Fallopia* species leaves and antioxidant properties of their non-cellulosic polysaccharides. *Chemical Papers*, *64*, 663–672. DOI: 10.2478/s11696-010-0054-2.

Jiménez, J. T., O'Connell, S., Lyons, H., Bradley, B., & Hall, M. (2010). Antioxidant, antimicrobial, and tyrosinase inhibition activities of acetone extract of *Ascophyllum nodosum*. *Chemical Papers*, *64*, 434–442. DOI: 10.2478/s11696-010-0024-8.

Kemal, C., Louis-Flamberg, P., Krupinski-Olsen, R., & Shorter, A. L. (1987). Reductive inactivation of soybean lipoxygenase 1 by catechols: a possible mechanism for regulation of lipoxygenase activity. *Biochemistry*, *26*, 7064–7072. DOI: 10.1021/bi00396a031.

Kiss, A. K., Mańk, M., & Melzig, M. F. (2008). Dual inhibition of metallopeptidases ACE and NEP by extracts, and iridoids from *Ligustrum vulgare* L. *Journal of Ethnopharmacology*, *120*, 220–225. DOI: 10.1016/j.jep.2008.08.015.

Kulkarni, A. P., Cai, Y., & Richards, I. S. (1992). Rat pulmonary lipoxygenase: dioxygenase activity and role of xenobiotic metabolism. *International Journal of Biochemistry*, *24*, 255–261. DOI: 10.1016/0020-711X(92)90255-Y.

Ma, S.-C., He, Z.-D., Deng, X.-L., But, P. P.-H., Ooi, V. E.-C., Xu, H.-X., Lee, S. H.-S., & Lee, S.-F. (2001). *In vitro* evaluation of secoiridoid glucosides from the fruits of *Ligustrum lucidum* as antiviral agents. *Chemical & Pharmaceutical Bulletin*, *49*, 1471–1473. DOI: 10.1248/cpb.49.1471.

Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). *The systematic identification of flavonoids*. New York, NY, USA: Springer-Verlag.

Mučaji, P., Nagy, M., Grančai, D., & Švajdlenka, E. (2006). Flavonoidné glykozidy *Ligustrum vulgare* L. *Farmaceutický Obzor*, *75*(10–11), 266–271.

Nagao, T., Abe, F., & Okabe, H. (2001). Antiproliferative constituents in the plants 7. leaves of *Clerodendron bungei* and leaves and bark of *C. trichotomum*. *Biological & Pharmaceutical Bulletin*, *24*, 1338–1342. DOI: 10.1248/bpb.24.1338.

Nagy, M., Križková, L., Mučaji, P., Kontšeková, Z., Šeršeň, F., & Krajčovič, J. (2009). Antimutagenic activity and radical scavenging activity of water infusions and phenolics from *Ligustrum* plants leaves. *Molecules*, *14*, 509–518. DOI: 10.3390/molecules14010509.

Nagy, M., Spilková, J., Vrchovská,V., Kontšeková, Z., Šeršeň, F., Mučaji, P., & Grančai, D. (2006). Free radical scavenging activity of different extracts and some constituents from the leaves of *Ligustrum vulgare* and *L. delavayanum*. *Fitoterapia*, *77*, 395–397. DOI: 10.1016/j.fitote.2006.04.010.

Pan, L. T., He, X. P., & Yanag, L. Y. (2002). Studies on chemical constituents in the leaf of *Ligustrum delavayanum*. *Zhongguo Zhong Yao Za Zhi*, *27*, 754–756.

Pieroni, A., & Pachaly, P. (2000a). An ethnopharmacological study on common privet (*Ligustrum vulgare*) and phillyrea (*Phillyrea latifolia*). *Fitoterapia*, *71*, Supplement 1, S89–S94. DOI: 10.1016/S0367-326X(00)00182-9.

Pieroni, A., & Pachaly, P. (2000b). Isolation and structure elucidation of ligustroflavone, a new apigenin triglycoside from the leaves *Ligustrum vulgare* L. *Pharmazie*, *55*, 78–80.

Scogin, R. (1992). The distribution of acteoside among angiosperms. *Biochemical Systematics and Ecology*, *20*, 477–480. DOI: 10.1016/0305-1978(92)90090-Z.

Shoemaker, M., Hamilton, B., Dairkee, S. H., Cohen, I., & Campbell, M. J. (2005). *In vitro* anticancer activity of twelve Chinese medicinal herbs. *Phytotherapy Research*, *19*, 649–651. DOI: 10.1002/ptr.1702.

Stojanović-Radić, Z., Comić, L., Radulović, N., Dekić, M., Ranđelović, V., & Stefanović, O. (2010). Chemical composition and antimicrobial activity of *Erodium* species: *E. ciconium* L., *E. cicutarium* L., and *E. absinthoides* Willd. (Geraniaceae). *Chemical Papers*, *64*, 368–377. DOI: 10.2478/s11696-010-0014-x.

Šeršeň, F., Mučaji, P., Grančai, D., Nagy, M., & Švajdlenka, E. (2006). Constituents of butanol extract from leaves of *Ligustrum vulgare* L. *Acta Facultatis Pharmaceuticae Universitatis Comenianae*, *53*, 253–261.

Šmejkal, K., Babula, P., Šlapetová, T., Brognara, E., Dall'Acqua, S., Žemlička, M., Innocenti, G., & Cvačka, J. (2008). Cytotoxic activity of *C*-geranyl compounds from *Paulownia tomentosa* fruits. *Planta Medica*, *74*, 1488–1491. DOI: 10.1055/s-2008-1081339.

Tattini, M., Galardi, C., Pinelli, P., Massai, R., Remorini, D., & Agati, G. (2004). Differential accumulation of flavonoids and hydroxycinnamates in leaves of *Ligustrum vulgare* under excess light and drought stress. *New Phytologist*, *163*, 547–561. DOI: 10.1111/j.1469-8137.2004.01126.x.

Wong, I. Y. F., He, Z.-D., Huang, Y., & Chen, Z.-Y. (2001). Antioxidative activities of phenylethanoid glycosides from *Ligustrum purpurascens*. *Journal of Agricultural and Food Chemistry*, *49*, 3113–3119. DOI: 10.1021/jf0100604.

Yim, T. K., Wu, W. K., Pak, W. F., & Ko, K. M. (2001). Hepatoprotective action of an oleanolic acid-enriched extract of *Ligustrum lucidum* fruits is mediated through an enhancement on hepatic glutathione regeneration capacity in mice. *Phytotherapy Research*, *15*, 589–592. DOI: 10.1002/ptr.878.

Young, R. N. (1999). Inhibitors of 5-lipoxygenase: a therapeutic potential yet to be fully realized? *European Journal of Medicinal Chemistry*, *34*, 671–685. DOI: 10.1016/S0223-5234(99)00225-1.