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Bioactive phenols in leaves of *Forsythia* species

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Summary: Nowadays a number of lignans (arctigenin, matairesinol, pinoresinol and phillygenin) have come to the fore in research due to their various biological activities. In this paper the accumulation of these constituents in leaf extracts of *Forsythia* plants (*F. intermedia*, *F. ovata* 'Robusta' and 'Tetragold', *F. suspensa*, *F. viridissima*) was quantified using a new isolation method, supercritical CO_2 fluid extraction. The total phenolic and flavonoid contents, the antioxidant capacity and the aglycone lignan profile were determined in leaf extracts of *Forsythia* species. Within the phenols, the flavonoids were only present in small quantities, but the amount of aglycone lignans was extremely high. *F. ovata* 'Robusta' had the highest total lignan content (103.8 mg/g) of all the *Forsythia* species. The main lignan in this species is arctigenin, which normally makes up about 60% of the total lignan content, but in the case of *F. ovata* 'Robusta' this value was 96.1%. Since this arctigenin content is outstanding compared to that of other *Forsythia* species, it could be promising to develop a fermentation technology for the production of this natural compound.

Key words: Antioxidant capacity, arctigenin, *Forsythia*, lignan, total flavonoid content, total phenol content

Introduction

Forsythia plants are popular ornamental shrubs containing a significant number of phenolic compounds. Phenolic compounds are one of the largest, most diverse group of secondary plant metabolites. They play an important role in plants, for example in protection against pathogens and insects, as a signal in plant-fungi and plant-plant interactions, in pollination and the spreading of fruits, and in reducing UV-B penetration in the cell wall (*Ayres & Loike*, 1991). Examinations have shown that the phenols isolated from the leaves of *Forsythia* species have diverse profiles and chemotaxonomical characteristics (*Kitagawa* et al., 1984).

Among the phenolic compounds, the dibenzylbutyrolactone lignans, such as matairesinol and arctigenin, and the furofuran lignans, such as pinoresinol and phillygenin, are the main lignans of the *Forsythia* species (*Kitagawa* et al., 1988). These compounds have a wide range of pharmaceutical properties, including anti-tumor (*Hausott* et al., 2003; *Takasaki* et al., 2000; *Awale* et al., 2006), anti-HIV (*Vlietinck*, 1998; *Ishida* et al., 2001) and antioxidative (*Chen* et al., 1999), as well as exhibiting anti-inflammatory (*Kang* et al., 2008), hepatoprotective (*Kim* et al., 2003), and neuroprotective activity (*Jang* et al., 2002).

Since the phenol profile varies according to the isolation procedures, an effective new method, supercritical CO_2 fluid extraction, modified and optimized by *Sedlák* et al. (2008), significantly enhanced the extraction yield due to the application mixture a 75/25 of carbon dioxide and 60% methanol.

The present work involved the determination of the total phenolic (Folin-Ciocalteu method), and flavonoid content ($AlCl_3$ method), the antioxidant capacity (Ferric Reducing Ability of Plasma assay) and the aglycone lignan profile (HPLC).

Materials and methods

Plant Material

Leaves of *Forsythia* x *intermedia* (Zabel), *Forsythia ovata* 'Robusta' and 'Tetragold', *F. suspensa* and *F. viridissima* were obtained from the Botanical Garden of Corvinus University, Faculty of Horticultural Science, Budapest.

Extraction

Samples consisting of 0.20 g of previously lyophilized and pulverized young leaves were extracted with SFE (Supercritical Fluid Extractor, ISCO SFX 2–10, Lincoln, USA) at 30.4 MPa and 40 °C using a 75/25 ratio of CO_2/60% (v/v) methyl alcohol mixture with a 30 minute stationary phase and a 30 minute dynamic phase. The final volumes were adjusted to 15.0 mL with 60% (v/v) methyl alcohol.

Colorimetric Measurements

Determination of Total Phenolic Compounds. The total phenolic content was estimated according to the Folin-Ciocalteu method (*Turkmen* et al., 2005) with some modifications. The data were expressed in mg gallic acid equivalents (GAE)/g dried matter (DM).

Determination of Total Flavonoid Content. For the determination of total flavonoid content the aluminium chloride colorimetric method was used (*Chang* et al., 2002). The results were expressed in mg quercetin equivalents (QE)/g DM.

Determination of the Antioxidant Capacity of the Leaf Extracts. To measure the antioxidant capacity of the leaf extracts, the Ferric Reducing Ability of the Plasma assay (FRAP) was applied (*Benzie & Strain,* 1996). The results were expressed in mg ascorbic acid equivalents (AAE)/g DM.

High Performance Liquid Chromatography with UV Detection (HPLC-UV)

The system consisted of two Pharmacia LKB HPLC pumps and a VWM 2141 UV-VIS detector (Pharmacia LKB Biochrom Ltd., Cambridge, UK) operating at λ=280 nm. Column: 150 × 4 mm MOS Hypesil BDS C18 5 m RP (Shandon Southern Products, Runcorn, UK). Injected amount: 20 µL. Gradient elutions were performed. Eluent A: acetonitrile: 0.02 M ammonium acetate buffer (pH 4)=15:85 (v/v). Eluent B: acetonitrile: 0.02 M ammonium acetate buffer (pH 4)=85:15 (v/v). Gradient 15% B→30% B over 5 min, 30% B→44% B over 7 min, 44% B›100% B over 5 min (*Sedlák* et al., 2008).

Statistical analysis

The results were presented as the mean values ± Standard Error of Mean (S.E.M.) of triplicate independent experiments. Analysis of variance was performed using InStat ANOVA procedures. The correlation (Pearson r) between means was determined by Gaussian distribution at the $P < 0.05$ level.

Results

The total phenolic content, determined by the Folin-Ciocalteu method, ranged from 5.21 ± 1.50 in *F. viridissima* to 31.07 ± 2.08 mg GAE/g DM in *F. ovata* 'Tetragold' (*Figure 1*). The total flavonoid content, determined by the $AlCl_3$ colorimetric method, varied between 0.47 ± 0.01 in *F. intermedia* and 0.69 ± 0.03 mg QE/g DM in *F. viridissima*. The antioxidant capacity, defined by the Ferric Reducing Ability of Plasma assay, ranged from 5.30 ± 0.18 in *F. ovata* 'Tetragold' to 11.89 ± 0.68 mg AAE/g in *F. ovata* 'Robusta'.

The total aglycone lignan content, consisting of arctigenin, matairesinol, pinoresinol and phillygenin, was detected in various amounts from 48.4 mg/g in *F. ovata* 'Tetragold' to 103.8 mg/g in *F. ovata* 'Robusta'.

The proportions of the four lignans differed (*Figure 2*). The main lignan of the species is arctigenin. In three cases (*F. intermedia, F. ovata* 'Tetragold', *F. viridissima*) this made up about 60% of the total lignan content, it had a value of 79.9% in the case of *F. suspensa* and 96.1% in *F. ovata* 'Robusta'.

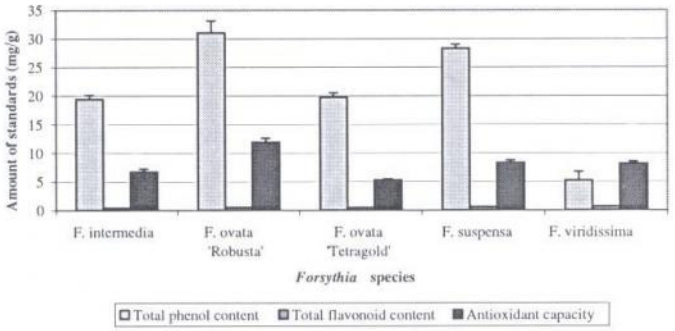

Figure 1 Colorimetric measurements on leaves of *Forsythia* species. The total phenoloid content is expressed as mg gallic acid equivalents (GAE)/g dried matter (DM), the total flavonoid content as mg quercetin equivalents (QE)/g DM and the antioxidant capacity as mg ascorbic acid equivalents (AAE)/g DM

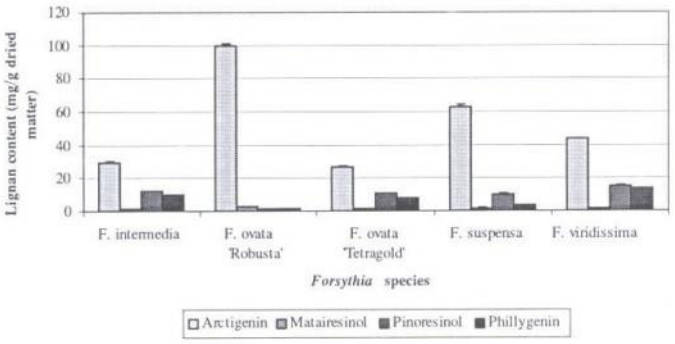

Figure 2 Aglycone lignan content in leaves of *Forsythia* species, determined by HPLC-UV

Discussion

The colorimetric measurements, total phenolic, flavonoid content and antioxidant capacity, were the first conducted using these strains.

Based on the total phenoloid and flavonoid contents and on the antioxidant capacity of leaf extracts of the *Forsythia* species, three important conclusions can be drawn. 1) There are significant differences in total phenoloid content between the species. 2) One of the main phenoloid groups, the flavonoids, mainly known for their excellent antioxidant effect, is only present in small quantities. 3) The profile of the antioxidant capacities of the leaf extracts is not similar to the total phenoloid content ($R^2 = 0.08812$, $P = 0.6277$), but corresponds well with the total aglycone lignan content ($R^2 = 0.9271$, $P = 0.0086$) (*Figures 3 and 4*).

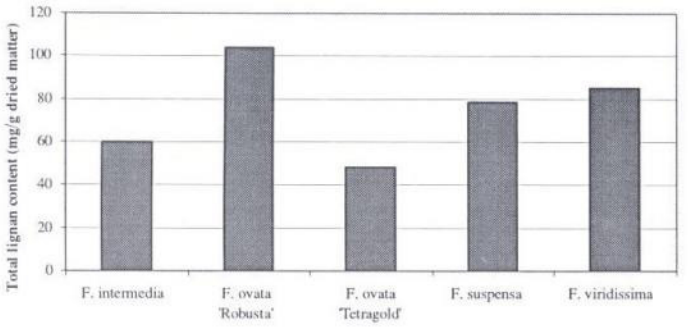

Figure 3 Total aglycone lignan content, consisting of arctigenin, matairesinol, pinoresinol and phillygenin, determined by HPLC-UV in *Forsythia* species

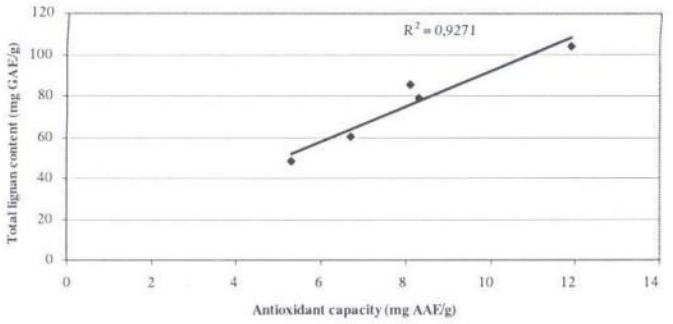

Figure 4 Correlation between the antioxidant capacity and total lignan content measured in *Forsythia* species

Lignans are the prominent phenoloid group in the leaf extracts, but there are significant differences between the *Forsythia* species.

In *F. ovata* 'Robusta' cultivar the amount of arctigenin was extremely high, compared to data in the literature (*Rahman* et al., 1990; *Choi* et al., 2003), not just in relative but also in absolute terms (99.7 mg/g). Since the literature suggests that arctigenin has significant biological activity, it could be promising to use *in vitro* cell culture of *F. ovata* 'Robusta' to develop a fermentation technology for the production of this compound.

References

Awale, S., Lu., J, Kalauni, S.K., Kurashima, Y., Tezuka, Y., Kadota, S. & Esumi, H. (2006): Identification of arctigenin as an antitumor agent having the ability to eliminate the tolerance of cancer cells to nutrient starvation. Cancer Res. 66 (3): 1751–1757.

Ayres, D.C. & Loike, J.D. (1990): Lignans, Chemical, Biological and Clinical Properties. University Press, Cambridge

Benzie, I.F.F. & Strain, J.J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power', FRAP assay. Analytical Biochemistry. 239: 70–76.

Chang, C.C., Yang, M.H., Wen, H.M. & Chern, J.C. (2002): Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. Journal of Food and Drug Analysis. 10: 178–182.

Chen, C.C., Chen, H.Y., Shiao, M.S., Lin, Y.L., Kuo, Y.H. & Ou, J.C. (1999): Inhibition of low density lipoprotein oxidation by tetrahydrofurofuran lignans from *Forsythia suspensa* and *Magnolia coco*. Planta Medica. 65: 709–711.

Choi, Y.H., Kim, J. & Yoo, K. (2003): Lignans and flavonoids of *Forsythia korena*. Chromatographia. 57: 73–79.

Hausott, B., Greger, H. & Marian, B. (2003): Naturally occurring lignans efficiently induce apoptosis in colorectal tumor cells. J. Cancer Res. Clin. Oncol. 129: 569–576.

Ishida, J., Wang, H.K., Oyama, M., Cosentino, M.L., Hu, C.Q. & Lee, K.H. (2001): Anti-AIDS agents. 46. Anti-HIV activity of harman, an anti-HIV principle from *Symplocos setchuensis*, and its derivatives. J. Nat. Prod. 64: 958–960.

Jang, Y.P., Kim, S.R., Choi, Y.H., Kim, J., Kim, S.G., Markelonis, G.J., Oh, T.H. & Kim, Y.C. (2002): Arctigenin protects cultured cortical neurons from glutamate-induced neurodegeneration by binding to kainate receptor. J Neurosci Res. 68 (2): 233–240.

Kang, H.S., Lee, J.Y. & Kim, C.J. (2008): Anti-inflammatory activity of arctigenin from *Forsythiae* Fructus. J. Ethnopharmacol. 116: 305–312.

Kim, S.H., Jang, Y.P., Shung, S.H., Kim, C.J., Kim, J.W. & Kim, Y.C. (2003): Hepatoprotective dibenzylbutyrolactone lignans of *Torreya nucifera* against CCl_4-induced toxicity in primary cultured rat hepatocytes. Biol. Pharm. Bull. 26 (8): 1202–1205.

Kitagawa, S., Hisada, S. & Nishibe, S. (1984): Phenolic compounds from Forsythia leaves I. Phytochemistry. 23: 1635–1636.

Kitagawa, S., Nishibe, S., Benecke, R. & Thieme, H. (1988): Phenolic compounds from *Forsythia* leaves II. Chem. Pharm. Bull. 36: 3667–3670.

Rahman, M.M.A., Dewick, P.M., Jackson, D.E. & Lucas, J.A. (1990): Lignans of *Forsythia intermedia*. Phytochemistry. 29: 1871–1880.

Sedlák, É., Boldizsár, I., Borsodi, L., Füzfai, Zs., Molnár-Perl, I., Preininger, É. & Gyurján, I. (2008): Identification and quantification of lignans, carboxylic acids and sugars in the leaves of *Forsythia* species and cultivars. Chromatographia (In press.)

Takasaki, M., Konoshima, T., Komatsu, K., Tokuda, H. & Nishino, H. (2000): Anti-tumor-promoting activity of lignans from the aerial part of *Saussurea medusa*. Cancer Letter. 158: 53–59.

Turkmen, N., Sari, F. & Velioglu, Y.S. (2005): Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chemistry. 99: 835–841.

Vlietinck, A.J., De Bruyne, T., Apers, S. & Pieters, L.A. (1998): Plantderived leadining compounds for chemotherapy of human immunodeficiency virus (HIV) infection. Planta Medica. 64 (2): 97–109.