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THE DETERMINATION OF COUMARINS IN EXTRACTS FROM PLANTS OF THE GENUS *PHILADELPHUS* L.

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Species of the genus *Philadelphus* L. (mock orange, Hydrangeaceae) are popular ornamental shrubs cultivated in Eastern Asia, Northern America, South-eastern Europe and the Caucasus. Mock orange was used to make soap, bows, arrows and cradles in the past. Aqueous extracts of *Philadelphus coronarius* L. flowers are used in traditional medicine and in homeopathy. Some members of the genus *Philadelphus* L. are known for their cytotoxic, antibacterial and immunomodulatory effects.

This study deals with quantitative determination of coumarins in different extracts (methanol, butanol, water) of leaves of *Philadelphus coronarius* L., *Philadelphus magdalenae* Rehd., *Philadelphus pekinensis* Rupr., *Philadelphus schrenkii* Rupr., *Philadelphus subcanus* Koehne, *Philadelphus tenuifolius* Rupr. et Maxim. and *Philadelphus zeyheri* Schrad. The content of coumarins was determined as umbelliferone using spectrophotometry.

The highest content of coumarins was recorded in methanol extract of species *Philadelphus schrenkii* Rupr., and butanol and water extract of *Philadelphus subcanus* Koehne. The results indicate higher content of coumarins in butanol and water extract compared to methanol extract.

The results obtained provide the basis for isolation and identification of biologically active substances from these extracts.

Keywords*: coumarins – quantitative determination – Philadelphus* L.

INTRODUCTION

Plants play an important role as a source of effective anti-cancer agents. Currently over 60% of anti-cancer agents are derived in one way or another from natural sources, including plants, marine organisms and microorganisms [1].

Some members of the genus *Philadelphus* L. (Hydrangeaceae) are known for their cytotoxic, antiproliferative, apoptotic, antibacterial, antioxidant, scavenger and immunomodulatory effects. Aqueous extract from the flowers of *P. coronarius* L. is

used in traditional medicine for the treatment of some gynaecological diseases and in homeopathy [2–9].

Coumarins (umbelliferone, scopoline), stigmasteryl-3-*β*-D-glucoside, uvaol, 3-*β*-28-dihydroxyoleanane-11(12),13(18)-diene and flavonoids (apigenine-7-*O*-glucoside, luteoline-7-*O*-glucoside, kaempferol-3-*O*-rutinoside, kvercetine-3-*O*-rutinoside) were isolated from leaves of *P. coronarius* L. Taraxerol, *α*- and *β*-amyrin, ursolic and oleanolic acid were reported in extract from branches of *P. coronarius* L. [9-13].

Stigmasteryl-3-*β*-D-glucoside, taraxerol, umbelliferone and aesculin were isolated from leaves of *P. tenuifolius* Rupr. et Maxim. [9].

Coumarins (1,2-benzopyrones) are ubiquitously found in higher plants where they originate from the phenylpropanoid pathway. They contribute essentially to the persistence of plants being involved in processes such as defence against phytopathogens, response to abiotic stresses, regulation of oxidative stress and probably hormonal regulation [14].

Some natural coumarins have been used as human therapeutics, while 4-hydroxycoumarins are prominent examples of microbial modification which gave rise to the first generation molecules developed along with aspirin and heparin as anticoagulants. Other applications appear possible in the course of new developments in various therapeutic fields, like symptomatic treatment of multiple sclerosis, photochemotherapy of T cell lymphoma, chemotherapy of multidrug resistant tumours, organ transplants or treatment of smokers for nicotine addiction [14]. It has been found that coumarin and its derivatives also show a wide range of bioactivities such as anticoagulant, oestrogenic, dermal photosensitizing, vasodilator, molluscicidal, anthelmintic, sedative, hypnotic, analgesic, hypothermic, antimicrobial, anti-inflammatory, antifungal and antiulcer [15].

This paper describes quantitative determination of coumarins in different extracts (methanol, butanol, water) of leaves of plants of the genus *Philadelphus* L.

MATERIAL AND METHODS

Leaves were collected at Arboretum Mlyňany, Institute of Dendrobiology, Slovak Academy of Science, in September 2005. All samples were identified by Ing. Hoťka and voucher specimens are deposited there. Plant material was dried at room temperature. Light petrol, chloroform and methanol extracts from each part (100 g of powdered leaves) were prepared in Soxhlet apparatus until the extracts became colourless. After methanol extraction the rest of the plant material was dried and extracted with hot water (3 × 500 ml). The butanol fraction was prepared with subsequent re-extraction of the water extract with butan-1-ol (3 × 200 ml). All freshly prepared extracts were evaporated *in vacuo* to full dryness at temperatures not exceeding 40°C. The water layer after the liquid–liquid extraction with butan-1-ol was evaporated to full dryness as well.

The dry material was stored at room temperature before further analyses. For the amounts of extracts from the selected plants obtained using different solvents, see Table 1.

Table 1. Weight of obtained extracts

	Light petrol	Chloroform	Methanol	Butanol	Water
P. coronarius	2.95	33.07	26.67	0.55	6.38
P. magdalenae	3.53	2.52	32.56	1.37	8.42
P. pekinensis	4.11	18.15	29.10	2.23	5.85
P. schrenkii	2.47	2.99	35.97	1.83	7.19
P. subcanus	3.84	1.95	31.55	0.38	8.23
P. tenuifolius	2.44	36.78	30.86	1.15	5.09
P. zeyheri	2.52	2.32	35.50	0.44	7.97

Content of coumarins was determined by using a spectrophotometric method with ammonium persulphate at λ = 495 nm. The percentage content of coumarins was calculated and expressed as umbelliferone [16].

All analyses were done in triplicate. The percentage of coumarins was calculated with reference to the drug dried at 105°C.

RESULTS AND DISCUSSION

The aim of this study was to compare the contents of coumarins in different extracts of seven species of the genus *Philadelphus* L. The quantification of coumarins was carried out using spectrophotometry and expressed as umbelliferone.

The results are shown in Figure 1.

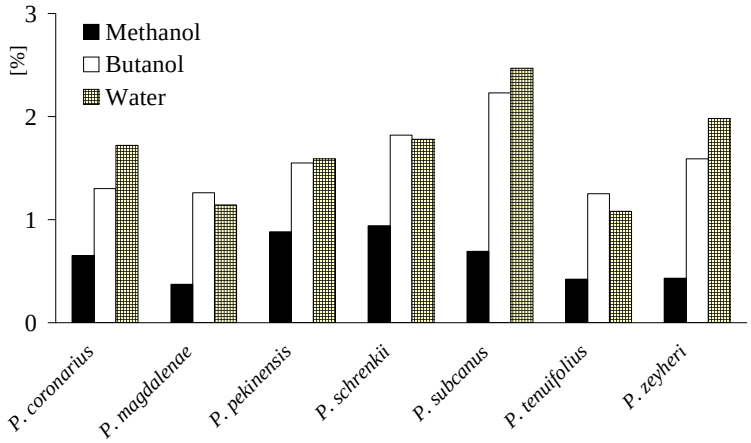

Figure 1. The percentage content of coumarins expressed as umbelliferone in 3 different extracts of different *Philadelphus* species leaves

The highest content of coumarins amongst methanol extracts was recorded in *Philadelphus schrenkii* Rupr. (0.94%), the lowest in species *Philadelphus magdalenae* Rehd. (0.37%).

The highest content of coumarins amongst butanol extracts was measured in extract of *Philadelphus subcanus* Koehne (2.23%), the lowest in *Philadelphus tenuifolius* Rupr. et Maxim. (1.26%) and *Philadelphus magdalenae* Rehd. (1.25%).

The highest content of coumarins amongst water extracts was determined in *Philadelphus subcanus* Koehne (2.47%), the lowest in species *Philadelphus tenuifolius* Rupr. et Maxim. (1.08%).

The results indicate higher content of coumarins in butanol and water extracts (1.25–2.23% resp. 1.08–2.47%) compared to methanol ones (0.37–0.94%).

In our previous study we found the highest content of coumarins in light petrol leaf extract of species *Philadelphus pekinensis* Rupr. and chloroform extract of *Philadelphus magdalenae* Rehd. [17].

The variation of coumarin content in the different species of the genus *Philadelphus* L. may be attributed to the geographical source, cultivation and age of the plants [18].

Based on these results of coumarin quantification we assume that the complex of coumarins in the studied plants of the genus *Philadelphus* L. is constituted predominantly by components with marked polarity, e.g. complex coumarin glycosides or polyhydroxy substituted compounds.

The results obtained provide the basis for isolation and identification of biologically active coumarins from these extracts.

CONCLUSION

This study deals with quantitative determination of coumarins in methanol, butanol and water extracts of leaves of seven species of *Philadelphus* plant. The content of coumarins was determined as umbelliferone using spectrophotometry. The highest content of coumarins amongst methanol extracts was recorded in *Philadelphus schrenkii* Rupr., the lowest one in species *Philadelphus magdalenae* Rehd. The highest content of coumarins amongst butanol extracts was measured in extract of *Philadelphus subcanus* Koehne, the lowest one in *Philadelphus tenuifolius* Rupr. et Maxim. and *Philadelphus magdalenae* Rehd. The highest content of coumarins amongst water extracts was determined in *Philadelphus subcanus* Koehne, the lowest one in species *Philadelphus tenuifolius* Rupr. et Maxim. The results indicate higher content of coumarins in butanol and water extracts compared to methanol ones.

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STANOVENIE KUMARÍNOV V EXTRAKTOCH RASTLÍN RODU *PHILADELPHUS* L.

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Druhy rodu *Philadelphus* L. (Hydrangeaceae) sú obľúbené okrasné kry pestované hlavne vo východnej Ázii, severnej Amerike, juhovýchodnej Európe a na Kaukaze.

Pajazmín sa v minulosti využíval na výrobu mydla, lukov, šípov a kolísok. Vodný extrakt kvetov *Philadelphus coronarius* L. sa používal v ľudovej medicíne a v homeopatii. Niektoré druhy rodu *Philadelphus* L. sú známe pre svoju cytotoxickú, antibakteriálnu a imunomodulačnú aktivitu.

Táto práca sa zaoberá stanovením obsahu kumarínov v extraktoch (metanolový, butanolový, vodný) z listov druhov *Philadelphus coronarius* L., *Philadelphus magdalenae* Rehd., *Philadelphus pekinensis* Rupr., *Philadelphus schrenkii* Rupr., *Philadelphus subcanus* Koehne, *Philadelphus tenuifolius* Rupr. et Maxim. a *Philadelphus zeyheri* Schrad. Obsah kumarínov bol stanovený spektrofotometrickou metódou ako umbeliferón.

Najvyšší obsah kumarínov bol zistený v metanolovom extrakte druhu *Philadelphus schrenkii* Rupr. a v butanolovom a vodnom extrakte druhu *Philadelphus subcanus* Koehne. Výsledky naznačujú vyšší obsah kumarínov v butanolovom a vodnom extrakte v porovnaní s metanolovým.

Závery tejto práce sú základom pre izoláciu a identifikáciu biologicky aktívnych zložiek týchto extraktov.

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