ANTIRADICAL ACTIVITY OF PETALS OF *PHILADELPHUS CORONARIUS L.*

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SUMMARY

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and nitroprusside assay were used for in vitro study of antiradical/scavenger activity of Philadelphus coronarius petal substances. The results obtained can be used as the basis for further studies of this type of the biological effects in the plant.

Key words: antiradical activity, *Philadelphus coronarius*

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INTRODUCTION

The problem of formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), that manifest negatively in pathogenesis of civilisation diseases, attracts the interest in substances showing the antiradical or antioxidant properties. Compounds with scavenging and antioxidant effects, e. g. flavonoids, phenolic acids and some triterpenes, are often available from natural sources. *Philadelphus coronarius* L. can be one of them.

Philadelphus coronarius L., family Philadelphaceae, order Saxifragales, is a shrub occurring in south and south-east Europe. Its leaves are 4-8 cm long, opposite, oval and dentate with petiole (1). Racemes are formed with 5-7 flowers, the flowers are tetramerous, 2.5-3.5 cm of the diameter and strongly aromatic. Sepals are oval. Petals are wide oval and yellowish white. It blossoms in June and July. Fruits are capsules.

Literature indicates their use in homeopathy. An aqueous extract is used in folk medicine for a treatment of some gynaecological diseases. This plant is cultivated in the Czech Republic as ornamental shrub in many varieties.

Flavonoids (rutin, quercetin, kaempferol, isorhamnetin, naringenin and eriodictyol) are known compounds in ethanolic extracts of leaves and flowers (2).

Further identified constituents include some triterpenes [e. g. taraxerol, β-amyrin, ursolic acid and oleanolic acid, uvaol and 3β, 28-dihydroxyolean-11(12), 13(18)-diene] obtained from light petrol extract of branches and leaves. Coumarins (scopolin, umbelliferone) have been isolated from chloroform extracts of branches (3). Phenolic acids (caffeic acid and protocatechic acid) have been identified in ethanolic extracts of branches and leaves, as well as some γ-glutamylpeptides (4). Volatile compounds (e.g. 2-aminobenzaldehyd, nopinone, myrtanal, myrtenal, myrtenol, indole, 2-formamidobenzaldehyde and veratraldehyde) have been determined from living flowers by gas chromatography--mass spectrometry (GC-MS) analysis (5).

Several biological effects of *Philadelphus* extract have been described in literature. The ethanolic extract of branches and leaves containing flavonoids and triterpenes possess very strong antibacterial activity when tested on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* (6). A cytotoxic activity test of the same extract employing the human cell line HeLa has been done. This activity was not proved (7).

Flowers have not been closely investigated, therefore a phytochemical analysis and testing of petal extract of *Philadelphus coronarius* was performed.

MATERIALS, METHODS AND RESULTS

Petals of *Philadelphus coronarius* L. were collected during the period of their spontaneous fall. Dry plant material was extracted in ethanol using ultrasonic bath (Sonorex Super 10 P sonicator; 22 °C, intensity 10, 20 min) and then lyophilised.

Identification of phenolic compounds in *Philadelphus* extract was mostly performed by an HPLC method with diode array detector. The glycoside of quercetin (rutin) and phenolic acids (caffeic acid and ferulic acid) were determined by comparison of their retention times and UV spectra with those of pure compounds. Ethanolic extract were analysed by HPLC using LiChrosorb RP-18 column (Merck, Germany; 250x4 mm; particle size 7 μm), flow rate 0.8 ml/min, and a linear gradient elution from 15% MeOH + 75% 0.01 M solution of H_3PO_4 to 70% MeOH + 15% 0.01 M solution of H_3PO_4 during 30 min (at 20 °C). UV spectra of phenolic compounds in the *Philadelphus* extract were in good agreement with the results obtained earlier for the leaf extract (8).

Antiradical activity of the ethanolic extract was tested by two standard *in vitro* assays (9, 10, 11) with standard of rutin. Starting concentration of the extract was 1.5 mg/ml. DPPH assay is based on a colour reaction of the samples with DPPH (2,2-diphenyl-1-picrylhydrazyl) stable radical, and the decrease in absorbance

Table 1. *Scavenger activity of Philadelphus extract in comparison with the standard of rutin*

	EC_{50} (DPPH assay)	EC_{50} (nitroprusside assay)
Rutin	3.95 μg/ml	$52.51x10^{-3}$ μg/ml
Philadelphus extract	67.55 μg/ml	$87.65x10^{-3}$ μg/ml

(λ=517 nm) is directly correlated to the content of a compound showing the free radical scavenger effect (9, 10). The antiradical activity can be expressed as EC_{50} and compared with the standard (rutin). The value of EC_{50} was calculated by non-linear regression of the dose-response curve (Table 1).

Nitroprusside assay for the determination of RNS scavengers is a spectrophotometric method based on measurement of RNS production. Nitric oxide, generated from sodium nitroprusside in aqueous solution at pH 7, interacts with oxygen to produce nitrite ions which are measured by Griess reaction (11). Components of samples with antiradical activity inhibit the interaction between nitric oxide and oxygen. The value of EC_{50} was calculated by non-linear regression of the dose-response curve (Table 1).

The extract exhibited positive antiradical activity in both assays (Table 1).

In DPPH assay, rutin was significantly more active in comparison with the extract, while in nitroprusside assay the antiradical activity of the extract was similar to rutin.

Biological testing on cytotoxicity and acute toxicity of *Philadelphus* extract was performed.

Acid phosphatase assay is a kinetic colorimetric method which evaluates in vitro activity of the proliferation of the cell lines (12). Results of this determination with acid phosphatase assay are interpreted as IC_{50} value (20.08 μg/ml), i.e. concentration of the extract decreasing the proliferation of pulmonary carcinoma cells to 50 % compared to control (pure medium + 1% DMSO).

Alternative test of acute toxicity applying the worms of *Tubifex tubifex* Müll. species was performed (13). This bioassay is based on the observation of the experimental worms immobilisation depending on the concentrations of the tested substance. Six worms placed in a Petri dish were treated with 2 ml of the aqueous solution of *Philadelphus* extract. The exposition time was 3 min. EC_{50} of the immobilisation was calculated for every experiment by statistical method developed by C. S. Weil (13). The sensitivity of the tested organism was specified by the solution of standard toxin – $MnCl_2$. The value of EC_{50} (95% confidence interval) of the extract was 7.1 (6.1 - 8.2) mg/ml. The value of EC_{50} (95% confidence interval) of the standard toxin was 24.3 (22.3 - 26.1) mg/ml.

The biological tests were performed as preliminary assays. All assays were repeated three times at least and under standard conditions.

CONCLUSION

Since the constituents of this species and their biological effects are not known well, the preliminary screening tests and analysis have been performed. The both tests of toxicity (acid phosphatase assay and alternative test of acute toxicity) were negative. The extract of *Philadelphus coronarius* showed significant antiradical/scavenger activity in both used assays. Further research of active constituents, more detailed analyses and also phytochemical studies should be performed for the deduction of more exact results and conclusions.

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