

EXUDATE FLAVONOIDS OF *Primula veris* LEAVES AND  
THEIR INHIBITORY ACTIVITY ON *Lolium perrene* SEED  
GERMINATION

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**Abstract**

In the present study exudate flavonoids from *P. veris* leaves were identified and their potential as inhibitors on *L. perrene* seed germination was evaluated. Leaves of *P. veris* were collected from natural population. Leaf exudate was obtained by dipping whole leaves into acetone for 2–3 min. Chemical composition of the exudate was analysed by GC/MS. The seed germination test was done in Petri dishes. Flavonoid aglycones were found as main components of the exudate (over 50% of all compounds). Unsubstituted flavone, 2'-methoxyflavone, 3'-methoxyflavone, 3',4'-dimethoxyflavone, 2',5'-dimethoxyflavone, 5,6,2',6'-tetramethoxyflavone were identified. Complete inhibition on *Lolium perenne* seed germination and root elongation by aqueous solution of the exudate at concentration 7 mg/mL was found. This is the first report about inhibitory activity of *P. veris* leaf exudate on seed germination.

**Key words:** bio-herbicide, surface flavonoids, *Primula veris*

**Introduction.** *Primula veris* L. (syn. *Primula officinalis* Hill) is a valuable medicinal plant whose roots and flowers are mainly used for their expectorant, anti-inflammatory, antimicrobial, secretolytic activity [1–3]. The leaves of the plant are less used as a therapeutic agent. Phenolic compounds have been reported

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as the main active compounds in the leaves of the species [4,5]. Distribution of exudate flavonoids in the leaves of many species in the genus *Primula* has been comprehensively studied mainly in connection with chemosystematics [6–10]. Nevertheless exudate flavonoids in the *Primula* species from Bulgaria have not been studied.

Besides their pharmacological properties plants are studied also as sources of biocidal activity. The potential of plant extracts and essential oils as an environmentally friendly alternative to synthetic pesticides has been an object of intensive studies in recent years. A lot of data on plant-based products with potential to be applied as bio-insecticides, bio-fungicides and bio-herbicides have been accumulated [11–14].

It has been reported that exudate compounds exhibited allelopathic activity and protective role: UV-protection, herbivore deterrence, antifungal [15,16]. Also strong cytostatic properties even at low concentrations have been established for *Primula* exudate flavonoids [17]. This is the reason our attention to be focused on the biological activity of an exudate fraction.

In the present study exudate flavonoids of *Primula veris* leaves were identified and their potential as inhibitors of seed germination was evaluated.

**Material and methods. Plant material.** Leaves of *P. veris* were collected from natural population from Struma Valley, in a pine forest above the village of Ilindentsi (N 41.680420, E 23.298180).

**Acetone exudate.** Dry not ground leaves (50 g) of *Primula veris* were rinsed for 2–3 min with acetone to dissolve the compounds accumulated on the plant surface. After that the obtained solution was filtered and evaporated to dryness (760 mg exudate) for future analysis. The procedure was repeated with new material several more times until the required amount of exudate for the analyses was obtained.

**Derivatization.** One hundred microlitres pyridine and 100  $\mu$ L of N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) were added to the dried sample of the exudate (50 mg) into vial. The prepared sample was heated at 70 °C for 2 h. After cooling, 300  $\mu$ L of chloroform were added and the samples were analyzed by GC/MS.

**Gas chromatography/mass spectrometry (GC/MS) analysis.** GC/MS analysis of acetone exudate was recorded on a Thermo Scientific Focus GC coupled with Thermo Scientific DSQ mass detector operating in EI mode at 70 eV. A DB-5MS column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) was used. The temperature programme was: 100–180 °C at 15 °C/min, 180–300 °C at 5 °C/min, and 10 min at 300 °C. The injector temperature was 250 °C. The flow rate of carrier gas (Helium) was 0.8 mL/min. The split ratio was 1:10. One microlitre of the solution was injected. The components were identified by comparing their mass spectra and retention indices (RI) with those of authentic standards from the National Institute of Standards and Technology (NIST) spectra library.

**Inhibitory activity on seed germination.** One hundred seeds of *Lolium perenne* L. were placed in a Petri dish on filter papers moistened with the test solutions. The exudate, in water–acetone mixture (99.5:0.5), was assayed at concentration of 3, 5 and 7 mg/mL. The control consisted only of a water-acetone mixture. The samples were incubated at room temperature for 7 days. At the end of the week, the degree of inhibition of germination [%] compared to the control was calculated according to ATAK et al. [12]. The reduction in root growth caused by the extracts was estimated as a percentage of the root length in the control using a formula after the source cited above. Seed germination assay was performed in three independent experiments.

**Statistical analysis.** Statistical analyses were performed using Microsoft Excel software. The results are presented as the mean of three repetitions with standard deviation (SD).

**Results and discussion. Phytochemical analysis.** Acetone exudate of *P. veris* leaves was analysed for its components by GC/MS. Flavonoid aglycones were identified as main bioactive compounds (Table 1, Fig. 1). The amount of flavonoids in the exudate reaches over 50% of all compounds. The identified flavonoids are in accordance with previously reported data for the species as well as other *Primula* species [6,7,9,18]. Two non-flavonoid-type constituents of exudate were detected in major quantity. According to their MS spectra they belong to straight-chain alkanes class.

**Inhibitory activity on seed germination.** The results from the evaluation on inhibitory activity of *Primula veris* acetone exudate on *Lolium perrene*

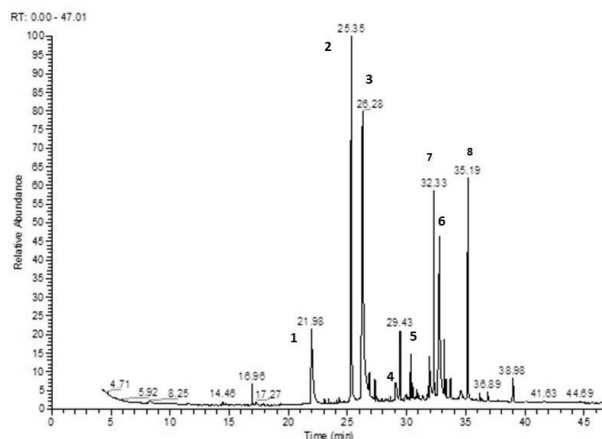


Fig. 1. GC/MS chromatogram of acetone exudate from *Primula veris* leaves; (1) unsubstituted flavone; (2) 2'-methoxyflavone; (3) 3'-methoxyflavone; (4) 2',5'-dimethoxyflavone; (5) 3',4'-dimethoxyflavone; (6) 5,6,2',6'-tetramethoxyflavone (zapotin); (7) straight-chain alkane 1 (8) straight-chain alkane 2

T a b l e 1

Identified flavonoids in leaf exudate of *P. veris*

Identified flavonoids	RT	Quantity Area [%]
unsubstituted flavone (1)	21.98	3.9 ± 1.5
2'-methoxyflavone (2)	25.35	18.1 ± 3.0
3'-methoxyflavone (3)	26.28	17.4 ± 3.5
2',5'-dimethoxyflavone (4)	29.06	5.0 ± 1.2
3',4'-dimethoxyflavone (5)	30.48	1.7 ± 1.0
5,6,2',6'-tetramethoxyflavone ( <i>zapotin</i> ) (6)	32.75	8.9 ± 2.6

seed germination and root elongation are presented in Table 2. Strong inhibitory activity was found at application of aqueous solution of leaf exudate at concentration of 5 mg/mL. Total inhibition was achieved by applying the solution at a concentration of 7 mg/mL.

The inhibitory effect on seed germination of acetone exudates from other aromatic plant species *Artemisia absinthium*, *Salvia sclarea*, *Tanacetum vulgare*, *Salvia officinalis* has been also reported [19]. LIM et al. [20] found that leaf extract from Dilleniaceae family at concentration 10 mg/mL showed high inhibition on *Eleusine indica* seeds germination.

T a b l e 2

Inhibitory activity of *P. veris* leaf exudate on *L. perrene* seed germination and root elongation

Inhibitory activity [%]	Concentration of <i>P. veris</i> exudate aqueous solutions [mg/mL]		
	3	5	7
on seed germination	29 ± 5	72 ± 14	100 ± 0
on root elongation	55 ± 12	93 ± 4	100 ± 0

**Conclusion.** Leaf exudate of *Primula veris* was found to be rich in flavonoid aglycones. Complete inhibition on seed germination and root elongation of *Lolium perenne* seed by aqueous solution of *P. veris* exudate at concentration 7 mg/mL was established. This is the first report about inhibitory activity of *P. veris* leaf exudate on seed germination. Flavonoid profile of acetone exudate of *P. veris* leaves of Bulgarian origin was reported for the first time as well.

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