9. SUMMARY

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This experimental work deals with pharmaco-botanical evaluation of the aerial part of taxon *Evolvulus alsinoides* L. The plant is a component of formulations with nootropic and adaptogenic activity. A subject of the investigation was a dried herb of the plant, which is of Indian origin. The aim of this work was to extend the knowledge about compounds of the taxon, to evaluate biological activity of the fractions, isolated substances and to determine characteristic metabolites of the plant.

In the first part of the project the crude extract of the plant *E. alsinoides* was prepared by percolation of 8.8 kg of herb with 95% ethanol. The extract was partitioned into three extracts by extracting by two solvents of different polarity (petroleum ether, ether). The first fraction of weight 92.1 g was obtained by extracting of methanolic solution of crude extract with petroleum ether. The second fraction of weight 69.7 g was obtained by extracting of water solution of the crude extract with ether. The remaining solvent was evaporated from the crude extract and this one represented the third part - polar residue, yield 231 g.

The fractions were processed by the methods of column chromatography (CC).

The bioassay-guided separation of selected parts of extract (test of acute toxicity, fototoxicity, antioxidant activity, anti-aggregation activity and antifungal activity) was performed during separation of the extract. The content of phenolic compounds of selected fractions was determined by reaction with Folin-Ciocalteu's test solution.

The quantitative content of isolated substances in crude extract was established by HPLC and GC/FID methods.

Fraction designated as polar residue (230 g) was subjected to polyamide CC.

The fraction of non-phenolic compounds (196.5 g) and the fraction of phenolic compounds (12.5 g) were obtained.

The fraction of phenolic compounds was subjected to Si-gel CC. In total 65 fractions was obtained which were combined into 26 qualitatively different fractions. 25 mg of scopoletin were isolated from combined fraction 6-7. 33 mg of umbelliferone were isolated from fraction 8 of primary extract. The rest of the fractions (1-5, 9-26) was combined again and subjected to new Si-gel CC. In total 143 fractions were obtained which were combined to 35 qualitatively different fractions. The significant amount of new crystals was not obtained from these fractions. The fractions were subjected to evaluation of antioxidant activity.

White crystals identified as Na_2SO_4 were crystallized from the fraction of nonphenolic compounds. This fraction was subjected to the crude separation on Si-gel CC and was fractioned in three parts designated as FPFLHD1 (31 g), FPFLHD2 (7 g),

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FPFLHD3 (5 g). Fraction FPFLHD1 was subjected to Si-gel CC. Obtained fractions 1-100 were combined to 22 qualitatively different fractions. 15 mg of scopolin were isolated from combined fraction 36-40. The large crystals of orthorhombic system of substance 4 (245 mg) crystallized from combined fraction 47-56. These crystals were identified as 2-methyl-1,2,3,4-butanetetrol.

For the purposes of biological tests an isolation of sufficient amount of scopolin from fraction FPFLHD1 was performed using diaione HP-20. 187 mg of crystals of scopolin were obtained from fraction FPFLHD1.

In total 145 fractions were obtained on Si-gel CC from ether fraction.

Scopoletin was isolated from combined fraction 26-50. Both coumarines (scopoletin and umbelliferone) were proven in combined fractions 51-62, 63-69, 70-85 and 86-92.

The presence of nitrogen substances of alkaloids structure was detected in subfraction 26-45 (from combined fraction 93-117). Only the mixture of very impure substances that's structure has not been identified yet was isolated. The proof

of alkaloids by Dragendorff˙s reagent was negative. The presence of alkaloids in this extract has not been confirmed yet.
The analysis of petroleum ether fraction was complicated by higher content of chlorophyll and non-polar substances. In total of 113 fractions were obtained CC. 8-methyldecanic acid, oleic acid and the mixture of unidentifiable polyunsaturated fatty acid and aliphatic hydrocarbon were isolated from combined fractions 3-4 and 5-7. The presence of esters of ferulic acid with linear alcohols C14-16 was proved in combined fraction 15-16. The mixture of palmitic, stearic and heptadecanic acid was obtained from combined fraction 36-40 by preparative chromatography.
The content of scopoletin (0.0271 %), umbelliferone (0.0257 %), scopolin (0.0090 %) and 2-methyl-1,2,3,4-butanetetrol (0.870 %) in plant was determined using HPLC and GC-FID methods.
The evaluation of antioxidant activity was performed by DPPH test with the use of Sequential Injection Analysis. Simultaneously the content of phenolic compounds in particular fractions of the extract was determined using Folin-Ciocalteu‘s reagent. The highest antioxidant activity at DPPH test was determined in fraction 31 (0.230 mg/ml, content of phenolic compounds 23.1 %). The highest content of phenolic compounds (28.9 %) was determined in fraction 28 (antioxidant activity 0.258 mg/ml). The antioxidant activity of partial fractions varied in the interval from 0.2 to 0.9 mg/ml. The antioxidant activity of partial fractions of the extract was higher than the activity of isolated compounds because isolated pure compounds did not show significant

activity at the tested concentrations. Only the ester of ferulic acid showed moderate antioxidant activity. Simultaneously coumarins and 2-methyl-1,2,3,4-butanetetrol were at the same time subjected to FRAP test of antioxidant activity. Scopoletin showed positive result 19.8 μM after 4 minutes of test in comparison to 14.18 μM of standard trolox.
The evaluation of thrombocyte anti-aggregation activity was performed via *in vitro* test using the optic aggregation method. Anti-aggregation activity of monitored fractions (polar residue, fraction of phenolic compounds, fraction of non-phenolic compounds, scopolin, 2-methyl-1,2,3,4-butanetetrol) was about 10 % that is very low effect. The ester of ferulic acid with higher alcohols showed higher anti-aggregation activity caused by collagen (over 80 %). Scopoletin and umbelliferone showed significant anti-aggregation activity (over 90 %).
The evaluation of acute toxicity and phototoxicity was performed using annelidans *Tubifex tubifex* Müll. Preliminary tests of acute toxicity did not show higher toxicity. The activity was found out in 3% water solution of polar residue, 1% water solution of fraction of phenolic compounds and 5% water solution of fraction of nonphenolic
compounds. Phototoxicity was evaluated at following compounds - scopolin, scopoletin, umbelliferone and 2-methyl-1,2,3,4-butanetetrol. Only scopoletin showed phototoxic activity. The damage of exposed individuals was 30 % in concentration 7.5 mM in contrast to 17 % of non-exposed individuals.
The antifungal activity was determined using M27-A test. The fraction polar residue, fraction of phenolic compounds, fraction of non-phenolic compounds and ether fraction were used for this test. Fraction of phenolic compounds and ether fraction had inhibited the growth of *Candida albicans*, *C. krusei*, *Absidia corymbifera* in concentration 10 mg/ml for 24 and 48 hours and *Trichophyton mentagrophytes* in concentration 1 mg/ml for 72 and 120 hours.