Bioactive secondary metabolites from Juncaceae species

Summary of PhD. Thesis

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INTRODUCTION

The family Juncaceae comprises about 500 species worldwide, which are distributed into seven genera. Species of the family are widely distributed on both hemispheres, the two largest, cosmopolitan genera of the family are *Juncus* L. (n = 347) and *Luzula* DC. (n = 115). There are 15 *Juncus* and 6 *Luzula* species native to Hungary.

The phytochemical investigations of *Juncus* plants resulted in the isolation of several different secondary plant metabolites (e.g. coumarins, flavonoids, phenanthrenes and terpenoids). Among them phenanthrenes and 9,10-dihydrophenanthrenes are the most characteristic constituents.

Phenanthrenes are considered to comprise a relatively small group of natural products. According to the most accepted hypothesis, the phenanthrene skeleton can be formed by oxidative coupling of the aromatic rings of stilbene precursors. These compounds have drawn considerable interest from the aspect of natural product drug discovery because of the wide range of their potentially valuable biological activities and their broad structural diversity. Up to date more than 450 compounds have been isolated from higher plants and liverwort species. As a result of the intense phytochemical examination on phenanthrene-containing plants almost 50% of the naturally occurring phenanthrenes were identified during the last ten years.

Until now only five species belonging to the genus *Juncus* were investigated extensively and almost one hundred novel phenanthrenes have been isolated from these species. There is a significant lack of information with regard to chemical constituents of the plants belonging to the genus *Luzula*. Previously only the flavonoid components of *Luzula* species were identified by the use of thin layer chromatography.

Various *Juncus* species are used in the traditional medicine for the treatment of numerous conditions (e.g. cold, sleep disorders). In several cases, the folk medicinal use of the plants was confirmed by pharmacological investigations. Phenanthrenes isolated from different Juncaceae species exert many different activities, including

antitumor, anti-inflammatory, antioxidant, anxiolytic, cell-protective, sedative, and spasmolytic effects.

Phenanthrenes are considered to be important taxonomic markers, because of their limited occurrence.

AIMS OF THE STUDY

A few years ago a research program has been started in the Department of Pharmacognosy, University of Szeged with the aim of investigating the secondary metabolites of plants belonging to the family Juncaceae. The objectives of the present work – as part of this project – were the isolation and structural characterization of phenanthrenes, and investigation of their pharmacological effects. In order to achieve the aims, the main tasks of the presented study were:

- Review the literature of the naturally occurring phenanthrenes.
- Overview the chemical and pharmacological properties of the plants of the family Juncaceae.
- Preparation of extracts with different polarity in order to perform pharmacological and chemical screening of the extracts of Juncaceae species (altogether 19 species).
- *In vitro* screening of the extracts for antibacterial effect on resistant bacterial strains, and determination of their MIC values.
- *In vitro* screening of the *Luzula* species for anti-inflammatory effects on fMLP/CB-induced human neutrophils.
- Detailed phytochemical analysis of *Juncus inflexus* L. and *Luzula luzuloides* (Lam.) Dandy & Wilmott, using a bioactivity-guided approach to identify active fractions and components.
- Provide characteristic spectral data on the isolated compounds, and structure elucidation of the isolated components.
- Determination of the absolute configuration of the chiral compounds.
- Prediction of possible biosynthetic pathways by semi-synthetic approaches.

- Evaluation of the pharmacological potential and chemotaxonomical relevance of the isolated compounds.

MATERIALS AND METHODS

Plants [*Juncus acutus* L., *J. alpinoarticulatus* Chaix, *J. articulatus* L., *J. compressus* Jacq., *J. conglomeratus* L., *J. effusus* L., *J. filiformis* L., *J. gerardii* Loisel., *J. inflexus* L., *J. maritimus* Lam., *J. monanthos* Jacq., *J. squarrosus* L., *J. tenuis* Willd., *J. trifidus* L., *Luzula campestris* (L.) DC., *L. forsteri* (Sm.) DC., *L. luzuloides* (Lam.) Dandy & Wilmott, *L. sudetica* (Willd.) Schult. and *L. sylvatica* (Huds.) Gaudin] were collected during the flowering period between May and September 2014, in several regions of the Carpathian Basin (Hungary, Croatia and Romania). Root parts of *J. inflexus* were collected in Hódmezővásárhely (Hungary) in May 2014, and *L. luzuloides* was collected in the flowering period in Pádis-terrace (Romania) in June 2014.

The compounds were isolated by multistep chromatographic methods, including open-column chromatography (OCC), vacuum-liquid chromatography (VLC), medium pressure liquid chromatography (MPLC), preparative layer chromatography (PLC), gel filtration (GF) and high-performance liquid chromatography (HPLC). Normal (NP) or reversed phase (RP) SiO_2, polyamide or Sephadex LH-20 gel were applied as stationary phases.

The isolated compounds were characterized and their structures were elucidated by means of different spectroscopic methods (UV, NMR, HR-MS). The absolute configuration of the chiral compounds was determined by chiral HPLC analysis and ECD calculations.

For the antibacterial screening the test microorganisms were one standard and nine clinical isolates with different antibiotic resistant profile. The standard strain was methicillin-resistant *Staphylococcus aureus* (ATCC43300). The clinical strains were multiresistant (MR) *Acinetobacter baumannii* (64060/2 and 61748/2), ESBL-positive *Citrobacter freundii* (63458), ESBL-positive *Enterobacter cloacae* (63033), ESBL-positive *Escherichia coli* (64663), ESBL-positive *Klebsiella pneumoniae* (63735),

MR *Pseudomonas aeruginosa* (61485/1 and 64658) and methicillin-resistant *Staphylococcus aureus* (64326).

The anti-inflammatory properties of the *Luzula* extracts and the pure compounds were determined by testing their inhibitory effects on superoxide anion generation and elastase release on fMLP/CB-induced human neutrophils.

RESULTS AND DISCUSSION

SCREENING OF JUNCACEAE SPECIES FOR ANTIBACTERIAL ACTIVITY

As part of our screening program for biologically active compounds in Juncaceae plants occurring in the Carpathian Basin 96 extracts with different polarity were prepared from 19 species of the family in order to evaluate their antibacterial effects. First methanol extracts were prepared from whole plants, or where it was possible from different plant parts (aerial part, root). After filtration and evaporation, the residues were dissolved in 50% aqueous MeOH and were subjected to solvent–solvent partition between *n*-hexane (extracts A), CH_2Cl_2 (extracts B) and EtOAc (extracts C); and the remaining H_2O extracts were named as extracts D.

All fractions were tested for their antibacterial activity against 10 multiresistant bacterial strains. The activities of extracts were screened at first for their inhibitory zones by disc-diffusion method at concentrations of 50 mg/mL. 16 Fractions (CH_2Cl_2 and remaining H_2O) from *Juncus* species and 3 CH_2Cl_2-soluble fractions from *Luzula* species possessed mild to strong inhibitory activities against MRSA strains (inhibition zones = 6.7 mm – 14.6 mm). A total of 6 extracts had diameters of inhibition zone ≥10 mm, therefore these were studied further to determine their minimal inhibitory concentrations (MICs) by the microdilution method. The most active fractions were exclusively fractions B (containing CH_2Cl_2-soluble lipophilic constituents). Among them, the CH_2Cl_2-soluble fraction of the roots of *J. inflexus* showed the highest activity (MIC = 9.75 μg/mL). Fraction B of the roots of *J. effusus* displayed significant anti-MRSA activity (MIC = 39 μg/mL). The CH_2Cl_2-soluble fraction of *J. maritimus* and *J. gerardii* possessed marked activity (MIC = 78 μg/mL, for both extracts), and mild

anti-MRSA activities were observed for fraction B of *J. acutus* and for fraction B prepared from the aerial parts of *J. tenuis* (MIC = 156 µg/mL, for both extracts). The antibacterial activity of the species belonging to the genus *Juncus* was more significant (inhibition zones = 7.6 mm – 14.6 mm) than the activity of the species of genus *Luzula* (inhibition zones = 6.7 mm – 7.3 mm). The difference of the chemical composition between the two genera could serve as an explanation for the different activities.

According to the preliminary screening results *J. inflexus* has been chosen for further studies.

SCREENING OF JUNCACEAE SPECIES FOR ANTI-INFLAMMATORY ACTIVITY

In the course of investigation of anti-inflammatory activity, 24 extracts (*n*-hexane CH_2Cl_2, EtOAc and remaining H_2O) of five species (*L. campestris*, *L. forsteri*, *L. luzuloides*, *L. sudetica* and *L. sylvatica*) were tested at 10 µg/mL concentration.

The apolar *n*-hexane-soluble fractions of *L. forsteri*, *L. luzuloides*, *L. sudetica* and *L. sylvatica* showed higher than 70% inhibition at the tested concentration in both assays. The *n*-hexane-soluble extract of *L. campestris* exerted the most selective elastase release inhibition. Among the fractions with different polarities, the CH_2Cl_2-soluble lipophilic fractions showed the most remarkable activities (> 93% inhibition). All of the CH_2Cl_2-soluble fractions displayed high activities on the inhibition of elastase release, and proved to be slightly less active on superoxide generation. The EtOAc-soluble fractions of *L. campestris*, *L. luzuloides*, *L. sudetica* and *L. sylvatica* (aerial part) possessed noteworthy (> 65%) inhibitory activities at concentration 10 µg/mL. The remaining H_2O fraction of *L. luzuloides* also showed notable anti-inflammatory activities (> 65%) in both methods.

On the basis of these results, *L. luzuloides* has been selected for further investigations.

ISOLATION OF BIOACTIVE COMPOUNDS FROM *J. INFLEXUS* AND *L. LUZULOIDES*

In the initial step of the phytochemical work, the dried plant materials were percolated with MeOH at room temperature, and then solvent–solvent partition was applied, which resulted in the CH_2Cl_2 phases. The CH_2Cl_2-soluble fractions were subjected to a multistep chromatographic procedure in order to isolate the compounds.

ISOLATION OF COMPOUNDS FROM *J. INFLEXUS*

The CH_2Cl_2 fraction of the roots of *J. inflexus* was chromatographed on a polyamide column (OCC) with mixtures of MeOH and H_2O (2:3, 1:1, 3:2, 4:1, 1:0; each eluent was collected as a fraction). The composition of the fractions obtained from the polyamide column with methanol–water 3:2 and 4:1 was similar, therefore these fractions were combined (J3). All of the fractions (J1–4) obtained from the polyamide column were subjected to antibacterial screening; among them J3 showed the strongest inhibitory activity against MRSA strains (ATCC43300 and 64326) (inhibition zones = 15.3 ± 0.6 mm and 14.3 ± 0.6 mm, respectively), while J2 (inhibition zones = 7.3 ± 0.6 mm, for both strains), J4 (inhibition zones = 7.6 ± 0.6 mm, for both strains) possessed moderate activity, and J1 was proved to be inactive.

Since fraction J3 showed the highest activity the preparative work was continued with this fraction. As it was demonstrated great chemical complexity, more selective methods (VLC, MPLC, GF, PLC and HPLC) were applied, with the use of normal and reversed phase silica gel or Sephadex LH-20 gel, eluted with solvent systems with different selectivity. The purification process led to the isolation of 12 (**JIN-1**–**5**, **7**, **8**, **11**–**13**, **15**, **19**) compounds (**Figure 1**).

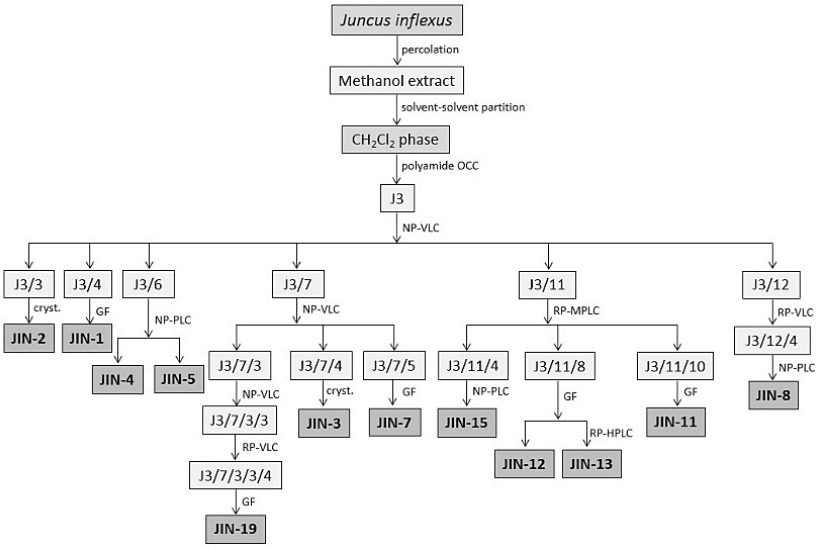


Figure 1. Isolation of compounds from *J. inflexus*

ISOLATION OF COMPOUNDS FROM *L. LUZULOIDES*

The purification of the CH_2Cl_2-soluble phase of *L. luzuloides* was performed with open column chromatography (OCC) on polyamide with mixtures of MeOH and H_2O (1:1, 4:1) to afford two main fractions (L1 and L2). Fraction L2 was separated by NP-VLC with a gradient system of cyclohexane–EtOAc–EtOH. During the purification process several compounds were crystallized (cryst.). Finally, NP- and RP-PLC separations and gel filtration were used for the isolation of the compounds (**LUB-1**, **3**, **4**, **6**, **9**, **10**) (**Figure 2**).

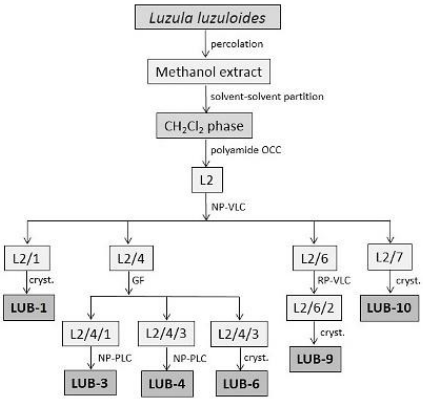


Figure 2. Isolation of compounds from *L. luzuloides*

CHARACTERIZATION AND STRUCTURE DETERMINATION OF THE ISOLATED COMPOUNDS

The chemical structures of the isolated compounds were determined by means of spectroscopic methods. The molecular masses and compositions were obtained from MS investigations. The most useful data concerning the structures were furnished by 1D and 2D NMR spectroscopy. The constitutions of the compounds were elucidated via ^{1}H NMR, JMOD, ^{1}H–^{1}H COSY, HSQC and HMBC experiments, and the relative configurations were then characterized with the aid of NOESY spectra. As a result of the NMR studies, complete ^{1}H- and ^{13}C-assignments were made for the new compounds and also in case of some known compounds, where previously published data were incomplete.

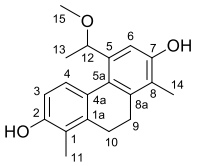
The absolute configurations of the chiral compounds were determined using chiral HPLC separation and circular dichroism (CD) experiments.

COMPOUNDS FROM *JUNCUS INFLEXUS*

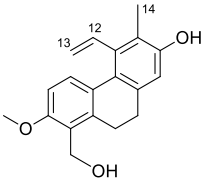
From the CH_2Cl_2-soluble fraction of *J. inflexus* 12 compounds, 11 phenanthrenes (**1**–**11**) and a flavonoid (**12**) were isolated with the combination of different chromatographic techniques.

Four phenanthrenes (jinflexins A–D, **1**–**4**) are new natural products. Jinflexin A (**JIN-7**, **1**) is a methoxyethyl substituted 9,10-dihydrophenanthrene, from which the

vinyl group is missing, but the position of the methoxyethyl group suggests that biogenetically this compound was also vinylated. Its zero specific rotation and the baseline ECD spectrum adding to the results of the chiral HPLC analysis confirmed that jinflexin A (**1**) is a racemic mixture. Jinflexin B (**JIN-12**, **2**) is also specific, as its 9,10-dihydrophenanthrene core is substituted with an oxymethylene group at C-1. Moreover, it has a methoxy substitution at C-2, which is a quite unusual position for this group among Juncaceae phenanthrenes. Jinflexin C (**JIN-15**, **3**) is a carbonyl (C-1) substituted 5,6,9,10-tetrahydrophenanthrene, not only its core is unusual, but the presence of a methyl and a vinyl group at the same carbon (C-7) also contributes to its unique structure. On the basis of the TDDFT-ECD calculations and chiral HPLC analysis, jinflexin C (**3**) is a 6*R*,7*R* enantiomer with 80% enantiomeric excess (ee). Jinflexin D (**JIN-19**, **4**) is a dimer with an unprecedented heptacyclic ring system, which may be considered to derive by the coupling of dehydrojuncuenin A (**9**) with 2,7-dihydroxy-1,8-dimethyl-5-vinyl-9,10-dihydrophenanthrene through their vinyl groups forming a unique structure. The chiral HPLC analysis of jinflexin D (**4**) showed 9% ee. The absolute configuration of the first-eluting enantiomer of **4** was identified as (*R*). The enantiomeric excess came from the second-eluting enantiomer, which was therefore determined as (*S*).



(±)-**1**



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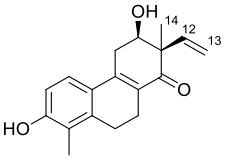
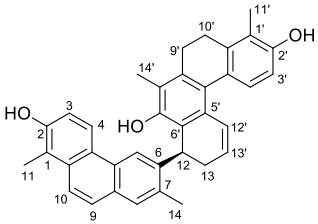
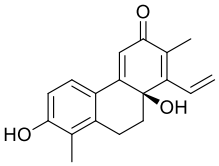


(6*R*,7*R*)-**3** (80% ee)

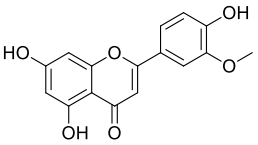
Besides the new compounds (**1–4**), dehydrojuncuenin A [**JIN-1** (**9**)], juncuenin A (**JIN-2**, **5**), juncuenin B (**JIN-3**, **6**), dehydrojuncusol (**JIN-4**, **10**), juncusol (**JIN-5**, **7**), dehydrojuncuenin B (**JIN-11**, **11**), juncuenin D (**JIN-13**, **8**) and chrysoeriol (**JIN-8**, **12**) were also isolated from the roots of *J. inflexus*. Among the known phenanthrenes, six compounds can be paired, based on the saturation of the C-9–C-10 bond.



(*S*)-**4** (9% ee)



(*S*)-**8** (4% ee)



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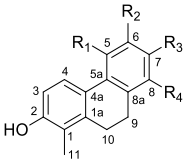
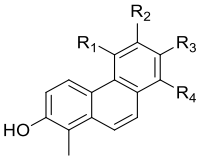


	R_1	R_2	R_3	R_4
5	H	$CH=CH_2$	CH_3	H
6	H	OH	CH_3	$CH=CH_2$
7	$CH=CH_2$	CH_3	OH	H



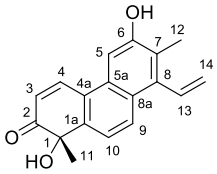
	R_1	R_2	R_3	R_4
9	H	$CH=CH_2$	CH_3	H
10	$CH=CH_2$	CH_3	OH	H
11	H	OH	CH_3	$CH=CH_2$

The substitution pattern of dehydrojuncuenin A (**9**) and juncuenin A (**5**); dehydrojuncuenin B (**11**) and juncuenin B (**6**); dehydrojuncusol (**10**) and juncusol (**7**) are the same, the first member of the pairs are phenanthrenes, and the second ones are their 9,10-dihydro analogues. Juncuenin D (**8**) is a phenanthrenequinone presumably derived from juncuenin B (**6**). The absolute configuration of juncuenin D (**8**) was also determined, and its (*S*) enantiomer has 4% of enantiomeric excess.

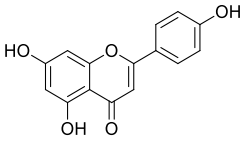
COMPOUNDS FROM *LUZULA LUZULOIDES*

The structure analysis of compounds isolated from *L. luzuloides* led to the identification of four phenanthrenes and two flavonoids. A new 1,6-dihydroxy-2-keto-1,7-dimethyl-8-vinyl-1,2-dihydrophenanthrene (luzulin A, **LUB-3**, **13**) was identified from the plant. Its chiral HPLC analysis and TDDFT-ECD calculations suggested that luzulin A (**13**) is an (*S*) enantiomer, with 25% ee. Three known phenanthrenes [juncusol (**LUB-1**, **7**), juncuenin B (**LUB-4**, **6**), and dehydrojuncuenin

B (**LUB-6**, **11**)] and two flavonoids [apigenin (**LUB-9**, **14**) and luteolin (**LUB-10**, **15**)] were also isolated from the apolar fraction of the plant. All of the compounds were identified for the first time in *L. luzuloides*, and this was the first time when phenanthrenes were isolated from a species belonging the genus *Luzula*.



(*S*)-**13** (25% ee)



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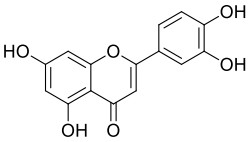

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BIOSYNTHETIC PATHWAYS

Considerable amount of juncuenin B (**6**) (850 mg) has been isolated from *J. inflexus*, hence it can be considered as the main compound of the root. In our studies juncuenin B (**6**) and its unsaturated form, dehydrojuncuenin B (**11**) have been isolated from both *J. inflexus* and *L. luzuloides*. Luzulin A (**13**) yielded from *L. luzuloides* and juncuenin D (**8**) isolated from *J. inflexus* have obvious structure similarity with the abovementioned two phenanthrenes [juncuenin B (**6**) and dehydrojuncuenin B (**11**)], suggesting that both phenanthrenequinones are possibly derived from the oxidation of juncuenin B (**6**). In case of luzulin A (**13**), ring A is oxidized, while in case of juncuenin D (**8**), ring C has changed. In order to confirm their possible biosynthetic connection, juncuenin B (**6**) was subjected to an oxidative reaction by the use of hypervalent iodine reagent.

The oxidative transformation of juncuenin B (**6**) led to the isolation of its possible biometabolites. The compounds were identified as juncuenin D (**8**), dehydrojuncuenin B (**11**) and luzulin A (**13**) comparing their ^{1}H NMR spectrum with literature data. These findings confirm that phenanthrenequinones, juncuenin D (**8**) and luzulin A (**13**), and the phenanthrene, dehydrojuncuenin B (**11**) can be formed by oxidation of juncuenin B (**6**), and most probably similar process may occur during their biosynthesis.

BIOACTIVITY OF THE ISOLATED COMPOUNDS

ANTIBACTERIAL ACTIVITY

In our preliminary screening, the CH_2Cl_2 fraction of *J. inflexus* exerted the most potent antibacterial activity against MRSA strains. Therefore, it was subjected to a comprehensive preparative phytochemical analysis. The compounds isolated from this fraction were tested for their anti-MRSA activity at concentration of 10 mg/mL (in **Table 1** only the active compounds were mentioned). Among the isolated phenanthrenes, noteworthy inhibitory activities were recorded for jinflexin B (**2**), juncusol (**7**), juncuenin D (**8**) and dehydrojuncuenin B (**11**).

Table 1. Anti-MRSA activity of isolated compounds

Compound	MRSA (ATCC43300) inhibitory activity	
	Inhibition (diameter of inhibition zone in mm)	MIC (μg/mL)
2 (jinflexin B)	7.0 ± 0.1	100
7 (juncusol)	12.0 ± 0.6	25
8 (juncuenin D)	12.0 ± 0.3	12.5
11 (dehydrojuncuenin B)	10.0 ± 0.2	25
vancomycin*	15.5 ± 0.6	2

*positive control at concentration 5 μg/disc

Juncuenin D (**8**, inhibition zone = 12.0 ± 0.3 mm, MIC = 12.5 μg/mL) and juncusol (**7**, inhibition zone = 12.0 ± 0.6 mm, MIC = 25 μg/mL) were the most potent in inhibition of MRSA (ATCC43300) growth. Moreover, dehydrojuncuenin B (**11**, inhibition zone = 10.0 ± 0.2 mm, MIC = 25 μg/mL) and jinflexin B (**2**, inhibition zone = 7.0 ± 0.1 mm, MIC = 100 μg/mL) possessed marked activity. The other compounds were proved to be inactive against MRSA.

ANTI-INFLAMMATORY ACTIVITY

Compounds from *L. luzuloides* and the semisynthetically obtained juncuenin D (**8**) were evaluated for their anti-inflammatory properties using superoxide anion generation and elastase release inhibition assays (**Table 2**). In the superoxide anion

generation assay, significant inhibitory activities were recorded for juncuenin B (**6**) (IC_{50} = 4.92 μM), juncusol (**7**) (IC_{50} = 3.11 μM), dehydrojuncuenin B (**11**) (IC_{50} = 3.17 μM), apigenin (**14**) (IC_{50} = 6.12 μM) and luteolin (**15**) (IC_{50} = 4.73 μM).

Table 2. Inhibitory effects of compounds on superoxide anion generation and elastase release on human neutrophils in response to fMLP/CB

Compound	Superoxide anion generation		Elastase release	
	IC_{50} (μM)	Inhibition (%)	IC_{50} (μM)	Inhibition (%)
6 (juncuenin B)	4.92 ± 0.27	81.54 ± 3.5***	5.47 ± 1.11	80.57 ± 4.15***
7 (juncusol)	3.11 ± 0.25	93.07 ± 0.48***	> 10	2.05 ± 2.07
8 (juncuenin D)	> 10	43.29 ± 5.77***	> 10	32.75 ± 6.98**
11 (dehydrojuncuenin B)	3.17 ± 1.19	82.90 ± 7.65***	> 10	25.58 ± 2.83**
13 (luzulin A)	> 10	12.26 ± 3.76*	> 10	40.50 ± 5.57**
14 (apigenin)	6.12 ± 0.72	73.92 ± 4.11***	> 10	46.14 ± 6.03**
15 (luteolin)	4.73 ± 0.49	79.77 ± 4.37***	6.91 ± 2.25	54.16 ± 4.66***
LY294002	1.29 ± 0.05	-	4.97 ± 0.80	-

Percentage of inhibition (Inhibition %) at 10 μM concentration. Results are presented as mean ± S.E.M. (n=3–5). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the control value. LY294002, a PI3K inhibitor, was used as positive control.

Furthermore, juncuenin B (**6**) and luteolin (**15**) inhibited effectively the elastase release with IC_{50}s of 5.47 μM and 6.91 μM, respectively, comparable to that of the positive control (LY294002, 4.79 μM). Juncusol (**7**), dehydrojuncuenin B (**11**) and apigenin (**14**) were considered to be inactive on the elastase release, while juncuenin D (**8**) and luzulin A (**13**) were proved to be inactive in both assays.

Juncuenin B (**6**) lost its effect on the elastase release by the unsaturation of ring B, but dehydrojuncuenin B (**11**) inhibited the superoxide generation slightly effectively than juncuenin B (**6**). The phenanthrenequinones, juncuenin D (**8**) and luzulin A (**13**), presumably derived from juncuenin B (**6**), showed significantly lower anti-inflammatory activities compared to their possible biosynthetic precursor.

HPLC-MS INVESTIGATION OF EXTRACTS

Bioassay-guided fractionation of *J. inflexus* resulted in the isolation of four phenanthrenes [jinflexin B (**2**), juncusol (**7**), juncuenin D (**8**), dehydrojuncuenin B (**11**)] with significant anti-MRSA activity. The presence of these compounds was investigated by HPLC-MS in the most active Juncaceae extracts (CH_2Cl_2 fractions of the roots of *J. effusus* and *J. inflexus*, the whole plants of *J. acutus*, *J. gerardii* and *J. maritimus*, and aerial parts of *J. tenuis*).

All of these compounds were detected in the CH_2Cl_2 fraction of *J. inflexus*, which suggests that these components were originally in the plant, instead of evolving during the purification process.

Juncusol (**7**) was identified in all the investigated extracts, and the presence of jinflexin B (**2**) was confirmed in the CH_2Cl_2-soluble extracts of *J. acutus* and *J. gerardii*. The detected active compounds could play an important role in the antibacterial effects of the extracts, which can involve different pharmacological mechanisms. However, the chemical composition of *J. gerardii*, *J. maritimus* and *J. tenuis* were not studied thoroughly; therefore, some other, unidentified compounds may also play role in the activities of these extracts.

CHEMOTAXONOMY

The constituents of *J. inflexus* and *L. luzuloides* have not been investigated previously. All of the isolated compounds were detected for the first time from the investigated plants. The chemical characterization of *L. luzuloides*, and the presence of vinylated phenanthrenes in the plant further confirm the close botanical relationship between the genera *Juncus* and *Luzula*.

Vinyl substituted derivatives can be considered as chemotaxonomic markers for plants belonging to family Juncaceae, since these specifically substituted phenanthrenes were reported previously only from *Juncus* species, and this was the first time when they were isolated from the genus *Luzula*. The isolation of phenanthrenes from *L. luzuloides* confirmed that phenanthrenes and flavonoids are

the characteristic constituents of this plant. The secondary metabolite profile of *L. luzuloides* showed great similarity to that of the species of genus *Juncus*. The isolation of phenanthrenes, including a novel one from *L. luzuloides* highlighted that not only *Juncus* species can produce phenanthrenes in family Juncaceae, and *Luzula* plants are also promising starting materials for further phytochemical investigations.

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THE THESIS IS BASED ON THE FOLLOWING PUBLICATIONS:

1. **Tóth B**; Liktor-Busa E; Kúsz N; Szappanos Á; Mándi A; Kurtán T; Urbán E; Hohmann J; Chang FR; Vasas A.
   Phenanthrenes from *Juncus inflexus* with antimicrobial activity against methicillin-resistant *Staphylococcus aureus*
   Journal of Natural Products 2016; **79**: 2814–2823. If: 3.662*

2. **Tóth B**; Liktor-Busa E; Urbán E; Csorba A; Jakab G; Hohmann J; Vasas A.
   Antibacterial screening of Juncaceae species native to the Carpathian Basin against resistant strains and LC-MS investigation of phenanthrenes responsible for the effect
   Fitoterapia 2016; **115**: 69–73. If: 2.408*

3. **Tóth B**; Chang FR; Hwang TL; Szappanos Á; Mándi A; Hunyadi A; Kurtán T; Jakab G; Hohmann J; Vasas A.
   Screening of *Luzula* species native to the Carpathian Basin for anti-inflammatory activity and bioactivity-guided isolation of compounds from *Luzula luzuloides* (Lam.) Dandy & Wilmott
   Fitoterapia 2017, **116**: 131–138. If: 2.408*

OTHER PUBLICATIONS:

1. **Tóth B**; Bartho L; Vasas A; Sándor Z; Jedlinszki N; Pinke G; Hohmann J.
   Dual excitatory and smooth muscle-relaxing effect of *Sideritis montana* extract on guinea-pig ileum
   Natural Product Communications 2015; **10**: 487–490. If: 0.884

*The impact factor for the year 2015 is given.

PRESENTATIONS HELD IN THE SAME THEME OF THE THESIS:

1. **Tóth B**; Liktor-Busa E; Urban E; Jakab G; Hohmann J; Vasas A.
   Antibacterial activity of 19 species from the Juncaceae family, and bioactivity guided fractionation of the most active species *Juncus inflexus*
   63rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA2015)
   Budapest, 23-27 August 2015.

2. **Tóth B**; Kúsz N; Liktor-Busa E; Csorba A; Urbán E; Hohmann J; Vasas A.
   A *Juncus inflexus* biológiailag aktív vegyületeinek izolálása és szerkezet-meghatározása
   MTA Alkaloid- és Flavonoidkémiai Munkabizottságának ülése
   Mátrafüred, 14-15 April 2016.

3. **Tóth B**; Kúsz N; Liktor-Busa E; Urbán E; Hunyadi A; Chang FR; Kurtán T; Hohmann J; Vasas A.
   Juncaceae fajok biológiailag aktív vegyületeinek izolálása és szerkezet-meghatározása
   Fiatal Gyógynövénykutatók Fóruma: A Magyar Gyógyszerésztudományi Társaság Gyógynövény Szakosztályának tudományos konferenciája
   Budakalász, 24 June 2016.

4. **Tóth B**; Kúsz N; Csorba A; Kurtán T; Hohmann J; Vasas A.
   Isolation and characterization of new phenanthrenes from *Juncus inflexus* and their chemotaxonomic significance
   9th Joint Natural Product Conference 2016 (Joint Meeting with ASP, AFERP, JSP, PSE and SIF) and 64th International Conference and Annual Meeting of GA
   Copenhagen, 24-27 July 2016.

5. Kuo CY; Schelz Z; **Tóth B**; Vasas A; Hohmann J; Zupkó I; Wang HC.
   Study the anticancer mechanism for compounds from *Juncus inflexus* root extract in cervical cancer cells
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6. Vasas A; **Tóth B**; Kúsz N; Hwang TL; Cheng YB; Chang FR; Wu YC; Hohmann J.
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   XIII. Clauder Ottó Emlékverseny
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