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Bioactive secondary metabolites from Juncaceae species

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I. **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*
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II. **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
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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.

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ABBREVIATIONS AND SYMBOLS

1D	one-dimensional
2D	two-dimensional
APCI	atmospheric pressure chemical ionization
COSY	correlated spectroscopy
cryst	crystallization
δ	chemical shift
ee	enantiomeric excess
fr	fraction
GF	gel filtration
HMBC	heteronuclear multiple-bond correlation spectroscopy
HSQC	heteronuclear single-quantum coherence spectroscopy
HPLC	high-performance liquid chromatography
HRE(S)IMS	high-resolution electron (spray) ionization mass spectroscopy
JMOD	*J*-modulated spin-echo experiment
NMR	nuclear magnetic resonance
MS	mass spectroscopy
MPLC	medium pressure liquid chromatography
NOE	nuclear *Overhauser* effect
NOESY	nuclear *Overhauser* enhancement spectroscopy
NP	normal phase
OCC	open-column chromatography
R_f	retention factor
RP	reversed phase
RPC	rotation planar chromatography
PLC	preparative thin-layer chromatography
TLC	thin-layer chromatography
TMS	tetramethylsilane
t_R	retention time
UV	ultraviolet
VLC	vacuum-liquid chromatography

1. INTRODUCTION

The family Juncaceae comprises about 500 species worldwide, which are distributed into seven genera.[1-3] Species of the family are widely distributed on both hemispheres, from arctic regions to the subtropics, but sporadically they are occurring in tropic regions as well.[4] Most of the Juncaceae species grow exclusively in wetland habitats (i.e. rice lands, marshes). The two largest, cosmopolitan genera of the family are *Juncus* L. (n = 347) and *Luzula* DC. (n = 115).[5] There are 15 *Juncus* and 6 *Luzula* species native to Hungary.[6]

Because of the global distribution of the Juncaceae plants, the species are used worldwide, but mainly in the Asian folk medicine. Various *Juncus* species (e.g. *J. effusus,* also known as "Dengxincao") are used in the traditional Chinese medicine for the treatment of numerous disorders. The spectrum of indications is quite wide; in the folk medicine the tranquilizing effects (e.g. sedative-hypnotic, anxiolytic) on the central nervous system are well known.[7-10] Furthermore, *Juncus* species are also used in the treatment of cold, fever and inflammation.[11] The seeds of *J. rigidus* are consumed in Egypt in order to treat diarrhoea and diuretic disorders.[12] In Basque regions, the stems of *J. conglomeratus*, *J. effusus* and *J. inflexus* were used in rituals against warts and other skin diseases.[13,14] The aerial parts of *J. balticus* and *J. effusus* and the roots of *J. ensifolius* were consumed as healthy foods by indigenous peoples in Canada.[15] The traditional usage of plants belonging to other genera of the family has not been reported yet.

Several Juncaceae species have economic significance. *Juncus* plants (e.g. *J. acutus*, *J. arabicus, J. rigidus*) have been used to make mats, mattresses, sandals and baskets throughout Africa since 895 B.C.[16-18] *J. kraussii* is a valuable source of fibre among Zulus; bridal sleeping mats and baskets are weaved from the culms of the plant.[19] Great deals of *Juncus* species are native to South-America, therefore these plants (e.g. *J. arcticus* var. *andicola*, *J. effusus*, *J. ramboi* subsp. *colombianus*) are commonly used in that region in order to prepare traditional craftworks.[20] Some species, such as *J. acutus*, *J. rigidus*, and few *Luzula* species were used in the paper industry.[21]

The most characteristic compounds identified in the species of family Juncaceae are phenanthrenes and 9,10-dihydrophenanthrenes.[22] To date, almost one hundred novel phenanthrenes have been isolated from the family, but only from *Juncus* species.[23-31] Although many phenanthrene-containing species have been used in folk medicine for hundreds of years in order to treat several diseases, however the chemical composition and the mechanism of action of many of these plants have not been studied thoroughly yet. Therefore, these plants and their secondary metabolites are worthy of future investigations. Phenanthrenes have drawn considerable interest from the aspect of natural product drug discovery because of the wide range of their potentially valuable biological activities (e.g. antitumor, antibacterial, anti-inflammatory, antioxidant, anxiolytic, cell-protective,

sedative, and spasmolytic activities) and their broad structural diversity.[7-9,11,23,27-35] Probably, the most investigated naturally occurring phenanthrene derivate is denbinobin, which displays remarkable anti-tumour activity against several cancer cell lines. Moreover, its anti-HIV property is also very promising.[36-38]

In 2014, a research group of the Department of Pharmacognosy, University of Szeged initiated a research program with the aim of investigating the secondary metabolites of Juncaceae species. This program involved the phytochemical and pharmacological investigation of 19 Juncaceae species, and preparative phytochemical work was performed with the most promising species, namely *Juncus inflexus* and *Luzula luzuloides*. The present thesis summarizes the results of this work.

2. AIMS OF THE STUDY

The family Juncaceae is an abundant source of bioactive phenanthrenes. 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 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.
- Collection of Juncaceae plant samples (altogether 19 species) from the Carpathian Basin.
- Preparation of extracts with different polarity in order to perform pharmacological and chemical screening of the extracts of Juncaceae species.
- *In vitro* screening of the extracts for antibacterial effect on resistant bacterial strains, and determination of MIC values of the active extracts.
- *In vitro* screening of *Luzula* species for anti-inflammatory effects on fMLP/CB-induced human neutrophils.
- Detailed phytochemical analysis of *Juncus inflexus* and *Luzula luzuloides*, using a bioactivity-guided approach to identify active fractions and components.
- Provide characteristic spectral data on the isolated compounds.
- Structure elucidation of the isolated components.
- Determination of the absolute configuration of the chiral compounds.
- Evaluation of the pharmacological potential and chemotaxonomical relevance of the isolated compounds.
- Prediction of possible biosynthetic pathways by semi-synthetic approaches.

3. LITERATURE OVERVIEW

3.1. BOTANY OF THE FAMILY JUNCACEAE AND THE INVESTIGATED SPECIES

The monocotyledonous Juncaceae plants are usually perennial herbs, but some representatives are annual. From the creeping rhizomes of the plants, short lived leafy shoots (culms) grow that end in an inflorescence, but the rhizome is sometimes reduced or lost. Several leaves cluster around the base of each culm, and sometimes along with it. The long, narrow leaves of the plants have sheathing base, but in some cases the leaves are reduced to scale-like structures on the rhizome. *Juncus* plants may have bifacial or unifacial leaves, and sometimes it can also be reduced to membranous sheaths. Species belonging to the genus *Luzula* can be recognised by the existence of closed-leaf sheaths and multicellular hairs on the leaves. Both genera have inflorescences with many flowers, the air-pollinated flowers are actinomorphic, hermaphrodite and protogynous. The flowers have six perianth-segments in two whorls. The number of ovules and type of placentation is also characteristic to *Luzula* species, because they have three basal ovules, whereas all of the other genera have numerous axile or parietal ovules. Juncaceae plants have dehiscent fruits and endospermic seeds.[6,39]

Both investigated plants belong to the family Juncaceae, in the order Poales. *Juncus inflexus* L. belongs to the subgenus *Juncus* of the genus *Juncus*, and *Luzula luzuloides* (Lam.) Dandy & Wilmott belongs to the subgenus of Antheleae of the genus *Luzula*.[39]

Juncus inflexus L. (hard rush, blue rush; syn. *Juncus glaucus* Sibth., *Juncus longicornis* Bast.) is a perennial, caespitose plant with a height of 50-120 cm. The basal sheaths are usually reddish-brown. The stems are grey-green in colour, and the pith is interrupted. The plant has only basal leaves, measuring 4-10 cm. The sheaths are brown to black in colour. The lax inflorescence is many flowered. The perianth-segments measure 2.5-4.0 mm, they are unequal, narrow ovate and acuminate. The flowers are green in colour. The brown seeds measure 0.5 mm; they are obliquely ovoid, reticulate. The shining brown capsules exceed or equal to the perinath, its shape may vary from trigonous-ovoid to trigonous-ellipsoid. The plant grows in fens, wet grasslands, and damp open habitats. It occurs throughout Europe, North-Africa and Asia.[6,39]

Luzula luzuloides (Lam.) Dandy & Wilmott (forest wood rush, white wood rush; syn. *Luzula albida* (Hoffm.) DC., *Luzula nemorosa* (Pollich) E.Mey.) is a perennial, laxly caespitose plant with a height of 30-50 cm. The basal leaves are 3-6 mm wide, 30 cm long, flat, long-ciliate shaped. The corymbose inflorescence is lax or condensed with divaricate branches with clusters of 2-10 flowers. The perianth-segments measure 2.5-3.5 mm; they are white, but sometimes their colour is suffused with red. The dark brown, shining ellipsoid seeds measure 1.1-1.2 mm. The ovoid capsules are as long as the perinath-segments. The plant occurs in Europe and North-America. It grows in woods and meadows.[6,39]

3.2. CHEMICAL CONSTITUENTS OF THE FAMILY JUNCACEAE

The Juncaceae family has been the subject of intense phytochemical examination in the past ten years. It can be explained with the fact, that the widely available plants of the family have been overlooked over decades. Despite of the comprehensive research focused on Juncaceae family, until now only species belonging to the genus *Juncus* were investigated extensively. The chemical constituents of the genus *Luzula* has not been discovered yet.

Phytochemical investigations of *Juncus* plants resulted in the isolation of different secondary metabolites. Numerous coumarins [e.g. juncusyl ester A, (2*S*)-1-*O*-*p*-coumaroyl glyceride, 6-hydroxy-7-methyl-5α,8α-benzocoumarin] and flavonoids (e.g. apigenin, apigenin 7-*O*-glucoronide, chrysoeriol, hydnocarpin, luteolin, nobiletin, quercetin) were identified from *Juncus* species.[22,27,40-42] Carotenoids and steroids (β-sitosterol, stigmasterol, α-spinasterol) were also isolated from *J. effusus*, *J. gerardii* and *J. bufonius*.[43,44] Several terpenoids, such as rosane-type diterpenes (e.g. effusenone A) and cycloartane triterpenes (juncusides I–V) were isolated from *J. effusus*.[24,45,46] Among the isolated phenolic compounds commonly occurring constituents (e.g. *p*-coumaric acid, vanillic acid) and more specific components, such as markhamioside F and canthoside B were reported.[44,47] Stilbenes are considered to be the biosynthetic precursors of phenanthrenes and 9,10-dihydrophenanthrenes, therefore it is worth noting that oxyresveratrol 2-*O*-*β*-D-glucopyranoside and resveratrol 3',4'-*O*,*O*'-di-*β*-D-glucopyranoside were isolated from *J. acutus*.[47] The most specific chemical constituents of the family Juncaceae are phenanthrenes and 9,10-dihydrophenanthrenes.[22] The isolated compounds were obtained from different parts (aerial parts, medullae, rhizomes, roots, root bark, stems) of the plants.

Genus *Luzula*, the second largest genus of the family, comprises around 115 species.[48] However, there is a significant lack of information regarding the chemical constituents of the plants belonging to this genus. Before our study on *L. luzuloides*, only the flavonoid components of *Luzula* species were confirmed. As a result of an extensive research, several flavonoids were identified by comparison of their R_f values and colours with standards from eleven *Luzula* species (*L. arcuata*, *L. campestris*, *L. confusa*, *L. forsteri*, *L. lactea*, *L. multiflora*, *L. nivea*, *L. nodulosa*, *L. nutans*, *L. pilosa*, *L. spicata* and *L. sylvatica*). Luteolin and luteolin 7-*O*-glucoside were detected from all of the investigated *Luzula* samples.[42] Quercetin, procyanidin and other luteolin derivatives (luteolin 5-methyl ether, luteolin 5-methyl ether 7-*O*-glucoside) were also identified in some species. So far, only luteolin and luteolin 7-*O*-glucoside were isolated by the use of preparative paper chromatography from *Luzula* species.[49] Therefore, flavonoids were considered to be the main secondary metabolites, and the most characteristic components of *Luzula* species.

Based on the previous literature data, flavonoids, phenanthrenes and 9,10-dihydrophenanthrenes are the most specific chemical constituents of the members of Juncaceae family.

3.2.1. Chemical properties of phenanthrenes

3.2.1.1. Biosynthesis of phenanthrenes

Phenanthrenes are considered to be important taxonomic markers, because of their limited occurrence.[22] These rare secondary metabolites are derived from a specific biosynthetic pathway. According to the most accepted hypothesis, the phenanthrene skeleton can be formed by oxidative coupling of the aromatic rings of stilbene precursors.[22] In 1983, FRITZEMEIER et al. proved that L-phenylalanine is the key precursor in the biosynthesis of dihydrostilbenes and dihydrophenanthrenes.[50] From L-phenylalanine, almost all higher plants are able to synthesize malonyl-CoA, and CoA-esters of cinnamic acid derivatives. These compounds are the precursors of stilbenoids, but the occurrence of stilbene synthase, which catalyses the formation of stilbene skeleton, is very limited. Stilbene synthase-related enzymes catalyse great deals of biosynthetic transformations in plants. The transformation of dihydro-*m*-coumaroyl-CoA into dihydrostilbenes (bibenzyls) is catalysed by bibenzyl synthase.[51]

The phenanthrene core could be generated from stilbenes by UV irradiation in the presence of oxidants.[52,53] Thus, the hydroxylation/methylation patterns of at least one of the two benzene rings in most phenanthrenes correspond to the 3,5-disubstitution pattern of stilbenes.[54] The biosynthesis of phenanthrenes can be induced by oxidative stress or fungal infection in higher plants. These cyclized metabolites are stronger antioxidant and anti-fungal agents than their stilbenoid precursors.[55] Fungal infections can also contribute to the high content of dihydrophenanthrenes in Juncaceae species.[56]

Besides stilbenoid origin, phenanthrenes could also have other biosynthetic precursors. In 1974, SZENDREI et al. presumed the existence of phenanthrene derivatives from morphine alkaloids.[57] Their presupposition was proved to be correct; thebaol, isolated from opium, shares the same core structural elements as thebain. Later, new phenanthrene alkaloids were isolated from *Cryptocarya crassinervia*.[58] These compounds are probably not derived through the polyketide pathway, because later, very similar aporphine alkaloids were isolated from the same plant.[59] A carboxamide substituted phenanthrene was isolated from *Aristolochia manshuriensis*, which is presumably derived from aristolochic acid.[60] Aristolochic acids form a special group of secondary metabolites; according to their structures these compounds are alkaloid-like phenanthrenes. During their biosynthesis from aromatic amino acids (e.g. tyrosine) two phenylethane units are formed.[61] The coupling of the phenylethane units results norlaudanosoline, as an intermedier, from which norlaudanosoline aristolochic acid and

3,4-methylenedioxy-8-methoxy-10-nitro-phenanthrene-1-carboxylic acid are derived.[62] Therefore, nitrogen containing phenanthrenes do not have a stilbenoid origin.

Euphorbiaceae family is a great source of diterpenes, but the occurrence of phenanthrenes is rather sporadic in the family.[22,63] It is presumed, that ethylated, oxymethylated and thiomethylated phenanthrenes reported from the family are not undoubtedly derived from stilbenes.[64] Although the highly aromatized norditerpene trigonochinene E possesses a phenanthrene skeleton, it seems very likely that biogenetically this compound is a norditerpene.[65]

3.2.1.2. Chemical characteristics of mono- and diphenanthrenes

Phenanthrenes are occurring in both monomeric and dimeric forms, around 80% of the plant synthesized phenanthrenes are monophenanthrenes.[22] The substituents and the saturation of the bond between C-9 and C-10 resulted in the diversity of the monomers. The structures of the dimers are determined by the types of the connecting monomers and the position of the linkage.

The wetland *Juncus* plants are considered as valuable sources of nitrogen-free alkylated monophenanthrenes; the first example of this class was juncusol isolated from *J. roemerianus*.[66] To date, more than 90 monomeric phenanthrenes have been identified from *Juncus* species, including *J. acutus*, *J. effusus*, *J. roemerianus*, *J. setchuensis* and *J. subulatus*.[22-31] Most of the phenanthrenes were isolated from *J. acutus* and *J. effusus*, and these plants are the most investigated species among the Juncaceae plants.

Among monomeric compounds phenanthrenes and 9,10-dihydrophenanthrenes are distributed almost equally, around 60% of the compounds are 9,10-dihydrophenanthrenes (**Figure 1**).[22] Reviewing the substitutions of the phenanthrenes, it can be recognized that all of the naturally occurring phenanthrenes are substituted. Most of the substituents are linked at C-2 and C-7, but C-3, C-4 and C-6 substitutions also occur. Substitution at positions C-9 and C-10 is very rare either in phenanthrenes and dihydrophenanthrenes.[22]

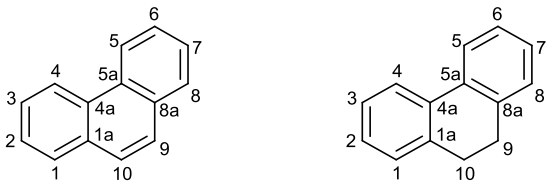


Figure 1. Phenanthrene and dihydrophenanthrene skeletons

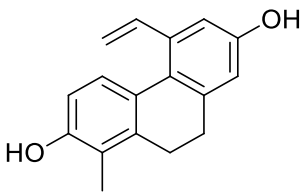
Almost all of the naturally occurring phenanthrenes are substituted with hydroxy group or they can be *O*-glycosides, which suggests that those compounds were also hydroxylated biogenetically.[67] The hydroxy group is generally linked at C-2 or C-7. The second most common substituent is the methoxy group, mainly at positions C-2, C-4, C-5 and C-6. Only phenanthrenes isolated from the members of Juncaceae family have vinyl substitution, habitually at C-5. Therefore, vinylated

phenanthrenes can be considered as chemotaxonomic markers of Juncaceae species.[22-31] Most of the Juncaceae phenanthrenes are substituted with methyl groups. Methyl substitutions were generally found at C-1 or C-7.[22-31] In some cases, uncommonly substituted phenanthrenes were also reported. Hydroxyalkyl substitution also occurs among *Juncus* phenanthrenes. Several hydroxymethyl substituted phenanthrenes were isolated from plants belonging to the genus *Juncus*.[26,31,69,71] Hydroxyethyl substituted 9,10-dihydrophenanthrenes were obtained as minor constituents from *J. acutus* and *J. effusus*.[68-71] A methoxyethyl derivative was isolated by DELLA GRECA et al. from *J. acutus* and *J. effusus*.[68,70,71] Carboxyl substitutions are also quite rare among the monophenanthrenes, and a few carboxylated compounds were reported from *J. acutus* and *J. effusus*.[9,68,71,72] Carbaldehyde and keto substituted dihydrophenanthrenes were also isolated from *J. effusus*.[9,28,68,72] Symmetric derivatives are rather uncommon among naturally occurring phenanthrenes, recently a symmetric tetracyclic molecule, juncutol was obtained from *J. acutus*.[23] Juncutol could be derived from effusol, which was previously reported from the same plant, through the coupling of C-4 and the methine carbon of the vinyl group.

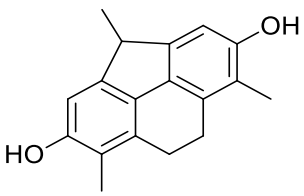
Monosaccharide (e.g. glucose) moiety can also link to the phenanthrene skeleton, but disaccharide substituted phenanthrenes, such as phenanthrene-glucoapioside and -glucorhamnoside were also reported from higher plants.[73] So far only five phenanthrene glucosides were isolated from the Juncaceae family (effusides I-V).[74]

Furan or pyran ring can attach to the phenanthrene core. DELLA GRECA et al. obtained a representative of phenanthrofurans from *J. effusus*.[68]

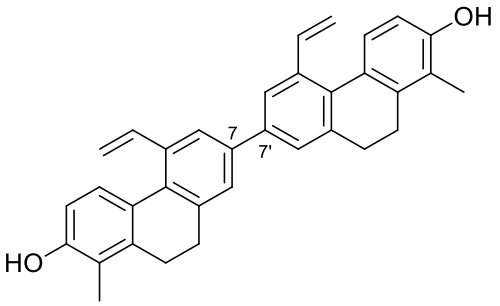
Phenanthrenequinones were also isolated from several higher plants.[75,76] The biosynthesis of these compounds is not fully established. Oxygenated phenanthrenes and abietane-type diterpenoids can provide analogous phenanthrenequinones and alkylated 1,4-phenanthrenequinones, respectively.[77,78] Despite of the great structural diversity of these compounds, only one phenanthrene-quinone was isolated from Juncaceae species so far.[25]



effusol



juncutol



effususin D

Monophenanthrenes can connect through their functional groups or directly via C–C coupling to form di- or triphenanthrenes. The monomers can be linked together at many different positions, usually C-1 is involved into the connection. The linking monomers have usually been isolated previously

from the same plant, or the same genus or the same family. Almost all of the monomer parts of the dimers have been isolated earlier, only a few examples are undiscovered yet. Understandably, these secondary metabolites are even more sporadic and unique than monophenanthrenes.

It is worth noting that lately several novel diphenanthrenes were reported from Juncaceae species. Until now seven compounds were isolated from *J. acutus*, and four dimers were obtained from *J. effusus*.[27,29,79,80] Among the Juncaceae diphenanthrenes, the hit rate of the symmetric compounds is very high. DELLA GRECA et al. isolated five dimeric dihydrophenanthrenes with unexpected structures from the rhizome of *J. acutus*.[79,80] The hepta- or octacyclic skeleton of these compounds are unique among naturally occurring phenanthrenes. These compounds are probably considered to derive by the coupling of various 5-vinyl-9,10-dihydrophenanthrene derivatives through a vinyl and a hydroxy group.[79,80] The dihydrophenanthrene dimers, effususin A and effususin D are homodimers of effusol. According to the proposed biosynthetic pathway effususin D is formed from two effusols, after losing their hydroxy group at C-7.[29] Other dimers of the *Juncus* genus have at least one unsaturated phenanthrene ring in their structure. A dimer of juncusol and dehydrojuncusol was isolated from the rhizomes of *J. acutus*.[80] Dehydroeffusol and effusol are linked at 8,3' positions in effususin B.[29] Presumably, effususin C is a dimer of two 7,7'-dehydroxylated dehydroeffusols linked at the positions of the former hydroxy groups. In case of 8,8'-bidehydrojuncusol, two dehydrojuncusol units form a symmetric homodimer through an 8,8' coupling.[29] Phenanthrene dimers were described from different parts of the plants, therefore it seems that there is no specific plant organ where their synthesis would be more expressed. Juncaceae dimers were reported from the roots, rhizomes and medullae of the species.

Some phenanthrenes (e.g. effusol, juncusol) have been isolated from several *Juncus* species, whereas others have a more limited distribution, and they have been isolated exclusively from one plant (e.g. effususins A–D from *J. effusus*).

3.2.2. Chemical constituents of *Juncus inflexus*

Extensive phytochemical investigations of this plant have not been reported previously. Only flavonoids were detected in the stem and leaf tissue of the plant. Luteolin and luteolin 7-*O*-glucoside, luteolin 5-methyl ether, luteolin 5-methyl ether 7-*O*-glucoside, luteolin 7-*O*-glucoside sulphate, chrysoeriol and chrysoeriol 7-*O*-glucoside sulphate were identified by comparing their R_f values and colours with standards.[42]

3.2.3. Chemical constituents of *Luzula luzuloides*

Earlier chemical investigations of the genus *Luzula* revealed the presence of several flavonoids, but comprehensive phytochemical investigations of the plant have not been reported previously.[42,49]

3.3. PHARMACOLOGY OF JUNCACEAE PHENANTHRENES

Phenanthrenes isolated from different Juncaceae species exert many different activities, including antitumor,[28-32] anti-inflammatory,[23,27,29,31] antioxidant,[23] anxiolytic,[7,9] cell-protective,[11] sedative,[8,9] and spasmolytic[32] effects.

3.3.1. Cytotoxic and antiproliferative activity

Several phenanthrenes isolated from *Juncus* species were screened for their *in vitro* cytotoxic activity in various cancer cell lines. The first example of cytotoxic Juncaceae phenanthrenes was juncusol, which was proved to be active against NCI 90 KB (human epidermoid nasopharynx carcinoma) cell line with an ED_{50} of 0.3 μg/mL.[66] The bioactivity of several 9,10-dihydrophenanthrenes from *J. effusus* was screened by the use of brine shrimp lethality and antitumor potato disc assays.[68] All of the compounds showed toxicity, especially the ones with vinyl or carbinol substitution at C-5, and hydroxy function in ring C. 2,7-Dihydroxy-1-methyl-5-aldehyde-9,10-dihydrophenanthrene and juncuenin E inhibited moderately the growth of MCF-7 (human breast adenocarcinoma) and HeLa (human cervix adenocarcinoma) cells compared to adriamycin as positive control by the use of sulforhodamine B assay.[9,28] Among the dimers, effususin A displayed marked activity, and effususin B possessed noteworthy activity against SHSY-5Y (human neuroblastoma), HepG2 (human hepatocellular liver carcinoma), HeLa and MCF-7 cell lines compared to paclitaxel in a CCK-8 assay.[29] ISHIUCHI et al. demonstrated the caspase-3-mediated cytotoxic activity of effusol, juncusol, juncuenin B, dehydrojuncuenin B and juncuenin D on HT22 (mouse hippocampal neuroblastoma) cell line, by the use of MTT assay and Western blot analysis.[30] Fifteen phenanthrenes isolated from *J. effusus* were screened on five cancer cell lines [SHSY-5Y, SMMC-7721 (human hepatocarcinoma), HepG2, HeLa and MCF-7] in order to evaluate their cytotoxic activities.[31] 5-(1-Methoxyethyl)-1-methyl-phenanthrene-2,7-diol showed selective and very promising inhibitory activity on MCF-7 cell line (IC_{50} = 10.87 μM) compared to paclitaxel (IC_{50} = 28.36 μM) in CCK-8 assay. Dehydroeffusal inhibited the growth of HepG2 (IC_{50} = 12.43 μM) and HeLa (IC_{50} = 13.07 μM) cells two-three times more effectively than the positive control paclitaxel (IC_{50} = 36.77 μM and 25.79 μM, respectively). The results indicated that the presence of a hydroxy group at C-7 is necessary for cytotoxic activity. Moreover, vinyl substitution at C-5, and an unsaturated C-9–C-10 bond increase the effect.

Dehydroeffusol was isolated by antitumor activity-guided fractionation from *J. effusus*, after screening 120 traditional Chinese medicinal herbs. The compound inhibited the growth of highly metastatic cancer cell lines [SGC-7901 (human gastric carcinoma) and AGS (human caucasian gastric adenocarcinoma)] in a dose-dependent manner.[32] The effective inhibition of gastric cell adhesion, migration, invasion and gastric cancer cell mediated vasculogenic mimicry with very low toxicity makes dehydroeffusol a very promising candidate for anti-gastric cancer drug development.

3.3.2. Antimicrobial activity

Juncusol, isolated from *J. roemerianus*, showed potential antimicrobial activity against gram-positive bacteria, including *Bacillus subtilis* and *Staphylococcus aureus*, but gram-negative strains (e.g. *Enterobacter aerogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*) were resistant towards the compound.[32] These findings suggested that juncusol may inhibit a specific system, or a specific reaction found only in gram-positive microorganisms, although the mechanism of action of juncusol was not established. In another study, the antibacterial activity of dehydroeffusol and juncusol isolated from *J. effusus* was tested against methicillin resistant and -sensitive *S. aureus*. It was observed that the activity of the compounds increased after UVA irradiation, with 16- and two-fold, respectively.[33]

3.3.3. Anti-algal activity

Various vinyl, methyl, and hydroxy substituted 9,10-dihydrophenanthrenes isolated from *J. acutus* and *J. effusus* displayed significant *in vitro* phytotoxicity against the microalga, *Selenastrum capricornutum*.[69-71] According to the results, 9,10-dihydrophenanthrenes were more active than their phenanthrene analogues. Dimeric dihydrophenanthrenes were also tested for their anti-algal activity, and they possessed more pronounced activity than the monophenanthrenes.[40] The anti-algal effect of mono- and diphenanthrenes was decreased with the reduction of the polarity. It can be concluded, that the anti-algal activity of phenanthrenes can contribute to the allelochemical toxicity of *Juncus* species.[81]

3.3.4. Anti-inflammatory activity

J. effusus has been used in folk medicine as a remedy for inflammation. Therefore, phenanthrenes from the plant were tested for their anti-inflammatory activity by the inhibition of LPS-induced NO production on RAW 264.7 cells. By the use of this assay, the strong anti-inflammatory activity of effususin B (IC_{50} = 7.42 μM) was demonstrated, while other dimers from the plant [effususin A (IC_{50} = 20.43 μM), effususin C (IC_{50} = 35.58 μM) and effususin D (IC_{50} = 29.14 μM)] possessed moderate activity compared to the positive control quercetin (IC_{50} = 6.63 μM).[29] MA et al. isolated sixteen phenanthrenes from the medulla of *J. effusus* and evaluated their anti-inflammatory properties by the abovementioned assay.[31] 8-Hydroxymethyl-2-hydroxy-1-methyl-5-vinyl-9,10-dihydrophenanthrene (IC_{50} = 14.42 μM), 5-(1-methoxyethyl)-1-methylphenanthrene-2,7-diol (IC_{50} = 11.10 μM), effusol (IC_{50} = 15.13 μM), dehydroeffusol (IC_{50} = 12.69 μM), dehydroeffusal (IC_{50} = 10.50 μM), 2,7-dihydroxy-5-hydroxymethyl-1-methyl-9,10-dihydrophenathrene (IC_{50} = 16.06 μM), 5-hydroxymethyl-1-methyl-phenanthrene-2,7-diol (IC_{50} = 16.30 μM), dehydrojuncusol (IC_{50} = 15.57 μM), and the mixture of 2,7-dihydroxy-1,8-dimethyl-5-vinyl-9,10-dihydrophenanthrene and juncusol (IC_{50} = 13.65 μM) showed remarkable activities in comparison with the positive control quercetin. Effususol A (IC_{50} = 33.12 μM) and juncuenin G (IC_{50} = 23.63 μM) were less active, while juncuenin F (IC_{50} > 50 μM), 2,7-dihydroxy-1-

methyl-5-aldehyde-9,10-dihydrophenanthrene (IC_{50} > 50 μM) and dehydrojuncuenin E (IC_{50} > 50 μM) were proved to be inactive.

The anti-inflammatory property of juncutol was evaluated by the inhibition of iNOS protein expression in LPS-stimulated RAW 264.7 cells.[23] Juncutol displayed noteworthy anti-inflammatory activity, the iNOS level was reduced to 11.2% from 100% after treating with 10 μM of the compound. Juncusol and dehydrojuncusol showed moderate activity, the expression of the protein was decreased to 35.0% and 59.0%, respectively. Among the studied phenanthrenes, 6-hydroxymethyl-1-methyl-5-vinyl-9,10-dihydrophenanthrene-2-ol was the less active, the iNOS level was decreased to 82.0%. None of the investigated four phenanthrenes influenced the COX-2 expression.

Dehydrojuncusol and its dimer (8,8'-bidehydrojuncusol) were also assayed by the abovementioned method.[27] According to the immunoblot analysis, the effect of dehydrojuncusol on the iNOS protein (41.0% inhibition at 10 μM) decreased dramatically after dimerization (< 12.0% inhibition at 10 μM).

3.3.5. Antioxidant activity

The antioxidant capacity of dehydrojuncusol and 8,8'-bidehydrojuncusol were measured by ABTS radical cation decolourisation assay. The diphenanthrene inhibited effectively the free radical scavenging generation, comparable to the positive control ascorbic acid, and its monomer also possessed moderate activity.[27]

In vitro cellular protective effect of dehydroeffusol was proved on human erythrocytes by the use of photohemolysis assay, which could be explained with the high antioxidant activity of the compound based on its high ROS scavenging activity.[11]

3.3.6. Sedative and anxiolytic activity

Juncus species are commonly used as sedative and anxiolytic agents in traditional Asian medicine.[7-9] The ethnopharmacological use of the plants was confirmed by the isolation of their CNS-depressing constituents. Dehydroeffusol was the first phenanthrene tested for its anxiolytic and sedative effects.[7] The compound increased the time spent with open arms and elevated the frequency of the entries in mice. The anxiolytic effect of dehydroeffusol was further confirmed with the hole-board test; 5 mg/kg orally administered dehydroeffusol enhanced the number of head-dips. The sedative property of the compound was proved by the open field test. In the rota-rod test, the phenanthrene did not increase the fall-down time, which indicates that the compound had no effects on the motor activity. In conclusion, dehydroeffusol reduced anxiety in mice, and its sedative activity was not linked with decreased motoric function.

WANG et al. proved the anxiolytic properties of effusol and juncusol on mice in the elevated plus-maze test; their sedative activity was also confirmed by the decreased locomotion in the open field

test.[9] The mechanism of action study revealed that effusol and dehydroeffusol inhibited dose-dependently the $\alpha_1\beta_2\gamma_2$s subtype of $GABA_A$ receptors.[8] The benzodiazepine antagonist flumazenil did not inhibit the action of these phenanthrenes. Therefore, it was assumed that effusol and dehydroeffusol were not attached to the benzodiazepine binding site of $GABA_A$ receptor.

3.3.7. Spasmolytic activity

Phenanthrene-containing plants were used by Native Americans in order to treat stomach aches, and to avoid abortion. The spasmolytic effects of numerous phenanthrenes have been demonstrated, but only dehydroeffusol was investigated for its antispasmodic properties among Juncaceae phenanthrenes so far.[22] Although, in high doses dehydroeffusol increased the contractions, in lower doses it inhibited the effects of several spasmogens (KCl, pilocarpin, histamine, Bay-K8644) on isolated rat jejunum.[32]

4. MATERIALS AND METHODS

4.1. PLANT MATERIAL

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). Botanical identification of the plant materials was performed by Gusztáv Jakab (Institute of Environmental Sciences, Szent István University, Szarvas, Hungary) and voucher specimens (No. 869–873) have been deposited at the Department of Pharmacognosy, University of Szeged, Szeged, Hungary. The plant material was dried at room temperature.

4.2. PREPARATION OF PLANT EXTRACTS FOR PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING

Extracts were prepared from 20 g of air-dried, powdered plant materials with 300 mL of MeOH with the use of an ultrasonic bath (3 × 15 min). After filtration, the solutions were evaporated to dryness under reduced pressure on a Büchi Rotavapor R-210 at 40 °C. The residues were dissolved in 50 mL of 50% aqueous MeOH and were subjected to solvent–solvent partition between *n*-hexane (3 × 100 mL) (extracts A), CH_2Cl_2 (3 × 100 mL) (extracts B) and EtOAc (3 × 100 mL) (extracts C); and the remaining H_2O extracts were named as extracts D. The fractions were concentrated and monitored by thin-layer chromatography (TLC), using cyclohexane–EtOAc–MeOH (20:10:1) as mobile phase. The TLC separations were monitored at 254 and 366 nm, and at daylight after spraying with vanillin-sulfuric acid reagent and heating at 120 °C for 5 min. Mobile phases in all types of chromatography methods are given in terms of volume ratio, v/v.

4.3. GENERAL EXTRACTION AND ISOLATION PROCEDURES

Open-column chromatography: OCC was performed on polyamide (MP Biomedicals 09602).

Vacuum-liquid chromatography: For normal phase VLC, silica gel 60 G (15 µm, Merck 1.11678) was used. LiChroprep RP-18 (40–63 µmm, Merck 113900) stationary phase was used for reversed phase VLC.

Gel filtration chromatography: Sephadex LH-20 (25–100 µm, Pharmacia Fine Chemicals) was used for gel filtration.

Medium pressure liquid chromatography: MPLC was performed by a Biotage SP1TM Purification System using a KP-C_{18}HS 40+M column.

Isocratic high-performance liquid chromatography: HPLC was carried out with a Shimadzu LC-10AT pump interface equipped with a Shimadzu SPD-20A UV-Vis detector using Luna® Phenyl-Hexyl column (250 × 10 mm, 5 μm).

Thin-layer chromatography: Preparative thin-layer chromatography (PLC) was performed on silica gel 60 F_{254} (Merck 105715). Separation was monitored in UV light at 254 nm and 366 nm. Compounds were eluted from the scraped adsorbent with CH_2Cl_2–MeOH (4:1). The OCC, VLC, PLC, MPLC fractions obtained were monitored by TLC on silica gel 60 F_{254} (Merck 105554) and on reversed phase silica gel 60 F_{254} (Merck 105559).

Visualization methods: UV light at 254 nm and 366 nm, and at daylight after spraying with vanillin-sulfuric acid reagent and heating at 120 °C for 5 min.

4.4. STRUCTURE DETERMINATION OF THE ISOLATED COMPOUNDS

Optical rotations were determined in MeOH at room temperature with a Perkin-Elmer 341 polarimeter.

NMR spectra were recorded in CD_3OD and DMSO-d_6 on a JEOL ECS 400 MHz FT-NMR at 400 MHz (^{1}H) and 100 MHz (^{13}C), with tetramethylsilane (TMS) as internal standard, and on a Bruker Avance DRX 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C). Chemical shifts were referenced to TMS (^{1}H) or to residual solvent resonances. Two-dimensional (2D) experiments were performed with standard JEOL or standard Bruker software. In the ^{1}H–^{1}H COSY, HSQC and HMBC experiments, gradient-enhanced versions were applied.

High-resolution MS data were recorded on a Waters-Micromass Q-TOF Premier mass spectrometer equipped with an electrospray source, and on a Thermo Scientific Q-Exactive Plus orbitrap mass spectrometer equipped with ESI ion source in positive ionization mode. The resolution was over 1 ppm.

APCI-MS measurements were performed on an API 2000 Triple Quad mass spectrometer (AB SCIEX, Framingham, MA, USA) with an atmospheric pressure chemical ionization (APCI) interface, using positive and negative polarity. The source temperature was 350 °C and the samples were dissolved in CH_3CN.

4.5. ABSOLUTE CONFIGURATION DETERMINATION OF THE CHIRAL COMPOUNDS

HPLC-ECD analysis

Chiral HPLC separations were performed with a JASCO HPLC system using Chiralpak IA column with 250 mm × 4.6 mm i.d., 5 μm (Daicel, Chemical Industries Ltd.) and *n*-hexane–propan-2-ol as eluent at a flow rate of 1.0 mL/min). HPLC-UV and optical rotation (OR) chromatograms were recorded with JASCO MD-910 multiwavelength and OR-2090Plus chiral detector, respectively. The on-line ECD and UV spectra were measured simultaneously by stopping the flow at the UV absorption maximum of

each peak. The values of the ECD ellipticity (ϕ) were not corrected for the concentration. Three consecutive scans were recorded and averaged for an HPLC-ECD spectrum with standard sensitivity, 2 nm bandwidth and 1 s response. The background HPLC-ECD spectrum of the eluent was recorded in the same way. ECD spectra were recorded on a J-810 spectropolarimeter.

Computational section

Mixed torsional/low-frequency mode conformational searches were carried out by means of the Macromodel 9.9.223 software using the Merck Molecular Force Field (MMFF) with an implicit solvent model for $CHCl_3$.[82] Geometry reoptimizations were carried out at the B3LYP/6-31G(d) level in vacuo, the B97D/TZVP [83,84] and the CAM-B3LYP/TZVP [85,86] levels with the PCM solvent model for CH_3CN or $CHCl_3$. TDDFT ECD calculations were run with various functionals (B3LYP, BH&HLYP, CAM-B3LYP, PBE0) and the TZVP basis set as implemented in the Gaussian 09 package with the same or no solvent model as in the preceding DFT optimization step.[87] ECD spectra were generated as sums of Gaussians with 3000 cm^{-1} widths at half-height (corresponding to ca. 24/41 nm at 280/370 nm), using dipole-velocity-computed rotational strength values.[88] Boltzmann distributions were estimated from the ZPVE-corrected B3LYP/6-31G(d) energies in the gas-phase calculations and from the B97D/TZVP and CAM-B3LYP/TZVP energies in the solvated ones. The MOLEKEL software package was used for visualization of the results [89-91]

4.6. HPLC-MS SCREENING METHODS

The HPLC-MS characterization was performed using a Shimadzu liquid chromatographic system (DGU-20A3 degasser, LC-20AD pumps, SIL-20A autosampler, CBM-20A controller, CTO-20AC column thermostat) coupled with API 2000 Triple Quad mass spectrometer, with turbo ESI ion source. Kinetex®(Phenomenex) 2.6 µm C18 column (50 × 2.1 mm, 100 Å) thermostated at 40 °C was used for the separation during the analysis. Mobile phase A was 0.1% formic acid in ultrapure water (R > 18.3 MΩ) and mobile phase B was 0.1% formic acid in gradient grade acetonitrile. The separation was performed by gradient elution with 500 µL/min flow rate. Mobile phase B ratio had a linear gradient from 5% to 95% in 10 minutes. The LC flow was split to 50 µL/min with a tee-connector (split ratio was measured at the initial LC conditions). MS detector worked in negative MRM mode. The turbo ion source nebulizer was set to 28 units (arbitrary), the auxiliary or turbo gas was set to 30 units and its temperature was 150 °C. Data acquisition and evaluation were performed by using Analyst 1.5.1 software.

4.7. PHARMACOLOGICAL TESTS

Pharmacological investigations were performed in cooperation with the Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary; the Graduate Institute of Natural Products,

Chang Gung University, Taoyuan, Taiwan; the Research Center for Industry of Human Ecology and Graduate Institute of Health Industry Technology, Chang Gung University of Science and Technology, Taoyuan, Taiwan; and the Department of Anesthesiology, Chang Gung Memorial Hospital, Taoyuan, Taiwan.

4.7.1. *In vitro* antibacterial investigations

Test microorganisms

The test microorganisms were one standard and nine clinical isolates with different antibiotic resistant profile, originated various departments of Albert Szent-Györgyi Health Center and identified in the Institute of Clinical Microbiology at University of Szeged by conventional method.

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). Microbial cultures were grown on standard Mueller-Hinton agar plates and maintained at 4 °C throughout the study to use as stock cultures.

Antibacterial screening

Antibacterial activities of the fractions and the pure compounds were first screened for their inhibitory zones by disc-diffusion method.[92] The plant extracts were prepared at 50 mg/mL, while pure compounds were diluted to 10 mg/mL using DMSO. The sterile filter paper discs (6 mm diameter) impregnated with the extracts (10 µL) were placed on the agar plate seeded with the respective bacterial suspension (inoculums 0.5 McFarland, 1-2 × 10^8 CFU/mL). The solvent was served as negative control. The plates were then incubated at 37 °C for 24 hours under aerobic conditions. The diameters of inhibition zones produced by the plant extracts and the pure compounds (including the disc) were measured and recorded. All experiments were carried out in triplicate.

Determination of the MIC values

The active compounds and the extracts with a diameter of inhibition zone ≥ 10 mm were subjected to determine their minimal inhibitory concentration (MICs) by the microdilution method.[93] Briefly, in the 96-well plates the stock solutions of the compounds (50 mg/mL in DMSO) were serially diluted with Mueller-Hinton broth to arrive at final concentration between 2.5 mg/mL and 4.9 µg/mL. 100 µL of inoculum (0.5 McFarland, 1-2 × 10^8 CFU/mL) were then added to the wells. A sterility check (medium and DMSO in amount corresponding to the highest concentration), negative control (medium, DMSO and inoculum) and positive control (medium, DMSO, inoculum and vancomycin) were included for

each experiment. The plates were then incubated at 37 °C for 24 hours under aerobic environment. The MIC of preparation was the lowest concentration that completely inhibited the visible bacterial growth. All experiments were performed twice in triplicate.

4.7.2. *In vitro* anti-inflammatory investigations

Preparation of human neutrophils

Blood was taken from healthy human donors (20–30 years old) by venipuncture using a protocol approved by the Institutional Review Board at Chang Gung Memorial Hospital. Neutrophils were isolated using a standard method.[94,95] Briefly, the cells were obtained from peripheral blood according to the standard method of dextran sedimentation, followed by centrifugation in a Ficoll-Hypaque gradient and the hypotonic lysis of the erythrocytes. The purified neutrophils contained > 98% viable cells, as determined by Trypan blue exclusion, and were suspended in calcium-free HBSS at 4 °C before used.

Measurement of superoxide generation

SOD inhibition was measured by the reduction of ferricytochrome *c*.[94,95] Neutrophils (6×10^5 cells/mL) in 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+} were equilibrated at 37 °C for 2 min and then incubated with drugs for 5 min. The cells were activated using formyl-methionyl-leucyl-phenylalanine (fMLP, 100 nM)/cytochalasin B (CB, 1 μg/mL) for 10 min. The absorbance was continuously monitored at 550 nm using a double-beam, six-cell positioned spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on the differences in absorbance with and without SOD (100 μg/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c* (ε = 21.1/mM/10 mm).

Measurement of elastase release

The degranulation of azurophilic granules was determined by elastase release.[94,95] Neutrophils (6×10^5 cells/mL) were equilibrated in MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), an elastase substrate, at 37 °C for 2 min and then incubated with drugs for 5 min. The cells were activated using fMLP (100 nM) in the presence of CB (0.5 μg/mL), and changes in the absorbance at 405 nm were continuously measured to monitor elastase release. The results are expressed as a percentage of elastase release in the fMLP/CB-activated, drug-free control system.

Statistical methods

Results of the anti-inflammatory assays were expressed as means ± S.E.M. Statistical analysis using Student's t-test was performed with SigmaPlot (Systat Software, San Jose, CA, USA). A value of $P < 0.05$ was considered statistically significant.

5. RESULTS

5.1. 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, 19 species of the family were investigated for their antibacterial effects. In our experiments 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 dry extracts were suspended in 50% aqueous MeOH and subjected to solvent–solvent partition with *n*-hexane, dichloromethane and EtOAc, to yield 96 fractions of different polarity.

All fractions were tested for their antibacterial activity against 10 multiresistant bacterial strains. The activities of extracts were screened firstly for their inhibitory zones by disc-diffusion method at concentrations of 50 mg/mL. According to the screening assay, several extracts showed anti-MRSA activity with mild to strong effectiveness. The results of the antibacterial assays are listed in **Table 1**.

Table 1. Anti-MRSA activity of plant extracts

Plant species	Plant part	Extract	Inhibition zone (mm) ± SD		MIC (μg/mL)
			MRSA (ATCC 43300)	MRSA (64326)	
J. acutus L.	Wp	B	10.0 ± 0.0	10.0 ± 0.0	156
J. articulatus L.	Wp	B	9.3 ± 0.5	9.0 ± 0.8	
J. compressus Jacq.	Wp	B	9.3 ± 0.6	9.3 ± 0.6	
J. effusus L.	R	B	13.0 ± 0.0	13.0 ± 1.0	39
	Ap	B	8.6 ± 0.6	8.6 ± 0.6	
J. filiformis L.	Wp	B	9.6 ± 0.6	8.6 ± 1.2	
	Wp	D	7.6 ± 0.6	8.6 ± 1.2	
J. gerardii Loisel.	Wp	B	10.3 ± 2.0	9.0 ± 1.0	78
J. inflexus L.	R	B	14.6 ± 1.2	13.0 ± 1.0	9.75
	Ap	B	8.0 ± 0.0	8.0 ± 0.0	
J. maritimus Lam.	Wp	B	12.3 ± 0.6	12.3 ± 0.6	78
J. monanthos Jacq.	Wp	B	9.0 ± 0.8	9.0 ± 0.8	
J. squarrosus L.	R	B	8.0 ± 0.0	8.3 ± 0.6	
	Ap	B	8.6 ± 0.6	8.3 ± 0.6	
J. tenuis Willd.	R	B	8.3 ± 0.6	8.6 ± 0.6	
	Ap	B	10.3 ± 0.6	9.6 ± 0.6	156
L. campestris (L.) DC.	Wp	B	6.7 ± 0.6	6.7 ± 0.6	
L. forsteri (Sm.) DC.	Wp	B	7.3 ± 0.6	7.3 ± 0.6	
L. sylvatica (Huds.) Gaudin	Ap	B	6.7 ± 0.6	6.7 ± 0.6	
Vancomycin*			15.5 ± 0.6	15.5 ± 0.6	

Data represents the mean value of clear inhibition zone (diameter of inhibition zone plus diameter of the disc) obtained from three independent experiments. As well as the minimal inhibitory concentration (MICs) of the extracts with diameter of inhibition zone ≥ 10 mm by the microdilution method. The extracts without any antibacterial activity were not presented in the table. Abbreviations: Ap = Aerial part; R = Root; Wp = Whole plant; B = CH_2Cl_2 fraction; D = remaining H_2O fraction. *Vancomycin was used as positive control at 5 μg/disc.

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; **Table 1**).

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* (whole plant) 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).

5.2. ANTI-INFLAMMATORY ACTIVITY OF *LUZULA* SPECIES

The anti-inflammatory activity of 24 extracts [*n*-hexane (A), CH_2Cl_2 (B), EtOAc (C) and remaining H_2O (D)] prepared from five species (*L. campestris*, *L. forsteri*, *L. luzuloides*, *L. sudetica* and *L. sylvatica*) were tested (**Table 2**).

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 (10 µg/mL) in both assays. The fraction A of *L. campestris* exerted the most selective elastase release inhibition. Among the fractions with different polarities, fractions B (containing CH_2Cl_2-soluble lipophilic constituents) had the most remarkable activities (> 93% inhibition) at a concentration of 10 µg/mL. 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 10 µg/mL. The remaining H_2O fraction of *L. luzuloides* also showed notable anti-inflammatory activities (> 65%) in both methods.

Regarding to the species, *L. luzuloides* was the most promising; all fractions of the plant showed mild to strong inhibitory activities in both assays, although the effects of the fractions were slightly more pronounced on the elastase release. On the basis of the results of the preliminary screening, the lipophilic extract of *L. luzuloides* was selected for more detailed phytochemical studies, with the aim of the identification of their anti-inflammatory constituents.

Table 2. Inhibitory effects of extracts with different polarity from *Luzula* species on superoxide anion generation and elastase release on human neutrophils in response to fMLP/CB

Plant species	**Extract**	**Superoxide anion inhibition (%)**	**Elastase inhibition (%)**
Luzula campestris (L.) DC.	A	50.04 ± 5.08***	106.98 ± 6.35***
	B	96.06 ± 0.91***	112.19 ± 6.16***
	C	77.45 ± 6.44***	84.22 ± 4.09***
	D	32.52 ± 5.43***	45.18 ± 6.47***
L. forsteri (Sm.) DC.	A	71.53 ± 7.75***	71.88 ± 7.07***
	B	93.14 ± 6.14***	113.51 ± 1.07***
	C	35.24 ± 1.43***	37.95 ± 6.88**
	D	32.80 ± 3.40***	54.52 ± 0.99***
L. luzuloides (Lam.)	A	70.65 ± 6.65***	92.50 ± 3.58***
	B	99.39 ± 0.37***	114.22 ± 3.13***
	C	62.84 ± 3.50***	68.39 ± 5.74***
	D	68.80 ± 5.92***	77.71 ± 4.99***
L. sudetica (Willd.) Schult.	A	72.85 ± 6.35***	76.08 ± 5.45***
	B	109.25 ± 6.01***	115.21 ± 4.14***
	C	77.95 ± 0.22***	91.41 ± 1.84***
	D	37.92 ± 5.39**	8.59 ± 3.19
L. sylvatica (Huds.) Gaudin (aerial part)	A	85.27 ± 4.20***	89.10 ± 6.41***
	B	96.09 ± 1.92***	111.90 ± 2.99***
	C	73.32 ± 2.91***	54.82 ± 4.11***
	D	26.90 ± 7.01**	48.83 ± 6.51**
L. sylvatica (Huds.) Gaudin (root part)	A	89.18 ± 6.58***	107.88 ± 3.96***
	B	95.28 ± 2.48***	104.23 ± 5.67***
	C	53.62 ± 7.13***	54.13 ± 3.03***
	D	41.60 ± 6.19***	43.02 ± 2.96***

A = *n*-hexane soluble fraction; B = CH_2Cl_2 fraction; C = EtOAc fraction; D = remaining H_2O fraction
Results are presented as mean ± S.E.M. (n=3–5). ** $P < 0.01$, *** $P < 0.001$ compared with the control value (DMSO).

5.3. ISOLATION AND PURIFICATION OF COMPOUNDS FROM *JUNCUS INFLEXUS*

The air dried roots of *J. inflexus* (3.5 kg) were grounded with a Retsch mill (model SM 100 comfort), and percolated with MeOH (60 L) at room temperature. The crude extract (393 g) was concentrated *in vacuo*. The extract was dissolved in 500 mL 50% MeOH, and liquid-liquid partition was performed with *n*-hexane (5 × 700 mL), then with CH_2Cl_2 (5 × 1000 mL), and finally with EtOAc (5 × 1000 mL).

The CH_2Cl_2 fraction (52.8 g) was chromatographed on a polyamide column (OCC) (sorbent: 540 g, column: 150 mm × 350 mm) with mixtures of MeOH and H_2O [2:3, 1:1, 3:2, 4:1, 1:0 (2.5, 5, 10, 40, and 5 L, respectively); fractions were collected according to the eluents]. The fractions were concentrated and monitored by thin-layer chromatography (TLC) using cyclohexane–EtOAc–EtOH (20:10:1) as solvent system. According to their TLC chromatograms the compositions of the fractions obtained from the polyamide column with methanol–water 3:2 and 4:1 were 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 two 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) and J4 (inhibition zones = 7.6 ± 0.6 mm, for both strains) possessed moderate activity, and J1 was proved to be inactive.

Fraction J3 (32.9 g) was further chromatographed by NP-VLC (sorbent: 250 g, column: 35 mm × 210 mm) with a gradient system of cyclohexane–EtOAc–EtOH [from 19:1:0 to 5:5:1 (1000 mL/eluent), and finally with EtOH (1500 mL); volume of collected fractions was 100 mL] to yield major fractions J3/1–14, according to their TLC patterns (**Figure 2**).

From fraction J3/3 (35.9 mg) **JIN-2** (16.2 mg) was crystallized. Fraction J3/4 (71.8 mg) was purified on Sephadex LH 20 column using MeOH as eluent [(120 mL); volume of collected fractions was 3 mL] to afford **JIN-1** (21.6 mg). **JIN-4** (35.5 mg) and **JIN-5** (12.2 mg) were purified from fraction J3/6 (60.2 mg) by NP-PLC using cyclohexane–EtOAc (7:3) as solvent system.

NP-VLC was used for the purification of fraction J3/7 (3.6 g), which was eluted with a gradient system of *n*-hexane–CH_2Cl_2–MeOH [from 3:7:0 to 0:97:3 (500 mL/eluent), and finally MeOH (500 mL); volume of collected fractions was 100 mL] to yield six subfractions. Subfraction J3/7/3 (667.7 mg) was further separated by NP-VLC using the gradient system of $CHCl_3$–acetone [from 99:1 to 4:1 (200 mL/eluent), and finally acetone (200 mL); volume of collected fractions was 20 mL] to yield six subfractions. Furthermore, subfraction J3/7/3/3 (84 mg) was purified by RP-VLC using a MeOH–H_2O gradient elution [from 3:2 to 17:3 (150 mL/eluent), and finally MeOH (150 mL); volume of collected fractions was 12 mL] to yield six subfractions. Subfraction J3/7/3/3/4 (12 mg) was purified on Sephadex LH-20 column, using MeOH–CH_2Cl_2 (1:1) as eluent [(90 mL); volume of collected fractions was 2 mL] to afford **JIN-19** (4.1 mg). Furthermore, from J3/7/4 **JIN-3** (850 mg) was crystallized. Subfraction J3/7/5 (324 mg) was separated on Sephadex LH-20 column, using MeOH as eluent [(80 mL); volume of collected fractions was 2 mL] to afford **JIN-7** (52.1 mg).

Fraction J3/11 (370.2 mg) was subjected to RP-MPLC using a gradient system of MeOH–H_2O [from 1:1 to 4:1, and finally with MeOH (300 mL each), at a flow rate of 6 mL/min; volume of collected fractions was 9 mL] to yield 12 subfractions. **JIN-15** (7.5 mg) was obtained from fraction J3/11/4

(11.0 mg) after using NP-PLC with CH_2Cl_2–MeOH (19:1) as a solvent system. Subfraction J3/11/8 (23.3 mg) was chromatographed by gel filtration using MeOH as mobile phase [(100 mL); volume of collected fractions was 3 mL] to get **JIN-12** (6.7 mg) and **JIN-13**. Final purification of **JIN-13** (5.2 mg) was performed by RP-HPLC, eluted with MeOH–H_2O (7:3) at a flow rate of 2 mL/min (t_R = 21.5 min). Subfraction J3/11/10 (25.2 mg) was further chromatographed on Sephadex LH-20 column using the mobile phase MeOH [(200 mL); volume of collected fractions was 4 mL] to get **JIN-11** (3.8 mg).

Fraction J3/12 (405.1 mg) was further separated by RP-VLC by a gradient system of MeOH–H_2O [from 3:7 to 9:1 (100 mL/eluent), and finally MeOH (200 mL); volume of collected fractions was 15 mL] to yield 10 subfractions. From subfraction 4 **JIN-8** (6.1 mg) was isolated by the use of NP-PLC, using CH_2Cl_2–MeOH (19:1) as solvent system.

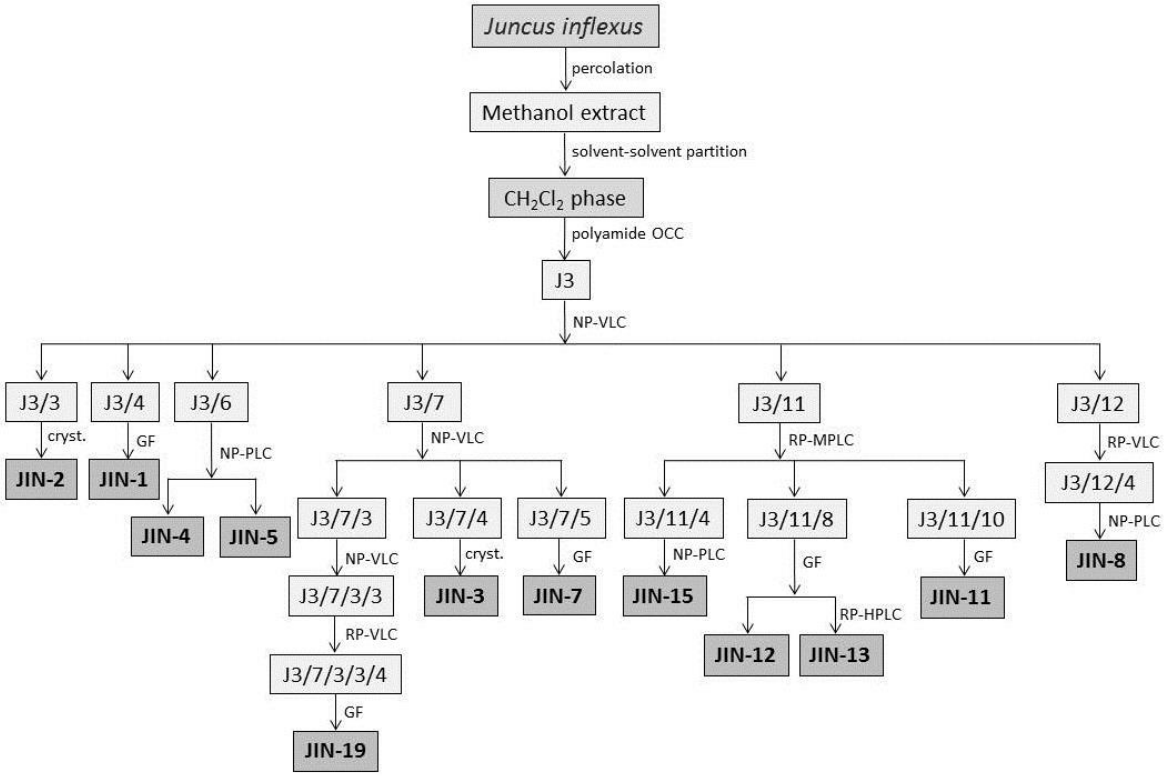


Figure 2. Isolation of compounds from *J. inflexus*

5.4. ISOLATION AND PURIFICATION OF COMPOUNDS FROM *LUZULA LUZULOIDES*

The air-dried whole plant of *L. luzuloides* (480 g) was powdered with a Retsch mill (model SM 100 comfort), and percolated with MeOH (20 L) at room temperature. The crude extract was concentrated (40 mL) *in vacuo*. The extract (76 g) was suspended in 200 mL of 50% MeOH and subjected to solvent–solvent partitioning with CH_2Cl_2 (5 × 500 mL).

After evaporation, the CH_2Cl_2 fraction (4.9 g) was chromatographed on a polyamide column (sorbent: 55 g, column: 45 mm × 200 mm) with mixtures of MeOH and H_2O [1:1, 4:1 (1 and 4 L, respectively); each eluent was collected as a fraction]. The fraction obtained from the polyamide

column with MeOH–H_2O 4:1 (1.1 g) was further chromatographed by NP-VLC (sorbent: 40 g, column: 35 mm × 150 mm) with a gradient system of cyclohexane–EtOAc–EtOH [from 9:1:0 to 5:5:1 (300 mL/eluent), and finally with EtOH (200 mL); volume of collected fractions was 20 mL] to yield twelve major fractions (L2/1–12) (**Figure 3**). The fractions were concentrated and monitored by TLC using cyclohexane–EtOAc–EtOH (20:10:1) as solvent system.

From fraction L2/1 (9.2 mg) **LUB-1** (5.2 mg) was crystallized. Fraction L2/4 (31.3 mg) was purified by gel filtration using MeOH as eluent [(100 mL); volume of collected fractions was 2 mL] to afford four subfractions. **LUB-3** (3.0 mg) was purified from subfraction L2/4/1 (10.6 mg) by NP-PLC using *n*-hexane–EtOAc (7:3) as solvent system. Subfraction L2/4/3 (14.1 mg) was also purified by NP-PLC, with cyclohexane–EtOAc–MeOH (20:10:1) as solvent system to afford **LUB-4** (5.1 mg). **LUB-6** (3.2 mg) was crystallized from subfraction L2/4/4 (8.1 mg).

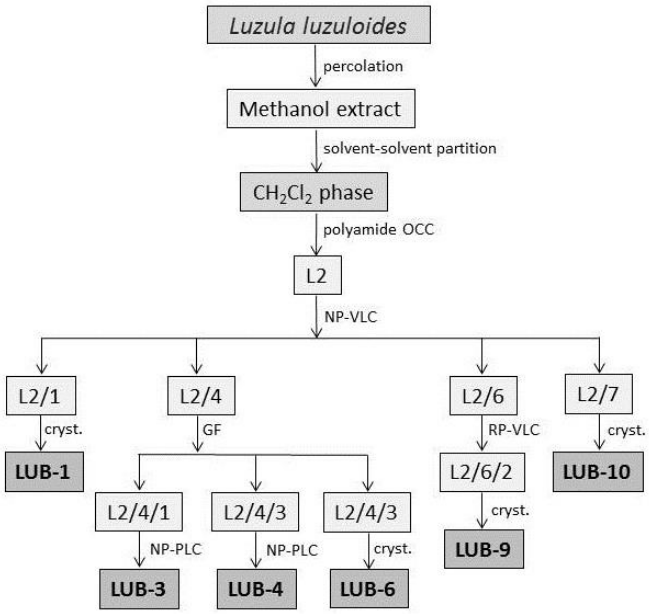


Figure 3. Isolation of compounds from *L. luzuloides*

Fraction L2/6 (25.1 mg) was separated by RP-VLC, with a gradient system of MeOH–H_2O [from 2:3 to 9:1 (150 mL/eluent), and finally MeOH (100 mL); volume of collected fractions was 10 mL] to yield five subfractions (L2/6/1–5). **LUB-9** (9.0 mg) was crystallized from subfraction L2/6/2 (12.4 mg). Furthermore, **LUB-10** (7.1 mg) was crystallized from fraction L2/7 (18.1 mg).

5.5. CHARACTERIZATION AND STRUCTURE DETERMINATION OF THE ISOLATED COMPOUNDS

The structure elucidation of the compounds was performed by means of MS and NMR measurements. HRESIMS measurements revealed the molecular masses and molecular compositions of the novel compounds. Information from 1D (^{1}H NMR and JMOD) and 2D (^{1}H–^{1}H COSY, NOESY, HSQC and HMBC) NMR experiments proved to be the most valuable for the structure determination.

5.5.1. Compounds from *Juncus inflexus*

From the CH_2Cl_2 fraction of *J. inflexus* 12 compounds (**1**–**12**) were isolated with the combination of different chromatographic techniques.

JIN-7 (**1**) was isolated as an amorphous powder with $[\alpha]_D^{26}$ 0 (*c* 0.1, MeOH). Its HRESIMS provided the molecular formula, $C_{19}H_{22}O_3$, through the presence of a peak at *m/z* 321.1532 $[M+Na]^+$ (calcd. for $C_{19}H_{22}O_3Na$, 321.1461). The ^{1}H NMR spectrum (**Annex II**) displayed signals of two *ortho*-coupled aromatic protons (δ_H 6.70 d and 6.84 d), and one aromatic proton as a singlet (δ_H 6.86), three methyls, two methylenes, one sp^3 methine, and signals of protons belonging to one methoxy and two hydroxy groups. In the JMOD spectrum, the presence of 19 carbon signals was detected. In the ^{1}H NMR spectrum, two methylene signals at δ_H 2.32 and 2.75 (2 × 2H) indicated this compound to be a 9,10-dihydrophenanthrene derivative. In the ^{1}H–^{1}H COSY spectrum, correlations were observed between the H-9, H-10 methylenes, and δ_H 6.70 d and 6.84 d (H-3/H-4), and δ_H 4.69 d and 1.47 d (3H) (H-12/H-13). The methyl doublet at δ_H 1.47 and a methine proton at δ_H 4.69 provided evidence for the presence of an isolated CH_3CH structural unit (C-12, C-13) in the molecule. According to the ^{1}H and ^{13}C NMR signals at δ_H 2.85 and δ_C 55.0, a methoxy group could be identified, which was connected to C-12 as confirmed by HMBC correlation between OCH_3 (δ_H 2.85) and C-12 (δ_C 74.5) (**Figure 4**). Moreover, on the basis of HMBC correlations between C-5/H_3-13, C-6/H-12, and C-5a/H-12, this side chain was placed at C-5. One of the methyl groups (δ_H 2.13 s) was placed at C-1 on the basis of its HMBC correlation with the quaternary carbons at δ_C 138.8 (C-1a), 120.2 (C-1) and 153.7 (C-2), and the other methyl group was assigned at C-8 on the basis of the correlations of H_3-14/C-8. The hydroxy groups linked to C-7 and C-2 were confirmed by the chemical shift of the quaternary carbons ($\delta_{C\text{-}7}$ 153.8 and $\delta_{C\text{-}2}$ 153.7).

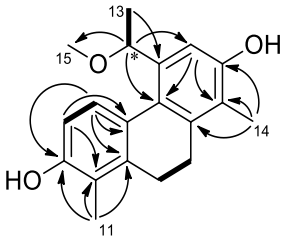
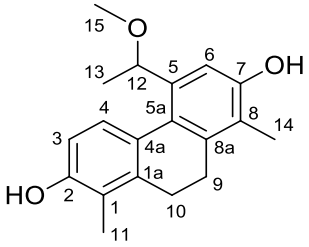


Figure 4. Diagnostic COSY (▬) and HMBC correlations (H→C) for **1**

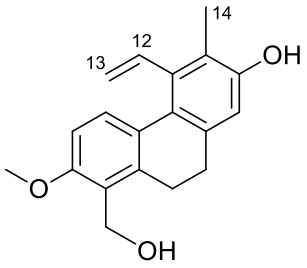
NOESY correlations confirmed the substitution pattern of compound **1**. Nuclear Overhauser effects were detected between H-3/H-4, H-4/H-12, H-4/H-13, H_3-OMe/H-12, H_3-13/H-12, H-6/H_3-13 and H-9/H_3-14, H-9/H-10. All of the above evidence confirmed the planar structure of **1** as 2,7-dihydroxy-1,8-dimethyl-5-(1-methoxyethyl)-9,10-dihydrophenanthrene, which has been named as jinflexin A.

The zero specific rotation and baseline ECD spectrum of jinflexin A (**1**) suggested it to be a racemic mixture, which was also confirmed by the separation of its enantiomers on a Chiralpak IA column using *n*-hexane–propan-2-ol (9:1) as eluent. The HPLC-ECD spectra of the separated enantiomers were recorded, which enabled the determination of absolute configuration by exploiting the solution TDDFT-ECD protocol.[86, 96] In a preliminary MMFF conformational search of (*S*)-**1**, 31 conformers were generated, which were then reoptimized at both the B3LYP/6-31G(d) in vacuo and B97D/TZVP [83,84] PCM/$CHCl_3$ levels, resulting in five and six low-energy conformers above a 2% Boltzmann population, respectively. Jinflexin A (**1**) has an inherently chiral substituted biphenyl chromophore, of which the conformational freedom is restricted by an ethylidene linker. Similar to atropisomeric biphenyls, the populations of the preferred *P*- or *M*-helicity conformers determines the features of the ECD spectra, which, in turn, are governed by the absolute configuration of the benzylic chirality center. According to literature data of substituted 9,10-dihydrophenanthrene derivatives with *ortho* tetrasubstituted biphenyl moiety, *P*- and *M*-helicity conformers can be interconverted at ambient temperature by flipping the C-9 and C-10.[97,98] The conformational analysis identified conformers with both *P*- and *M*-helicity of the biphenyl moiety, which showed near mirror-image computed ECD curves. The lowest-energy B3LYP/6-31G(d) in vacuo conformer (conf. A, 63.5%) of (*S*)-**1** had *P*-helicity with 30.78° $\omega_{C1a,C4a,C5a,C8a}$ torsional angle (in **Appendix I**), and the C-12–H-12 bond of the benzylic chirality center was near coplanar with the benzene ring ($\omega_{12H,C12,C5,C5a}$ = 12.18°). The computed ECD spectra of this conformer reproduced well the experimental HPLC-ECD curve of the first-eluting enantiomer and conformers B (12.0%) and D (5.3%) differing only in the orientation of the OH-2 and OH-5 protons, showed near congruent computed ECDs. In contrast, conformers C (8.6%) and E (3.3%), having *M*-helicity with −32.78° and −31.20° $\omega_{C1a,C4a,C5a,C8a}$ torsional angles, respectively, showed mirror-image computed ECD curves of the experimental ECD (in **Appendix I**). Thus, the proper estimation of the population for the *P*- and *M*-helicity conformers is fundamental for the unambiguous assignment of the absolute configuration.[91] It was found that the distance of the H-4 and H-13 protons is 2.13 Å in the three *P*-helicity conformers (A, B and D), while this distance is larger than 4.10 Å for the *M*-helicity conformers (C and E). Since NOE correlation could be observed between H-4 and H-13 protons and there was no correlation between H-4 and H-15, which would be expected from conformers C and E, the *P*-helicity conformers were also confirmed experimentally as the dominant ones in solution. The B97D/TZVP (PCM/$CHCl_3$) method showed an even smaller contribution of the *M*-helicity forms with a

total population of 4.5% from two conformers (in **Appendix I**). The ECD spectra of (*S*)-**1** computed at B3LYP/TZVP, BH&HLYP/TZVP and PBE0/TZVP levels reproduced the experimental HPLC-ECD spectrum of the first-eluting enantiomer and thus the (*S*) absolute configuration could be assigned to the enantiomer with a negative Cotton effect (CE) at 275 nm.

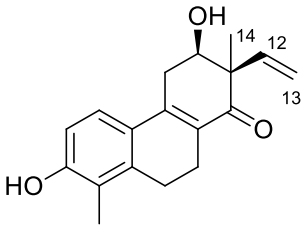


(±)-**1**

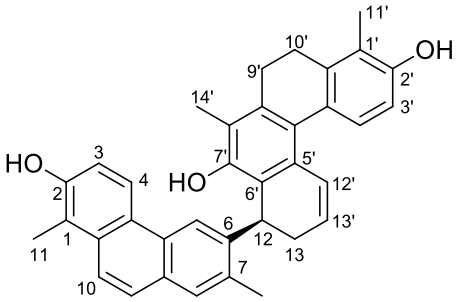


2

JIN-12 (**2**) was obtained as an amorphous solid; it gave a molecular formula $C_{19}H_{20}O_3$ as determined by HRESIMS, through the presence of a peak at *m/z* 297.1392 $[M+H]^+$ (calcd. for $C_{19}H_{21}O_3$, 297.1380). The ^1H and ^{13}C NMR spectra (**Annex II**) suggested a 9,10-dihydrophenanthrene skeleton with vinyl substitution, similar to that of juncusol (**7**), isolated previously from other *Juncus* species (*J. acutus*, *J. effusus* and *J. roemerianus*).[66] The differences found were that a hydroxymethyl group instead of the methyl group is linked to the skeleton at position C-1, and methoxy group (δ_H 3.84, δ_C 56.1) could be placed at C-2 according to its HMBC correlation with C-2 (δ_C 157.4). The position of the hydroxymethyl group was determined by the HMBC cross-peaks between methylene protons (H-11) and C-1, C-1a and C-2. Nuclear Overhauser effects were observed between OCH_3-2/H-3, H-3/H-4, H-12/H_3-14, H_2-13/H_3-14, H-8/H-9 and H-10/H_2-11. All this evidence confirmed the structure of **2** (jinflexin B) as 7-hydroxy-6-methyl-1-hydroxymethyl-2-methoxy-5-vinyl-9,10-dihydrophenanthrene.



3



4

JIN-15 (**3**) was isolated as an amorphous yellow powder with $[\alpha]_D^{26}$ –19 (*c* 0.2, MeOH). Its HRESIMS provided a molecular formula of $C_{18}H_{20}O_3$ through the presence of a peak at *m/z* 285.1498 $[M+H]^+$ (calcd. for $C_{18}H_{21}O_3$, 285.1485). The ^1H and ^{13}C NMR chemical shifts at δ_H 2.24, 2.65, 2.57 and 2.84 and δ_C 21.1 and 25.1 (C-9, C-10) confirmed a dihydrophenanthrene structure of **3**. The ^1H NMR spectrum exhibited two methyl singlets at δ_H 1.32 and 2.15, two *ortho*-coupled aromatic protons at δ_H 7.23 (J = 8.6 Hz) and 6.71 (J = 8.5 Hz), and a vinylic system at δ_H 5.13, 4.90 and 6.10 (C-12, C-13)

(**Annex III**). The ^{1}H–^{1}H COSY correlations of the protons at δ_H 2.72, 2.98 and 3.88, and the chemical shifts of their carbons (δ_C 33.6 and 74.4) indicated a –CH_2-CH(OH)– unit (–C-5–C-6–) in the molecule. This structural part was found to form a keto-substituted six-membered ring together with quaternary carbons at δ_C 149.4, 128.8 and 55.5, according to the long-range correlations between H-5 and C-7, C-4a, C-8a, C-5a; H-6 and C-12, C-14; and H-12/C-8. The methyl and vinyl substitution at C-7 was indicated by the HMBC cross-peaks between H_2-13/C-12 and C-7 as well as H_3-14 and C-6, C-7, C-8, C-12, respectively. In the HSQC spectrum the protons of the methyl group at δ_H 2.15 (H_3-11) were correlated to the carbon at δ_C 11.4 (C-11), and had HMBC correlations with C-1, C-1a and C-2. There was a hydroxy group at position C-2, which was suggested by the HMBC correlations between C-2 (δ_C 159.4)/H-3 and H-4. All the above evidence confirmed the planar structure of jinflexin C (**3**). The (6*S**,7*S**) relative configuration of **3** was determined on the basis of NOE correlations observed between α-oriented H-5 (δ_H 2.98 m) and H-6, and β-oriented H-5 (δ_H 2.72 m) and H-12.

In contrast to jinflexin A (**1**), jinflexin C (**3**) was found to be optically active with a negative specific rotation, and a distinct ECD spectrum could be recorded as well (in **Appendix I**). In order to check on the possibility of partial racemization, chiral HPLC analysis of **3** was carried out under the same conditions used for jinflexin A (**1**) (Chiralpack IA, *n*-hexane–propan-2-ol 9:1), which showed 80% enantiomeric excess. HPLC-ECD measurements and HPLC-ECD traces confirmed the enantiomeric relationship of the separated components. For the determination of the absolute configuration, the same ECD calculation protocol was carried out as for jinflexin A (**1**). The initial MMFF conformational search of the arbitrarily chosen (6*S*,7*S*) enantiomer yielded 96 conformers, which were reoptimized at the B3LYP/6-31G(d) level in vacuo and the B97D/TZVP PCM/CH_3CN levels resulting in ten and 12 conformers above 2%, respectively. In the lowest-energy B3LYP/6-31G(d) conformer, the C-7 vinyl group adopted an axial orientation, while OH-6 has an equatorial orientation. This conformation of ring C was represented by seven other computed conformers totalling 71.1% at the population, and an equatorial C-7 vinyl group was found in conformers C and J with a total population of 10.1%. The structures of the low-energy conformers were in agreement with the observed NOE correlations of jinflexin C (**3**). The ECD spectra of (6*S*,7*S*)-**3** computed for the gas-phase or solvent model conformers gave mirror-image agreement of the experimental ECD recorded in acetonitrile allowing for the assignment of the absolute configuration for the major enantiomer (second-eluting enantiomer in the chiral HPLC analysis) of jinflexin C (**3**) as (6*R*,7*R*).

JIN-19 (**4**) was isolated as an amorphous solid with $[\alpha]_D^{26}$ 0 (*c* 0.1, MeOH). The HRESIMS (*m/z* 513.2448 $[M+H]^+$) established a molecular formula $C_{36}H_{32}O_3$ (calcd. for $C_{36}H_{33}O_3$ 513.2424), suggesting the dimeric nature of compound **4**. In the JMOD spectrum the presence of 36 signals was detected (**Annex IV**). The ^{1}H NMR spectrum showed the sharp singlets for the H-11, H-11′, H-14 and H-14′ methyls, the doublets for H-3, H-4, H-9, H-10, H-3′ and H-4′, the aromatic singlets for the H-5 and H-8

protons, and signals for H-12′ and H-13′ (**Annex IV**). According to the NMR data, one of the monomers is a tetrasubstituted phenanthrene containing two methyls and one hydroxy groups and a trisubstituted carbon, while the other part of the molecule is a dihydrophenanthrene substituted with two methyls and two hydroxy groups. The linkage between these units was determined on the basis of $^{1}H–^{1}H$ COSY and HMBC experiments (**Figure 5**).

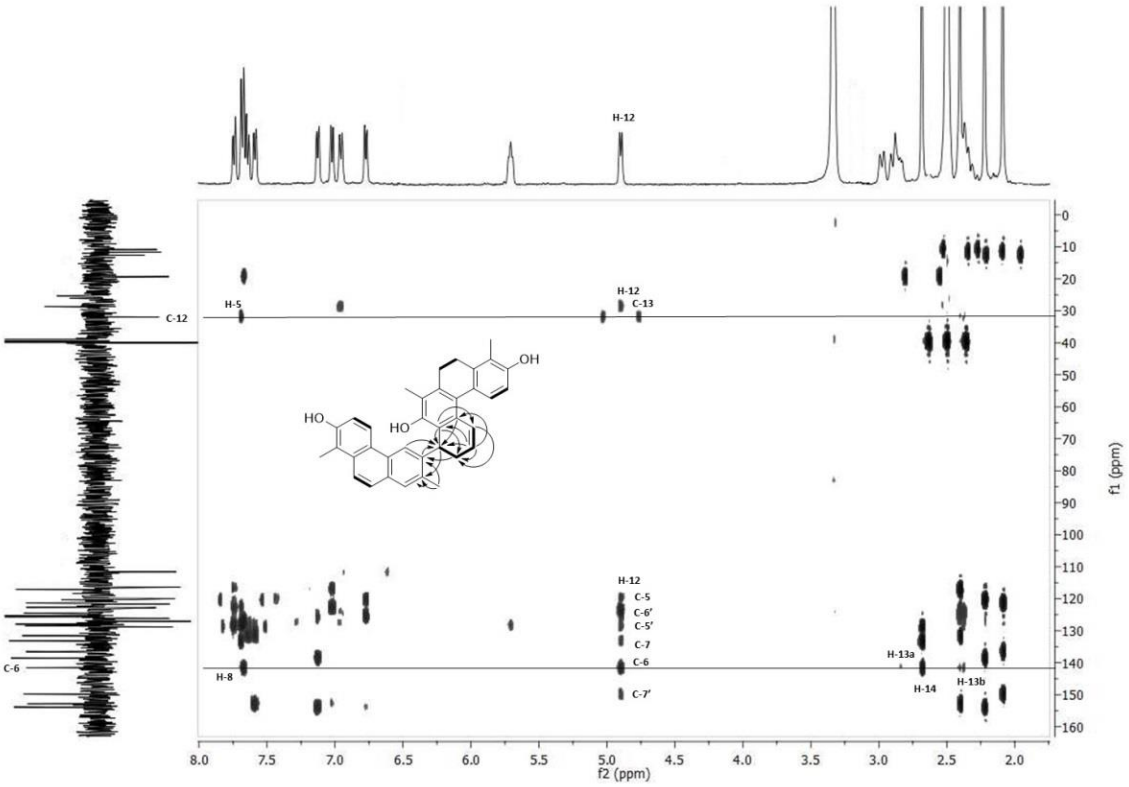


Figure 5. HMBC spectrum, $^{1}H–^{1}H$ COSY (**—**) and HMBC (H→C) correlations of jinflexin D (**4**)

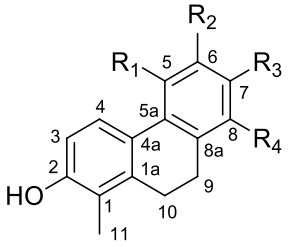
The $^{1}H–^{1}H$ COSY spectrum defined the structural fragment –$CHCH_2$–CH=CH– with the relevant correlated protons (H-12-H-13-H-13′-H-12′). The H-12 proton was correlated in the HMBC spectrum with C-5, C-5′, C-6, C-6′, C-7, C-7′, and C-13′. The long-range correlations of the H_3-13 protons with the C-6, C-6′, C-12, C-12′ and C-13′ carbons also indicated linkages to occur between C-12/C-6′ and C-13/C-13′ of the units, forming a new ring system containing a tri- and a tetracyclic unit. Most probably, during the biosynthesis process, two vinyl substituted phenanthrene monomers were connected through their vinyl groups producing a new ring. The HMBC long-range correlations observed between H_3-11/C-1, C-1a and C-2, and H_3-14/C-8, C-8a and C-7, and H_3-11′/C-1′, C-1a′ and C-2′, and H_3-14′/C-8′, C-8a′ and C-7′ indicated the C-1, C7, C-1′ and C-7′ positions of methyl groups and the C-2, C-2′ and C-7′ positions of hydroxy groups, respectively.

The NOESY correlations between H-3/H-4, H-4/H-5, H-8/H_3-14, H-9/H-10, H-10/H_3-11, H_3-14/H-12 and H-13, H-13/H-13′, H-12′/H-13′, H-4′/H-12′, H-11′/H-10′, H-9′/H-10′ and H-9′/H_3-14′ further

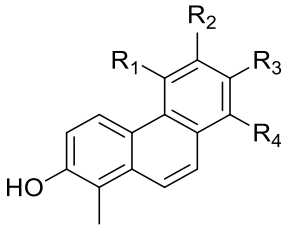
confirmed the proposed structure of the molecule. On the basis of the above findings, the structure of this compound (jinflexin D) was established as depicted in structural formula **4**.

Although a distinct ECD spectrum for jinflexin D (**4**) could be recorded in acetonitrile, the chiral HPLC analysis showed only 9% enantiomeric excess and the HPLC-ECD spectra of the separated enantiomers were also recorded. DFT reoptimization of the initial 40 MMFF conformers generated for the arbitrarily chosen (*R*)-**4** yielded nine and nine low-energy conformers (≥ 2%) at B3LYP/6-31G(d) in vacuo and B97D/TZVP PCM/$CHCl_3$ levels, respectively. The B97D/TZVP PCM/$CHCl_3$ conformers differed only in the orientation of the OH protons, while the relative arrangement of the fused tricyclic and tetracyclic units was near identical (in **Appendix I**). The ECD spectra of (*R*)-**4** computed for the B97D/TZVP PCM/$CHCl_3$ conformers and the Boltzmann-weighted ECD curve reproduced well the HPLC-ECD spectrum of the second-eluting enantiomer of jinflexin D (**4**) and it was a mirror image of the experimental solution ECD spectrum. Thus, the absolute configuration of the first-eluting enantiomer of jinflexin D (**4**) was determined unambiguously determined as (*R*), with the enantiomeric excess derived from the (*S*) enantiomer (second-eluting enantiomer).

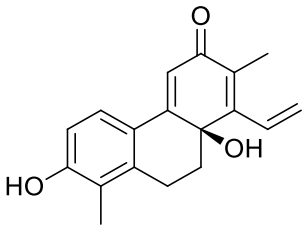
Besides the new compounds (**1**–**4**), dehydrojuncuenin A [**JIN-1** (**9**)], juncuenin A [**JIN-2** (**5**)], juncuenin B [**JIN-3** (**6**)], dehydrojuncusol [**JIN-4** (**10**)], juncusol [**JIN-5** (**7**)], dehydrojuncuenin B [**JIN-11** (**11**)], juncuenin D [**JIN-13** (**8**)] and chrysoeriol [**JIN-8** (**12**)] were also isolated from the roots of *J. inflexus* (**Annex I**). Their structures were determined by comparison of their APCI-MS and ^1H and ^{13}C NMR data with those published in the literature.[25,99-101] In the case of jucuenin B (**6**), juncusol (**7**), juncuenin D (**8**), dehydrojuncusol (**10**), and dehydrojuncuenin B (**11**) the previously published NMR data were completed with ^1H and ^{13}C NMR data determined in methanol or dimethyl sulfoxide (in **Appendix I**).



	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	$CH=CH_2$



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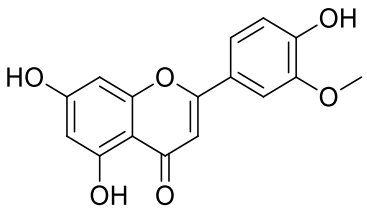

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Although juncuenin D (**8**) is a chiral derivative, it had been isolated previously as a racemic mixture and thus characterization of its enantiomers has not been reported yet.[25] The chiral HPLC analysis of juncuenin D (**8**) showed 4% enantiomeric excess, which however was not sufficient to record an acceptable solution ECD spectrum. Thus, the HPLC-ECD spectra of the separated enantiomers were recorded and ECD calculations were performed to assign the absolute configuration. The 13 initial MMFF conformers of the arbitrarily chosen (*S*)-**8** were reoptimized at B3LYP/6-31G(d) in vacuo and B97D/TZVP PCM/$CHCl_3$ levels resulting in six and nine low-energy conformers above 2%, respectively. Two groups of the low-energy B3LYP/6-31G(d) in vacuo conformers could be distinguished based on the relative orientation of the vinyl group. Conformers of the two groups had substantially different computed ECD curves as represented by conformers A and B. The Boltzmann-weighted ECD spectra of the B3LYP/6-31G(d) in vacuo conformers of (*S*)-**8** reproduced well the HPLC-ECD spectrum of the second-eluting enantiomer of juncuenin D (**8**), allowing the determination of absolute configuration for the separated enantiomers (in **Appendix I**).

5.5.2. Compounds from *Luzula luzuloides*

From the CH_2Cl_2 extract of *L. luzuloides* six compounds [**LUB-1** (**7**), **LUB-3** (**13**), **LUB-4** (**6**), **LUB-6** (**11**), **LUB-9** (**14**) and **LUB-10** (**15**)] were isolated with the combination of different chromatographic techniques.

LUB-3 (**13**) was obtained as an amorphous solid with $[\alpha]_D^{26}$ 0 (*c* 0.1, MeOH). Its HRESIMS provided the molecular formula $C_{18}H_{16}O_3$ through the presence of a peak at *m/z* 281.1175 $[M+H]^+$ (calcd. for $C_{18}H_{17}O_3$, 281.1172). The ^{1}H NMR spectrum (**Annex III**) displayed signals of two pairs of *ortho*-coupled aromatic protons (δ_H 6.25 d/8.35 d and δ_H 8.12 d/7.63 d), and one aromatic proton as a singlet (δ_H 7.52), two methyl singlets at δ_H 1.53 and δ_H 2.35, and a vinylic system at δ_H 5.81, 5.35 and 7.05 (C-14, C-13). In the JMOD spectrum, the presence of 18 carbon signals was detected (**Annex III**). In the ^{1}H NMR spectrum the presence of *ortho*-coupled aromatic protons at δ_H 8.12 d and 7.63 d could be assigned to the H-9 and H-10 protons of a phenanthrene derivative. In the ^{1}H–^{1}H COSY spectrum four correlations were observed between δ_H 8.12 d and 7.63 d (H-9/H-10), between δ_H 6.25 d and 8.35 d (H-3/H-4), between δ_H 7.05 dd and 5.81 d (H-13/H-14a), and between δ_H 7.05 dd and 5.35 d (H-13/H-14b). The two *ortho*-coupled doublets at δ_H 6.25 and 8.35 were assigned to the H-3 and H-4 protons. According to the ^{13}C NMR signals at δ_C 207.1 one carbonyl group was identified, which was placed to

C-2 as confirmed by HMBC correlations of the H_3-11 (δ_H 1.53) and H-4 (δ_H 8.35 d) with C-2 (δ_C 207.1) (**Annex III**). The location of one of the methyl groups (δ_H 1.53 s) was confirmed at C-1 on the basis of its HMBC correlation with the quaternary carbons δ_C 146.6 (C-1a), 78.1 (C-1) and 207.1 (C-2) (**Figure 6**). The linkage of hydroxy groups to C-1 and C-6 was confirmed by the chemical shifts of the quaternary carbons at $\delta_{C\text{-}1}$ 78.1 and $\delta_{C\text{-}6}$ 157.0, respectively. In the HMBC spectrum another methyl group was assigned at C-7 by the correlation of H_3-12/C-7. The vinyl substitution at C-8 was indicated by the HMBC cross-peaks between H-14a/C-8 and H-14b/C-8.

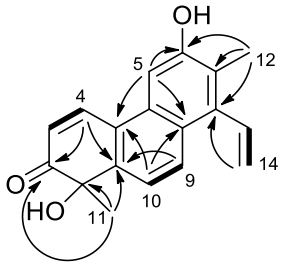


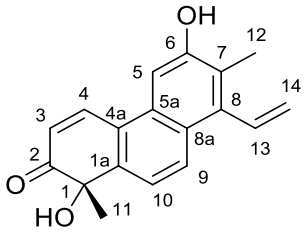
Figure 6. Diagnostic COSY (**—**) and HMBC correlations (H→C) for **13**

The NOESY correlations further confirmed the structure of compound **13**. *Overhauser* effects were detected between H-3/H-4, H-4/H-5, H-9/H-10, H-9/H-13, H-9/H-14b, H-13/H_3-12, H-14b/H_3-12. All of the above evidence confirmed the planar structure of **13** named as luzulin A.

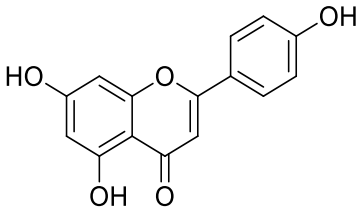
Luzulin A (**13**) is a chiral natural product but it had a very weak ECD spectrum in acetonitrile and zero specific rotation, which suggested that it had low enantiomeric excess. Thus chiral HPLC analysis of luzulin A (**13**) was carried out, which resulted in the separation of the two enantiomers on a Chiralpak IA column and determined the enantiomeric excess as 25%.

In order to elucidate the absolute configuration of the enantiomers and identify the major enantiomer, HPLC-ECD spectra of the separated enantiomers were recorded and the solution TDDFT-ECD protocol was applied on the arbitrarily chosen (*R*) enantiomer (in **Appendix III**).[90] The initial MMFF conformational search resulted in five conformers in a 21 kJ/mol energy window, which were reoptimized at various DFT levels [B3LYP/6-31G(d) in vacuo, B97D/TZVP PCM/$CHCl_3$ and CAM-B3LYP/TZVP PCM/$CHCl_3$] and ECD spectra were computed with B3LYP, BH&HLYP, CAM-B3LYP and PBE0 functionals and TZVP basis set. The computed conformers differed in the orientation of the 6-OH proton and the C-8 vinyl group, the latter of which influenced the ECD spectra as well. The 1-OH group adopted equatorial position in all the computed conformers. All the applied combinations for all conformers reproduced the first two transitions at ca 422 and 375 nm and Boltzmann-weighted spectra acquired at all applied levels resembled also the high-energy transitions at ca. 227 and 206 nm allowing the elucidation of the absolute configuration as (*R*) for the first-eluting enantiomer (minor component) and (*S*) for the second-eluting enantiomer (major component). Interestingly, the 312 nm Cotton effect (CE) could not be reproduced by any applied combination of theoretical levels, which can

be attributed to difficulties in estimating the proper orientation of the conjugating quite freely rotating vinyl chromophore and the overlapping transitions of this region.[91]



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



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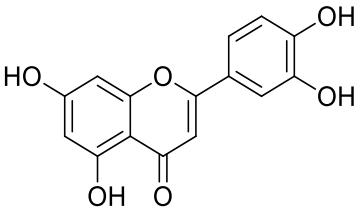

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Besides the new compound, luzulin A (**LUB-3**, **13**), two dihydrophenanthrenes [juncusol (**LUB-1**, **7**) and juncuenin B (**LUB-4**, **6**)], one phenanthrene [dehydrojuncuenin B (**LUB-6, 11**)] and two flavonoids [apigenin (**LUB-9**, **14**) and luteolin (**LUB-10**, **15**)] were obtained from *L. luzuloides* (**Annex I**). Their structures were identified by analyses of MS, 1D and 2D NMR data, and by comparison with literature data.[25,99,102,103]

Juncuenin B (**JIN-3**, **LUB-4**, **6**), juncusol (**JIN-5**, **LUB-1**, **7**) and dehydrojuncuenin B (**JIN-11**, **LUB-6**, **11**) were isolated from both *J. inflexus* and *L. luzuloides*.

5.6. OXIDATION OF JUNCUENIN B

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, occurring up to 0.0243%. 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**) from *L. luzuloides* and juncuenin D (**8**) 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, [bis(trifluoroacetoxy)iodo]benzene (commonly known as PIFA)].

50.0 mg of juncuenin B (**6**) was dissolved in $CH_3CN–H_2O$ (9:1, 50 mL), then 2 eq. PIFA (106.1 mg) was added to the solution in a careful manner to prevent sudden boiling. The reaction was monitored by TLC using cyclohexane–EtOAc–EtOH (20:10:1) as solvent system. After 30 min of stirring at room temperature all of the starting material, juncuenin B (**6**) was converted. Therefore, the mixture was cooled down in an ice bath, and evaporated at room temperature. Three major compounds were isolated from the mixture by NP-VLC, Sephadex LH-20 gel filtration and preparative TLC.

5.7. SEPARATION OF THE OXIDATIVE PRODUCTS OF JUNCUENIN B

The concentrated mixture was chromatographed by NP-VLC on silica gel (sorbent: 10.0 g, column: 25 mm × 100 mm) with a gradient system of CH_2Cl_2–acetone [from 99:1 to 7:3 (200 mL/eluent), and finally with MeOH (150 mL); volume of each collected fraction was 20 mL] to yield seven major fractions (1–7). Dehydrojuncuenin B (**11**) (8.9 mg) was purified from fraction 2 after gel filtration using MeOH–CH_2Cl_2 (3:1) as eluent [(60 mL); volume of collected fractions was 3 mL]. Fraction 4 was also separated by gel filtration with MeOH as eluent [(50 mL); volume of collected fractions was 2 mL] to afford three subfractions (4/1–3). From subfraction 4/2 luzulin A (**13**) (8.1 mg) was obtained by the use of NP-PLC using toluene–acetone (4:1) as solvent system. Juncuenin D (**8**) (11.2 mg) was isolated from fraction 6 after gel filtration using MeOH–CH_2Cl_2 (3:1) as eluent [(50 mL); volume of collected fractions was 2 mL]. The compounds were identified according to their TLC patterns and comparison of their ^{1}H NMR spectrum with literature data.[25]

6. DISCUSSION

6.1. SCREENING STUDIES

The aim of the work was to perform a screening study on the members of the Juncaceae family native to the Carpathian Basin for antibacterial and anti-inflammatory activity. Although some experimental evidence and ethnobotanical data have accumulated concerning the biological activity of Juncaceae species, no comprehensive screening studies have yet been published on the plants of this family.[7-12]

The investigated Juncaceae species were collected in several regions of Hungary, Croatia and Romania. Among the selected species, previously only *J. acutus* and *J. effusus* were studied comprehensively from both phytochemical and pharmacological aspects.[9,22-24,27-31] Moreover, carotenoids (lutein and β-carotin) and triterpenoids (β-sitosterol and stigmasterol) were obtained from *J. gerardii*.[43] The flavonoid content of the stems and leaves of *J. acutus*, *J. articulatus*, *J. conglomeratus*, *J. effusus*, *J. gerardii*, *J. inflexus*, *J. maritimus*, *J. squarrosus*, *J. tenuis*, *J. trifidus*, *L. campestris*, *L. forsteri*, *L. sudetica* and *L. sylvatica* was reported earlier, but none of the plants (except *J. acutus* and *J. effusus*) were investigated extensively before our studies.[42,49]

In the presented work, the antibacterial effects of 19 species belonging the genera *Juncus* (14) and *Luzula* (5) of the family Juncaceae were tested *in vitro* against ten multiresistant bacterial strains, by the use of disc-diffusion and microdilution methods. The plant samples, where it was possible, were separated into different plant organs, and extracted with an amphipolar solvent (MeOH), which permitted the isolation of lipophilic and polar components. Solvent–solvent partition was performed between *n*-hexane (extract A), CH_2Cl_2 (extract B), EtOAc (extract C) and the remaining H_2O extract were named as extract D, to get altogether 96 fractions with different polarity. All fractions were screened for their antibacterial activity. According to the assays, several extracts showed anti-MRSA activity with mild to strong effectiveness. Sixteen extracts (extracts B and D) from *Juncus* species and three extracts B from *Luzula* species showed strong to moderate inhibitory activities against MRSA strains. Among them, extracts B of the roots of *J. inflexus*, and the roots of *J. effusus* showed remarkable activities. Extracts B of the whole plants of *J. maritimus* and *J. gerardii* displayed marked activities, and mild antibacterial activities were observed for the extract B of *J. acutus* and for the extract B of the aerial parts of *J. tenuis*. Weak antibacterial activities were recorded for *L. campestris*, *L. forsteri* and *L. sylvatica*. The differences between the chemical composition of genera *Juncus* and *Luzula* could serve as an explanation for the different activities. Up to now, much more phenanthrenes were reported from the species of genus *Juncus*, however the genus *Luzula* is far less studied than the genus *Juncus*. Previously, phenanthrenes were mainly isolated from the apolar fractions of the plants, therefore it was assumed that the majority of the antibacterial activity can be related to these compounds, but other compounds may also have influence on the overall activity.[22]

Extracts prepared from five *Luzula* species were evaluated for their anti-inflammatory activities by measuring their superoxide anion generation and elastase release inhibitory effects on human neutrophils. All of the investigated plants were found to be active. Among the fractions with different polarities, the CH_2Cl_2-soluble fractions had the most remarkable activities. All of the prepared fractions of *L. luzuloides* displayed remarkable activities in both assays.

According to the preliminary screening results *J. inflexus* and *L. luzuloides* were chosen for further studies.

Our comprehensive screening investigations on the selected Juncaceae species provided valuable data on the antibacterial and anti-inflammatory activities of numerous plants, which may contribute to the selection of other species for future pharmacological and phytochemical work.

6.2. INVESTIGATION OF *JUNCUS INFLEXUS AND LUZULA LUZULOIDES*

Chemical investigations of *J. inflexus* and *L. luzuloides* resulted in the isolation of 15 compounds, including five new (**1**–**4** and **13**) natural products. The structures were identified by means of spectral methods as phenanthrenes, 9,10-dihydrophenanthrenes, a diphenanthrene and flavonoids. Pharmacological analysis confirmed that some of the isolated compounds possess biological activity.

6.2.1. Isolation of bioactive compounds

Previous pharmacological investigations of the extracts with different polarity prepared from Juncaceae species (see sections **5.1** and **5.2**) and reviewing the literature data led to the conclusion that mainly the lipophilic CH_2Cl_2 extracts contain the bioactive secondary metabolites.

The air-dried plant materials were percolated with MeOH at room temperature and then solvent–solvent partition was performed to yield the apolar *n*-hexane and CH_2Cl_2 phases. In both cases, the CH_2Cl_2 phases were subjected to a series of chromatographic steps as well as a combination of several chromatographic techniques in order to isolate the compounds responsible for the pharmacological effects.

The first fractionation of the CH_2Cl_2-soluble phase of *J. inflexus* was carried out by OCC, to afford four main fractions (J1–4); among them, fraction J3 was the most interesting from both phytochemical and pharmacological aspects. Since this fraction demonstrated great chemical complexity, more selective methods (VLC, MPLC) were applied on normal and reversed phase silica gel, and different solvent systems were used for gradient elution. The final purification of the pure compounds was performed by the use of NP-PLC, RP-PLC, GF, and RP-HPLC (**1**–**12**).

The CH_2Cl_2 phase of the methanol extract of *L. luzuloides* was separated by polyamide OCC. The fraction obtained with MeOH–H_2O 4:1 was subjected to multiple chromatographic separations, including VLC, Sephadex LH-20 gel chromatography and PLC, to yield six compounds (**6**, **7**, **11**, **13**–**15**).

The preparative work was completed with analytical TLC on silica gel with various solvent systems. The detection was carried out in UV light at 254 and 366 nm, followed by spraying with vanillin-sulfuric acid reagent and heating at 120 °C for 5 min.

6.2.2. Structure elucidation

The chemical structures of the isolated compounds were determined by means of spectroscopic methods. The molecular masses and compositions were obtained from MS investigations; optical rotation measurements provided further important information for characterization of the compounds. The most useful data concerning the structures were provided 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 the case of some known compounds, where previously published data were incomplete. The absolute configuration of the new compounds was determined by TDDFT-ECD calculations and enantiomeric purity was checked by chiral HPLC analysis.

Eleven phenanthrenes were isolated from *J. inflexus*. Four compounds (jinflexins A–D, **1**–**4**) are new natural products. Jinflexin A (**1**) is a methoxyethyl substituted 9,10-dihydrophenanthrene, which does not have a vinyl substitution, but the position of the methoxyethyl group suggests that biogenetically this component is originated from a vinylated compound. 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 (**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 (**3**) is a carbonyl (C-1) substituted 5,6,9,10-tetrahydrophenanthrene, not only its core is unusual, but also the presence of a methyl and a vinyl group at the same carbon (C-7). On the basis of the TDDFT-ECD calculations and chiral HPLC analysis, jinflexin C (**3**) is a 6*R*,7*R* enantiomer with 80% ee. Jinflexin D (**4**) is a dimer with an unprecedented heptacyclic ring system, which may be considered to derive by the coupling of 2-hydroxy-1,7-dimethyl-6-vinyl-phenanthrene (dehydrojuncuenin A, **9**) with 2,7-dihydroxy-1,8-dimethyl-5-vinyl-9,10-dihydrophenanthrene through their vinyl groups forming a unique structure. Distinct ECD spectrum could be recorded for jinflexin D (**4**), and the chiral HPLC analysis showed a 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*) enantiomer. Previously, phenanthrene dimers were published from *J. acutus* and *J. effusus*, but in those compounds neither or only one of the vinyl groups took part in the dimerization.[23,29,79,80] Among the known phenanthrenes, six compounds can be paired, based on the saturation of the C-9–C-10

bond. 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**) (see section **5.7**). The absolute configuration of juncuenin D (**8**) was also determined, and its (*S*) enantiomer has 4% of enantiomeric excess. All of the phenanthrenes were isolated for the first time from the plant. In accordance with the previous screening focused on the flavonoid contents of the plant, chrysoeriol was also isolated.[42]

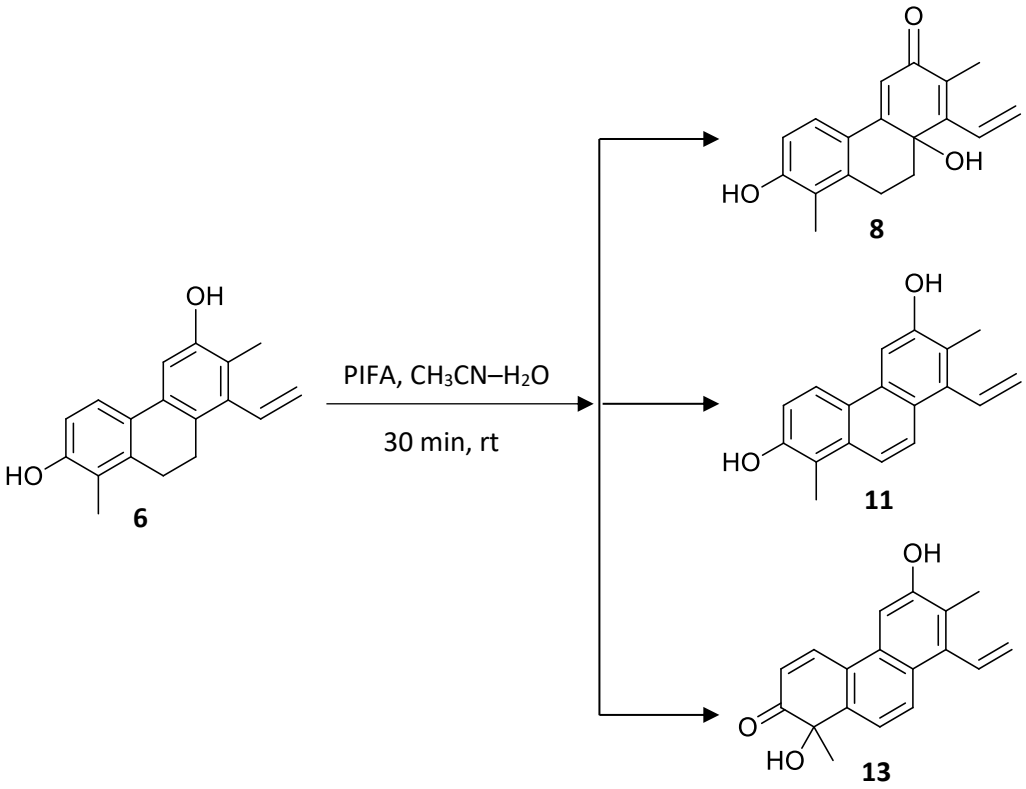
The structure analysis of compounds isolated from *L. luzuloides* led to the identification of four phenanthrenes and two flavonoids. A new 1,6-dihydroxy-2-keto-1,7-dimethyl-8-vinyl-1,2-dihydrophenanthrene (luzulin A, **13**) was identified from the plant. Its chiral HPLC analysis and TDDFT-ECD calculations suggested that luzulin A (**13**) is an (*S*) enantiomer, with 25% ee. Three known phenanthrenes [juncuenin B (**6**), juncusol (**7**) and dehydrojuncuenin B (**11**)] and two flavonoids [apigenin (**14**) and luteolin (**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*.

Among the isolated chiral compounds, jinflexin C (**3**), jinflexin D (**4**) and luzulin A (**13**) have quaternary stereogenic centers, which do not allow the inversion of the absolute configuration during the isolation. Therefore, the low enantiomeric excess can be attributed to the low enantioselectivity of the biosynthetic step, which has been observed for jinflexin D (**4**) and luzulin A (**13**) as well.

6.2.3. Biosynthetic pathways

The oxidative transformation of juncuenin B (**6**) led to the isolation of its possible biometabolites (**Scheme 1**). The compounds were identified as juncuenin D (**8**, 22%), dehydrojuncuenin B (**11**, 18%) and luzulin A (**13**, 16%) according to their TLC patterns and comparison of their ^{1}H NMR spectrum with literature data.[25] These findings confirm that the phenanthrenequinones, luzulin A (**13**) and juncuenin D (**8**), and the phenanthrene, dehydrojuncuenin B (**11**) can be formed by oxidation of juncuenin B (**6**), and most probably similar process may happen during their biosynthesis.

Scheme 1. Oxidation of juncuenin B (**6**)



6.2.4. Chemotaxonomy

The isolation of vinylated 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. Our results confirmed the close chemotaxonomical relationship of the two genera.

6.3. BIOACTIVITY OF THE ISOLATED COMPOUNDS

6.3.1. *Juncus inflexus*

In our preliminary screening the apolar extract 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 (**Table 3**). Among the isolated phenanthrenes, noteworthy inhibitory activities were recorded for jinflexin B (**2**), juncusol (**7**), juncuenin D (**8**), and dehydrojuncuenin B (**11**). 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, in accordance with previously reported studies.[32,33] 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. Other compounds were proved to be inactive against MRSA.

Table 3. Anti-MRSA activity of isolated compounds

Compound	MRSA (ATCC43300) inhibitory activity	
	Inhibition (diameter of inhibition zone in mm)	MIC (μg/mL)
1 (jinflexin A)	inactive	
2 (jinflexin B)	7.0 ± 0.1	100
3 (jinflexin C)	inactive	
4 (jinflexin A)	inactive	
5 (juncuenin A)	inactive	
6 (juncuenin B)	inactive	
7 (juncusol)	12.0 ± 0.6	25
8 (juncuenin D)	12.0 ± 0.3	12.5
9 (dehydrojuncuenin A)	inactive	
10 (dehydrojuncusol)	inactive	
11 (dehydrojuncuenin B)	10.0 ± 0.2	25
12 (chrysoeriol)	inactive	
vancomycin*	15.5 ± 0.6	2

*Positive control: vancomycin (5 μg/disc)

6.3.2. *Luzula luzuloides*

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). 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 (IC_{50} = 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.

These results indicated that the unsaturation of ring B in case of juncuenin B (**6**) resulted in the loss of the effect of the compound on the elastase release, but dehydrojuncuenin B (**11**) inhibits the superoxide generation slightly more effective 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.

Table 4. Inhibitory effects of the 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.

6.4. HPLC-MS INVESTIGATION OF THE OCCURRENCE OF COMPOUNDS

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 (**Table 3**). The presence of these compounds was investigated by HPLC-MS in the most active Juncaceae extracts (CH_2Cl_2-soluble fractions of the roots of *J. effusus* and *J. inflexus*, whole plants of *J. acutus, J. gerardii* and *J. maritimus*, and aerial parts of *J. tenuis*) (**Table 1**).

The detected active compounds could play an important role in the antibacterial effects of the extracts, which can involve different pharmacological mechanisms (**Figure 7**). Although juncusol (**7**) was detected 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 chemical composition of *J. gerardii*, *J. maritimus* and *J. tenuis* were not studied thoroughly; therefore, some other, unidentified compounds may play a role in the activities of these extracts.

All of the isolated 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.

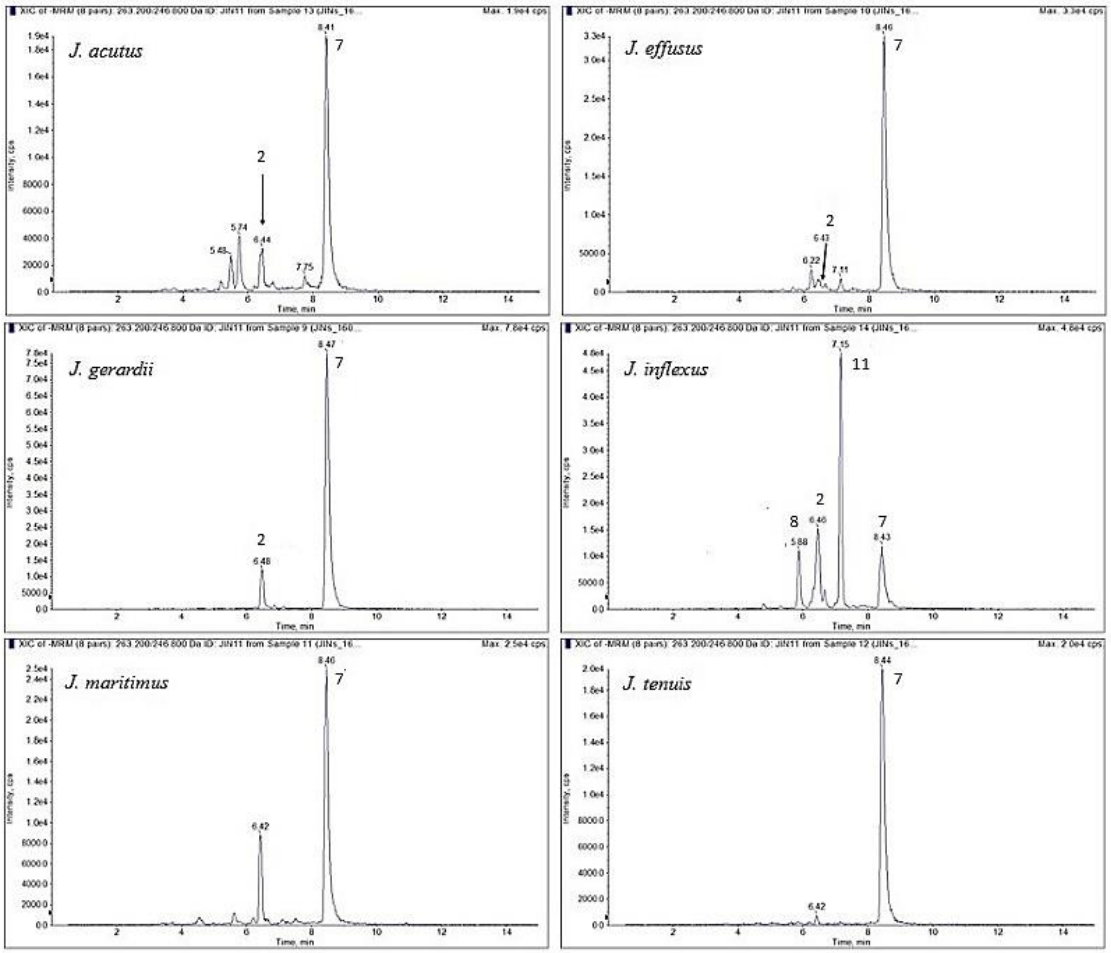


Figure 7. HPLC chromatograms of plant extracts and identification of the bioactive compounds (**2**, **7**, **8**, **11**)

7. SUMMARY

The primary aim of the present work was the evaluation of antibacterial and anti-inflammatory effects of Juncaceae species native to the Carpathian Basin, and the isolation and structure determination of biologically active compounds from the most promising species, *Juncus inflexus and Luzula luzuloides*.

Extracts with different polarity of 19 Juncaceae species were screened *in vitro* for their antimicrobial effects against methicillin-resistant *S. aureus*, extended-spectrum β-lactamase (ESBL)-producing *C. freundii*, *E. coli*, *E. cloacae*, *K. pneumoniae*, multiresistant *A. baumannii* and *P. aeruginosa* by disc-diffusion and microdilution methods. According to the results *Juncus inflexus* was chosen for further studies.

The *Luzula* species were assayed for their anti-inflammatory properties. The CH_2Cl_2-soluble fraction of *Luzula luzuloides* showed the most remarkable anti-inflammatory activities, it significantly inhibited the superoxide anion generation and the elastase release on fMLP/CB-induced human neutrophils.

Pharmacological evaluations of the extracts with different polarity prepared from the collected species led to the conclusion that the lipophilic extracts (CH_2Cl_2) contain the most valuable bioactive secondary metabolites. Isolation of the compounds from these fractions was carried out by multistep separation procedures, including OCC, VLC, MPLC, PLC, GF and RP-HPLC. The structures of the isolated compounds were elucidated by means of spectroscopic methods (HRMS, MS and NMR). In addition, complete ^{1}H and ^{13}C NMR assignments were made for the characterization of the compounds.

Four new natural phenanthrenes [jinflexin A (**JIN-7**, **1**), jinflexin B (**JIN-12**, **2**), jinflexin C (**JIN-15**, **3**) and jinflexin D (**JIN-19**, **4**)] were isolated from the root parts of *J. inflexus*. Jinflexin C (**JIN-15**, **3**) is a novel unusually substituted phenanthrenequinone, and jinflexin D (**JIN-19**, **4**) is a dimer with a novel chemical skeleton containing a unique heptacyclic ring system.

Besides the four new compounds, four dihydrophenanthrenes [juncuenin A (**5**), juncuenin B (**6**), juncusol (**7**), juncuenin D (**8**)], three phenanthrenes [dehydrojuncuenin A (**9**), dehydrojuncusol (**10**), dehydrojuncuenin B (**11**)], and the flavonoid chrysoeriol (**12**) were also obtained from the plant. In cases of jinflexin A (**1**), jinflexin C (**3**), jinflexin D (**4**) and juncuenin D (**8**) the absolute configurations were also determined.

Four phenanthrenes possessed remarkable inhibitory activities against MRSA strains. Juncusol (**7**) and juncuenin D (**8**) displayed significant activity, moreover marked activity were recorded for jinflexin B (**2**) and dehydrojuncuenin B (**11**). The HPLC-MS investigation of the chemical composition of other significantly active Juncaceae extracts revealed the presence of juncusol (**7**) in all the investigated extracts, and the presence of jinflexin B (**2**) in some cases. Species belonging to the genus *Juncus*,

especially *J. gerardii*, *J. maritimus* and *J. tenuis* are promising candidates for further activity-guided fractionation in the search for new active antibacterial natural compounds.

The apolar extract of *L. luzuloides* exerted noteworthy anti-inflammatory activity *in vitro*. Comprehensive preparative phytochemical work resulted in the isolation of a new phenanthrenequinone, luzulin A (**13**), three known phenanthrene derivatives [juncuenin B (**6**), juncusol (**7**) and dehydrojuncuenin B (**11**)] and two flavonoids [apigenin (**14**) and luteolin (**15**)] from the plant. This was the first time that phenanthrenes were detected from a *Luzula* species, which could confirm that besides flavonoids, phenanthrenes could also serve as chemotaxonomic markers for *Luzula* species and prove the close relationship of genera *Juncus* and *Luzula*.

The oxidative semi-synthetic transformation of juncuenin B (**6**) led to the isolation of its possible biometabolites: juncuenin D (**8**), dehydrojuncuenin B (**11**) and luzulin A (**13**).

The superoxide anion generation and the elastase release inhibitory activities of the isolated compounds (**6**–**8**, **11**, **13**–**15**) were tested on fMLP/CB-induced human neutrophils. Noteworthy anti-inflammatory activities were recorded for juncuenin B (**6**), juncusol (**7**), dehydrojuncuenin B (**11**), apigenin (**14**) and luteolin (**15**). Although great deals of phenanthrenes were assayed for their anti-inflammatory properties, this was the first time when the anti-inflammatory activity of the isolated phenanthrenes was evaluated. The traditional use of Juncaceae plants against inflammatory diseases seems to be supported by our data.

All of the isolated compounds were detected for the first time from the investigated plants. The chemical constituents of *J. inflexus* and *L. luzuloides* have not been investigated previously.

The methyl group at C-1, a hydroxy group at C-2 and vinyl, methyl and hydroxy substitution on ring C are characteristic features of the isolated phenanthrenes. In compounds **3**, **8** and **13**, a carbonyl group can be found in the molecule.

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 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*.

Our preliminary screen has provided important data on the antibacterial and anti-inflammatory properties of numerous Juncaceae species native to the Carpathian Basin, which promotes the selection of further species for future pharmacological and phytochemical work. The results reveal that secondary metabolites of Juncaceae species can be regarded as promising starting materials in the search for new pharmaceutical discoveries, in consequence of their pharmacological potential, and in particular their noteworthy antibacterial and anti-inflammatory activities.

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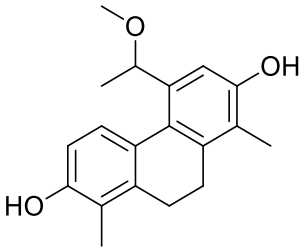
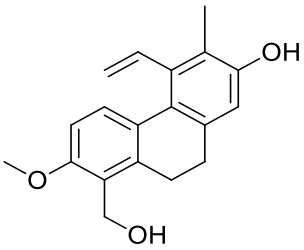
I would like to extend my special thanks to my family and friends. I could not have carried out this work without their support and love.

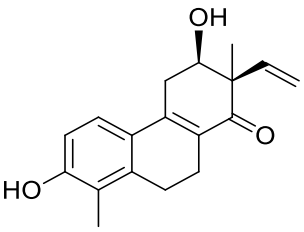
Annex I. Structure of the isolated compounds



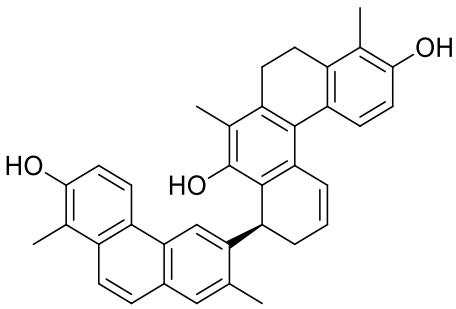
jinflexin A (**1**)



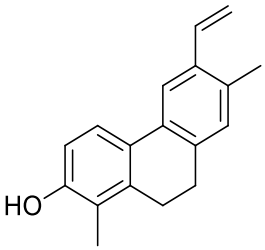
jinflexin B (**2**)



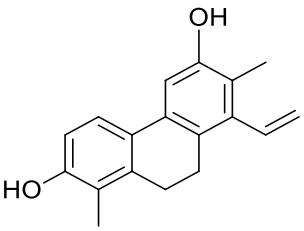
jinflexin C (**3**)



jinflexin D (**4**)



juncuenin A (**5**)



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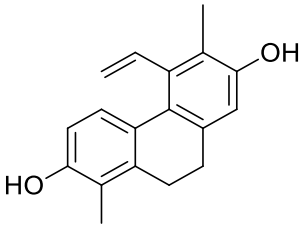
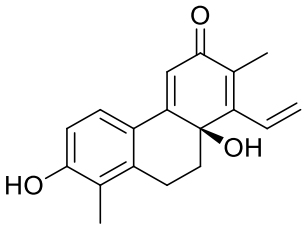
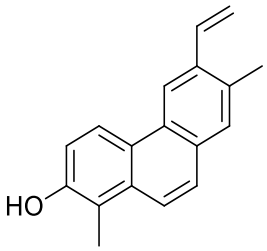


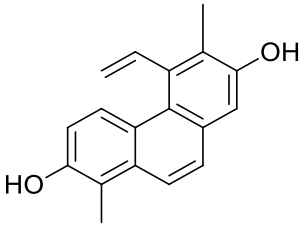
juncusol (**7**)



juncuenin D (**8**)



dehydrojuncuenin A (**9**)



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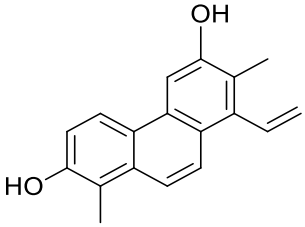

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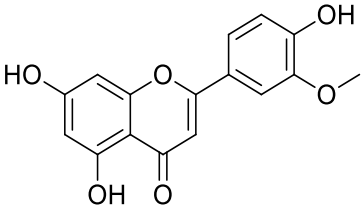
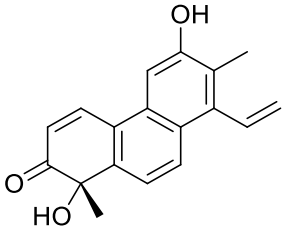
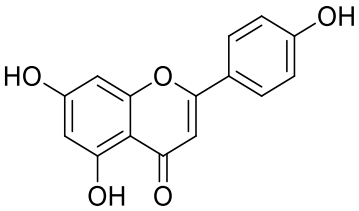


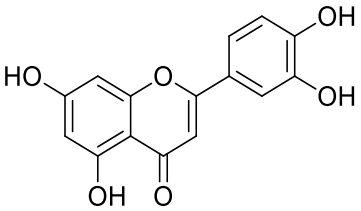
chrysoeriol (**12**)



luzulin A (**13**)



apigenin (**14**)



luteolin (**15**)

Annex II. NMR data of jinflexin A (**1**) [DMSO-d_6, 500 MHz (^{1}H), 125 MHz (^{13}C), δ (ppm), J = Hz], and jinflexin B (**2**) [CD_3OD, 400 MHz (^{1}H), 100 MHz (^{13}C), δ (ppm), J = Hz] isolated from *J. inflexus*

position	**1**			**2**	
	^{1}H	**^{13}C**	**HMBC**[a]	**^{1}H**	**^{13}C**
1		120.2, C			125.9, C
1a		138.8, C			141.7, C
2		153.7, C			157.4, C
3	6.70, d (8.0)	111.6, CH	1, 4a	6.78, d (9.2)	108.2, CH
4	6.84, d (8.0)	126.0, CH	1a, 2	7.65, d (8.7)	131.8, CH
4a		125.3, C			129.4, C
5		137.5, C			137.9, C
5a		126.8, C			127.0, C
6	6.86, s	110.5, CH	5a, 8		122.2, C
7		153.8, C			155.1, C
8		119.6, C		6.64, s	113.7, CH
8a		138.1, C			138.6, C
9	2.32, 2H, m	26.3, CH_2		2.59, 2H, m	31.4, CH_2
10	2.75, 2H, m	25.3, CH_2		2.80, 2H, m	26.6, CH_2
11	2.13, s	11.6, CH_3	1, 1a, 2	4.76, 2H, s	55.9, CH_2
12	4.69, d (4.9)	74.5, CH	5a, 6, OMe-12	6.83 dd (18.6, 11.6)	139.2, CH
13	1.47, d (4.9)	23.2, CH_3	5, 12	5.43 dd (11.4, 1.1), 5.13 dd (18.0, 1.0)	119.5, CH_2
14	2.10, s	11.7, CH_3	7, 8, 8a	2.21, s	13.6, CH_3
OH	9.23, s				
OH	9.17, s				
2-OMe				3.84, s	56.1, OCH_3
12-OMe	2.85, s	55.0, CH_3	12		

[a]HMBC correlations are from proton(s) stated to the indicated carbon.

Annex III. NMR data of jinflexin C (**3**) [CD_3OD, 400 MHz (^1H), 100 MHz (^{13}C), δ (ppm), J = Hz] isolated from *J. inflexus*, and luzulin A (**13**) [CD_3OD, 500 MHz (^1H), 125 MHz (^{13}C), δ (ppm), J = Hz] isolated from *L. luzuloides*

position	**3**		**13**		
	^1H	**^{13}C**	**^1H**	**^{13}C**	**HMBC[a]**
1		123.1, C		78.1, C	
1a		140.1, C		146.6, C	
2		159.4, C		207.1, C	
3	6.71, d (8.5)	113.6, CH	6.25, d (10.2)	123.1, CH	1, 4a
4	7.23, d (8.6)	125.2, CH	8.35, d (10.1)	141.9, CH	1a, 2
4a		126.4, C		122.6, C	
5	2.98, m; 2.72, m	33.6, CH_2	7.52, s	103.3, CH	4a, 6, 8a
5a		149.4, C		132.2, C	
6	3.88, dd (9.6, 4.5)	74.4, CH		157.0, C	
7		55.5, C		126.3, C	
8		201.9, C		138.3, C	
8a		128.8, C		127.7, C	
9	2.65, m; 2.24, m	21.1, CH_2	8.12, d (8.5)	129.8, CH	1a, 5a
10	2.84, m; 2.57, m	25.1, CH_2	7.63, d (8.7)	121.0, CH	4a, 8a
11	2.15, s	11.4, CH_3	1.53, s	32.1, CH_3	1, 1a, 2
12	6.10, dd (17.7, 10.8)	138.1, CH	2.35, s	13.4, CH_3	6, 7, 8
13	5.13, d (10.8), 4.90, d (17.7)	116.5, CH_2	7.05, dd (17.9, 11.5)	135.7, CH	
14	1.32, s	20.2, CH_3	5.81, d (11.5), 5.35, d (18.0)	122.2, CH_2	8

[a]HMBC correlations are from proton(s) stated to the indicated carbon.

Annex IV. NMR data of jinflexin D (**4**) isolated from *J. inflexus* [DMSO-d_6, 500 MHz (^{1}H), 125 MHz (^{13}C), δ (ppm), J = Hz]

position	4		
	^{1}H	^{13}C	HMBC[a]
1		117.1, C	
1a		131.6, C	
2		152.9, C	
3	7.02, d (8.9)	116.4, CH	1, 2, 4a
4	7.59, d (9.0)	119.9, CH	1a, 2, 5a
4a		122.7, C	
5	7.69, s	120.3, CH	4a, 7, 12
5a		128.0, C	
6		141.6, C	
7		133.2, C	
8	7.67, s	128.8, CH	5a, 6, 9, 14
8a		128.1, C	
9	7.64, d (9.2)	126.1, CH	1a, 5a
10	7.74, d (9.2)	121.7, CH	1, 1a, 4a
11	2.41, s	10.9, CH_3	1, 1a, 2
12	4.90, d (8.6)	32.0, CH	5, 5′, 6, 6′, 7, 7′, 13, 13′
13	2.85, m; 2.40, m	28.7, CH_2	6, 6′, 12, 12′, 13′
14	2.68, s	19.3, CH_3	6, 7, 8
1′		120.3, C	
1a′		138.5, C	
2′		153.9, C	
3′	6.77, d (8.3)	111.6, CH	1′, 2′, 4a′
4′	7.13, d (8.3)	127.1, CH	1a′, 2′, 5a′
4a′		125.4, C	
5′		128.4, C	
5a′		125.7, C	
6′		124.5, C	
7′		149.8, C	
8′		121.4, C	
8a′		136.5, C	
9′	2.91, dt (4.3) 2.36, m	26.1, CH_2	1a′, 5a′, 8′, 8a′, 10′
10′	2.98, dt (4.1) 2.50, m	25.3, CH_2	1′, 1a′, 4a′, 8a′, 9′
11′	2.26, s	11.7, CH_3	1′, 1a′, 2′
12′	6.96, dd (9.7, 2.7)	128.0, CH	6′, 13
13′	5.71, ddd (9.7, 7.1, 2.1)	122.8, CH	5′, 12, 13
14′	2.09, s	12.6, CH_3	7′, 8′, 8a′

[a]HMBC correlations are from proton(s) stated to the indicated carbon.

APPENDIX

The thesis is based on the following publications:

I. **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.

II. **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.

III. **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.