

Phenanthrenes and Other Phenolic Compounds with α -Glucosidase Inhibitory Activity from *Luzula campestris* and *Juncus kraussii*

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ABSTRACT: The present study aimed to identify natural compounds with potential relevance to diabetes management. Two members of the Juncaceae family, *Luzula campestris* and *Juncus kraussii*, were investigated for compounds with α -glucosidase inhibitory activity. Multistep chromatographic separation of the methanolic extracts led to the isolation of juncusol, effusol, luteolin, dehydrovomifoliol, *trans-p*-coumaric acid, salicylaldehyde, and 1-*O*-feruloyl-3-*O-p*-coumaroylglycerol from *L. campestris*, and juncusol, effusol, dehydrojuncusol, dehydroeffusol, 2-hydroxy-7-(hydroxymethyl)-1-methyl-5-vinyl-9,10-dihydrophenanthrene, and 2,7-dihydroxy-1,8-dimethyl-5-vinyl-9,10-dihydrophenanthrene from *J. kraussii*. Eight of the isolated compounds were evaluated for their α -glucosidase inhibitory activity. Luteolin ($IC_{50} = 4.92 \mu M$) and 2-hydroxy-7-(hydroxymethyl)-1-methyl-5-vinyl-9,10-dihydrophenanthrene ($IC_{50} = 7.99 \mu M$) exhibited the strongest inhibitory effects. *In silico* analyses elucidated enzyme interactions and ADMET properties, highlighting the potential of these species as sources of bioactive compounds relevant to diabetes research.

Keywords: *Luzula campestris*, *Juncus kraussii*, Juncaceae, phenanthrene, phenolic compounds, α -glucosidase inhibition.

INTRODUCTION

Diabetes mellitus (DM) represents a major and rapidly growing global health challenge, affecting millions of people worldwide and contributing substantially to morbidity and mortality. The disease is characterized by impaired insulin secretion and/or insulin resistance, leading to persistent hyperglycemia and severe microvascular and macrovascular complications that progressively damage multiple organ systems. Despite the availability of several therapeutic approaches, the sustainable management of diabetes remains difficult due to the continuous emergence of decreased drug efficacy and limitations associated with current treatments. Consequently, there is an increasing demand for novel therapeutic agents with improved pharmacological properties, and recent research has increasingly focused on safe and effective phytochemicals as promising alternatives for diabetes management.^{1,2} Among the currently investigated therapeutic targets, α -glucosidase inhibitors have gained considerable attention due to their ability to delay carbohydrate digestion and reduce postprandial hyperglycemia. By inhibiting intestinal α -glucosidase enzymes, these compounds contribute to improved glycemic control and represent an important strategy in the management of type 2 diabetes mellitus.^{3,4}

The genus *Luzula* comprises approximately 125 species distributed worldwide.⁵ *Luzula campestris* (L.) DC. is a perennial flowering plant in the family Juncaceae, native to Europe and northwestern Africa. It primarily occurs in temperate biomes and grows to a height of around 10 cm. The species prefers acidic to neutral soils but can also tolerate chalky conditions. Its life form is hemicryptophyte. Morphological studies have demonstrated root responses to experimentally induced soil heterogeneity.⁶ Common names, such as chimney sweepers, sweeps, smuts, and blackcaps, refer to the sooty-colored florets characteristic of the species.⁷ *L. campestris* is palatable to livestock, particularly sheep, and historically, its sweet-tasting flower heads and seeds were given to children as "rabbit bread".

Similar to other species in the genus *Luzula*, *L. campestris* is not considered a widely recognized medicinal plant, and reliable recipes or documented applications in traditional medicine are largely absent from major botanical and ethnobotanical sources.⁸ Scientific research on its pharmacological properties is limited. Antimicrobial investigations against methicillin-resistant *Staphylococcus aureus* (MRSA) strains ATCC43300 and 64326 revealed only marginal activity. Testing *Luzula* species of the Carpathian basin, superoxide anion and elastase inhibitory activity of extract with different polarity were assayed. The *n*-hexane fraction of *L. campestris* exerted a selective

elastase release inhibition, while the dichloromethane-soluble fraction displayed significant activities on the inhibition of elastase release, and proved to be slightly less active on superoxide generation.⁹ To date, no phytochemical studies have been conducted on *L. campestris*.

Juncus kraussii Hochst. (Juncaceae), commonly known as matting rush (English) or incema (Zulu), is a perennial species that grows primarily in temperate biomes. It is native to southern Africa (from South Africa to Mozambique), Australia, and parts of South America, including Brazil.⁵ *J. kraussii* can reach a height of up to 1.5 m and typically forms large, dense colonies in suitable habitats. The species is highly tolerant of saline conditions, as it is capable of germination, growth, and survival in environments containing up to 70% seawater. In addition, *J. kraussii* is able to take up and compartmentalize large quantities of metal(loid)s in its aboveground tissues.¹⁰

J. kraussii plays an important ecological role in erosion control and provides a high-quality natural fiber. Traditionally, the strong and pliable stems have been widely used for weaving durable sleeping mats, baskets, and other craft items, particularly in South Africa within Zulu culture, as well as by Indigenous Australian communities.^{11,12} There are no documented traditional medicinal uses of *J. kraussii*, and its secondary plant metabolites have not been previously investigated. Nevertheless, the occurrence of phenolic constituents, particularly phenanthrene derivatives, in the *Juncus* and *Luzula* genera provided a reasonable basis to hypothesize that some constituents might exhibit α -glucosidase inhibitory activity. As only a limited number of α -glucosidase-inhibitory phenanthrenes have been reported to date,¹³ this compound class remains relatively unexplored in this context. Moreover, Juncaceae species are known sources of vinylphenanthrenes, whose polyhydroxylated and aromatic features may provide favorable structural characteristics for enzyme binding.

Continuing our study towards phenanthrene-containing Juncaceae species, the present study aimed to isolate phenolic compounds of *L. campestris* and *J. kraussii*. The isolated compounds were investigated

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for α -glucosidase inhibitory activity with the goal of identifying natural compounds with potential antidiabetic properties. Finally, *in silico* investigations of the most promising compounds were conducted through molecular docking and ADMET analysis techniques.

RESULTS AND DISCUSSION

Methanolic extracts of the whole plants of *L. campestris* and *J. kraussii* were subjected to solvent-solvent partition resulting *n*-hexane, dichloromethane (*L. campestris*) or chloroform (*J. kraussii*), ethyl acetate and aqueous methanolic fractions. TLC investigations indicate that the dichloromethane/chloroform fractions contain phenanthrenes and other phenolic compounds, therefore they were separated by multiple chromatography, including open column chromatography (OCC), gel filtration (GF), high pressure liquid chromatography (HPLC), preparative TLC, and centrifugal planar chromatography (CPC). Preparative phytochemical work afforded the isolation of two phenanthrenes, three other phenolic compounds and a megastigmane from *L. campestris*, and five phenanthrenes from *J. kraussii*. The structure of the isolated compounds was determined using NMR investigations, including ^1H NMR, ^{13}C NMR JMOD, ^1H - ^1H COSY, NOESY, HSQC, and HMBC experiments.

Juncusol (1), effusol (2), luteolin (7), the megastigmane dehydrovomifoliol (8), *trans*-*p*-coumaric acid (9), salicylaldehyde (10), 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol (11) were isolated from the dichloromethane fraction of *L. campestris*. Among the isolated compounds luteolin (7) was obtained in the highest yield. Chromatographic separation of *J. kraussii* chloroform extract resulted in the isolation of juncusol (1), effusol (2), dehydrojuncusol (3), dehydroeffusol (4), 2-hydroxy-7-(hydroxymethyl)-1-methyl-5-vinyl-9,10-dihydrophenanthrene (5), and 2,7-dihydroxy-1,8-dimethyl-5-vinyl-9,10-dihydrophenanthrene (6) (Fig. 1).

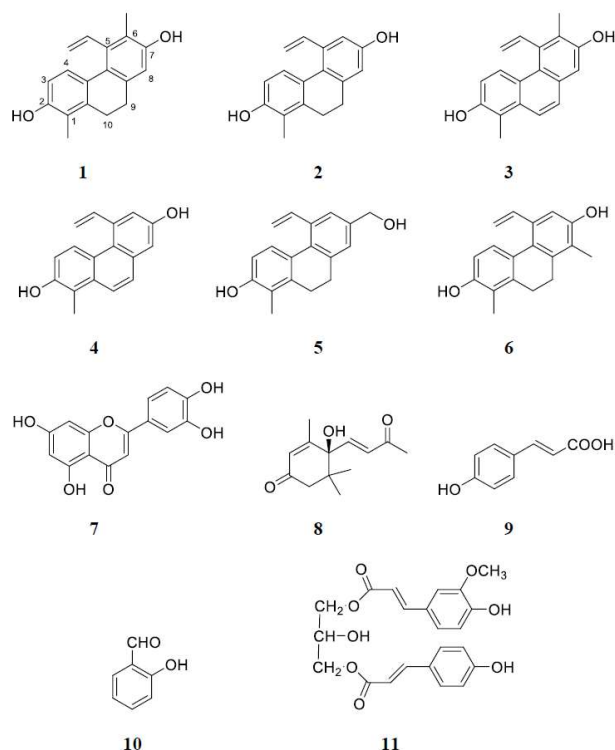


Figure 1. Structure of the isolated compounds 1–11.

The compound identification was made by comparison of the NMR data with those published for juncusol (1),¹⁴ effusol (2),¹⁵ dehydrojuncusol (3),¹⁶ dehydroeffusol (4),¹⁷ 2-hydroxy-7-(hydroxymethyl)-1-methyl-5-vinyl-9,10-dihydrophenanthrene (5),¹⁸ 2,7-dihydroxy-1,8-dimethyl-5-vinyl-9,10-dihydrophenanthrene (6),¹⁹ luteolin

(7),²⁰ dehydrovomifoliol (8),²¹ *trans*-*p*-coumaric acid (9),²² salicylaldehyde (10),²³ 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol (11).²⁴

All compounds were isolated from the given species for the first time. In *L. campestris*, dihydrophenanthrenes were detected, whereas in *J. kraussii* both phenanthrenes and their corresponding dihydroderivatives were identified. The isolated phenanthrenes (1–6) are substituted with vinyl group at C-5, as it is characteristic to the Juncaceae species.²⁴ With the exception of juncusol (1), all phenanthrenes (2–6) were obtained from the *Luzula* genus for the first time. Dehydrovomifoliol (8), *trans*-*p*-coumaric acid (9), salicylaldehyde (10), and 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol (11) have not previously been reported from *Juncus* or *Luzula* species. Juncusol (1) and effusol (2) are among the most widespread phenanthrene derivatives having previously been isolated from twelve and nine Juncaceae species, respectively. The dehydro derivatives of these compounds (3 and 4) are somewhat less widespread, being recorded in seven and five species, respectively.^{25,26}

Compound 5 was reported from *J. effusus*,¹⁸ *J. gerardii*,²⁷ *J. compressus*,²⁸ *J. subulatus*,²⁹ *J. acutus*.³⁰ Compound 6 has been identified in *J. effusus*,¹⁹ *J. acutus*,³¹ *J. tenuis*,³² and *J. maritimus*.³³ Metabolite spectrum of *J. kraussii* exhibited close similarity with that of *J. effusus*, all six phenanthrenes (1–6) are detected in both species.

Eight of the isolated compounds (1–3, 5, 7–9, and 10) that were available in sufficient quantities, including phenanthrenes, other phenolic constituents, and a megastigmane derivative, were evaluated for their α -glucosidase inhibitory activity using acarbose, the most widely prescribed drug, as a positive control. The results are shown in Table 1. The IC_{50} value of acarbose varies considerably across the literature, due to the varying reaction conditions used such as concentrations (reactant, inhibitor, buffer), incubation time, or even the source of the enzyme (many cases not even specified, *Geobacillus stearothermophilus*, *Rattus norvegicus*, *Homo sapiens* or as in our case *Saccharomyces cerevisiae*).³⁴ In our experimental conditions acarbose was determined to have an IC_{50} value of 0.00190 μM , consistent with multiple values reported in refs.^{35–37} Among the isolated compounds, luteolin (7) exhibited the strongest inhibitory effect against α -glucosidase, with an IC_{50} value of 4.92 μM . Similarly high inhibitory potency was observed for 2-hydroxy-7-(hydroxymethyl)-1-methyl-5-vinyl-9,10-dihydrophenanthrene (5), which showed an IC_{50} value of 7.99 μM . Although the activities of juncusol (1) and dehydrojuncusol (3) were lower compared to the aforementioned compounds, they still demonstrated considerable inhibition of α -glucosidase activity, reducing enzyme activity by 75.6% and 62.4%, respectively, at a concentration of 100 μM . Effusol (2) and *trans*-*p*-coumaric acid (9) displayed moderate inhibitory activity, whereas dehydrovomifoliol (8) and salicylaldehyde (10) were found to be inactive under the applied assay conditions.

Table 1. α -Glucosidase inhibitory activity of compounds 1–3, 5, 7–9, and 10.

Compound	Inhibition % at 100 μM		Inhibition % at 10 μM		IC_{50} (μM)	95% CI
	mean	SD	mean	SD		
Juncusol (1)	75.6	2.8	9.7	5.0		
Effusol (2)	55.0	5.3	1.5	5.2		
Dehydrojuncusol (3)	62.4	1.5	26.1	6.2		
Compound (5)	80.3	2.0	55.2	2.5	7.99	5.172 to 12.52
Luteolin (7)	94.7	3.6	50.1	2.6	4.92	4.290 to 5.633
Dehydrovomifoliol (8)	26.0	5.0	1.1	1.5		
<i>Trans</i> - <i>p</i> -coumaric acid (9)	48.9	1.3	12.6	2.0		
Salicylaldehyde (10)	11.1	1.6	2.0	7.2		
Acarbose	90.4	1.9	89.6	1.5	0.00190	0.001618 to 0.002224

This is the first report describing the α -glucosidase inhibitory activity of vinyl-substituted phenanthrenes. Previously, only a few methoxylated phenanthrenes and dihydrophenanthrenes isolated from *Dendrobium scabringue* and *Pleione maculata* were reported to

exhibit α -glucosidase inhibitory activity, with IC_{50} values ranging from 96.2 – 131.4 μM ³⁸ or inhibition rates between 11.59% and 58.13% at a concentration of 200 $\mu g/mL$.³⁹ Furthermore, San et al. described methoxylated phenanthropyran derivatives from *Gastrochilus bellinus* as effective α -glucosidase inhibitors, with IC_{50} values ranging from 45.92 to 301.12 μM .⁴⁰

In the present study, 2-hydroxy-7-(hydroxymethyl)-1-methyl-5-vinyl-9,10-dihydrophenanthrene (5) exhibited markedly stronger inhibitory activity against α -glucosidase, with an IC_{50} value of 7.99 μM , indicating substantially higher potency than the previously reported phenanthrene derivatives. Comparison of the activities of juncusol (1) and dehydrojuncusol (3) suggests that the presence or absence of unsaturation between C-9 and C-10 does not significantly influence α -glucosidase inhibitory activity. Moreover, the different activities observed for compounds 1, 2, and 5 indicate that the structural features at positions C-6 and C-7 play an important role in enzyme inhibition. Based on these observations, it can be concluded that a 7-hydroxymethyl substitution in the absence of a substituent at C-6 is favorable for α -glucosidase inhibitory activity.

Based on the *in vitro* α -glucosidase inhibition assay results, compounds 1, 2, 3, 5 and 7 were selected for further *in silico* investigations to better understand the molecular basis of the interaction and to predict the ADMET properties; acarbose was used as a standard. The molecular docking study was performed using a grid box that included the amino acids reported in literature. All tested compounds docked into the active site of the enzyme, which represents a groove lined by amino acids that included Tyr 158, Arg 315 and Asn 415;⁴¹ the standard acarbose was an exception as it interacts with amino acids located out of the binding groove (Fig. 2), this goes in agreement with the previous reports indicating the presence of allosteric sites that are present within the protein structure.⁴² Interestingly, many of these compounds interacted with one or more of these amino acids or nearby residues. Notably, the unsaturated side chain of phenanthrenes 1, 2, 3 and 5 interacted with Tyr 158 through Pi-alkyl or Pi-Pi stacked bonds (Figs. 3 and 4, Table S1).

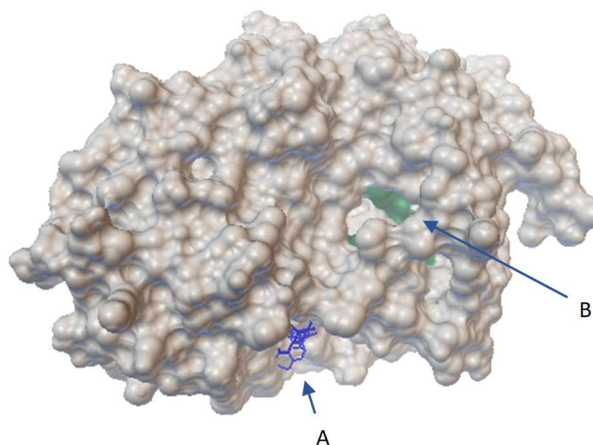


Figure 2. Molecular surface representation of the α -glucosidase enzyme (PDB ID: 3A4A) complexed with acarbose (A shown in blue sticks) docked out of the active site (B shown in green). Molecular surface representation of the α -glucosidase enzyme and its complexes with ligands is generated via the GUI of AutoDock4 software (v.1.5.7).

In accordance with the results of the *in vitro* inhibitory assay of the α -glucosidase enzyme, luteolin (7) showed the best interaction with the enzyme active site (the lowest binding energy, -9.64 Kcal/mol) with hydrogen bond interactions with the amino acids Gln 239, Lys 156, Asn 415, Pro 312 and also through carbon hydrogen bond, Alkyl, Pi-alkyl, Pi-anion to other amino acid present in the binding groove (Fig 4_B), while compound 2 was the least in ranking among the selected compounds (-8.21 Kcal/mol). Despite the presence of the hydroxymethyl group in

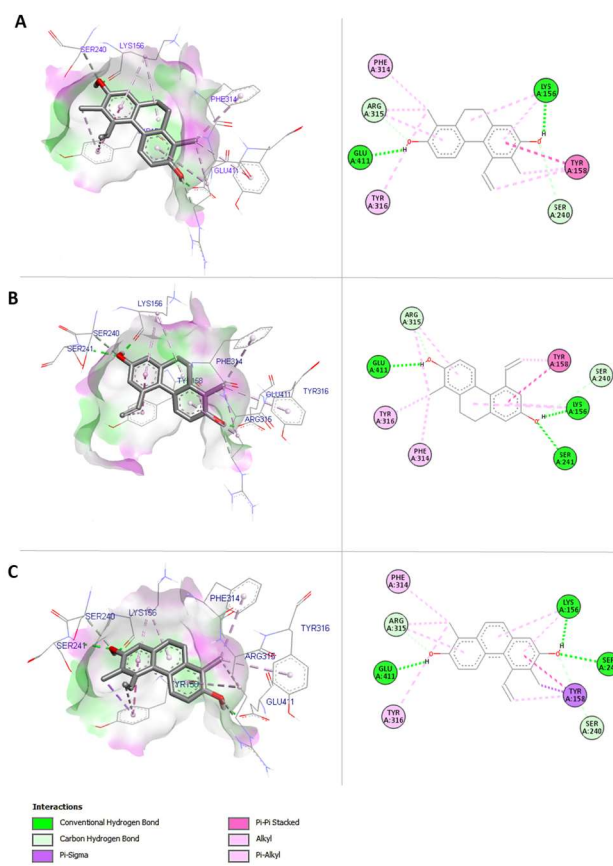


Figure 3. The 2D (right) and 3D (left) representations of the interactions of compounds 1 (A), 2 (B) and 3 (C) with α -glucosidase enzyme (PDB ID: 3A4A), visualized using Discovery studio visualizer (21.1.0.20298).

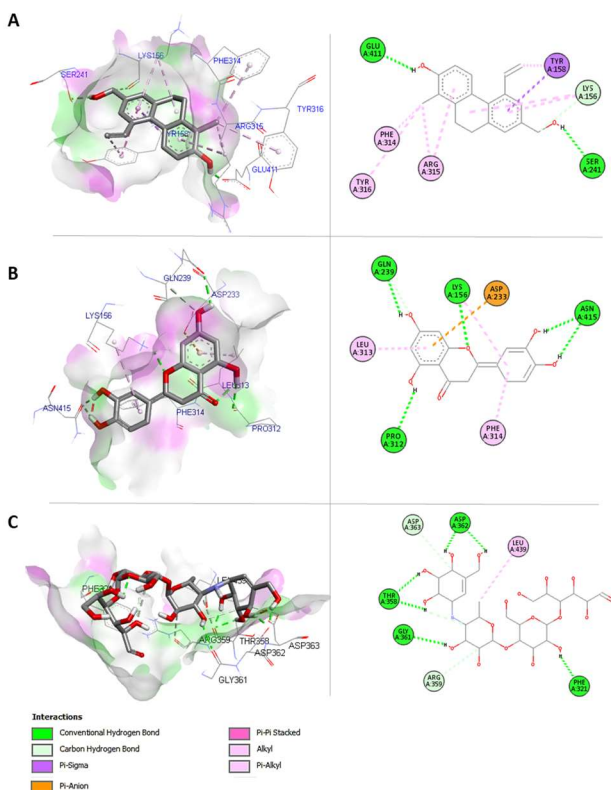


Figure 4. The 2D (right) and 3D (left) representations of the interactions of compounds 5 (A), 7 (B) and acarbose (C) with α -glucosidase enzyme (PDB ID: 3A4A) visualized using Discovery studio visualizer (21.1.0.20298).

Table 2. Summary of the pharmacokinetic parameters of 1–3, 5, 7 and acarbose calculated by pkCSM software

Absorption	1	2	3	5	7	acarbose
Water solubility (log mol/L)	-4.12	-4.002	-4.185	-4.277	-3.083	-1.175
Caco2 permeability (Log Papp)	1.102	1.1	1.023	1.496	1.001	-0.819
Intestinal absorption (human) (%)	94.241	94.444	93.835	93.963	78.805	0
Skin Permeability (log Kp)	-2.87	-2.871	-2.782	-2.748	-2.735	-2.735
P-glycoprotein inhibitor	No	No	No	No	No	No
Distribution						
VDss (human) (log L/Kg)	0.38	0.343	0.166	-0.007	0.102	-0.902
Fraction unbound (human) (Fu)	0.033	0.043	0.035	0.108	0.156	0.568
BBB permeability (Log BB)	-0.106	-0.119	-0.014	0.299	-1.307	-2.527
CNS permeability (Log PS)	-1.392	-1.406	-1.364	-1.429	-2.514	-7.598
Metabolism						
CYP2C9 inhibitor	Yes	Yes	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	Yes	No
Excretion						
Total Clearance (log ml/min/kg)	0.097	0.048	0.14	0.145	0.66	0.655
Renal OCT2 substrate	No	No	No	No	No	No
Toxicity						
AMES toxicity	Yes	Yes	Yes	No	No	No
Max. tolerated dose (human) (log mg/kg/day)	0.327	0.334	0.544	1.325	0.839	0.696
hERG I inhibitor	No	No	No	No	No	No
hERG II inhibitor	Yes	No	Yes	Yes	No	Yes
Oral Rat Acute Toxicity (LD50)	0.052	2.022	2.682	2.115	2.235	2.744
Oral Rat Chronic Toxicity (LOAEL)	2.052	1.866	1.758	2.408	2.052	5.426
Hepatotoxicity/Skin Sensitization	No	No	No	No	No	No
T. pyriformis toxicity	0.738	0.742	0.414	1.837	0.361	0.285
Minnow toxicity (mol/Kg)	-0.611	-0.514	-1.101	0.324	0.87	14.641

compound **5** might appear to decrease the chances of hydrogen bonding interaction possibility with Ser 241, nevertheless, this might not be totally inferred from the present docking results, considering compound **1** interaction profile though it lacks this group. Compound **5** outperformed compound **3** in experimental *in vitro* results, it showed a less favorable docking score (−8.29 Kcal/mol) than compound **3** (−8.95 Kcal/mol), this might be attributed to the limitations of conventional docking scoring functions, as factors like protein conformational flexibility, solvation and receptor adaptation might not be adequately represented.⁴³

The pharmacokinetic profile analysis of the selected compounds was performed using SwissADME (Tables S2–S6) and pkCSM (Table 2) descriptor algorithm procedures to get insights into the pharmacokinetic *in vivo* behavior of these compounds. With the exception of acarbose, all compounds comply with the Lipinski's rule of five, indicating favorable physicochemical properties,⁴⁴ they are also anticipated to have good GI absorption, indicated by their Caco2 permeability values. Apart from luteolin (**7**), all compounds are expected to cross the BBB, with compound **5** standing out in this regard. Interestingly, luteolin (**7**) is anticipated to possess good oral bioavailability as predicted from the results of the bioavailability radar (Table S6), with all parameters confined to the pink area of the plot.

Drug-drug interactions seem to be a less concerning issue for these compounds as no inhibition is predicted for the P-gp glycoprotein, as well as for CYP3A4 (except for luteolin) and CYP2D6, however, some inhibitory effects could be related to compounds **1–3** against CYP2C9 enzyme. According to the results, no predicted mutagenic (AMES), hepatotoxic, or cardiotoxic (hERG I and II inhibitor) effects could be noted for luteolin (**7**), though compound **5** is expected to have some cardiotoxic properties.

EXPERIMENTAL

General experimental procedures: The NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer (Bruker, Billerica, MA, USA) operating at 500 MHz for ¹H and 125 MHz for ¹³C. The residual signals of the deuterated solvents were taken as references. Two-dimensional NMR experiments were performed using standard Bruker software (TopSpin 3.6.2), employing gradient-enhanced techniques for COSY, HSQC, and HMBC spectra. Open column chromatography (OCC) was performed on polyamide (MP Polyamide, 50–160 µm, MP Biomedicals, Irvine, CA, USA). Vacuum liquid chromatography (VLC) was carried out on silica gel (silica gel GF254, 15 µm, Merck, Darmstadt, Germany). Sephadex LH-20 (25–100 µm, Sigma-Aldrich, Budapest, Hungary) was employed for gel filtration. Rotational planar chromatography (RPC) was performed on silica gel 60 GF254 using a

Chromatotron (Harrison Research). Preparative thin-layer chromatography (prepTLC) was conducted using reversed-phase silica plates (20 × 20 cm 60 RP-18 F254s MS-grade, Supelco 1510150001). Reversed-phase high performance liquid chromatography (RP-HPLC) separations were performed using a Shimadzu LC-20AD instrument equipped with a PDA detector (Shimadzu, Co., Ltd., Kyoto, Japan), and reversed-phase columns. In the *L. campestris* experiment Kinetex C18 column (5 µm, 100A, 150 × 4.6 mm) was used, while in the *J. kraussii* experiment Phenomenex Kinetex Phenyl-Hexyl (5 µm, 100A, 150 × 4.6 mm) and the semipreparative column Phenomenex Luna Phenyl-Hexyl (5 µm, 100 A, 250 × 10 mm) were applied. Sigma-Aldrich Kft. (Budapest, Hungary) and Molar Chemicals (Halásztelek, Hungary) supplied the chemicals used in this research. All of the solvents used for column chromatography were at least analytical grade, and those used for HPLC were of HPLC grade.

Plant material: The whole plant of *Luzula campestris* (L.) DC. was collected in April 2022 in Vizvár and identified by Dragica Purger (Department of Pharmacognosy, University of Pécs). A voucher specimen No. 960 was deposited at the Herbarium of Institute of Pharmacognosy, University of Szeged, Hungary. *Juncus kraussii* Hochst. was collected in April 2021 in South Africa and identified by Gerhard Prinsloo. Voucher specimen was preserved with accession number 961 at the Herbarium of the Institute of Pharmacognosy, University of Szeged, Hungary.

Extraction, and isolation: The dried plant material (802.7 g) of *L. campestris* was ground in a blender and then percolated with MeOH (15 L) at room temperature. After evaporation of the solvent, the dry residue (251 g) was dissolved in 50% aqueous MeOH and subjected to solvent–solvent partitioning with *n*-hexane (10 × 500 mL), dichloromethane (5 × 500 mL), and ethyl acetate (8 × 500 mL). The dichloromethane phase (3.654 g), which was richest in phenolic compounds, was fractionated by VLC on silica gel using a gradient solvent system of cyclohexane–ethyl acetate–ethanol (1:0:0, 98:2:0, 95:5:0, 9:1:0, 85:15:0, 8:2:0, 75:25:0, 7:3:0, 60:30:1, 60:30:3, 60:30:5, 6:3:1, and 0:0:1) to yield 16 fractions. Juncusol (**1**) (16.8 mg) crystallized upon standing from Fr 3. Fr 4 was separated by RP-HPLC using a linear gradient system of H₂O–MeOH (0–1 min: 60% MeOH, 1–7 min: 60%→72%). The peak at RT = 4.8 min revealed to be effusol (**2**) (0.5 mg). Fr 9 was purified by prepTLC on RP-silica gel with MeOH–H₂O (8:2) as developing system. Bands at R_f = 0.75 (Fr 9a) and 0.65 (Fr 9b) were eluted and further purified by RP-HPLC. For Fr 9a, isocratic elution with 25% MeOH was applied to yield dehydrovomifoliol (**8**) (RT = 9.2 min) (1.4 mg), while for Fr 9b a gradient system (0–1 min: 40% MeOH, 1–5 min: 40%→72% MeOH) was used, to afford salicylaldehyde (**10**) (RT = 4.5 min) (0.6 mg). Fr 13 (482.5 mg) was subjected to OCC on polyamide eluted with mixtures of MeOH–H₂O (6:4, 7:3, 8:2, 1:0). The fraction obtained with the first eluent (Fr 13a) was separated by RPC on 2 mm silica gel layer using CHCl₃–MeOH (95:5) as the eluent. Subfraction Fr 13a₉ from this chromatography was further purified by RP-HPLC using an isocratic system of MeCN–H₂O (32:78), yielding 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol (**11**) (RT = 7.2 min) (20 mg). Upon crystallization, subfraction Fr 13/a₁₁ yielded *trans*-*p*-coumaric acid (**9**) (2 mg) as a white crystalline material. Luteolin (**7**) (1 g) crystallized from the fractions obtained from the polyamide OCC using 70% and 80% MeOH.

Dried, grinded whole plants of *J. kraussii* (290 g) were extracted with MeOH. The dried extract (33.2 g) was dissolved in 560 mL of 50% aqueous MeOH and partitioned successively with *n*-hexane (10 × 300 mL), chloroform (8 × 300 mL), and ethyl acetate (3 × 300 mL). The chloroform phase (2.10 g), which contained the phenanthrene-like compounds, was subjected to VLC on silica gel using a gradient system of cyclohexane–ethyl acetate (95:5, 9:1, 85:15, 8:2, 75:25, 7:3, 6:4, 1:1), followed by MeOH as the final eluent, affording nine fractions based on TLC monitoring. Fraction 3 was separated by gel filtration on

Sephadex LH-20 column using dichloromethane–MeOH (1:1) as eluent to give three subfractions (Fr 3a–c). Subfraction 3a was purified by semi-preparative RP-HPLC using 80% MeOH as eluent to afford the isolation of compound **5** (RT = 9.6 min) (6.3 mg). Subfraction 3b yielded compound **6** (2 mg) after purification by RP-HPLC with 65% MeOH (RT = 8.4 min). Dehydrojuncusol (**3**) (2 mg) was obtained from Fr 3c by semipreparative RP-HPLC using a gradient system of MeOH and H₂O (0–1 min: 75% MeOH, 1–9 min: 75%→100% MeOH) (RT = 9.3 min). Fractions 4 and 5 were subjected to gel filtration on Sephadex LH-20 eluted with dichloromethane–MeOH (1:1), to afford five (Fr 4a–e) and seven (Fr 5a–g) subfractions, respectively. Fr 4a contained pure dehydroeffusol (**4**) (2 mg). Repeated RP-HPLC purification of Fr 5d let to the isolation of juncusol (**1**) (4 mg) and effusol (**2**) (2.4 mg) [eluent: 55% MeOH; RT = 17.3 min (**1**); RT = 13.1 min (**2**)].

α-Glucosidase inhibition assay: The α-glucosidase inhibition assay was performed following the methodology described by Suhail et al.⁴⁵ A pH = 6.8 potassium phosphate buffer (0.1 M) was prepared to be used as medium. Samples were first dissolved in DMSO (6.4 mM), and diluted with buffer to get the appropriate concentrations in the well during the reaction. On a 96 well plate to 120 µL of the sample solutions 20 µL of α-glucosidase enzyme (HY-P2802, MedChemExpress, Monmouth Junction, NJ, USA) (from *Saccharomyces cerevisiae*, 1 unit/mL in buffer) was added and the mixture was incubated at 37 °C for 15 minutes. The reaction was initiated by the addition of 20 µL of the substrate (5 mM *para*-nitrophenyl-α-D-glucopyranoside in buffer). After 15 minutes of incubation at 37 °C, the reaction was stopped by the addition of 80 µL of 0.2 M Na₂CO₃ in buffer. As blanks appropriate amount of DMSO in buffer was used. The absorbance was measured using a BMG Labtech FluoStar Optima plate reader at 405 nm. The activity of the compounds was calculated as inhibition (%) = (A_{blank} – A_{sample}) / A_{blank} × 100, and the IC₅₀ values were calculated using GraphPad Prism. In case of the flavonoid, the result was corrected by their own absorbance without the substrate, in the same alkaline conditions. Acarbose (PHR1253, Merck Sigma-Aldrich, St. Louis, MO, USA) was used as a reference inhibitor.

Molecular docking: Adopting the method described before^{46,47}, molecular docking studies were performed using AutoDockTools version 1.5.7 from the Scripps Research Institute, San Diego, California, USA.⁴⁸ The crystalized 3D structure of *Saccharomyces cerevisiae* α-glucosidase enzyme was retrieved from the PDB database (ID: 3A4A) with 1.6 Å resolution. Ligand structures were either retrieved from the PubChem database or sketched using ChemDraw 12.0.2 software (ACD/LABS, Advanced Chemistry Development, Inc.). Protein and ligand preparations were conducted using the graphical user interface of AutoDock4 software. The grid box dimensions were adjusted to include the interacting amino acids reported in literature^{41,49}. Parameters were set to the default values and ligands were docked via the Lamarckian algorithm. The binding energies for the selected compounds were obtained from the resulting DLG files, and Biovia (Discovery Studio visualizer, version 21.1.0.20298; Dassault Systèmes, Vélizy-Villacoublay, France) was used for visualization of the protein–ligands' interactions.

ADMET profiling: The ADMET properties and the drug-likeness prediction for the selected compounds was conducted using the pkCSM <https://biosig.lab.uq.edu.au/pkcsml/> and Swiss ADME <https://www.swissadme.ch/> free online servers (accessed on Aug 14, 2026).⁵⁰

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