

Goldenrod Root Compounds Active against Crop Pathogenic Fungi

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ABSTRACT: Root extracts of three goldenrods were screened for antimicrobial compounds. 2Z,8Z- and 2E,8Z-matricaria esters from European goldenrod (*Solidago virgaurea*) and E- and Z-dehydromatricaria esters from grass-leaved goldenrod (*Solidago graminifolia*) and first from showy goldenrod (*Solidago speciosa*) were identified by high-performance thin-layer chromatography combined with effect-directed analysis and high-resolution mass spectrometry or nuclear magnetic resonance spectroscopy after liquid chromatographic fractionation and isolation. Next to their antibacterial effects (against *Bacillus subtilis*, *Aliivibrio fischeri*, and *Pseudomonas syringae* pv. *maculicola*), they inhibited the crop pathogenic fungi *Fusarium avenaceum* and *Bipolaris sorokiniana* with half maximal inhibitory concentrations (IC₅₀) between 31 and 107 μg/mL. Benzyl 2-hydroxy-6-methoxybenzoate, for the first time found in showy goldenrod root, showed the strongest antifungal effect, with IC₅₀ of 25–26 μg/mL for both fungal strains.

KEYWORDS: *Fusarium avenaceum*, *Bipolaris sorokiniana*, HPTLC–EDA, antifungal assay, *Solidago* species

INTRODUCTION

The phytopathogenic fungal species *Fusarium avenaceum* (teleomorph: *Gibberella avenacea*) and *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) are in the phylum of the Ascomycetes and can cause significant economic losses. *F. avenaceum* is a generalist plant pathogen that mainly causes head blight of cereals, root rot of legumes, and dry rot of potato.^{1,2} It produces different terpenes, indole-diterpene-type compounds, alkaloids, and various mycotoxins harmful for humans, such as fusarin C, moniliformin, enniatins, and beauvericin.^{3,4} *B. sorokiniana* has a wide host range in the Poaceae family, with the greatest economic importance as the causal agent of common root rot, seedling blight, and foliar spot blotch of barley and wheat in warm and humid environments.^{5,6} There is a wide spectrum of secondary metabolites of *B. sorokiniana*, including many biologically active compounds, like terpenes, quinones, and xanthenes. Several of them are specific for this pathogen, such as the phytotoxins sorokinol and sorokinianin as well as the crop-destroying helmintosporal acid.^{7–10}

As a result of the increasing concerns about fungicide resistance, the continued effort to improve the disease control practices with more effective and environmentally friendly but less harmful agents is imperative. Hyphenated analytical–microbiological techniques are very useful for effect screening.^{11–14} High-performance thin-layer chromatography (HPTLC) combined with microbiological detection (direct bioautography) is widely used, but the number of introduced microscopic fungal species is limited.¹⁵ Thus, there is still room for improvement in this field that could lead to the discovery of new antifungal agents. For examination of agriculturally prominent fungal strains, several direct bioautography methods have been introduced¹⁵ and also for *F. avenaceum*.¹⁶ The latter method is suitable for non-targeted and effect-directed analysis (EDA) of antimicrobial compounds with considerable time

and cost savings, if compared to status quo analyses for detection and isolation of individual antimicrobial compounds in complex plant extracts.^{12,16,17} In the past decade, HPTLC analyses have benefited from their hyphenation to high-resolution mass spectrometry (HRMSⁿ), particularly through the use of elution head-based TLC–MS interfaces.^{13,18,19} This supports the characterization and identification of the separated compounds of interest, e.g., the compounds responsible for the desired effect.^{17,20}

Most of the about 120 goldenrod (*Solidago*) species are native in North America, and three of them, i.e., *Solidago canadensis* L., *Solidago gigantea* Ait., and *Solidago graminifolia* (L.) Salisb., were introduced as ornamental flowers in Europe in the 19th century and have been naturalized. *S. graminifolia* [synonym for *Euthamia graminifolia* (L.) Nutt., grass-leaved goldenrod] is a less successful invader occurring only sporadically in Central Europe.^{21,22} Its first spontaneous occurrence in Hungary was reported in 2008 without the identification of its source.²³ Showy goldenrod (*Solidago speciosa* Nutt.) is widely merchandised and generally not considered as an invasive species. Both *S. graminifolia* and *S. speciosa* have been used as traditional medicine by Native Americans. The decoction of their roots has been applied to treat lung ailments, and their aboveground parts have been applied to cure fever and pains.²⁴ *Solidago virgaurea* L. (European goldenrod) is native in Eurasia and North Africa, and its aerial part is listed in the European Pharmacopoeia as

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Solidaginis virgaureae herba and also used in traditional medicine against urinary tract disorders for its anti-inflammatory and antibacterial effects.^{25,26} The broad spectrum of biological activities possessed by the goldenrod species is attributed,^{26,27} among others, to their contents of phenolic acids, flavonoids, sesquiterpenes, diterpenes, and acetylenes.^{12,16,17,25,26} Thus far, the latter two were established as antimicrobial components of the goldenrod roots.^{12,16,17} The biological activity of the roots has been rarely studied, e.g., Anžlovar et al. in 2014.²⁸ The diverse biological effects indicate that acetylenes^{29,30} and diterpenes³¹ are potential candidates for natural, plant-derived pesticides. Recently, the enzyme inhibitory profile of *S. graminifolia* and *S. virgaurea* root extract was reported by us.¹⁷ On the basis of this, polyacetylene esters (2Z,8Z-matricaria ester and 2E,8Z-matricaria ester) in *S. virgaurea* root were identified as antimicrobials and inhibitors of cholinesterase, glucosidase, and α -amylase.^{12,17}

To our knowledge, there is no report yet on the antibacterial and antifungal activities of *S. graminifolia* and *S. speciosa* roots. Hence, in this study, the antimicrobial profiles of *S. virgaurea*, *S. graminifolia*, and *S. speciosa* roots were compared with focus on the characterization of the antifungal components. For the first time, HPTLC was combined with the *B. sorokiniana* assay. HPTLC–ultraviolet/visible (UV/vis)/fluorescence detection (FLD)–EDA, HPTLC–heated electrospray ionization (HESI)–HRMSⁿ, flash chromatography, and high-performance liquid chromatography–ESI–quadrupole–time-of-flight MS (HPLC–ESI–qTOFMS) were used as techniques. The main antifungal compounds were isolated and identified by nuclear magnetic resonance (NMR) spectroscopy. Their antifungal activity was specified as the half maximal inhibitory concentration (IC₅₀) in the liquid phase.

MATERIALS AND METHODS

Chemicals. The 20 × 10 cm silica gel 60 F₂₅₄ HPTLC plates were from Merck (Darmstadt, Germany). The analytical-grade solvents used for HPTLC and flash chromatography and gradient-grade methanol were from Molar Chemicals (Halásztelek, Hungary) or Th. Geyer (Renningen, Germany). Distilled water was produced by a Millipore Direct-Q 3 UV system (Merck). Methanol-*d*₄ (99.8 atom % D) was purchased from VWR (Budapest, Hungary). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was from Sigma (Budapest, Hungary). Sodium chloride was acquired from Reanal (Budapest, Hungary), tryptone from Microtrade (Budapest, Hungary), yeast extract from Scharlau (Barcelona, Spain), and benomyl (Fundazol 50WP, Chinoïn, Budapest, Hungary) from Chinoïn (Budapest, Hungary).

Microorganisms. The Gram-positive *Bacillus subtilis* soil bacterium (F1276) was from József Farkas, Central Food Research Institute, Budapest, Hungary; the Gram-negative, naturally luminescent marine bacterium *Aliivibrio fischeri* (DSM 7151) was from Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures, Berlin, Germany); and the Gram-negative luminescent reporter gene-tagged paprika pathogen *Pseudomonas syringae* pv. *maculicola* was from Jun Fan (John Innes Center, Department of Disease and Stress Biology, Norwich, U.K.). *F. avenaceum* (Fr.) Sacc. IMI 319947 was from the CABI-IMI Culture Collection, Egham, U.K., and *B. sorokiniana* (Sacc.) Shoemaker H-299 (NCBI GenBank accession number MH697869) was collected from barley in Hungary.

Sample Preparation. Roots of fully flowered goldenrod species were collected in July 2019 in Hungary: *S. virgaurea* in a wood of sessile oaks and Turkey oaks in the Buda hills, *S. graminifolia* in the Bükk Mountains at Eger-Almár, and *S. speciosa* in the National Botanical Garden, Vácrtót. Voucher plant specimens were deposited at the Herbarium of the Hungarian Natural History Museum, Budapest, Hungary, under the accession numbers HNHM-

TRA00009950 (*S. virgaurea*), HNHM-TRA00035004 (*S. graminifolia*), and HNHM-TRA00027110 (*S. speciosa*). The fresh roots were cleaned, chopped, dried at room temperature, and ground with a coffee grinder (Sencor SCG 2050, Japan). The pulverized roots were extracted with *n*-hexane 3 times by maceration in an Erlenmeyer flask for 24 h (100 mg/mL). The combined filtrates (Whatman No. 2 filter paper, Sigma) were concentrated and filtered again [0.22 μ m polytetrafluoroethylene (PTFE) syringe filter, FilterBio, Nantong, China] to obtain the *n*-hexane crude extracts.

Fractionation by Flash Chromatography and Isolation by HPLC. Part (5 mL each) of the *S. speciosa* and *S. graminifolia* *n*-hexane crude extracts was fractionated with a Combiflash NextGen 300 (Teledyne Isco, Lincoln, NE, U.S.A.) flash chromatography system on a RediSep Rf Gold silica gel column (20–40 μ m, 12 g, Teledyne Isco) at a flow rate of 30 mL/min with a gradient of *n*-hexane (A) and acetone (B): 0–1 min, 0% B; 1–2 min, 0–5% B; 2–9 min, 5% B; 9–14 min, 5–60% B; and 14–15 min, 100% B. The absorbance was measured at 200 nm. The active fractions (selected by HPTLC–EDA) were further purified by HPLC using a LC-MS-2020 system (Shimadzu, Kyoto, Japan) with a diode array detector (DAD), ESI–MS, and installed Gemini C18 columns (Phenomenex, Torrance, CA, U.S.A.). The separation on the analytical column (250 × 4.6 mm inner diameter, 5 μ m particle size) used an isocratic elution (76.25% aqueous methanol) at 35 °C at a flow rate of 0.8 mL/min. MS conditions were as follows: nitrogen as nebulizer gas, flow rate of 1.5 L/min, drying gas (nitrogen) flow rate of 15 L/min, interface temperature of 350 °C, heat block temperature of 400 °C, desolvation line temperature of 250 °C, and detector voltage of 4.5 kV. Full scan mass spectra in the range of *m/z* 100–900 were recorded in the positive and negative ionization modes. The analogous separation on the semi-preparative column (250 × 10 mm inner diameter, 10 μ m particle size) used a flow rate of 4 mL/min. Instrument control and data acquisition were achieved with the LabSolutions 5.42v program (Shimadzu). The methanol was evaporated from the collected fractions with a rotary evaporator (Rotavapor R-134, Büchi, Flawil, Switzerland) at 40 °C. Then, using liquid–liquid extraction, each aqueous residue was transferred into ethyl acetate (2 × 5 mL).

HPTLC–UV/vis–EDA. Another part of the *n*-hexane crude extracts was dried with a rotary evaporator and redissolved in ethanol (1 mg/mL). These still crude extracts (1–5 μ L) and the HPLC-isolated compounds in ethyl acetate (0.2–1 μ L/band for antibacterial and 2–3 μ L/band for fungal assays) were applied as 5 mm bands onto the HPTLC plate at a 8 mm distance from the bottom and 8–10 mm track distance using the Automated TLC Sampler (ATS3, CAMAG, Muttenz, Switzerland) or applied manually with a 10 μ L microsyringe (Hamilton, Bonaduz, Switzerland). HPTLC plates were developed with *n*-hexane–acetone (17:3, v/v) up to a migration distance of 70 mm (Twin Trough Chamber 20 × 10 cm, CAMAG). The dried chromatograms were detected under UV/vis (UV lamp and digital camera Cybershot DSC-HX60, Sony, Neu-Isenburg, Germany), cut into identical segments, and subjected to the various assays as follows.

Bacterial cell suspensions were prepared, and the direct bioautographic assays were performed as described previously for the *B. subtilis* F1276,³² *A. fischeri*,³³ and *P. maculicola* bioassays.³⁴ Briefly, the developed and dried HPTLC plates were immersed into the bacterial suspensions. The bioautograms of the luminescent bacterial strains of *A. fischeri* and *P. maculicola* were immediately recorded with exposition times of 40–80 s (iBright FL1000 Imaging System, Thermo Fisher Scientific, Budapest, Hungary). On the bioautograms, the antibacterially active zones appear dark against a bright bioluminescent background. *B. subtilis* bioautograms were incubated in a humidity chamber at 28 °C for 2 h, then dipped into an aqueous MTT solution (1 mg/mL), and further incubated for 30 min. The antibacterial compounds produce bright zones against a purple background.

The HPTLC–antifungal assays were carried out with *F. avenaceum*¹⁶ and *B. sorokiniana* plant pathogens. Briefly, *F. avenaceum* and *B. sorokiniana* were grown in lysogeny broth (LB, 20 mL; 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L sodium chloride) in the dark

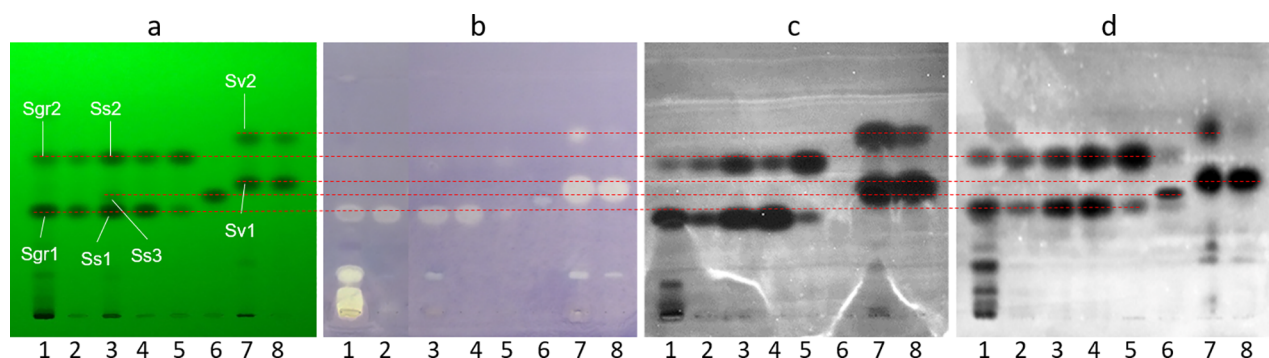


Figure 1. HPTLC chromatogram and bioautograms of *S. graminifolia* (1), *S. speciosa* (3), and *S. virgaurea* (7) root extracts and isolated compounds **Sgr1** (2), **Ss1** (4), **Ss2** (5), **Ss3** (6), and **Sv1** (8), developed with *n*-hexane–acetone (17:3, v/v) and detected (a) at UV of 254 nm and after applying the antibacterial (b) *B. subtilis*, (c) *P. maculicola*, and (d) *A. fischeri* bioassays.

at 120 rpm and 21 °C for 3 days. The washed mycelium in LB was cut to small pieces with a FastPrep-24 Classic homogenizer (MP Bio, Beograd, Serbia), using 7.2 mm glass beads in a 2 mL Eppendorf tube at 4.5 m/s speed for 20 s. The mycelium suspension was diluted with LB to an optical density (OD_{600}) of 0.4 for *F. avenaceum* or 0.2 for *B. sorokiniana*. HPTLC plates were dipped in the respective mycelium suspension and incubated in a vapor chamber at 21 °C for 48–72 h. The lack of visible white (*F. avenaceum*) or dark gray (*B. sorokiniana*) fungal mycelia indicated the inhibition zones on the bioautograms.

HPTLC–HRMSⁿ. Bioactive zones of interest were eluted with methanol (MS-grade) via an elution head-based interface (PlateExpress, Advion, Ithaca, NY, U.S.A., or TLC-MS Interface 2, CAMAG, equipped with an oval elution head of 4 × 2 mm) coupled to a quaternary pump (Ultimate LPG-3400 XRS, Dionex Softron, Germering, Germany, with a flow rate of 0.1 mL/min) into the heated electrospray probe (HESI-II) of a hybrid quadrupole-Orbitrap mass spectrometer (Q Exactive Plus, Thermo Fisher Scientific, Bremen, Germany). The HESI-II was set to a spray voltage of 3.5 kV, capillary temperature of 270 °C, probe heater temperature of 200 °C, N₂ sheath gas flow of 20 arbitrary units (au), and N₂ auxiliary (drying) gas flow of 10 au. Nitrogen was produced by a SF2 compressor (Atlas Copco Kompressoren and Drucklufttechnik, Essen, Germany). HRMS spectra were recorded in the positive ionization mode as full scan in the range of m/z 50–750, and the automatic gain control (AGC) target, maximum injection time (IT), and resolution were 3×10^6 , 100 ms, and 280 000, respectively. HRMSⁿ spectra were acquired as parallel reaction monitoring with mass isolation of the target molecule, fragmentation energy (collision energy) of 15–100 eV, resolution of 35 000, AGC target at 1×10^5 , maximum IT of 100 ms, and isolation window of m/z 0.4 from m/z 50. Operation and data processing were performed by Xcalibur 3.0.63 software (Thermo).

HPLC–ESI–qTOFMS. For the exact mass determination of the isolated compound **Ss3**, an Agilent 1200 Series HPLC system was used with an Agilent 6520A qTOFMS (Agilent Technologies, Santa Clara, CA, U.S.A.) equipped with a dual ESI ion source operated in negative ionization mode. The mobile phase consisted of 0.1 vol % formic acid (A) and acetonitrile (B). The solvent flow rate was 0.5 mL/min, and the column temperature was set to 25 °C. The injection volume was 5 μ L. Nitrogen was applied as drying gas at the temperature of 325 °C at 5 L/min, and the nebulizer pressure was 30 psi. Full-scan mass spectra were recorded in the negative ion mode in the range of m/z 45–1700. For collision-induced dissociation (CID), the collision energy varied between 10 and 30 eV. As collision gas, high-purity nitrogen was used. The fragmentor voltage was set to 175 V, and the capillary voltage was 3500 V. Product ion mass spectra were recorded in the negative ion mode in the range of m/z 45–700. Reference masses of m/z 112.985587 and 1033.988109 were used to calibrate the mass axis during analysis. Mass spectra were processed using the MassHunter Qualitative Analysis 10.0 software (Agilent).

Determination of IC₅₀. The 96-well microtiter plates were used for the determination of IC₅₀ of the isolates (2 mg/mL in ethanol)

against the mycelial growth of *F. avenaceum* and *B. sorokiniana*. Benomyl (25 mg/mL in ethanol) was used as the positive control, and ethanol was used as the negative control. A 2-fold dilution series of 10 μ L of the isolates in ethanol was prepared in microtiter plates. After the evaporation of the ethanol in a sterile laminar flow, 70 μ L of LB and then 50 μ L of a mycelium suspension (OD_{600} of 0.2 in LB prepared as mentioned for EDA above) were added to each well. OD_{600} of the suspensions was measured by a spectrophotometer (Labsystems Multiscan MS 4.0, Thermo Scientific) immediately and after 72 h of incubation at 21 °C. Each experiment was repeated on different days, and the results were averaged ($n = 3$).

NMR Spectroscopy. The isolated fractions were dried with a rotary evaporator and redissolved in deuterated methanol for NMR measurement. All NMR experiments were carried out on a 600 MHz Varian DDR NMR spectrometer equipped with a 5 mm inverse-detection gradient probehead maintained at 298 K using standard 5 mm NMR tubes. Standard pulse sequences and processing routines available in the VnmrJ 3.2 C/Chempack 5.1 software were used for structure identifications. The complete resonance assignments were established from direct ¹H–¹³C, long-range ¹H–¹³C, and scalar spin–spin connectivities using one-dimensional (1D) ¹H, ¹³C, ¹H–¹H gradient correlation spectroscopy (gCOSY), ¹H–¹H total correlation spectroscopy (TOCSY, NOESY), ¹H–¹³C gradient heteronuclear single-quantum correlation with adiabatic pulses (gHSQCAD) ($J = 140$ Hz), and ¹H–¹³C gradient heteronuclear multiple-bond correlation with adiabatic pulses (gHMBCAD) ($J = 8$ Hz) experiments, respectively. The ¹H chemical shifts were referenced to the applied NMR solvent CD₃OD [δ (CD₂HOD) = 3.310 ppm], and ¹³C chemical shifts were referenced to 49.00 ppm.

RESULTS AND DISCUSSION

In our previous work,¹⁷ the root extracts of *S. virgaurea* and *S. graminifolia* provided distinct chemical HPTLC profiles. However, the compounds at higher hR_F values showed similar chromatographic properties and detectabilities. For HPTLC separation, *n*-hexane–acetone (17:3, v/v) was used to obtain an appropriate resolution of compounds **Sv1** (hR_F of 48), **Sv2** (hR_F of 70), **Sgr1** (hR_F of 39), and **Sgr2** (hR_F of 61) (Figure 1). Meanwhile, the root of another goldenrod species, *S. speciosa*, was found to contain the compounds **Ss1** and **Ss2** with the same hR_F values as those in *S. graminifolia* (**Sgr1** and **Sgr2**, respectively). To screen for antimicrobial compounds, HPTLC was combined with antibacterial (*B. subtilis*, *P. maculicola*, and *A. fischeri*) and antifungal (*B. sorokiniana* and *F. avenaceum*) assays (Figures 1 and 2). In HPTLC–antibacterial assays, the zones of the mentioned compounds showed inhibitory activity; however, **Sgr2** and **Ss2** had very weak activity against Gram-positive *B. subtilis*. The zones **Sgr1**, **Ss1**, **Sv1**, and **Sv2** also displayed antifungal effects against both

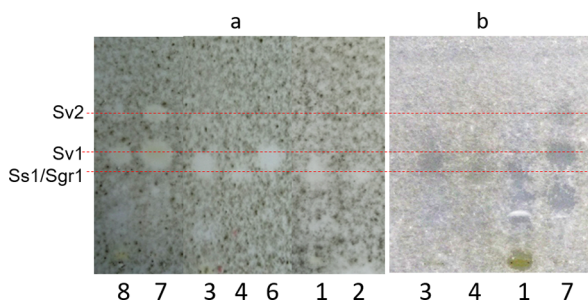


Figure 2. HPTLC bioautograms of *S. graminifolia* (1), *S. speciosa* (3), and *S. virgaurea* (7) root extracts and isolated compounds **Sgr1** (2), **Ss1** (4), **Ss3** (6), and **Sv1** (8), developed with *n*-hexane–acetone (17:3, v/v) and detected after applying the antifungal (a) *B. sorokiniana* and (b) *F. avenaceum* bioassays.

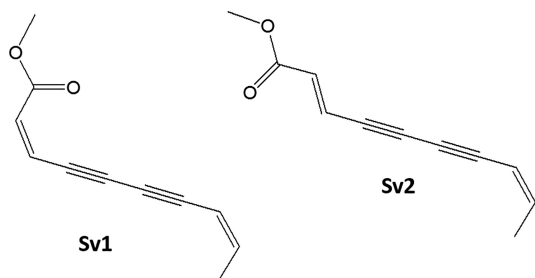


Figure 3. Chemical structures of the identified *S. virgaurea* root compounds, 2Z,8Z-matricaria ester (**Sv1**) and 2E,8Z-matricaria ester (**Sv2**).

test organisms (Figure 2). It has to be noted that the marked compound **Ss3** at h_{RF} of 43 was hardly detected at UV of 254 nm, and in the separated *S. speciosa* extract, a clear inhibition zone (above compound **Ss1**) was not evident.

HPTLC–HRMS and the application of the previously isolated *S. virgaurea* root compound confirmed the identity of compounds **Sv1** and **Sv2** as 2Z,8Z-matricaria ester and 2E,8Z-matricaria ester, respectively (Figure 3). Both isomers were detectable by HPTLC–HESI–HRMS in the positive ionization mode, and their sodium adducts at m/z 197.0575 and 197.0571 (calculated m/z 197.0573 for $C_{11}H_{10}O_2Na^+$) were dominant; however, the protonated molecules at m/z 175.0756 and 175.0755 (calculated m/z 175.0754 for

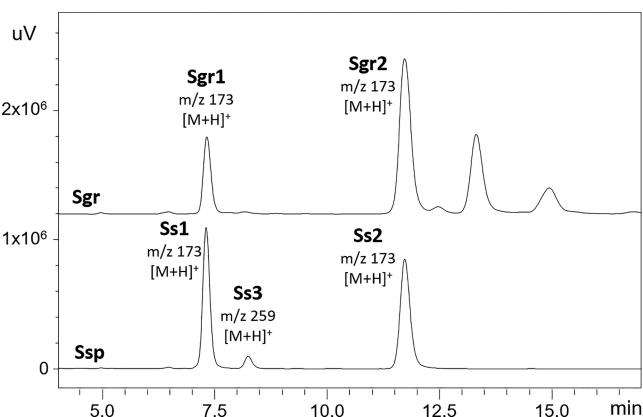


Figure 5. HPLC–DAD chromatograms at UV of 254 nm of the flash fractions of *S. graminifolia* (**Sgr**) and *S. speciosa* (**Ssp**) root extracts (labeled with the corresponding m/z values obtained by HPLC–DAD–ESI–MS).

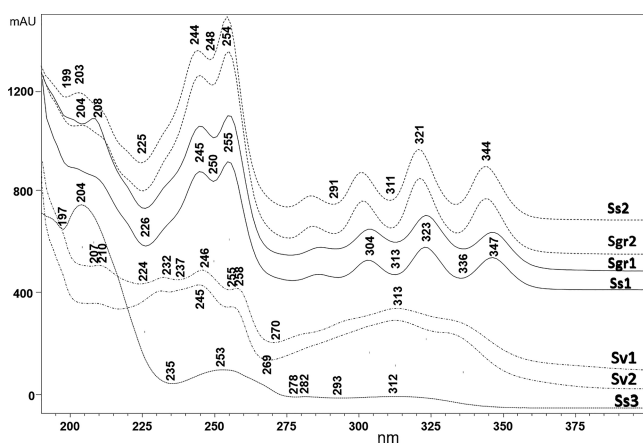


Figure 6. UV spectra of the bioactive goldenrod compounds obtained by HPLC–DAD.

$C_{11}H_{11}O_2^+$) and sodium adduct of the dimers at m/z 371.1257 and 371.1248 (calculated m/z 371.1254 for $C_{22}H_{20}O_4Na^+$) were also recorded, respectively. Similarly, in the spectra of compounds **Sgr1** and **Sgr2**, the sodium adducts at m/z 195.0424 and 195.0425 (calculated m/z 195.0417 for $C_{11}H_8O_2Na^+$) were dominant and the protonated molecules at

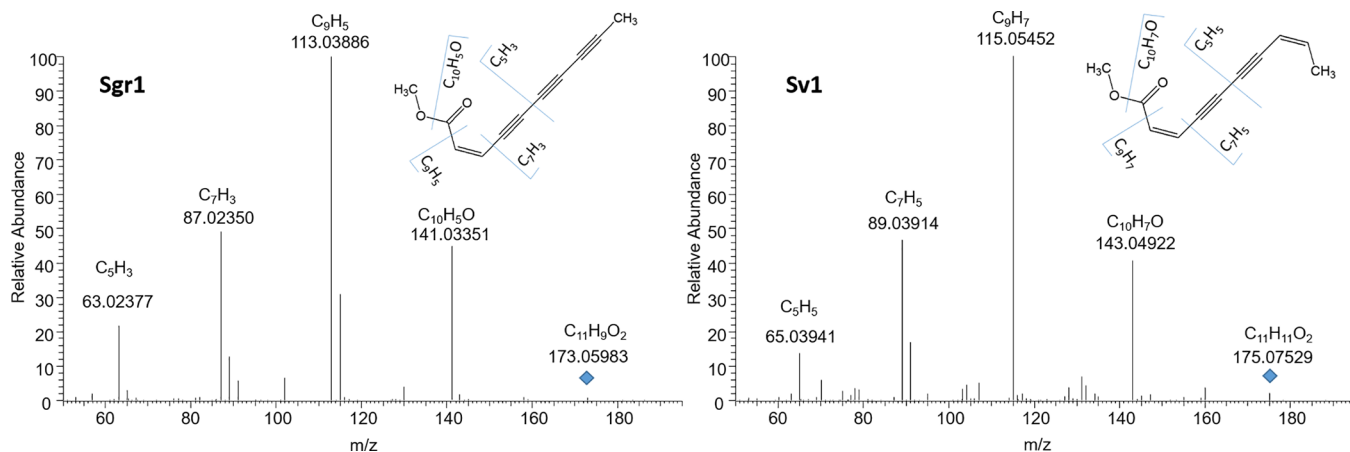


Figure 4. HPTLC–HESI–HRMS spectra of the determined protonated molecule of compounds **Sgr1** and **Sv1** (blue diamonds), obtained via the elution head-based TLC–MS interface 2.

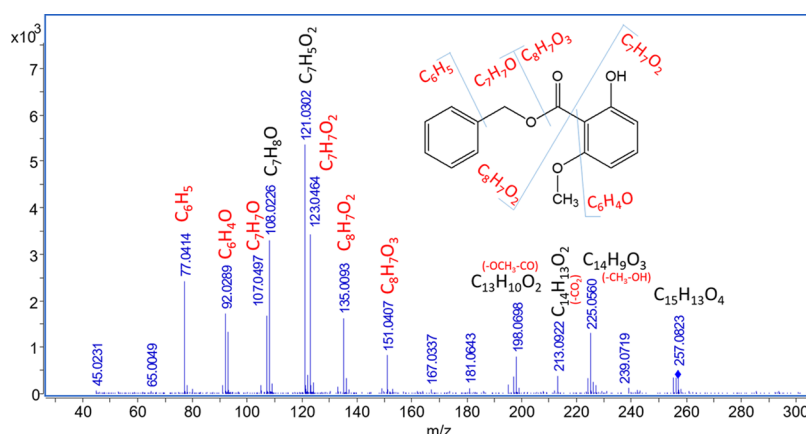


Figure 7. HPLC–ESI–qTOFMS spectra of the deprotonated molecule of compound **Ss3** (blue diamond) obtained at 20 eV collision energy.

m/z 173.0604 and 173.0598 (calculated m/z 173.0597 for $C_{11}H_9O_2^+$) and the sodium adduct of the dimers at m/z 367.0953 and 367.0952 (calculated m/z 367.0941 for $C_{22}H_{16}O_4Na^+$) had low signal intensity. Furthermore, the HPTLC–HESI–HRMSⁿ investigation provided a very similar fragmentation pattern of the protonated molecules of compounds **Sv1** and **Sgr1** with the sequential loss of CH_4O , CO , C_2H_2 , and C_2 groups (Figure 4). After flash chromatography, each fraction with the concentrated compounds of interest of *S. speciosa* and *S. graminifolia* root extracts were compared. Their HPLC–DAD–ESI–MS chromatograms showed the same retention time for the pairs of compounds **Ss1** and **Sgr1** as well as compounds **Ss2** and **Sgr2** (Figure 5), while the MS and UV spectra of compounds **Sgr1**, **Sgr2**, **Ss1**, and **Ss2** were very similar (Figure 6). Thus, it was assumed that compounds **Sgr1** and **Ss1** as well as compounds **Sgr2** and **Ss2** are identical and compounds **Sgr1** and **Sgr2** are isomers. Together with the already mentioned compound **Ss3**, altogether five compounds (**Sgr1**, **Sgr2**, **Ss1**, **Ss2**, and **Ss3**) were isolated as flash chromatographic fractions by semi-preparative HPLC. The isolated compounds were subjected to NMR spectroscopy (Figures S-1–S-36 of the Supporting Information) and compound **Ss3** were also subjected to HPLC–qTOF–MS analysis. Compound **Ss3** was detected in both the positive and negative ionization modes as sodium adduct at m/z 281.0822 and deprotonated molecule at m/z 257.0828 corresponding to the compound with the sum formula $C_{15}H_{14}O_4$. The MS/MS spectrum (Figure 7) recorded at 20 eV collision energy was helpful to elucidate the structure based on the NMR spectrum. The NMR analysis proved that compounds **Sgr1** and **Ss1** as well as compounds **Sgr2** and **Ss2** were identical and determined as 2*Z*-dehydromatricaria ester and 2*E*-dehydromatricaria ester, respectively (Table 1). The NMR spectral data of the isomers were essentially identical with those reported before.^{35–38} The NMR assignment of compound **Ss3** allowed its identification as benzyl 2-hydroxy-6-methoxybenzoate (Table 1) that was also confirmed by comparison to reported spectral data.^{39,40}

The antibacterial activity of the pure isolated compounds was tested via HPTLC–direct bioautography. On the basis of the HPTLC fingerprints (Figure 1a) of the isolates, none of the isomeric forms of matricaria and dehydromatricaria esters could be obtained in pure form because they transformed into each other, even though the isolated forms dominated the respective isolates. It was confirmed that the isolates were responsible for the observed antibacterial effects. The benzyl 2-

hydroxy-6-methoxybenzoate **Ss3** showed a pronounced activity against *A. fischeri*, weak activity against *B. subtilis*, and no activity against *P. maculicola* (Figure 1). In the bioautograms, the 2*Z*,8*Z*-matricaria ester **Sv1** and the *Z*-dehydromatricaria ester **Ss1** inhibited both *B. sorokiniana* and *F. avenaceum*, while compound **Ss3** inhibited *B. sorokiniana* (Figure 2). IC₅₀ determined by the microdilution assay of the isolates against fungal (mycelial) growth of both species was lowest for compound **Ss3**, followed by compounds **Sv1** and **Ss1** (Table 2). Note that the *E*-dehydromatricaria ester **Ss2** did not reach IC₅₀ at the maximum concentration used for *F. avenaceum*. The previous comparison of the bioactivity of the isomers indicated similarly that the 2*Z*,8*Z*-matricaria ester was more active than the 2*E*,8*E*-matricaria ester against plant pathogenic fungal species.⁴¹ The *Z*,*Z* form also had a similar or stronger antimycobacterial effect than the *E*,*Z* form.⁴²

C_{10} -polyacetylenes, like the identified esters, are allelochemicals with strong inhibitory activities on other plants.⁴³ Matricaria and dehydromatricaria esters have been found in the roots of several goldenrod species, including *S. virgaurea* and *S. graminifolia*.^{12,44–46} However, to our knowledge, this is the first report about the presence of dehydromatricaria esters in the root of *S. speciosa*. There are few literature data regarding the bioactivity of the isolated compounds. Essential oils of *Conyza* species rich in matricaria ester (74 and 88%) were found to be antifungal against *Cryptococcus neoformans*, *Rhodotorula glutinis*, *Microsporum canis*, and *Candida* and *Trichophyton* species, antibacterial against *Staphylococcus aureus*, and phytotoxic against *Raphanus sativus*, with inhibition of the seed germination.^{47,48} Both *Z*,*Z*- and *E*,*Z*-matricaria esters inhibited *Mycobacterium tuberculosis* and *Mycobacterium avium*⁴² and showed remarkable nematocidal⁴⁹ and α -glucosidase, β -glucosidase, α -amylase, acetylcholinesterase, and butyrylcholinesterase inhibitory activity.¹⁷ *Z*,*Z*-Matricaria ester exhibited antifungal effects against *Botrytis cinerea*, *Colletotrichum acutatum*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *Cladosporium cucumerinum*, and *Pyricularia oryzae* plant pathogenic fungi,^{41,50} antibacterial activity against *B. subtilis*, *P. syringae maculicola*, *Xanthomonas euvesicatoria*, *Lactobacillus plantarum*, and *A. fischeri*,¹² and molluscicidal activity against *Planorbella trivolvis*.⁴¹ *E*,*Z*-Matricaria ester displayed antileishmanial activity.⁵¹ Moreover, *Z*- and *E*-dehydromatricaria esters exhibited nematocidal activity and cytotoxicity against human cancer cell lines,^{46,49,52} but *Z*-dehydromatricaria ester also inhibited the growth of normal mammalian cells.⁴⁶ Furthermore, *Z*-dehydromatricaria ester

Table 1. NMR Spectral Data and Structures of Compounds Ss1, Ss2, and Ss3

2Z-Dehydromatricaria ester			2E-Dehydromatricaria ester			Benzyl 2-hydroxy-6-methoxybenzoate		
Ss1			Ss2			Ss3		
number	¹ H δ (ppm)	¹³ C δ (ppm)	¹ H δ (ppm)	¹³ C δ (ppm)	¹ H δ (ppm)	¹³ C δ (ppm)	¹ H δ (ppm)	¹³ C δ (ppm)
1		166.13						108.36
2	6.37 (d, ³ J _{H2,H3} (cis) = 11.5 Hz, 1H)	133.92	6.44 (d, ³ J _{H2,H3} (trans) = 15.9 Hz, 1H)	135.14				160.92
3	6.32 (dd, ³ J _{H2,H3} (cis) = 11.5 Hz, ⁹ J _{H10,H3} = 0.5 Hz, 1H)	122.44	6.79 (dd, ³ J _{H2,H3} (trans) = 15.9 Hz, ⁹ J _{H10,H3} = 0.5 Hz, 1H)	124.20	6.53 (d, J = 8.3 Hz, 1H)			103.54
4		72.20			7.28 (t, J = 8.3 Hz, 1H)			134.49
5		85.98			6.51 (dd, ³ J _{H5,H4} = 8.3, ⁵ J _{H5,H8} = 0.9 Hz, 1H)			110.13
6		58.78						161.00
7		72.32						170.62
8		64.88			3.81 (br s, 3H)			56.48
9		82.24						
10	2.030 (d, ⁹ J _{H10,H3} = 0.5 Hz, 3H)	4.02	2.026 (d, ⁹ J _{H10,H3} = 0.5 Hz, 3H)	3.98				
11	3.761 (s, 3H)	52.18	3.755 (s, 3H)	52.56				
1'								137.54
2', 6'					7.47 (d, J = 7.5 Hz, 2H)			128.78
3', 5'					7.37 (t, J = 7.3 Hz, 2H)			129.45
4'					7.31 (t, J = 7.6 Hz, 1H)			129.02
7'					5.37 (s, 2H)			67.78

Table 2. IC₅₀ Values of the Isolated Compounds and the Fungicide Benomyl against Fungal Strains in $\mu\text{g/mL}$

	<i>B. sorokiniana</i>	<i>F. avenaceum</i>
Z-dehydromatricaria ester	69.5 \pm 3.3	83.3 \pm 5.7
E-dehydromatricaria ester	106.7 \pm 6.2	
benzyl 2-hydroxy-6-methoxybenzoate	25.1 \pm 2.3	26.0 \pm 3.1
Z,Z-matricaria ester	31.1 \pm 2.4	47.5 \pm 1.1
benomyl	80.4 \pm 5.2	5.1 \pm 2.9

showed antimycobacterial activity against *M. tuberculosis* and *M. avium*.⁴² However, without the improvement of the chemical stability of polyacetylenes, e.g., by formulation or modification, their application as pesticides or drugs is limited.⁴¹ Benzyl 2-hydroxy-6-methoxybenzoate has been isolated from various plants, among others, from roots of *Lindera fruticosa*,⁵³ *Securidaca longipedunculata*,⁵⁴ and *Securidaca diversifolia*⁴⁰ and aerial parts of *Ageratina deltoidea*⁵⁵ but not from *Solidago* species so far. This benzyl benzoate exhibited cytotoxic (against HeLa cell lines), antiviral (against Herpes simplex virus type 1 and poliovirus Sabin 1), and human acyl-CoA:cholesterol acyltransferase inhibitory effects,^{40,53} but it was not active against *S. aureus*, *Escherichia coli*, and *Candida albicans*.⁵⁵

In conclusion, HPTLC bioassays developed for crop pathogenic fungi enabled the screening and selection of fungicidal goldenrod root compounds. The targeted, effect-directed HPTLC–HESI–HRMSⁿ characterization, isolation, and NMR analysis resulted in the identification of Z,Z- and E,Z-matricaria esters, Z- and E-dehydromatricaria esters, and benzyl 2-hydroxy-6-methoxybenzoate. To our knowledge, dehydromatricaria esters and benzyl 2-hydroxy-6-methoxybenzoate were found in *S. speciosa* for the first time. All five compounds inhibited the mycelial growth of *F. avenaceum* and *B. sorokiniana*. Benzyl 2-hydroxy-6-methoxybenzoate was the most effective, followed by Z,Z-matricaria and Z-dehydromatricaria esters. These showed potential to become agrochemical agents or lead compounds after appropriate modification and formulation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.1c03676>.

NMR spectroscopy of the isolated compounds (Figures S-1–S-36) (PDF)

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Notes

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■ REFERENCES

- (1) Vogelgsang, S.; Sulyok, M.; Hecker, A.; Jenny, E.; Krska, R.; Schuhmacher, R.; Forrer, H.-R. Toxicogenicity and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* on wheat. *Eur. J. Plant Pathol.* **2008**, *122* (2), 265–276.
- (2) Pollard, A. T.; Okubara, P. A. Real-time PCR quantification of *Fusarium avenaceum* in soil and seeds. *J. Microbiol. Methods* **2019**, *157*, 21–30.
- (3) *The Fusarium Laboratory Manual*; Leslie, J. F., Summerell, B. A., Eds.; Blackwell Publishing: Ames, IA, 2006; DOI: [10.1002/9780470278376](https://doi.org/10.1002/9780470278376).

- (4) Lysoe, E.; Harris, L. J.; Walkowiak, S.; Subramaniam, R.; Divon, H. H.; Riiser, E. S.; Llorens, C.; Gabaldón, T.; Kistler, H. C.; Jonkers, W.; Kolseth, A.-K.; Nielsen, K. F.; Thrane, U.; Frandsen, R. J. N. The genome of the generalist plant pathogen *Fusarium avenaceum* is enriched with genes involved in redox, signaling and secondary metabolism. *PLoS One* **2014**, *9* (11), No. e112703.
- (5) *Compendium of Barley Diseases*, 2nd ed.; Mathre, D. E., Ed.; APS Press: St. Paul, MN, 1997; pp 120.
- (6) Kumar, J.; Schäfer, P.; Hückelhoven, R.; Langen, G.; Baltruschat, H.; Stein, E.; Nagarajan, S.; Kogel, K.-H. *Bipolaris sorokiniana* a cereal pathogen of global concern: Cytological and molecular approaches towards better control. *Mol. Plant Pathol.* **2002**, *3* (4), 185–195.
- (7) Berestetskii, A. O.; Dalinova, A. A.; Dubovik, V. R.; Grigoryeva, E. N.; Kochura, D. M.; Senderskiy, I. V.; Smirnov, S. N.; Stepanycheva, E. A.; Turaeva, S. M. Analysis and isolation of secondary metabolites of *Bipolaris sorokiniana* by different chromatography techniques and the spectrum of their biological activity. *Appl. Biochem. Microbiol.* **2020**, *56* (5), 569–582.
- (8) Ali, L.; Khan, A. L.; Hussain, J.; Al-Harrasi, A.; Waqas, M.; Kang, S.-M.; Al-Rawahi, A.; Lee, I.-J. Sorokinol: A new enzymes inhibitory metabolite from fungal endophyte *Bipolaris sorokiniana* LK12. *BMC Microbiol.* **2016**, *16* (1), 103.
- (9) Nakajima, H.; Isomi, K.; Hamasaki, T.; Ichinoe, M. Sorokinianin: A novel phytotoxin produced by the phytopathogenic fungus *Bipolaris sorokiniana*. *Tetrahedron Lett.* **1994**, *35* (51), 9597–9600.
- (10) Qader, M. M.; Kumar, N. S.; Jayasinghe, L.; Araya, H.; Fujimoto, Y. Bioactive Sesquiterpenes from an endophytic fungus *Bipolaris sorokiniana* isolated from a popular medicinal plant *Costus speciosus*. *Mycology* **2017**, *8* (1), 17–20.
- (11) Morlock, G.; Schwack, W. Hyphenations in planar chromatography. *J. Chromatogr. A* **2010**, *1217* (43), 6600–6609.
- (12) Móricz, Á. M.; Ott, P. G.; Häbe, T. T.; Darcsi, A.; Böszörményi, A.; Alberti, A.; Krüzselyi, D.; Csontos, P.; Béni, S.; Morlock, G. E. Effect-directed discovery of bioactive compounds followed by highly targeted characterization, isolation and identification, exemplarily shown for *Solidago virgaurea*. *Anal. Chem.* **2016**, *88* (16), 8202–8209.
- (13) Morlock, G. E. High-performance thin-layer chromatography combined with effect-directed assays and high-resolution mass spectrometry as an emerging hyphenated technology: A tutorial review. *Anal. Chim. Acta* **2021**, *1180*, 338644.
- (14) Schreiner, T.; Morlock, G. E. Non-target bioanalytical eight-dimensional hyphenation including bioassay, heart-cut trapping, online desalting, orthogonal separations and mass spectrometry. *J. Chromatogr. A* **2021**, *1647*, 462154.
- (15) Móricz, Á. M.; Ott, P. G. Conventional and modern bioassays—Detection, isolation, identification. In *Forced-Flow Layer Chromatography*; Tyihák, E., Ed.; Elsevier: Amsterdam, Netherlands, 2016; Chapter 6, pp 347–395, DOI: 10.1016/B978-0-12-420161-3.00006-X.
- (16) Móricz, Á. M.; Krüzselyi, D.; Ott, P. G.; Garádi, Z.; Béni, S.; Morlock, G. E.; Bakonyi, J. Bioactive clerodane diterpenes of giant goldenrod (*Solidago gigantea* Ait.) root extract. *J. Chromatogr. A* **2021**, *1635*, 461727.
- (17) Móricz, Á. M.; Jamshidi-Aidji, M.; Krüzselyi, D.; Darcsi, A.; Böszörményi, A.; Csontos, P.; Béni, S.; Ott, P. G.; Morlock, G. E. Distinction and valorization of 30 root extracts of five goldenrod (*Solidago*) species. *J. Chromatogr. A* **2020**, *1611*, 460602.
- (18) Morlock, G.; Schwack, W. Coupling of planar chromatography to mass spectrometry. *TrAC, Trends Anal. Chem.* **2010**, *29* (10), 1157–1171.
- (19) Morlock, G. E.; Brett, N. Correct assignment of lipophilic dye mixtures? A case study for high-performance thin-layer chromatography—mass spectrometry and performance data for the TLC–MS interface. *J. Chromatogr. A* **2015**, *1390*, 103–111.
- (20) Krüger, S.; Hüskens, L.; Fornasari, R.; Scainelli, I.; Morlock, G. E. Effect-directed fingerprints of 77 botanical extracts via a generic high-performance thin-layer chromatography method combined with assays and mass spectrometry. *J. Chromatogr. A* **2017**, *1529*, 93–106.
- (21) Weber, E. The Dynamics of plant invasions: A case study of three exotic goldenrod species (*Solidago* L.) in Europe. *J. Biogeogr.* **1998**, *25* (1), 147–154.
- (22) Skrzypczak, L.; Wesolowska, M.; Thiem, B.; Budzianowski, J. *Solidago* L. Species (Goldenrod): In Vitro Regeneration and Biologically Active Secondary Metabolites. In *Medicinal and Aromatic Plants XI*; Bajaj, Y. P. S., Ed.; Springer: Berlin, Germany, 1999; Biotechnology in Agriculture and Forestry, Vol. 43, pp 384–403, DOI: 10.1007/978-3-662-08614-8_23.
- (23) Schmotzer, A. Occurrence of *Solidago graminifolia* (L.) Salisb. in Hungary. *Flora Pannonica* **2008**, *6*, 59–77.
- (24) Densmore, F. *Strength of the Earth: The Classic Guide to Ojibwe Uses of Native Plants*; Minnesota Historical Society: Saint Paul, MN, 2005.
- (25) Fursenco, C.; Calalb, T.; Uncu, L.; Dinu, M.; Ancuceanu, R. *Solidago virgaurea* L.: A review of its ethnomedicinal uses, phytochemistry, and pharmacological activities. *Biomolecules* **2020**, *10* (12), 1619.
- (26) Toiu, A.; Vlase, L.; Vodnar, D. C.; Gheldiu, A.-M.; Oniga, I. *Solidago graminifolia* L. Salisb. (Asteraceae) as a valuable source of bioactive polyphenols: HPLC profile, in vitro antioxidant and antimicrobial potential. *Molecules* **2019**, *24* (14), 2666.
- (27) Kolodziej, B. Antibacterial and Antimutagenic Activity of extracts aboveground parts of three *Solidago* species: *Solidago virgaurea* L., *Solidago canadensis* L. and *Solidago gigantea* Ait. *J. Med. Plants Res.* **2011**, *5* (31), 6770–6779.
- (28) Anžlovar, S.; Koce, J. D. Antibacterial and antifungal activity of aqueous and organic extracts from indigenous and invasive species of goldenrod (*Solidago* spp.) grown in Slovenia. *Phyton (Horn, Austria)* **2014**, *54* (1), 135–147.
- (29) Konovalov, D. A. Polyacetylene compounds of plants of the Asteraceae family (Review). *Pharm. Chem. J.* **2014**, *48* (9), 613–631.
- (30) Negri, R. Polyacetylenes from terrestrial plants and fungi: Recent phytochemical and biological advances. *Fitoterapia* **2015**, *106*, 92–109.
- (31) Veneziani, R. C. S.; Ambrósio, S. R.; Martins, C. H. G.; Lemes, D. C.; Oliveira, L. C. Antibacterial potential of diterpenoids. *Stud. Nat. Prod. Chem.* **2017**, *54*, 109–139.
- (32) Móricz, Á. M.; Häbe, T. T.; Böszörményi, A.; Ott, P. G.; Morlock, G. E. Tracking and identification of antibacterial components in the essential oil of *Tanacetum vulgare* L. by the combination of high-performance thin-layer chromatography with direct bioautography and mass spectrometry. *J. Chromatogr. A* **2015**, *1422*, 310–317.
- (33) Móricz, Á. M.; Häbe, T. T.; Ott, P. G.; Morlock, G. E. Comparison of high-performance thin-layer with overpressured layer chromatography combined with direct bioautography and direct analysis in real time mass spectrometry for tansy root. *J. Chromatogr. A* **2019**, *1603*, 355–360.
- (34) Móricz, Á. M.; Tyihák, E.; Ott, P. Usefulness of transgenic luminescent bacteria in direct bioautographic investigation of chamomile extracts. *J. Planar Chromatogr.-Mod. TLC* **2010**, *23* (3), 180–183.
- (35) Hearn, M. T. W.; Turner, J. L. The carbon-13 nuclear magnetic resonance spectra of the antibiotic polyacetylenic nitrile, diatretyne 2 (7-cyanohept-trans-2-ene-4,6-dienoic acid), and related compounds. *J. Chem. Soc., Perkin Trans. 2* **1976**, No. 9, 1027.
- (36) Wang, Q.; Hao, J.; Gong, J.; Bao, W. Isolation and structure elucidation of two new compounds from *Artemisia ordosica* Krasch. *Nat. Prod. Res.* **2020**, *34* (13), 1862–1867.
- (37) Drake, D. Polyacetylenes of *Artemisia vulgaris*. *Phytochemistry* **1974**, *13* (2), 455–457.
- (38) Tsao, R.; Eto, M. Light-activated plant growth inhibitory activity of cis-dehydromatricaria ester, rose bengal and fluoren-9-one on lettuce (*Lactuca saliva* L.). *Chemosphere* **1996**, *32* (7), 1307–1317.
- (39) Kodpinid, M.; Sadavongvivad, C.; Thebtaranonth, C.; Thebtaranonth, Y. Benzyl benzoates from the root of *Uvaria purpurea*. *Phytochemistry* **1984**, *23* (1), 199–200.

- (40) Casu, L.; Solinas, M. N.; Saba, A. R.; Cottiglia, F.; Caboni, P.; Floris, C.; Laconi, S.; Pompei, R.; Leonti, M. Benzophenones from the roots of the popoluca amerindian medicinal plant *Securidaca diversifolia* (L.) S.F. Blake. *Phytochem. Lett.* **2010**, *3* (4), 226–229.
- (41) Meepagala, K. M.; Sturtz, G.; Wise, D.; Wedge, D. E. Molluscicidal and antifungal activity of *Erigeron speciosus* steam distillate. *Pest Manag. Sci.* **2002**, *58* (10), 1043–1047.
- (42) Lu, T.; Cantrell, C.; Robbs, S.; Franzblau, S.; Fischer, N. Antimycobacterial matricaria esters and lactones from *Astereae* species. *Planta Med.* **1998**, *64* (7), 665–667.
- (43) Kobayashi, A.; Morimoto, S.; Shibata, Y.; Yamashita, K.; Numata, M. C10-Polyacetylenes as allelopathic substances in dominants in early stages of secondary succession. *J. Chem. Ecol.* **1980**, *6* (1), 119–131.
- (44) Lam, J.; Christensen, L. P.; Färch, T.; Thomasen, T. Acetylenes from the roots of *Solidago* species. *Phytochemistry* **1992**, *31* (12), 4159–4161.
- (45) Lam, J. Polyacetylenes of *Solidago virgaurea*: Their seasonal variation and NMR long-range spin coupling constants. *Phytochemistry* **1971**, *10* (3), 647–653.
- (46) Matsunaga, H.; Katano, M.; Tasaki, M.; Yamamoto, H.; Mori, M.; Takata, K. Inhibitory effect of cis-dehydromatricaria ester isolated from *Solidago altissima* on the growth of mammalian cells. *Chem. Pharm. Bull.* **1990**, *38* (12), 3483–3484.
- (47) Veres, K.; Csopor-Löffler, B.; Lázár, A.; Hohmann, J. Antifungal activity and composition of essential oils of *Conyza canadensis* herbs and roots. *Sci. World J.* **2012**, *2012*, 1–5.
- (48) Mabrouk, S.; Salah, K. B. H.; Elaissi, A.; Jlaiei, L.; Jannet, H. B.; Aouni, M.; Harzallah-Skhiri, F. Chemical composition and antimicrobial and allelopathic activity of Tunisian *Conyza sumatrensis* (Retz.) E. Walker essential oils. *Chem. Biodiversity* **2013**, *10* (2), 209–223.
- (49) Kimura, Y.; Mori, M.; Suzuki, A.; Kobayashi, A. Isolation and identification of two nematocidal substances from roots of *Erigeron philadelphicus* L. and nematocidal activities of their related compounds. *Agric. Biol. Chem.* **1981**, *45* (12), 2915–2917.
- (50) Vidari, G.; Abdo, S.; Gilardoni, G.; Ciapessoni, A.; Gusmeroli, M.; Zanon, G. Fungitoxic metabolites from *Erigeron apiculatus*. *Fitoterapia* **2006**, *77* (4), 318–320.
- (51) Chandra Pandey, S.; Dhami, D. S.; Jha, A.; Chandra Shah, G.; Kumar, A.; Samant, M. Identification of trans -2- cis -8-matricaria-ester from the essential oil of *Erigeron multiradiatus* and evaluation of its antileishmanial potential by in vitro and in silico approaches. *ACS Omega* **2019**, *4* (11), 14640–14649.
- (52) Lone, S. H.; Bhat, K. A.; Naseer, S.; Rather, R. A.; Khuroo, M. A.; Tasduq, S. A. Isolation, cytotoxicity evaluation and HPLC-quantification of the chemical constituents from *Artemisia amygdalina* Decne. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2013**, *940*, 135–141.
- (53) Song, M.-C.; Nigussie, F.; Jeong, T.-S.; Lee, C.-Y.; Regassa, F.; Markos, T.; Baek, N.-I. Phenolic compounds from the roots of *Lindera fruticosa*. *J. Nat. Prod.* **2006**, *69* (5), 853–855.
- (54) Dibwe, D. F.; Awale, S.; Kadota, S.; Morita, H.; Tezuka, Y. Hepta-oxygenated xanthenes as anti-austerity agents from *Securidaca longepedunculata*. *Bioorg. Med. Chem.* **2013**, *21* (24), 7663–7668.
- (55) Arciniegas, A.; Pérez-Castorena, A. L.; Meléndez-Aguirre, M.; Ávila, J. G.; García-Bores, A. M.; Villaseñor, J. L.; Romo de Vivar, A. Chemical composition and antimicrobial activity of *Ageratina deltoidea*. *Chem. Biodiversity* **2018**, *15* (3), No. e1700529.