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PHYTOCHEMICAL CHARACTERISTICS OF COMFREY (*SYMPHYTUM OFFICINALE* L.) GROWN EX SITU: POLAR METABOLITES AND ROOT INULIN

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Abstract
Comfrey (Symphytum officinale L.) roots are a known source of phenolic compounds and inulin-type fructans, but most data come from commercial dried roots of unknown origin. In this study, roots and leaves of comfrey from the ex situ collection of the Institute for Roses and Aromatic Plants (Kazanlak, Bulgaria) were characterized. The polar metabolite profile was determined by GC-MS after methoximation and silylation. Inulin was isolated from defatted roots by microwave-assisted water extraction and analysed by FTIR and ^{1}H NMR spectroscopy. Thirty compounds were quantified: sugars, polyols, sugar phosphates and organic, phenolic, amino and fatty acids. Sugars dominated in all samples. Sucrose was the main compound (88.6–164.8 mg/g dw), followed by fructose and glucose. Roots harvested in 2024 had the highest total of identified metabolites (370 mg/g dw), The FTIR spectrum of the isolated polysaccharide showed the bands typical of inulin-type fructans at 1122, 1019, 932, 870 and 816 cm^{-1}. ^{1}H NMR confirmed the terminal α-D-glucopyranosyl unit and gave a number-average degree of polymerization of 32. Comfrey roots grown ex situ are a good source of long-chain inulin.

Keywords: *Symphytum officinale*, *ex situ* cultivation, GC-MS, sugars, inulin, FTIR, ^{1}H NMR.

INTRODUCTION

Comfrey (*Symphytum officinale* L., Boraginaceae) grows in Europe, Asia and South America. Its roots have been used for centuries to treat bone fractures, sprains, wounds, joint pain and haematomas [1–3]. These effects are linked to allantoin, rosmarinic acid and other hydroxycinnamic acid derivatives, mucilage polysaccharides, triterpene saponins and tannins [1, 2]. The roots also contain hepatotoxic pyrrolizidine alkaloids, which is why comfrey is used mainly externally [3, 4].

Most studies on comfrey roots deal with polyphenols [2, 5, 6]. Carbohydrates have received less attention, although fructans are the main reserve carbohydrates of the root. Their content varies between 27 and 47 % dw, depending on harvest time and plant development [7]. In our earlier work, comfrey roots contained 27–32 g/100 g dw fructans [8], and inulin isolated from commercial roots had a degree of polymerisation (DP) of 29–33 [9]. We also

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described the GC-MS profile of four successive solvent fractions from commercial roots [10]. Comfrey root polysaccharides have been studied for their physicochemical properties and antioxidant and hypoglycaemic activity as well [11, 12].

Inulin is a linear chain of β-(2→1)-linked fructofuranosyl units, usually ending with an α-D-glucopyranosyl unit. It is a prebiotic, and its solubility, gelling ability and technological use depend strongly on chain length [13]. This makes DP one of the key parameters when a new inulin source is evaluated.

Commercial dried roots are convenient, but their origin, soil and harvest date are usually unknown. Plants grown in an *ex situ* collection give a better defined material. The aim of this study was to determine the polar metabolite profile of roots and leaves of comfrey grown *ex situ* and to characterise inulin isolated from its roots by FTIR and ^{1}H NMR spectroscopy.

MATERIALS AND METHODS

Plant material. Roots of *S. officinale* were collected at the end of October 2024 and at the beginning of November 2025 from the *ex situ* collection of the Institute for Roses and Aromatic Plants, Kazanlak, Bulgaria (372 m a.s.l.), located in a transitional continental climate zone. The plants were grown on cinnamonic forest soil formed on old deluvial deposits, with low base saturation, low cation exchange capacity and low humus content (1.8 %). The soil texture is dominated by fine sand, which gives it low water-holding capacity and high aeration and permeability. The soil pH is moderately acidic (pH 4.9). A voucher specimen was deposited in the herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOM 179645).

GC-MS analysis of polar metabolites. The dry residue of each extract was derivatised in two steps: with methoxyamine hydrochloride (20 mg/mL in pyridine, 150 µL, 1 h at 70 °C and 300 rpm) and then with 100 µL BSTFA (40 min at 70 °C and 300 rpm). Methoximation converts the carbonyl groups of reducing sugars into oximes and limits the number of anomeric peaks, while silylation replaces the active hydrogens of hydroxyl, carboxyl and amino groups with trimethylsilyl (TMS) groups. The analysis was carried out on a DB-5ms capillary column (30 m × 0.25 mm, 0.25 µm film), helium at 1.0 mL/min and a temperature program from 100 to 300 °C. Mass spectra were recorded in EI mode (70 eV) in the range 50–550 m/z. Compounds were identified by comparing their mass spectra with the NIST 08 library and their Kováts retention indices (RI), calculated against a C8–C40 n-alkane mixture run under the same conditions. Quantification was done with an internal standard, and the results are expressed as mg/g dry weight (dw).

Isolation of inulin. Inulin was isolated from roots harvested in 2025 by microwave-assisted water extraction, as described earlier [9]. The ground roots were first washed successively with hexane, chloroform and ethyl acetate to remove lipids and polyphenols. The dried residue (30 g) was extracted with 300 mL boiling water in a microwave device at 700 W for 5 min. The filtered extract was precipitated with acetone (1:4, v/v), the precipitate was recrystallised from boiling water, precipitated again with acetone (1:5, v/v) and dried at 40 °C.

FTIR spectroscopy. The isolated polysaccharide (2 mg) was pressed into a KBr tablet and the spectrum was recorded in the range 4000–400 cm^{-1}.

^{1}H NMR spectroscopy. The ^{1}H NMR spectrum was recorded in D_2O. The number-average DP was calculated following Barclay et al. [14]. The anomeric proton of the terminal glucose (about 5.44 ppm) was integrated and set to 1. The region 3.30–4.40 ppm, which holds all other glucose and fructose protons, was then integrated ($I_{3.30–4.40}$). Since the glucose unit contributes six of these protons and each fructose unit seven, DP was calculated as:

$$DP = (I_{3.30–4.40} − 6) / 7 + 1 \quad (1)$$

RESULTS AND DISCUSSION

Polar metabolite profile of roots and leaves. Thirty compounds were identified and quantified in the three samples (Table 1). They belong to nine groups: monosaccharides, sucrose, polyols, sugar phosphates, organic acids, phenolic acids, amino acids, fatty acids and phosphoric acid. The total of identified compounds was 276.7 mg/g dw in roots A, 370.1 mg/g dw in roots B.

Table 1. *Polar metabolites in roots of S. officinale grown ex situ, GC-MS (mg/g dw)*

No.	Compound	RI	Roots A	Roots B (2024)
1	Oxalic acid 2TMS	1122	1.47	0.74
2	Phosphoric acid 3TMS	1274	2.51	5.86
3	Succinic acid 2TMS	1313	0.33	1.60
4	Glyceric acid 3TMS	1319	0.40	1.77
5	Maleic acid 2TMS	1333	nd	1.90
6	Pipecolic acid 2TMS	1362	nd	1.42
7	Malic acid 3TMS	1478	1.29	4.85
8	Adipic acid 2TMS	1498	0.78	1.84
9	Salicylic acid 2TMS	1505	0.64	0.71
10	γ-Aminobutyric acid 3TMS	1522	1.41	nd
11	Cinnamic acid TMS	1549	1.21	1.67
12	Glutamic acid 3TMS	1609	2.13	1.79
13	Xylose oxime 4TMS	1633	2.43	1.50
14	Xylose oxime 4TMS	1640	0.85	0.24
15	Arabinose oxime 4TMS	1648	3.59	3.89
16	Xylitol 5TMS	1690	0.94	nd
17	Shikimic acid 4TMS	1793	nd	2.84
18	Citric acid 4TMS	1815	1.99	3.37
19	Fructose oxime 5TMS	1855	28.67	49.35
20	Fructose oxime 5TMS	1864	18.16	37.76
21	Glucose oxime 6TMS	1881	19.04	42.24
22	Glucose oxime 6TMS	1901	2.84	11.66
23	Mannitol 6TMS	1914	3.49	19.58
24	Glucose 1-phosphate 6TMS	1937	11.87	26.96
25	Palmitic Acid 1TMS	2040	1.70	2.54
26	Myo-Inositol 6TMS	2079	1.96	1.11
27	Stearic acid 1TMS	2241	2.23	3.33
28	myo-Inositol-1-phosphate 7TMS	2487	nd	17.04
29	Maltitol 9TMS	2601	nd	33.94
30	Sucrose 8TMS	2620	164.75	88.60
	Total identified		**276.68**	**370.10**

nd – not detected; RI – Kováts retention index; Roots A and Roots B – two root samples, Roots B harvested in October 2024.

Free sugars dominated all samples. Together, monosaccharides and sucrose made up 87 % of the identified compounds in roots A and about 63 % in roots B and leaves. Sucrose was the main single compound everywhere: 164.8 mg/g dw in roots A, 88.6 mg/g dw in roots B. Fructose (two oxime isomers) reached 46.8 and 87.1 mg/g dw in the two root samples. Glucose was lower, 21.9 and 53.9 mg/g dw in roots. In both root samples free fructose exceeded glucose. Sucrose is the substrate for fructan synthesis, and fructose is released when fructans are broken down by fructan exohydrolases [15]. The high levels of both in the roots fit well with the role of the root as a fructan storage organ. Xylose and arabinose were found in all samples (3.4–6.9 mg/g dw together) and probably come from cell wall polysaccharides.

Roots B differed from roots A mainly in their polyol and sugar phosphate content. Mannitol (19.6 mg/g dw), maltitol (33.9 mg/g dw) and myo-inositol 1-phosphate (17.0 mg/g dw) were present at notable levels only in this sample. Shikimic, maleic and pipecolic acids were also found only in roots B, while xylitol and γ-aminobutyric acid were detected only in roots A. The two root samples come from different harvests, and these differences most likely reflect harvest year and plant age. Replicate samples from each harvest are needed to confirm this.

Organic acids (succinic, glyceric, malic, adipic, citric, oxalic) were found in all samples, with a total of 6.3–18.9 mg/g dw. Amino acids (glutamic acid, γ-aminobutyric acid, pipecolic acid) and fatty acids (palmitic and stearic) were minor components, below 6 mg/g dw. Low fatty acid levels are expected, since the derivatisation method targets polar metabolites. In our earlier GC-MS study of commercial roots, fructose oximes and sucrose were also among the main compounds in the polar ethyl acetate and ethanol fractions [10].

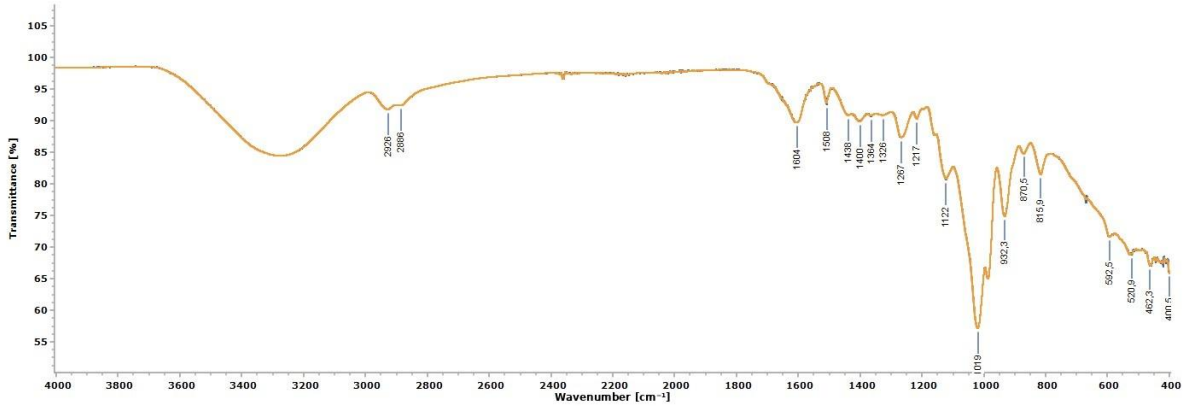


Fig. 1. *FTIR spectrum of inulin isolated from roots of S. officinale grown ex situ (harvest 2025) by microwave-assisted extraction*

FTIR spectrum of the isolated inulin. The FTIR spectrum of the polysaccharide isolated from roots harvested in 2025 is shown in Fig. 1, and the band assignment is given in Table 2. The spectrum shows a broad O–H stretching band centred at about 3280 cm^{-1}, due to inter- and intramolecular hydrogen bonds. The bands at 2926 and 2886 cm^{-1} correspond to asymmetric and symmetric C–H stretching of CH_2 groups.

Table 2. *Main FTIR bands of the isolated comfrey inulin*

Observed band, cm^{-1}	Comfrey root inulin [9], cm^{-1}	Assignment
≈3280 (broad)	3396	ν O–H, inter- and intramolecular H-bonds
2926	2930	ν_{as} C–H (CH_2)
2886	2889	ν_s C–H (CH_2)
1604	–	δ H–O–H of bound water; ν_{as} COO^- (uronic acids)
1508	–	Aromatic C=C (residual phenolics)
1438, 1400	1445	δ C–H (CH_2), δ O–H
1267, 1217	1218	δ O–H
1122	1129	ν_{as} C–O–C, glycosidic linkage
1019	1024	ν C–O
932	937	α-D-Glc*p* residue in the chain
870	873	β-anomeric C1–H bending, 2-ketofuranose
816	816	2-ketose in furanose ring

The region 1200–800 cm^{-1} is the fingerprint region of carbohydrates. The strongest band at 1019 cm^{-1} is due to C–O stretching, and the band at 1122 cm^{-1} to C–O–C stretching of the glycosidic bond. The bands at 932, 870 and 816 cm^{-1} are typical of inulin-type fructans [16]. They indicate an α-D-glucopyranosyl residue in the chain, β-anomeric C1–H bending of 2-ketofuranose, and the furanose ring of 2-ketose, respectively. The same three bands (937, 873 and 816 cm^{-1}) were found for inulin from commercial comfrey roots and chicory inulin [9].

Two bands are not typical of pure inulin. The band at 1604 cm^{-1} may come from bound water and from carboxylate groups of uronic acids, which are present in comfrey root polysaccharides [11]. The weak band at 1508 cm^{-1} points to aromatic rings, most likely from phenolic compounds that remained after the defatting steps. Together they show that the isolate contains small amounts of other compounds. This agrees with the moderate purity reported earlier for comfrey inulin obtained by the same method [9].

^{1}H NMR spectrum and degree of polymerisation. The ^{1}H NMR spectrum of the isolated inulin is shown in Fig. 2. The signal at 5.43 ppm belongs to the anomeric proton (H-1) of the terminal α-D-glucopyranosyl unit, and the large signal at 4.79 ppm to residual HDO. The signals at 4.25 and 4.12 ppm correspond to H-3 and H-4 of the fructofuranosyl units, and the overlapping signals between 3.95 and 3.65 ppm to H-5, H-6 and H-1 of fructose and the remaining glucose protons. This pattern is typical of inulin in D_2O [14]. Two small signals at 5.10 and 4.61 ppm were also seen. They may come from free reducing sugars or other glycans co-precipitated with inulin and were not included in the calculation.

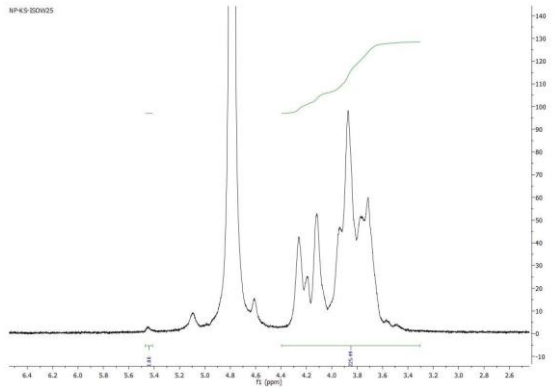


Fig. 2. *^{1}H NMR spectrum (D_2O) of inulin isolated from roots of S. officinale grown ex situ (harvest 2025)*

With the anomeric glucose signal set to 1.00, the integral of the region 3.30–4.40 ppm was 225.49. Equation (1) gives DP = (225.49 − 6) / 7 + 1 = 32.4, i.e. a number-average DP of about 32. This means that one inulin chain consists on average of one glucose and 31 fructose units, with a molar mass of about 5.2 kDa. The value is close to the DP of 29–33 found by spectrophotometric and HPLC-SEC methods for inulin from commercial comfrey roots [9], and to DP 33 reported for inulin from *S. officinale* callus cultures [17]. Chen et al. also found a fraction of about 5.8 kDa in hot water extracts of comfrey roots [12]. One limitation should be noted. If other carbohydrates contribute to the 3.30–4.40 ppm region, the NMR method overestimates DP. Still, the agreement with the earlier chromatographic data supports the result.

Inulin with DP above 23 is considered long-chain inulin, like the commercial high-performance chicory products. It is less soluble and forms stronger gels than short-chain inulin, which makes it suitable as a fat replacer and texturing agent [13]. Comfrey roots grown *ex situ* can therefore be seen as an alternative source of this type of inulin.

CONCLUSION

The polar metabolite profile of comfrey roots grown *ex situ* in Kazanlak was described for the first time with quantitative GC-MS data. Thirty compounds were quantified. Free sugars dominated all samples, with sucrose as the main compound, and the roots contained more free fructose than glucose, in line with their role in fructan storage. Inulin isolated from the roots by microwave-assisted extraction showed the typical FTIR bands of inulin-type fructans, and ^{1}H NMR gave a DP of 32. Comfrey roots from controlled *ex situ* cultivation are a defined and reliable source of long-chain inulin. Further work should include more harvests with replicates and a purity assessment of the isolated inulin.

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