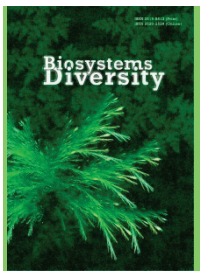

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Phytochemical profiles and antimicrobial activity of the inflorescences of *Sorbus domestica*, *S. aucuparia*, and *S. torminalis*

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The genus *Sorbus* L. is known for its extremely complex taxonomical relationships and health-promoting phytochemicals included in the composition of its floral constituents. The inflorescences of three Sorbus species (rowans), characterized by distinct molecular-genetic traits, were studied in order to examine the possible chemotaxonomic and antimicrobial value of their metabolites. GC–MS profiling of the hexane extracts of *S. domestica*, *S. aucuparia*, and *S. torminalis* inflorescences identified a total of 87 components, which represented six chemical classes (hydrocarbons, alcohols, esters, fatty acid, aldehydes, and ketones) and miscellaneous minor floral constituents (1-methylinosine, 5-amino tetrazole, 1,4-dimethylbenzene, 3,5-bis(1,1-dimethylethyl)-phenol, 3-acetoxy-7,8-epoxylanostan-11-ol, cycloeucalenol acetate, etc.). Principal component analysis (PCA) of the qualitative and quantitative heterogeneity of the floral metabolites determined 1-hentetracontanol, nonacosane, pentadecyl acrylate, 1-methylhexacosane, cycloeucalenol acetate, butyl acetate, and urs-12-ene as the main components which contributed to the differences between *S. domestica*, *S. aucuparia* and *S. torminalis* and resulted in the distinction between the rowan species. Disc-diffusion assays showed variability in activity of inflorescence extracts against Gram-negative (*Enterobacter dissolvens*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) and Gram-positive (*Micrococcus lysodeikticus*, *Staphylococcus aureus*, and *S. epidermidis*) bacterial and clinical fungal (*Candida albicans*) strains. The effect of *S. torminalis* was high against *S. epidermidis* and *P. aeruginosa*, while it was at its lowest against clinical *C. albicans* strains. Inflorescences of *S. domestica* showed the highest inhibition of *P. aeruginosa*, and moderate effects against *S. epidermidis* and *C. albicans*. Inflorescences of *S. aucuparia* caused low to moderate growth inhibition of both Gram-positive and Gram-negative bacterial strains, while it showed the highest effect on *C. albicans*. Antimicrobial properties of rowan inflorescences may be attributed to oleic, linoleic, arachidic, hexadecanoic, and pentadecanoic acids, 24-norursa-3,12-diene, hexahydrofarnesyl acetone, cycloeucalenol acetate, and other compounds which have known bioactivity. These findings indicated rowan inflorescences as a rich source of valuable secondary metabolites and allow us to assume an application of the floral constituents as chemotaxonomic markers of the genus *Sorbus* species.

Keywords: rowans; inflorescences; floral metabolites; GC-MS profiling; chemotaxonomy; antimicrobial ability.

Introduction

More than 250 species of the genus *Sorbus* L. (Rosaceae) are widespread in the Northern Hemisphere under different ecological conditions (Sołtys et al., 2020). The genus *Sorbus* L. is a polyphyletic taxon, known for its exceptional genetic and morphological diversity and extremely complex taxonomical relationships (Robertson et al., 1991) due to the ability of plants to backcrossing and apomixis, repeated polyploidization, homo- and heteroploidy hybridization (Hamston et al., 2018). In recent years, studies of the chloroplast genomes (Tang et al., 2022) confirmed the monophyletic nature of six segregate groups within the genus *Sorbus* L. (i.e., *Sorbus* sensu stricto, *Aria*, *Torminalis*, *Cormus*, *Miromeles*, and *Chamaemespilus*) and the expediency of considering them as separate genera. The species of the genus *Sorbus* L. naturally occurring in the flora of Ukraine were assigned by Fedoronchuk (2017), in accordance with the latest taxonomic innovations, to four taxa in the rank of genera: *Sorbus* L., 1753; *Torminalis* Medik., 1789; *Aria* (Pers.) Host, 1831; *Cormus* Spach, 1834.

The genus *Sorbus* plants (rowans) have a long ethno-pharmacological history of use, thanks to their various preventive and curative effects and undoubted benefits for human health. Today, some species and varieties of the genus *Sorbus* have been studied for their phytochemical composition (Tahirovic et al., 2019), which confirms their reputation as a rich source of biologically active compounds with high antioxidant capacity (Gaivelyte et al., 2014). However, above all the effectiveness of rowans recognized by folk medicine has been reproduced in biological studies conducted not only in *in vitro* tests, but also in *in vivo* assays (Sołtys et al., 2020). Currently, the well documented medicinal significance of *Sorbus* plants can be attributed to the anti-inflammatory (Yu et al., 2011) and vasorelaxant (Sohn et al., 2005) activity of the *Sorbus commixta* bark extracts, and to the antidiabetic activity of *Sorbus decora* bark (Vianna et al., 2011; Shang et al., 2015). Therefore, a significant number of the genus *Sorbus* representatives still remain understudied and promising objects for revelation and application of their biologically active constituents.

The phytochemical significance of rowans is assessed, as a rule, by the content and composition of polyphenols (Olszewska & Michel, 2009; Orsavová et al., 2023), and the radical-quenching ability as well (Hukkanen et al., 2006; Olszewska et al., 2010; Raudonis et al., 2014). Most studies directed attention to the chemical features of *Sorbus* leaves (Raudonė et al., 2015), bark (Choi et al., 2018), and fruits (Kylli et al., 2010; Mikulic-Petkovsek et al., 2017; Zymone et al., 2018), while inflorescences are the plant material with limited phytochemical scientific evidence (Zymone et al., 2022). Comparative studies of rowan inflorescences showed the superiority of *S. aucuparia* over 16 selected species from the genus *Sorbus* (Olszewska et al., 2010) in total phenolic levels and antioxidant activity.

However, plant secondary metabolites are not limited to polyphenols and include, in particular, the volatile fraction of phytochemicals, which also can exhibit biological activity. Recent findings discovered the chemical diversity of the floral volatile compounds which can indicate their chemotaxonomic relevance as well (Fahim et al., 2023). Therefore, the most complete definition of the qualitative and quantitative phytochemical profiles of the inflorescences of rowans is necessary. The aim of this study was profiling the inflorescence hexane extracts of three *Sorbus* species, which have distinct molecular-genetic characteristics, and examining the possible chemotaxonomic and antimicrobial significance of the floral metabolites.

Materials and methods

The fully blooming inflorescences of *Sorbus domestica* L., *S. aucuparia* L., and *S. torminalis* (L.) Crantz. were collected in the Botanical Garden of Oles Honchar Dnipro National University (48°26'7" N, 35°2'34" E; Dnipro city, Ukraine) during 12–15 May 2022, packed in plastic containers, transferred to the laboratory and air-dried in the shade under room conditions. The inflorescence extracts were prepared by cold maceration of air-dried and crushed plant material in hexane (1:10 w/v) during 24 h with occasional stirring. Further, filtered extracts were evaporated at 40 ºC using a rotary evaporator (IKA® RV 10, Germany), and the obtained solid fractions stored at 4 ºC prior to analysis.

GC-MS analyses were performed using Shimadzu-GC-MS (QP 2020 El, Japan) equipped with Rxi®-5ms column (30 m × 0.25 mm, film thickness 0.25 μm) containing 5% diphenyl/95% dimethyl polysiloxane as a fixed liquid phase. The oven temperature was programmed from 50 °C (with 2 min initial hold) to 300 °C at a rate of 15 °C per min, and kept constant at 300 °C for 5 min. The carrier gas helium passed at a total flow 28.2 and column flow 1.2 ml/min. Injector temperature was 280 °C; sample volume was 1 μL. The identification of the separated compounds was achieved based on Mass Spectrum Library 2014 for GC-MS (O2125401310) by their mass spectra comparison with those in the National Institute of Standards and Technology (NIST14.lib, NIST14s.lib) spectral database with a matching probability ≥80%. The content of individual compounds was estimated using the corresponding peak area and expressed as a percentage of the total sum, as previously described (Lykholat et al., 2021). Antimicrobial ability of the rowan inflorescences was evaluated by disc diffusion method (Mujeeb et al., 2014). The test strains of microorganisms were taken from the culture collection of the Microbiology, Virology and Biotechnology Department of Oles Honchar DNU. Three Gram-negative bacterial strains (*Escherichia coli* B906, *Pseudomonas aeruginosa* B907, and *Klebsiella pneumoniae* B920), and four Gram-positive strains (*Micrococcus lysodeikticus* 2665, *Staphylococcus aureus* B209, *Staphylococcus epidermidis* ATCC149, and *Staphylococcus epidermidis* B919) were selected. Two different clinical strains of *Candida albicans* were involved to test the antifungal potential of the rowan extracts. In each case, Petri plates containing meat-peptone agar (MPA) medium were seeded with 10^9 CFU (colony forming units) suspension of microorganisms. Sterile paper discs (6 mm diameter) were impregnated with 10 μL of hexane extracts and placed on the agar surface; plates incubated at 37 °C for 24 h. Norfloxacin (5.0 μg per disc) was used as the positive control for the bacterial strains; fluconazole 10.0 μg was used as the positive control for the fungal strains. Antimicrobial activity of the inflorescence extracts was expressed as the diameter of the inhibition zone (mm) around the discs along with disc diameter.

The taxonomic names of the studied rowan species are given in accordance with The World Flora Online Plant List (WFO). *Sorbus* L. www.worldfloraonline.org/taxon/wfo-4000035797

All bioassays were performed in five replications. Results of the experiment were statistically processed by analysis of variance (ANOVA). The data obtained were expressed as the mean value ± standard deviation (SD), and the differences between means were compared using Tukey's HSD. All differences were considered statistically significant at $P < 0.05$. Multivariate statistical analysis of the obtained results was performed by principal component analysis (PCA) using the software package Statistica 10.1 (StatSoft Inc., USA). To do this, a data matrix was created where rows represented the samples and columns described the features.

Results

The solid residue yields of the inflorescence hexane extracts of *S. domestica*, *S. aucuparia*, and *S. torminalis* were 0.38%, 0.40% and 0.40% respectively. Using the GC-MS technique, a total of 86 components were identified with a confidence of at least 80%. Among them, 46, 48, and 37 compounds were characterized from *S. domestica*, *S. aucuparia*, and *S. torminalis* inflorescences, which represented 98.2%, 97.5%, and 97.7% of their total pool, respectively (Table 1).

Table 1

Chemical constituents of the hexane extracts derived from the inflorescences of rowans as identified by GC-MS

Compound name	Formula	RT, min	Area, % of total		
			S. domestica	*S. aucuparia*	*S. torminalis*
Butyl acetate	$C_6H_{12}O_2$	1.738	–	–	6.0
1,4-Dimethyl benzene	C_8H_{10}	1.947	–	–	1.24
Butyrolactone	$C_4H_6O_2$	2.475	–	0.15	–
Uramil-N, N-diacetic acid	$C_8H_9N_3O_7$	3.720	–	0.20	–
Diethyl 2-cyano-2,3-dimethylbutanedioate	$C_{11}H_{17}NO_4$	4.438	0.30	0.34	–
5-Amintetrzole-	CH_3N_5	5.105	0.15	0.15	0.12
Cyclododecane	$C_{12}H_{24}$	5.454	–	0.26	–
2-Methylundecane-2-thiol	$C_{12}H_{26}S$	5.559	0.15	–	–
2-Decanol	$C_{10}H_{22}O$	5.651	0.45	–	–
Allyl pentadecyl oxalate	$C_{20}H_{36}O_4$	5.746	–	0.31	–
3,5-Bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$	5.885	–	0.64	–
1-Methylinosine	$C_{11}H_{14}N_4O_5$	5.895	0.18	0.34	0.26
6-Methyl octane	$C_{19}H_{40}$	6.365	0.78	0.42	0.76
1,1-Dimethyl-2-octyl-cyclobutane	$C_{14}H_{28}$	6.442	0.16	–	0.35
8-Azidoadenosine	$C_{10}H_{12}N_8O_4$	6.651	0.12	–	–
D-Homo-24-nor-17-oxachola-20,22-diene-3,7,16-trione	$C_{26}H_{32}O_6$	6.824	0.16	–	–
2-Methyl(phenylundecyl) oxalate	$C_{20}H_{30}O_4$	6.890	–	–	0.18
Ethylene dimethacrylate	$C_8H_{10}O_4$	6.906	–	4.07	12.61
1,2-Octadecanediol	$C_{18}H_{38}O_2$	6.924	–	0.48	–
Pentadecyl acrylate	$C_{18}H_{34}O_2$	6.940	13.71	–	–
Trans-2-Dodecen-1-ol	$C_{12}H_{24}O$	7.063	0.48	0.30	–
Isopropyl heneicosanoate	$C_{24}H_{48}O_2$	7.617	–	0.24	0.38
13-Heptadecyn-1-ol	$C_{17}H_{32}O$	7.714	1.68	1.72	1.56
2-Nonadecanol	$C_{19}H_{40}O$	7.750	0.26	–	–
Octadecanal	$C_{18}H_{36}O$	7.901	0.48	0.36	–
(2-phenyl-1,3-dioxolan-4-yl) methyl octadecenoate	$C_{28}H_{44}O_4$	8.085	0.41	–	0.13
Hexadecanoic acid (syn. Palmitic acid)	$C_{16}H_{32}O_2$	8.151	–	1.22	0.48

Compound name	Formula	RT, min	Area, % of total		
			S. domestica	*S. aucuparia*	*S. torminalis*
Dodecyl 3-chloropropionate	$C_{15}H_{29}ClO_2$	8.197	0.64	–	0.49
Butyl undecyl phthalate	$C_{23}H_{36}O_4$	8.241	–	0.41	1.14
2-Methyltetracosane	$C_{25}H_{52}$	8.349	1.45	2.08	1.38
Butyl tetradecyl phthalate	$C_{26}H_{42}O_4$	8.411	0.84	–	–
Pentadecanoic acid	$C_{15}H_{30}O_2$	8.478	–	0.44	0.38
1-Methylethyl hexanoate	$C_{19}H_{38}O_2$	8.531	0.23	–	–
Dodecyl 3-mercaptopropionate	$C_{15}H_{30}O_2S$	8.625	–	0.84	–
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-dien-2,8-dione	$C_{17}H_{24}O_3$	8.774	0.88	–	–
Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	8.804	–	0.30	–
Heneicosane	$C_{21}H_{44}$	8.958	5.54	–	–
9-Octadecenoic acid (Z), (syn. Oleic acid)	$C_{18}H_{34}O_2$	8. 993	0.33	–	–
Eicosanoic acid (syn. Arachidic acid)	$C_{20}H_{40}O_2$	9.068	–	0.25	0.59
9,12-Octadecadienoic acid (Z, Z) (syn. Linoleic acid)	$C_{18}H_{32}O_2$	9.129	–	–	7.61
3-Hydroxydodecanoic acid	$C_{12}H_{24}O_3$	9.165	–	0.20	0.22
Docosanol (syn. Behenic alcohol)	$C_{22}H_{46}O$	9.191	–	0.27	–
1-Docosene	$C_{22}H_{44}$	9.203	–	0.34	1.58
Docosanoic acid (syn. Behenic acid)	$C_{22}H_{44}O_2$	9.277	0.16	–	–
Pentadecanal	$C_{15}H_{30}O$	9.546	–	0.87	–
Decyl cyclohexane carboxylate	$C_{17}H_{32}O_2$	9.700	0.19	–	–
Eicosane	$C_{20}H_{42}$	9.743	13.58	12.52	17.81
Tetracosane	$C_{24}H_{50}$	9.796	–	2.76	–
2-(Acetyloxy)-1-[(acetyloxy)methyl] ethyl lilolenate	$C_{25}H_{40}O_6$	9.861	0.15	–	–
Undecyl undec-10-ynoate	$C_{22}H_{40}O_2$	9.907	–	3.29	–
Heptacosane	$C_{27}H_{56}$	9.953	1.22	–	1.34
Allyl tetradecyl oxalate	$C_{19}H_{34}O_4$	10.105	0.16	–	–
Heptadecanal	$C_{17}H_{34}O$	10.178	–	2.46	–
2-Methylhexacosane	$C_{27}H_{56}$	10.231	3.18	4.53	0.30
1-Heptacosanol	$C_{27}H_{56}$	10.344	0.36	0.40	–
7-Hexadecenal	$C_{16}H_{30}O$	10.433	–	0.30	–
9-Hexacosene	$C_{26}H_{52}$	10.476	–	1.58	–
13-Octadecenal	$C_{18}H_{34}O$	10.546	0.65	0.77	0.13
Hexacosane	$C_{26}H_{54}$	10.636	4.66	–	–
Octacosane	$C_{28}H_{58}$	10.671	–	–	7.74
Cyclooctacosane	$C_{28}H_{56}$	10.740	0.31	–	–
18-Oxohexacontanoic acid	$C_{60}H_{118}O_3$	10.822	–	–	0.10
Di-n-octyl phthalate	$C_{24}H_{38}O_4$	10.920	–	–	1.99
Diisooctyl phthalate	$C_{24}H_{38}O_4$	10.939	2.87	2.41	–
Z, Z-3,13-Octadecedien-1-ol	$C_{18}H_{34}O$	10.985	0.15	–	–
Tetratetracontane	$C_{44}H_{90}$	11.470	5.94	3.86	4.35
n-Tetracosanol	$C_{24}H_{50}O$	11.530	–	1.94	–
Nonacosane	$C_{29}H_{60}$	11.720	13.12	11.75	3.74
1-Octacosanol	$C_{28}H_{58}O$	11.842	3.62	1.97	–
Olean-12-ene-3,28-diol	$C_{30}H_{50}O_2$	12.012	–	–	2.22
Didodecyl thiodipropionate	$C_{30}H_{58}O_4S$	12.292	–	–	1.44
Methyl 3,11-bis(acetyloxy)-12-hydroxyandrostane-17-carboxylate	$C_{25}H_{38}O_7$	13.134	–	0.89	–
3-Hydroxy-21-methoxy-20-oxo-30-nor-lupan-28-oic acid methyl ester	$C_{31}H_{50}O_5$	13.182	0.34	–	1.60
Tridecyl-2-ynyl undec-10-ynoate	$C_{24}H_{40}O_2$	13.565	0.20	–	–
Tetradecyl undec-10-ynoate	$C_{25}H_{46}O_2$	13.632	–	0.63	–
1-Hentetracontanol	$C_{41}H_{84}O$	13.920	12.16	21.62	–
Triarachine	$C_{63}H_{122}O_6$	14.105	–	0.36	–
4-Methylcholesta-8,24-dien-3-ol	$C_{28}H_{46}O$	14.168	–	–	0.41
3,5-dehydro-6-methoxypivalate-cholest-22-ene-21-ol	$C_{33}H_{54}O_3$	14.190	0.43	–	–
Cholest-1-eno-[2,1-a] naphthalene-3,4-dihydro	$C_{35}H_{52}$	14.297	3.57	–	–
Cycloeucalenol acetate	$C_{32}H_{52}O_2$	14.352	–	–	3.77
Lup-20(29)-en-3-ol (3. beta) (syn. Lupeol)	$C_{30}H_{50}O$	14.373	–	4.13	–
Urs-12-ene	$C_{30}H_{50}$	14.532	0.41	1.05	11.02
24-Norursa-3,12-diene	$C_{29}H_{48}$	14.988	1.41	–	2.07
3-Acetoxy-7,8-epoxylanostan-11-ol	$C_{32}H_{54}O$	15.107	–	0.24	–
Urs-12-ene -3-ol (syn. alpha-Amyrin)	$C_{30}H_{50}O$	15.299	0.20	0.83	0.22

Note: RT – retention time; data on compounds content are expressed as peak area (percentage of total).

Pentadecyl acrylate (13.71%), eicosane (13.58%), nonacosane (13.12%), and hentetracontan-1-ol (12.16%) were found to be major components in the floral extracts of *S. domestica*. The chemical constituents of *S. aucuparia* inflorescences were dominated by hentetracontan-1-ol (21.62%), eicosane (12.52%), nonacosane (11.75%), 2-methylhexacosane (4.53%), and lupeol (4.13%). In the floral extracts of *S. torminalis* the most abundant components were eicosane (17.81%), ethylene dimethacrylate (12.61%), urs-12-ene (11.02%), octacosane (7.74%), linoleic acid (7.61%), and butyl acetate (6.0%).

GC-MS-identified constituents of the inflorescence's hexane extracts of the studied *Sorbus* species were categorized and compared on their chemical classes (Fig. 1). The total pool of hydrocarbons (20 individual components in the range of chain length C_8–C_{44}), which was found to be the main constituent of the analyzed *Sorbus* inflorescence extracts, was further divided in some different groups in accordance with compounds' structure. Straight-chain alkanes eicosane and nonacosane were the general aliphatic hydrocarbons in the extracts of all three rowan species, followed by alkanes tetratetracontane and 2-methylhexacosane, and triterpene urs-12-ene (Table 2).

Alcohols and esters constituted the second and third main classes of the rowan floral phytochemicals comparable in their relative compound contents. Alcohols (a total of 14 compounds) accounted for 19.36%, 33.66%, and 4.00% of the chemical constituents of *S. domestica*, *S. aucuparia*, and *S. torminalis* inflorescences respectively, encompassing the compounds in the range of chain length C_{10}–C_{41} (Table 3). The most abundant were primary alcohol hentetracontan-1-ol, followed by octaco-

san-1-ol, the terpenoids lupeol and olean-12-ene-3,28-diol, and 13-heptadecyn-1-ol.

The esters identified in the hexane floral extracts of *S. domestica*, *S. aucuparia*, and *S. torminalis* were represented, respectively, by 12, 11, and 10 compounds with the chain length ranged from C_6 to C_{63}, and accounted for 20.04%, 13.79%, and 25.95% of total. Among the 25 esters derived from the rowan inflorescences of all three species, ethylene dimethacrylate and pentadecyl acrylate were dominant, followed by undecyl undec-10-ynoate, 3-hydroxy-21-methoxy-20-oxo-30-nor-lupan-28-oic acid methyl ester, and diisooctyl phthalate. On the whole, content of different phthalic acid esters reached 3.71%, 2.82%, and 3.13% of overall esters' content, respectively in the inflorescence extracts of *S. domestica*, *S. aucuparia*, and *S. torminalis*.

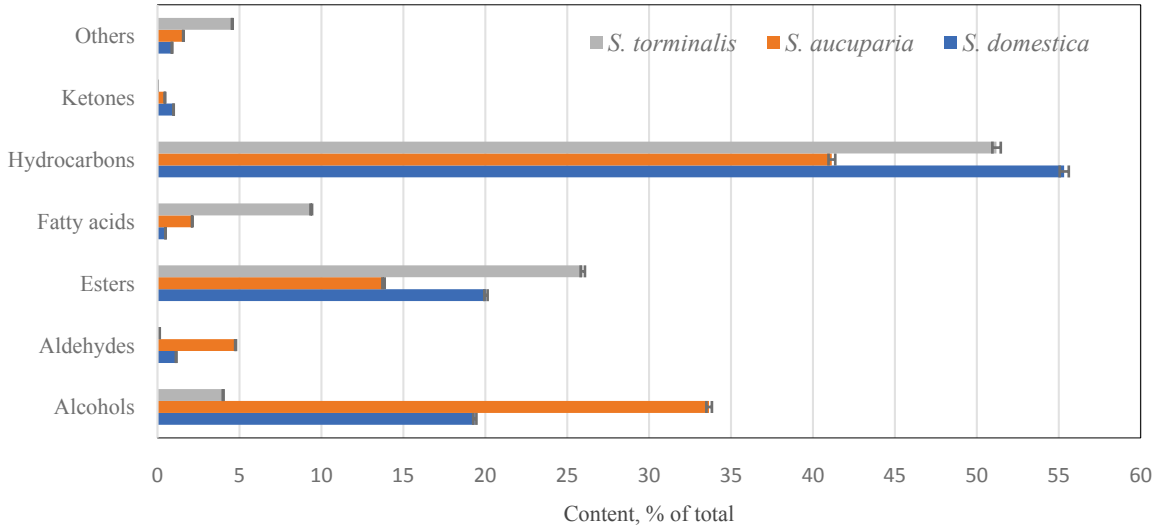


Fig. 1. Relative content (percentage of total) of different chemical classes in the hexane extracts derived from the inflorescences of *S. domestica*, *S. aucuparia*, and *S. torminalis*

Table 2
Hydrocarbons distribution in the rowan inflorescence extracts

Group of the hydrocarbons	Content of compounds, %		
	S. domestica	*S. aucuparia*	*S. torminalis*
A. Aliphatic:			
– straight-chain alkanes	44.06 (6)	32.81 (6)	36.56 (6)
– branched alkanes	5.41 (3)	7.03 (3)	2.44 (3)
– cyclic alkanes	0.47 (2)	0.26 (1)	0.35 (1)
Total:	49.94 (11)	40.10 (10)	39.35 (10)
B. Aromatic	–	–	1.24 (1)
C. Triterpenes	0.41 (1)	1.05 (1)	11.02 (1)
D. Terpenoids	4.98 (2)	–	2.07 (1)
Total:	55.33 (14)	41.15 (11)	53.68 (13)

Note: the numbers in brackets indicate the number of components.

Table 3
Diversity of alcohols in the rowan inflorescence extracts

Group of the alcohols	Content of compounds, %		
	S. domestica	*S. aucuparia*	*S. torminalis*
Primary	19.16 (8)	28.22 (7)	1.56 (1)
Diols	–	0.48 (1)	–
Terpenoids	0.20 (1)	4.96 (2)	2.44 (2)
Total alcohols	19.36 (9)	33.66 (10)	4.00 (3)

Note: see Table 2.

Fatty acids were derived in the greatest quantity from the *S. torminalis* inflorescences, including six compounds in a range C_{12}–C_{60} dominated by linoleic acid and contributed 9.38% of overall amount. The floral extracts of the other two rowans had a poorer fatty acids' composition containing two and four components and contributing 0.49% and 2.11% to the total, respectively, in *S. domestica* and *S. aucuparia*.

Aldehydes in the rowan inflorescence extracts were identified as a small class (only five compounds in the range C_{15}–C_{18}), dominated by heptadecanal and pentadecanal, and contributing 1.13%, 4.76%, and 0.13% to the total amount in the inflorescences of *S. domestica*, *S. aucuparia*, and *S. torminalis*, respectively.

Ketones in the *Sorbus* species inflorescences were found to be the smallest class of phytochemicals, representing 0.98% of total (two components only) in *S. domestica*, and 0.45% (two components) in *S. aucuparia* extract, while no ketone was detected in the floral extracts of *S. torminalis*. The highest content was provided by 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien-2,8-dione and hexahydrofarnesyl acetone.

Other compounds were the group of miscellaneous minor floral constituents, that united nitrogenous (amines and nucleosides), steroids, and phenolic compounds, accounted for 0.88%, 1.57%, and 5.8% of total, respectively in *S. domestica*, *S. aucuparia*, and *S. torminalis* inflorescence extracts.

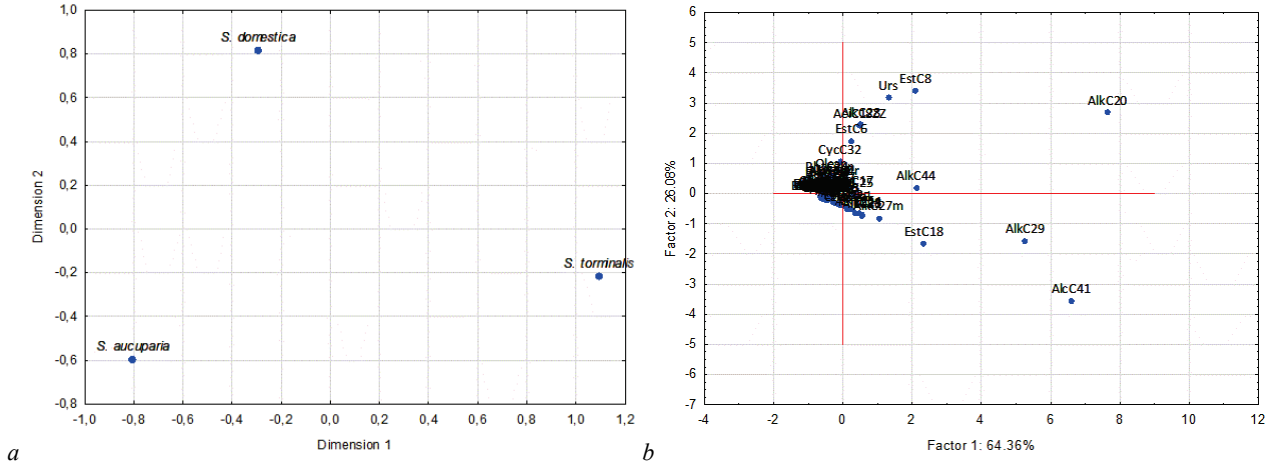
In addition, the multivariate analysis of the rowan floral constituents by the principal component analysis (PCA) was applied to establish the statistically significant differences between the studied rowan species. Two main factors explained 64.36% and 26.08% of the floral metabolites' variation, respectively, and the studied species were designated as distinct (Fig. 2a). The projection of the cases on the factor-plane (Fig. 2b) reflects the scatter of component composition due to the influence of each factor characterizing the contribution of specific metabolites.

Phytochemical compounds, derived from the inflorescences of the studied *Sorbus* species, exhibited low to moderate activity against bacterial and fungal strains when studied by disc-diffusion assay (Table 4).

Discussion

Until now, rowan inflorescences remain an object with insufficient phytochemical scientific data (Zymone et al., 2022), so the profiling floral hexane extracts of the selected rowan species can fill the gap to some extent. Previous studies of the phytochemical complexity of rowan inflorescences mostly focused on content and distribution of polyphenols (Olszewska & Michel, 2009; Olszewska et al., 2010), which gave grounds for considering *S. aucuparia* as a model antioxidant *Sorbus* species and the effective sources for natural health products as well. However, the floral pool of the secondary metabolites obviously covers a much wider variety of phytochemical compounds than polyphenols only. In particular, this applies to the volatile substances which are crucially essential for the plant-environment interaction, including attraction of pollinators, seed disseminators, and protection against abiotic (e.g. oxidative stress) and biotic (e.g. pathogens and pests) stressors (Xu et al., 2022). Additionally, the floral metabolite 1-methylinosine, which was common for profiles of all rowan species studied in our work, was reported (Jin et al., 2019) as important for vegetative and reproductive growth of plants.

The inflorescence hexane extracts of *S. domestica*, *S. aucuparia*, and *S. torminalis* showed remarkable differences in both qualitative and quantitative content of the chemical constituents considering the great phytochemical diversity of the genus *Sorbus* L. as a whole. Similarly, high quantitative heterogeneity of the phenolic profiling of inflorescence extracts was revealed by Zymone et al. (2022) across the various *Sorbus* species, genotypes, and cultivars. Comparison of *S. torminalis* and *S. aucuparia* inflorescences in terms of antioxidant activity and phenolic content suggested that the distinctive phenolic constituent's chemistry determined the different antioxidant power of two species (Olszewska, 2011). The same way, exploring the floral volatile metabolites of different *Chorisia* species revealed that their chemical composition is largely genus- and species-dependent (Fahim et al., 2023), which gives reasonable grounds to assume their biological and chemotaxonomic relevance. In this regard, the opinion of Wink (2003) is relevant, that the distribution of secondary metabolites within the same genus has taxonomic value but their occurrence reflects the species adaptations and particular life strategies embedded in a given phylogenetic framework.



a *b*

Fig. 2. Study of the inflorescence extract constituents of *S. domestica*, *S. aucuparia* and *S. torminalis* by principal component analysis (PCA): *a* – scatter plot 2D, *b* – double plot; the various short abbreviations on the double graph represent chemical compounds: eicosane (AlkC20), nonacosane (AlkC29), tetratetracontane (AlkC44), urs-12-ene (Urs), methylhexacosane (AlkC27m), hentetracontanol (AlcC41), butyl acetate (EstC6), ethylene dimethacrylate (EstC8), pentadecyl acrylate (EstC18), cycloeucalenol acetate (CycC32)

Table 4
Growth inhibiting effects (zones diameter, mm) of the rowan inflorescence extracts on the collection bacterial strains and clinical fungal strains (x ± SD, n = 5)

Test-culture	*S. domestica*	*S. torminalis*	*S. aucuparia*	Positive control[1]
Escherichia coli B906	13.21 ± 0.27^{a}	$10{,}32 \pm 0.35^{b}$	11.82 ± 0.29^{ab}	29.33 ± 1.53^{c}
Pseudomonas aeruginosa B 907	19.93 ± 0.40^{a}	19.07 ± 0.35^{a}	15.81 ± 0.30^{b}	28.37 ± 0.65^{c}
Klebsiella pneumoniae B 920	13.32 ± 0.29^{a}	11.31 ± 0.29^{b}	12.23 ± 0.76^{ab}	14.84 ± 0.29^{c}
Micrococcus lysodeikticus 2665	11.72 ± 0.58^{a}	11.83 ± 0.76^{a}	9.47 ± 0.57^{a}	26.53 ± 1.73^{b}
Staphylococcus aureus B209	13.54 ± 0.87^{a}	12.17 ± 0.76^{a}	12.20 ± 0.27^{a}	23.51 ± 0.90^{b}
S. epidermidis ATCC149	16.32 ± 0.76^{a}	18.81 ± 0.99^{a}	12.37 ± 0.51^{b}	24.17 ± 1.53^{c}
S. epidermidis B919	12.63 ± 0.21^{a}	17.73 ± 0.42^{b}	15.82 ± 0.46^{c}	26.73 ± 0.38^{d}
Candida albicans-1	16.34 ± 0.94^{a}	15.31 ± 0.21^{a}	18.63 ± 0.17^{b}	NA
C. albicans-2	14.90 ± 0.10^{a}	13.92 ± 0.32^{b}	18.51 ± 0.32^{c}	NA

Notes: [1] – norfloxacin (5.0 μg) and (fluconazole 10.0 μg) were used as positive control; the diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as x ± SD; different letters within a line indicate the significantly differing mean values in accordance with the Tukey test ($P < 0.05$); NA – no activity.

In accordance with the results of principal component analysis (PCA) of the component composition of the rowan inflorescence phytochemicals, the differences between *S. domestica* and *S. aucuparia* on the one hand, and *S. torminalis*, on the other hand, were mostly contributed by hentetracontan-1-ol, nonacosane, pentadecyl acrylate, 1-methylhexacosane, cycloeucalenol acetate, butyl acetate, and urs-12-ene. As for the floral metabolites located in the zone of positive influence of both factors, such as eicosane, ethylene dimethacrylate, and tetratetracontane, it can be assumed that they contribute less to the differences between the studied rowan species. These findings are coincident with the data of Al-Hajj et al. (2014), that a single group of secondary metabolites, as a rule, dominates within a given taxon, and a few major compounds are often accompanied by several minor components. The study results allow us to assume the potential possibility of application of the floral metabolites as chemotaxonomic markers of the genus *Sorbus* species.

It is expected that some floral metabolites can be synthesized by plants to attract pollinating insects. Among these, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, revealed in the inflorescences of *S. domestica*, was also detected by Lipińska et al. (2022) in the floral extract of black orchid *Brasiliorchis schunkeana* as the attractant with the potential antimicrobial activity. Attractive function, possibly, may be performed by the common for the studied *Sorbus* inflorescences saturated hydrocarbon 2-methylhexacosane, which is an insect pheromone (Spikes et al., 2010). According to the data of "The Pherobase: Database of pheromones and semiochemicals". El-Sayed (2023) www.pherobase.com, attractive properties can be also attributed to the common floral metabolites of all studied rowans 6-methyl octane, eicosane and nonacosane, as well as to eicosanoic acid (founded in *S. aucuparia* and *S. torminalis* inflorescences), heptacosane (*S. domestica* and *S. torminalis*), 2-decanol (*S. domestica* and *S. aucuparia*), 1,4-dimethyl benzene (*S. torminalis*), and tetracosane (founded in *S. aucuparia* inflorescences). On the other hand, several chemical constituents of the rowan's inflorescences are obviously called upon to perform the opposite role, preventing the contact of plants with unfriendly organisms. The repellent function is indicated (The Pherobase) for butyl acetate and linoleic acid (both the floral metabolites of *S. torminalis*), pentadecanoic and hexadecenoic acids (*S. aucuparia* and *S. torminalis* inflorescences).

Disc-diffusion assays showed the variability in the antimicrobial activity of the inflorescences of the studied rowan species. Growth-inhibiting effect of *S. torminalis* was the highest against the both *S. epidermidis* gram-positive strains and high against *P. aeruginosa* gram-negative strain, while it was the lowest against the both clinical fungal strains. Inflorescences of *S. domestica* showed the highest inhibition of all tested gram-negative bacterial strains, *P. aeruginosa* especially, while they showed moderate

effects against the both gram-positive *S. epidermidis* strains and the fungal strains *C. albicans* as well. Inflorescences of *S. aucuparia* caused low to moderate growth inhibition of both gram-positive and gram-negative bacterial strains, while it exerted the highest effect on both *C. albicans* clinical strains. Antibacterial properties of the rowan inflorescence extracts may be attributed to the fatty acids, including oleic acid (in *S. domestica*), linoleic acid (in *S. torminalis*), arachidic acid (in both *S. aucuparia* and *S. torminalis*), because of the known (Zheng et al., 2005) ability of long-chain unsaturated fatty acids for the inhibition of bacterial fatty acid synthesis. Versatile activities of linoleic and oleic acids were found against bacteria (Dilika et al., 2000), plant pathogenic fungi (Walters et al., 2004), and even in cancer prevention (Diab et al., 2021).

The saturated palmitic and pentadecanoic acids, detected in *S. aucuparia* and *S. torminalis* inflorescences, have reported antibacterial (Fernandes et al., 2013) and antimetastatic (Zhu et al., 2021) ability, and broad anti-inflammatory and antiproliferative (Venn-Watson & Butterworth, 2022) activities. Sesquiterpenoid hexahydrofarnesyl acetone, founded in *S. aucuparia* inflorescences, seemed to be endowed with antibacterial, anti-inflammatory, and cytotoxic activities (Xu et al., 2022). Among the esters, identified in the rowan inflorescences, the known antimicrobial activity was linked with pentadecyl acrylate (Mujeeb et al., 2014) and butyl acetate (Lens et al., 2016). Cycloeucalenol acetate from *S. torminalis* inflorescences was noted (Kandasamy et al., 2016) in the extracts exhibited strong antimicrobial activity and cytotoxicity towards human liver cancer (HepG2) cells. Terpenoid 24-norursa-3,12-diene having antimicrobial, antidiabetic, and antioxidant significance (Ullah et al., 2022), was identified in *S. domestica* and *S. torminalis* inflorescences. Thus, profiling the floral phytochemicals of the three selected rowan species convincingly indicate the inflorescences of genus *Sorbus* plants as a rich source of biologically active compounds with potentially beneficial properties for humans.

Conclusion

The inflorescence hexane extracts of three selected *Sorbus* species were comprehensively profiled using the GC–MS technique. In total, 87 components were identified, among which 47, 48, and 37 compounds were characterized from *S. domestica*, *S. aucuparia*, and *S. torminalis* inflorescences, which represented 98.2%, 97.47%, and 97.7% of the total content, respectively. Phytochemicals were divided into six chemical classes (hydrocarbons, alcohols, esters, fatty acid, aldehydes, and ketones) and one group of miscellaneous minor floral constituents. Hydrocarbons were found to be the main volatile constituents of *Sorbus* inflorescence, accounting for 55.33%, 41.15%, and 53.68% of total, respectively in *S. domestica*, *S. aucuparia*, and *S. torminalis* inflorescences. Alcohols (primary, secondary, diols, and terpenoids) constituted the second main class with 19.36%, 33.66%, and 4.0% in the floral phytochemicals of *S. domestica*, *S. aucuparia*, and *S. torminalis*. The esters identified in the hexane floral extracts accounted for 20.04%, 13.79%, and 25.95% of total, in the inflorescences of *S. domestica*, *S. aucuparia*, and *S. torminalis*, respectively.

The qualitative and quantitative heterogeneity of the constituents of inflorescence extracts was statistically assessed by principal component analysis (PCA), which determined hentetracontan-1-ol, nonacosane, pentadecyl acrylate, 1-methylhexacosane, cycloeucalenol acetate, butyl acetate, and urs-12-ene as the components which mostly contributed to the differences between *S. domestica*, *S. aucuparia* and *S. torminalis* which resulted in the species distinction. Disc-diffusion assays showed the variability of low to moderate antibacterial and antifungal activity of studied rowan species. The results obtained suggested the potential possibility of the application of the inflorescence constituents as chemotaxonomic markers of species of the genus *Sorbus*. The wide range of potential biological activities of the identified floral phytochemicals confirmed the rowan inflorescences as a rich source of different valuable secondary metabolites.

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