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Distribution of triterpene acids and their derivatives in organs of cowberry (*Vaccinium vitis-idaea* L.) plant★

Anna Szakiel✉ and Agnieszka Mroczek

Department of Plant Biochemistry, Institute of Biochemistry, Warsaw University, Warszawa, Poland

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Wild berries of the genus *Vaccinium* have become increasingly popular in human health promotion due to their nutritional and medicinal properties. Some striking divergence of opinion about the content of triterpenoids in these plants still exists, meanwhile, this very large class of natural isoprenoids exhibits a wide range of biological activities and hence is of growing research interest. An investigation of triterpenoidal constituents from the cowberry (*Vaccinium vitis-idaea* L.) plant led to the isolation of two isomeric acids : oleanolic and ursolic and the occurrence of their derivatives in this plant was demonstrated for the first time. Free triterpene acids as well as small amounts of their bound forms (presumable glycosides and glycoside esters) occur in fruits and the vegetative part of the plant, however, in various amounts and different ratios. The total content of both acids was the highest in organs regarded as traditional herbal resources, namely fruits and leaves (1 and 0.6% of dry mass, respectively), whereas it was markedly lower in stems and rhizomes. However, the rhizomes were in turn the plant organ containing relatively the highest amount of the bound forms of both acids (0.01% of dry mass). Ursolic acid was dominant in the whole plant, but the ratio of oleanolic to ursolic acid was significantly different in individual organs, decreasing from the upper (fruits 1 : 2.4, leaves 1 : 2) to the lower (stems 1 : 3.5, rhizomes 1 : 5.2) parts of the plant. This pattern of distribution of triterpenoids in the plant may have an important physiological and ecological meaning.

Keywords : *Vaccinium vitis-idaea* L., *Ericaceae*, allelopathic activity, cowberry, oleanolic acid, triterpenoids, ursolic acid

INTRODUCTION

Cowberry (*Vaccinium vitis-idaea* L., family *Ericaceae*) is a small evergreen shrub found in the herbaceous layer of circumboreal forests in Northern Eurasia and North America (Gwin *et al.,* 2003; Jędrzejko, 2001). It is sometimes cultivated, mainly in gardens, but more commonly its edible fruits are collected from the native habitats. Wild-collected cowberries are popular in Northern Europe, notably in Scandinavia, where they are harvested for home or commercial use and consumed mainly in the form of jam, compote, juice or syrup (Yang *et al.,* 2003, http : //en.wikipedia.org/wiki/Cowberry). Moreover, fruits and leaves of cowberry have a well-established role in pharmacognosy and they are used in herbal medicine. Several pharmacological activities have been documented for cowberry, including counteracting urinary- and digestive-tract infections (Kontiokari *et al.,* 2004).

The chemical composition of cowberry, as of other plants from the genus *Vaccinium* bearing edible fruits, has been investigated regarding mainly common nutritive compounds like sugars, vitamins, organic acids, mineral salts and also phenolic compounds, which are currently considered

★This paper is dedicated to Professor Tadeusz Chojnacki from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences in Warsaw on the occasion of the 50th anniversary of his scientific activity and 75th birthday.

✉**Corresponding author**: Anna Szakiel, Department of Plant Biochemistry, Institute of Biochemistry, Warsaw University, Miecznikowa 1, 02-096 Warszawa, Poland; tel.: (48) 22 554 3316; fax: (48) 22 554 3221; e-mail address: szakal@biol.uw.edu.pl

Abbreviations: AC, acid hydrolysis; AL, alkaline hydrolysis; CC, column chromatography; DW, dry weight; GLC, gas-liquid chromatography; MS, mass spectrometry; OL, oleanolic acid; TLC, thin-layer chromatography; UR, ursolic acid.

as one of the most promising groups of potential dietary antioxidants and anticarcinogens (Latza *et al.*, 1996; Törrönen *et al.*, 1997; Rauha *et al.*, 2000; Liu *et al.*, 2003). Other groups of compounds playing an important role in human health maintenance, which have been investigated in *Vaccinium* species, including cowberry, are sterols and fatty acids (Johansson *et al.*, 1997; Yang *et al.*, 2003). It has also been reported that cowberry contains high amounts of benzoic acid, one of the oldest but still commonly used chemical preservatives (Viljakainen *et al.*, 2002). However, although the list of constituents present in cowberry seems to be established, some striking discrepancies about the content of another very important group of compounds, i.e. triterpenoids in this plant still exist, namely, the data concerning the occurrence of triterpene acids (ursolic acid, oleanolic acid, or both?) in fruit and leaves are inconsistent (Duke, 1992). Other vegetative organs of cowberry which have no nutritional or medicinal meaning have not been investigated in detail. Surprisingly, triterpenoids in cowberry are very rarely mentioned in pharmaceutical handbooks or popular herbalist's guide-books (Bisset & Wichtl, 2001; Barnes *et al.*, 2002) despite their supposed properties in promoting human health (Liu, 1995; 2005; Patočka, 2003; Chołuj & Janiszowska, 2005). Meanwhile, this very large class of natural isoprenoids occurring in higher plants exhibits various biological activities and hence is subject to the intensive miscellanous research. Among the physiological functions of triterpenoids, plant chemical defense against pathogens and herbivores is reported (Brown, 1998). Thus, the aim of the present work was to establish what kind of triterpenoids and their derivatives (if any) occur in various organs of the cowberry plant (leaves, stems, rhizomes and fruits).

Plants of several *Vaccinium* species, including bilberry (*V. myrtillus*) and cowberry (*V. vitis-idaea*) are often dominating shrubs in the herbaceous layer of boreal forests, very often forming wide-spread, practically one-species biotopes. It has been observed that they have a capacity to strongly restrict the growth of other plants, including seedlings of trees; hence the importance of this phenomenon in forest management. In recent years, phenolic compounds of bilberry litter have been investigated in studies on allelopathic relationships in Scandinavian ecosystems (Jäderlund *et al.*, 1998). However, there are also some data pointing to triterpenoids as potent allelochemicals (Oleszek *et al.*, 1992; Ohara & Ohira, 2003). Therefore, the aim of this work was also to investigate the profile of triterpenoids in the soil obtained from native cowberry habitat and to examine the influence of triterpenoid-containing extracts on the germination and growth of test model plants.

MATERIAL AND METHODS

Plant and soil material. Plant material and soil samples were randomly collected from the natural habitat in White Forest, central Poland (52° 44′ N, 21° 35′ E) in August 2004. Fresh whole plants were manually divided into organs : leaves, stems and rhizomes. Berries were harvested separately as ripe fruits. All collected material was dried at 40°C and weighed. Plant parts were powdered in a laboratory mill.

Extraction. Cowberry fruits, leaves, stems and rhizomes as well as soil samples were extracted in a Soxhlet apparatus for 6 h at first with ethyl ether, afterwards with methanol. Ether extracts were evaporated to dryness immediately, whereas to the obtained methanol extracts equal volumes of water were added, methanol was evaporated and the remaining aqueous solutions were extracted four times with n-butanol.

Separation. The obtained mixtures were fractionated on a column (CC) using silica gel, 30–70 mesh ASTM (Merck) in an increasing-polarity step gradient of hexane/ethyl ether (for the ether extracts) or chloroform/methanol (for butanol extracts) and afterwards rechromatographed by TLC (silica gel; chloroform/methanol, 98 : 2, v/v; chloroform/methanol, 85 : 15, v/v; chloroform/methanol/water 61 : 32 : 7, by vol.). Compounds were localized on plates by comparison to standards. Free triterpene acids were eluted from the gel with ethyl ether. Fractions of derivatives were eluted with methanol and subjected to hydrolysis.

Hydrolysis. Acid hydrolysis (AC) were performed with the Kiliani mixture (hydrochloric acid/acetic acid/water, 10 : 35 : 55, by vol.) at 100°C for 2 h. Subsequently, a 5-fold volume of water was added to each hydrolysate and extracted four times with ethyl ether. The liberated aglycones were purified by TLC (chloroform/methanol, 98 : 2, v/v).

Alkaline hydrolysis (AL) was performed with 10% KOH in 80% methanol at 100°C for 2.5 h. An equal volume of water was added to each hydrolysate, pH was neutralized, obtained solutions were extracted with ethyl ether and afterwards analysed by TLC.

MS and GLC analysis. Mass spectrometric analyses were conducted using a Waters Themabeam Mass Detector equipped with Wiley reference libraries. GLC analysis were performed with a Varian 3300 instrument equipped with flame ionization detection (FID) and fitted with a 2 m × 1/8′′ column SS (1.5% OV-A/1.95% OV-210; CWHP 80/100). As a carrier gas nitrogen was used at a velocity of 120 $cm \cdot min^{-1}$, the temperature of the injector and detector was 275°C, the temperature of the column 259°C. Samples of triterpene acids were methylated with

diazomethane dissolved in ethyl ether. The quantities of individual acids were determined in the presence of cholestan as an internal standard.

Allelopathic bioassays. Sheets of Whatman No. 1 filter paper were placed in 10 cm diameter Petri dishes and impregnated with 10 ml of ether or butanol extract obtained from soil samples or a 1 : 2 mixture of both extracts, at concentrations of 10, 50 or 150 $\mu l \cdot ml^{-1}$. After evaporation of the solvents, 10 ml of water was added to each dish and 30 seeds of test plants : cress *Lepidium sativum* L., lettuce *Lactuca sativa* L., wheat *Triticum aestivum* L., or pine *Pinus sylvestris* L. were distributed evenly on the prepared sheets. For each plant control dishes were prepared with sheets of filter paper moisturized with 10 ml of pure water. Afterwards, the dishes were closed and placed in the dark in a thermostat (22°C). Germinating seeds were counted after 3 days and the length of radicles and hypocotyls was measured after 7 days of experiment.

RESULTS AND DISCUSSION

MS and GLC analysis

Plant material harvested in native forest habitat was selected, dried, powdered and extracted with ethyl ether and methanol. Preliminary chromatographic analysis of ether extracts with reference to standards of triterpenoids showed the presence of bands with R_F similar to those of oleanolic acid and ursolic acid. In the methanol extracts, several weaker bands of presumable triterpenoidal derivatives with R_F lower than free aglycones were observed. To isolate the compounds, the extracts were subjected to fractionation with the use of silica gel column (CC) and repeated chromatographic purification on silica gel plates (TLC).

Compounds isolated from the ethyl extracts were analyzed by MS. The obtained results, compared with the Wiley Library database, could suggest the presence of ursolic acid solely. However, the main base peaks of the mass spectrum (*m/z* 175, 203, 204, 207, 248, 410) are common for both isomers, i.e. ursolic acid and oleanolic acid. Oleanolic acid (3β-hydroxy-olea-12-en-28-oic acid) and its isomer 3β-hydroxy-urs-12-en-28-oic acid (Fig. 1) are triterpenoids occurring widely in many plants, separately or together (Liu, 1995). Despite their wide distribution and pharmacological importance, the literature contains relatively little information about their distribution and amounts even in well-known plant species. The obvious reason is the difficulty in separation, identification and quantitative determination of these compounds. The fundamental difference between both isomers consists in the position of methyl groups at ring E at C-29 and C-30. However, this difference is too subtle to distinguish easily those compounds not only by TLC, but even by MS. Therefore, the results of the analysis obtained by these two methods did not allow us to state explicitly the occurrence of only one isomer in the *V. vitis-idaea* plant.

In recent years several methods of qualitative and quantitative analysis of oleanolic acid and ursolic acid have been described, including reversed phase high performance liquid chromatography (Claude *et al.*, 2004), micellar electrokinetic capillary chromatography (Liu *et al.*, 2003) and gas chromatography (Janicsák *et al.*, 2003). Gas chromatography was the first method enabling parallel determination of both isomers in samples derivatized by methylation, acetylation or silylation; and it still remains very useful due to the relatively simple procedure and lower cost of instrumentation. Therefore, the GLC technique was adopted to identify the compounds isolated from cowberry. Purified samples and appropriate standards of oleanolic and ursolic acids were methylated and separated with satisfactory efficacy (Fig. 2). The obtained results indicated that in all organs of cowberry plant as well as in the analyzed sample of soil not only ursolic acid, but also oleanolic acid occurred. Thus it can be concluded that the fraction of triterpenoids of *V. vitis-idaea* plant contains both isomers – oleanolic and ursolic acids.

Quantitative determination and distribution of free triterpene acids in cowberry plant organs

The amounts of individual compounds were evaluated by GLC. In all studied organs of *V. vitis-idaea* plant both identified acids occurred, however, in different amounts and ratios (Table 1). The total content of both free acids was the highest in fruits, where it amounted to 1% of DW, and also relatively high in leaves: 0.6% of DW. Those findings are of great interest since fruits and leaves are exactly those organs which are harvested and used as herbal resources. By contrast, in other parts of the *V. vitis-idaea* plant, i.e. stems and rhizomes, the content of triterpene acids was significantly lower and

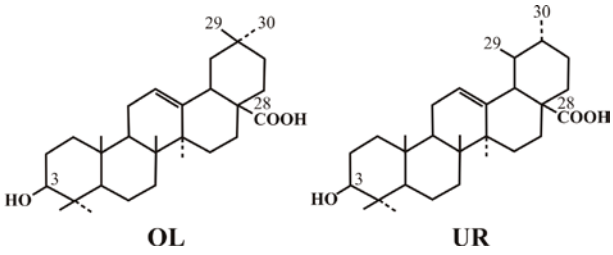

Figure 1. The structure of two triterpene isomers : oleanolic acid (OL) and ursolic acid (UR).

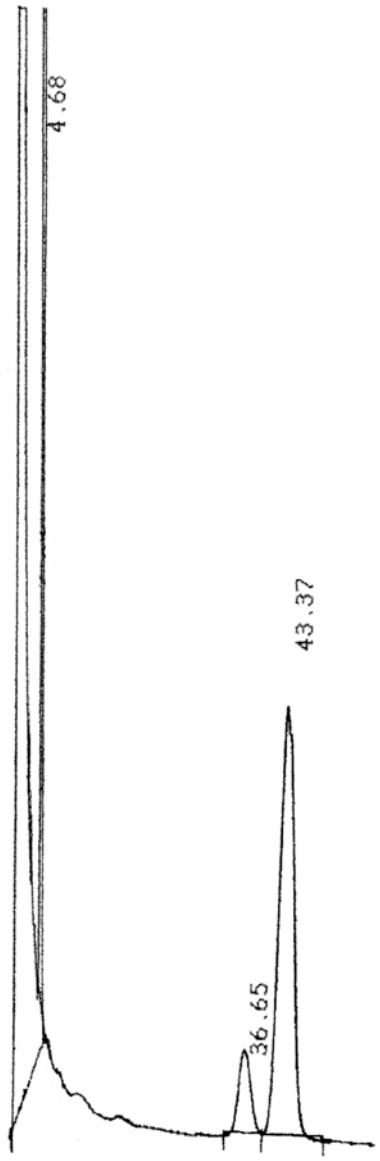

Figure 2. GLC chromatogram of triterpene acids isolated from *V. vitis-idaea* rhizomes.
R_T of cholestan (internal standard) 4.68, OL 36.65, UR 43.37.

amounted to 0.32 and 0.35% of DW, respectively, which was three times less than in fruits and almost two times less than in leaves.

The relatively high amount of free triterpene acids in fruits and leaves of cowberry might be related to some biological properties of those compounds, mainly antioxidant and preservative (Liu 1995; Hung & Yen 2001). Those organs are covered by a thick layer of surface waxes, thus it seems possible that those compounds can occur, at least partially, in those structures. Such epicuticular localization of triterpenes has been demonstrated for some other fruits, like apples and pears (Lu & Bramlage 2001; Claude *et al.*, 2004), and for leaves of various plants (Jetter *et al.*, 2000; Wollenweber *et al.*, 2000; Oliveira *et al.*, 2003). A high amount of free triterpene acids in the *V. vitis-idaea* evergreen leaves can be favorable for their permanence.

A comparison of the content of oleanolic and ursolic acid in individual organs of *V. vitis-idaea* indicated that in all parts of the plant ursolic acid distinctly predominated over oleanolic acid. However, what deserves attention, the ratio of both isomers was different in the examined organs. The highest content of oleanolic acid was detected in fruits and leaves, where it amounted to 0.3% and 0.2% of DW, respectively. The ratio of oleanolic to ursolic acid was also the highest in those two organs, equaling 1 : 2.08 in leaves and 1 : 2.38 in fruits. Markedly less oleanolic acid was found in stems (0.073% DW), and the least in rhizomes (0.056%). The ratio of oleanolic to ursolic acid in those plant parts was also low, equaling 1 : 3.45 in stems and 1 : 5.26 in roots. Thus, the ratio of oleanolic to ursolic acid was decreasing gradually from the upper to the lower parts of the plant, equaling in fruits and leaves approximately 1 : 2, and in rhizomes 1 : 5. Again, it deserves attention that oleanolic acid is the most abundant in the plant organs traditionally regarded as medicinal.

Distribution of derivatives of triterpene acids

A CC and TLC analysis of methanol extracts obtained from the tested organs of cowberry plant indicated that both identified acids — oleanolic and ursolic — occurred also, although in small amounts, in bound forms. In order to obtain preliminary information about the type of derivatives and to determine their amounts, the fractions were subjected to acid and alkaline hydrolysis. The obtained aglycones were identified, separated and determined quantitatively by GLC (Table 2). According to the results obtained after analysis of the products of acid hydrolysis, derivatives of triterpenic isomers (presumably 3-O-glycosides and 28-O-glycoside esters) were the most abundant in rhizomes (almost 0.01% DW), then in fruits and leaves (about 0.007% DW each), and the least abundant in stems (0.004% DW). If one considers the ratio of derivatives to free acids (Table 3) the highest one was in rhizomes (1 : 37.88), in stems and leaves over two-fold less (1 : 88.50 and 1 : 90.91, respectively), and in fruits – the least (1 : 142.86). Thus, it seems that since rhizomes are the part of the plant containing relatively the highest amount of the bound forms of both acids, it should be either the place of the biosynthesis of those compounds, or the site of their accumulation as a result of transport from other organs of the plant. Both those mechanisms, i.e. transport of saponins from green organs of the plant to roots, and lately also their independent biosynthesis in this organ, were demonstrated in

Table 1. Contents of free triterpene acids (oleanolic and ursolic) in organs of cowberry plant (*V. vitis-idaea*) and soil containing cowberry litter.

Values are means (± S.D.) of three determinations.

	Both acids		Oleanolic acid (OL)		Ursolic acid (UR)		The ratio OL : UR
	mg · g^{-1} DW	%DW	mg · g^{-1} DW	%DW	mg · g^{-1} DW	%DW	
Fruits	10.05 ± 0.13	1.005	2.96 ± 0.21	0.3	7.09 ± 0.44	0.71	1 : 2.38
Leaves	6.11 ± 0.24	0.61	1.99 ± 0.02	0.2	4.11 ± 0.26	0.41	1 : 2.08
Stems	3.17 ± 0.04	0.32	0.73 ± 0.01	0.07	2.45 ± 0.13	0.24	1 : 3.45
Rhizomes	3.52 ± 0.12	0.35	0.56 ± 0.04	0.06	2.96 ± 0.22	0.29	1 : 5.26
Soil	0.68 ± 0.03	0.07	0.110 ± 0.001	0.01	0.57 ± 0.05	0.06	1 : 5.26

marigold (*Calendula officinalis* L.) (Ruszkowski *et al.*, 2003; Szakiel *et al.*, 2005). The occurrence of relatively high amounts of bound forms of triterpene acids in underground parts of the plant can have important ecological meaning, because, as it has been shown in the case of marigold (Ruszkowski *et al.*, 2004; Szakiel *et al.*, 2005) and bilberry *V. myrtillus* (Szakiel, 2004), those compounds are partially responsible for allelopathic activity.

In all vegetative organs of cowberry oleanolic and ursolic acids released after acid hydrolysis prevailed over these aglycones liberated after alkaline hydrolysis, pointing to the predominance of putative 3-O-glycosides over 28-O-glycoside esters, especially in rhizomes (threefold) and stems (fourfold) (Table 2). In contrast, in fruits products of both types of hydrolysis were detected in similar amounts, suggesting that this organ synthesizes and accumulates mainly 28-O-glycoside esters.

The ratio of oleanolic to ursolic acid released after acid hydrolysis in individual plant organs was generally different from the corresponding ratio of free acids (Tables 2 and 3). Only in leaves the ratio of free oleanolic to ursolic acid was the same as the ratio of both acids as aglycones released as a result of acid hydrolysis.

Among the derivatives of both acids, those of ursolic acid predominated markedly, in fruits over fourfold, in stems six times, in rhizomes twice. It is interesting since in rhizomes the ratio of free oleanolic to ursolic acid equals 1 : 5.26, whereas the corresponding ratio of aglycones obtained after acid hydrolysis is 1 : 1.12. It points to the accumulation of especially large amounts of the derivatives of oleanolic acid in the underground part of the plant.

Occurrence of triterpene acids and their derivatives in the soil

Some amounts of free oleanolic and ursolic acids as well as traces of their derivatives were detected in extracts obtained from the soil of a forest

Table 2. Contents of bound triterpene acids released as a result of acid (AC) and alkaline (AL) hydrolysis.

Values are means (± S.D.) of three determinations.

	Both acids		Oleanolic acid (OL)		Ursolic acid (UR)		The ratio OL : UR
	mg · g^{-1} DW	%DW	mg · g^{-1} DW	%DW	mg · g^{-1} DW	%DW	
Fruits							
AC	0.070 ± 0.003	0.0070	0.017 ± 0.0006	0.0016	0.057 ± 0.002	0.0057	1 : 4.35
AL	0.068 ± 0.005	0.0067	0.013 ± 0.001	0.0013	0.051 ± 0.004	0.0051	1 : 3.13
Leaves							
AC	0.0670 ± 0.0008	0.0067	0.022 ± 0.001	0.0022	0.045 ± 0.003	0.0045	1 : 2.08
AL	0.046 ± 0.002	0.0046	0.014 ± 0.0005	0.0013	0.032 ± 0.002	0.0032	1 : 2.38
Stems							
AC	0.036 ± 0.002	0.0036	0.005 ± 0.0004	0.0005	0.0310 ± 0.0009	0.0031	1 : 5.88
AL	0.0080 ± 0.0006	0.0008	0.004 ± 0.0002	0.0004	0.0040 ± 0.0004	0.0004	1 : 1.08
Rhizomes							
AC	0.093 ± 0.005	0.0093	0.043 ± 0.0008	0.0043	0.050 ± 0.002	0.0050	1 : 1.12
AL	0.027 ± 0.002	0.0027	0.011 ± 0.0002	0.0011	0.016 ± 0.001	0.0016	1 : 1.45
Soil							
AC	0.0140 ± 0.0003	0.0014	0.007 ± 0.001	0.0007	0.0007 ± 0.0002	0.0008	1 : 1.12
AL	0.0040 ± 0.0003	0.0004	0.004 ± 0.0003	0.0004	–	–	1 : 0

Table 3. The ratio of bound forms (AC, released after acid; AL, alkaline hydrolysis) to the free form of triterpene acids.

	AC			AL		
	OL	UR	OL+UR	OL	UR	OL+UR
Fruit	1 : 227.27	1 : 123.46	1 : 142.86	1 : 178.57	1 : 138.89	1 : 147.06
Leaves	1 : 91.74	1 : 91.41	1 : 90.91	1 : 147.06	1 : 126.58	1 : 133.33
Stems	1 : 136.99	1 : 80.00	1 : 88.50	1 : 178.57	1 : 555.56	1 : 400.00
Rhizomes	1 : 13.02	1 : 59.17	1 : 37.88	1 : 51.55	1 : 188.68	1 : 129.87
Soil	1 : 16.26	1 : 75.76	1 : 49.02	1 : 27.17	–	1 : 172.41

Table 4. The influence of soil extracts on the germination and growth of model plants.

Values are means (± S.D.) of three determinations.

		Germination	Radicle	Hypocotyl
	Concetration μg/ml	%	%	%
Cress				
control		100 ± 1.97	100 ± 2.23	100 ± 6.59
	10	93.18 ± 1.97	92.52 ± 5.92	89.63 ± 1.62
ether extract	50	92.05 ± 6.82	80.10 ± 13.81	88.26 ± 5.48
	150	89.77 ± 1.97	74.80 ± 3.76	90.24 ± 2.69
	10	97.73 ± 1.97	82.58 ± 1.76	85.42 ± 5.70
butanol extract	50	94.32 ± 8.21	78.13 ± 0.94	93.53 ± 1.57
	150	90.91 ± 3.94	69.27 ± 1.93	78.07 ± 6.45
	10	93.18 ± 5.21	82.87 ± 1.38	96.29 ± 2.89
mixed extracts	50	92.05 ± 5.90	76.78 ± 6.12	92.29 ± .,05
	150	90.91 ± 1.97	66.49 ± 5.24	81.96 ± 4.47
Wheat				
control		100 ± 2.01	100 ± 0.74	100 ± 0.50
	10	94.19 ± 3.49	96.97 ± 16.31	96.75 ± 6.04
ether extract	50	84.88 ± 6.02	92.90 ± 6.63	98.10 ± 2.61
	150	81.40 ± 2.01	75.64 ± 3.76	88.83 ± 0.31
	10	84.88 ± 4.01	87.87 ± 11.58	92.03 ± 5.09
butanol extract	50	83.72 ± 3.49	85.48 ± 1.24	89.79 ± 1.93
	150	79.07 ± 2.01	71.47 ± 9.69	83.37 ± 15.22
	10	93.02 ± 3.90	102.98 ± 3.48	96.90 ± 1.72
mixed extracts	50	88.37 ± 2.47	99.21 ± 15.09	94.92 ± 1.71
	150	86.05 ± 2.93	86.52 ± 1.57	84.38 ± 5.96
Pine				
control		100 ± 4.49	100 ± 12.42	
	10	107.94 ± 14.66	128.12 ± 17.43	
ether extract	50	79.37 ± 7.33	60.92 ± 12.06	
	150	60.29 ± 9.70	43.85 ± 0.70	
	10	95.24 ± 14.41	152.87 ± 8.84	
butanol extract	50	79.37 ± 7.33	94.30 ± 12.15	
	150	19.05 ± 6.35	24.83 ± 3.55	
	10	104.76 ± 8.98	106.58 ± 5.80	
mixed extracts	50	60.32 ± 9.70	49.67 ± 7.53s	
	150	0	0	

cowberry habitat in central Poland (Tables 1 and 2). The ratio of free oleanolic acid to ursolic acid was identical in rhizomes and in the soil, equaling in both cases 1 : 5.26. Therefore it seems that both free acids occur in the soil as a result of direct exudation or simple decay of cowberry rhizomes, since in other parts of the plant that ratio is significantly different. Besides, as an evergreen plant, cowberry does not lose huge amounts of leaf matter every vegetative season, thus their decay cannot be the main source of substances detected in the litter, as it is in the case of other plants including bilberry *V. myrtillus* (Jäderlund *et al.*, 1996). However, another source of substances accumulating in the soil, i.e. leaching from epicuticular leaf waxes cannot be ruled out without further investigation, although the significance of this phenomenon seems rather low, regarding the ratio of both acids in the leaves (1 : 2) markedly different from that in the soil and rhizomes (1 : 5.26).

Allelopathic activity

Since triterpene acids and their derivatives were detected in the soil obtained from a *V. vitis-idaea* native habitat, these compounds could be at least partially responsible for the plant growth regulation effects exerted by cowberry. Therefore, the influence of triterpenoid-containing extracts of soil on the germination of seeds and growth of young seedlings of model plants (cress *Lepidium sativum* L., lettuce *Lactuca sativa* L., wheat *Triticum aestivum* L., and the main tree of the forest ecosystems in central Poland — pine *Pinus sylvestris* L.) was investigated.

Ethyl ether extract containing free triterpene acids and butanol extract containing their derivatives were supplied to seeds separately or in a 1 : 2 mixture imitating the real ratio in the soil. Results of bioassays on *L. sativum*, *T. aestivum* and *P. sylvestris* are presented in Table 4, the results obtained for *L. sativa*, practically identical to those obtained for *L. sativum*, were omitted.

The germination of cress and lettuce were slightly inhibited (by 6–10% as compared to the control) in all examined conditions. The inhibitory effect exerted on the germination of wheat was more significant (about 20% by the highest concentration of mixed extracts). However, the most dramatic effect was exerted on the germination of pine seeds, which was inhibited by 40% by ether extract, by 80% by butanol extract and by 100% by the mixed extracts at the highest applied concentration. By contrast, the lowest concentrations of soil extracts exerted a slightly stimulatory effect, which is often observed as a typical phenomenon for many compounds with allelopathic activity (Inderjit *et al.*, 1999).

Further growth of cress, lettuce and wheat seedlings, measured as length of radicles and hypocotyls, was generally reduced in all tested conditions. With increasing concentrations of the extracts, the growth of cress radicles was inhibited by 25–34%, of wheat — by 15–30%. The growth of hypocotyls, in the majority of plants less sensitive to allelochemicals than radicles (Ohara & Ohira, 2003), was reduced by 10–22% for cress and by 12–17% for wheat.

The influence of the examined soil extracts on the growth of pine seedlings was different. The lowest concentrations of extracts exerted remarkable stimulatory effect on the radicle growth (by more than 50% in the case of butanol extract), whereas the highest concentration sharply reduced the length of pine radicles (ether extract by 56%, butanol extract by 75%, and the mixture of extracts by 100%). In the applied duration of experiments, hypocotyls of pine seedlings were still not sprouted.

The obtained results suggested that soil extracts containing free triterpene acids were less active than those containing their derivatives. Moreover, if one considers the influence on the germination and growth of pine, the most dramatic effects were exerted by the mixture of the two fractions, leading to a total inhibition of pine development. It could be due to the described detergent-like action of free ursolic acid, which facilitates the penetration of other compounds into seeds (Harborne, 1993).

The observed particularly strong allelopathic activity against pine seems to have an obvious ecological justification. In native habitats, germinating pine seeds are dangerous competitors of shrubs of *Vaccinium* species, limiting accessibility to water, mineral salts and progressively also to light. The significant allelopathic potential of cowberry and bilberry can efficiently restrict the development of new stands of trees in some boreals ecosystems, where those plants induce serious perturbations in the revival of forest communities (Jäderlund *et al.*, 1996; 1998).

CONCLUSIONS

The presence of two isomeric triterpene acids : oleanolic and ursolic was demonstrated in the cowberry (*V. vitis-idaea*). These compounds, mainly in a free form but also as derivatives, were detected in different amounts in all parts of this plant, including the edible fruits. Apart from the presumable influence on human health, the identified triterpenoids can be regarded as plant defensive weapons, and thus their high abundance in cowberry fruits and leaves may confer chemical

protection against herbivores and pathogens. In turn, the high content of triterpenoid derivatives in rhizomes may be responsible for the allelopathic potential of cowberry.

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