*Biuletyn Ogrodów Botanicznych*, **10**: *101-109, 2001*

# THE SEARCH FOR PLANT POLYPRENOLS: SCREENING POLISH RARE AND ENDANGERED SPECIES

## Poszukiwanie poliprenoli roślinnych: przegląd polskich gatunków rzadkich i zagrożonych

Urszula A.GOLAS[1], Olga K. MOZGAWA[1], Anna GASEK[2], Wiesław GAWRYŚ[2], Roman MURANYI[2], Ewa SWIEZEWSKA[3] and Tadeusz CHOJNACKI[3]

[1] *Institute of Biochemistry, University of Warsaw, ul. Żwirki i Wigury 93, 02-089 Warszawa*
[2] *Botanical Garden – Centre for Biological Diversity Conservation, Polish Academy of Sciences, ul. Prawdziwka 2, 02-973 Warszawa;*
[3] *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Pawińskiego 5A, 02-106 Warszawa.*

### ABSTRACT

The long chain polyprenols were shown to be rather uncommon constituents in leaves of several herbaceous plants and of shrubs and trees classified as rare and endangered species. From among the over 100 studied species of vascular plants representing over 50 systematic families in only few of them the accumulation of long chain polyprenols was observed. The types of accumulated polyprenols mixtures were similar to the mixtures characteristic for members of *Rosaceae* family or for many gymnosperms (Swiezewska *et al.*, 1994).The accumulation of polyprenols composed of 10,11 and 12 isoprene residues was rare in this group of plants. A new type of polyprenol mixture was found to be characteristic for leaves of some species belonging to *Graminae* (e.g.*Melica ciliata*). They contain a group of polyprenols composed of 14 and 15 isoprene residues as dominating prenologues and a large number of longer chain molecules composed of several tens of isoprene residues. The polyprenols of *Graminae* occur in the form of carboxylic esters and account for up to ca. 2% of leaf tissue. The results of this search suggest no correlation between the vulnerability of a species and accumulation of polyprenols.

KEY WORDS: polyprenols, endangered plants, *Graminae*

### INTRODUCTION

The „Red Data List" of Polish vascular plant taxa comprises 339 species amounting to 14.7% of the polish flora (Zarzycki *et al.*,1992). They are grouped in five classes according to the category of the threat: 1. Extinct and probably extinct; 2.Endangered, whose survival is unlikely if the causal factors continue operating; 3. Vulnerable, taxa believed likely to move in the near future in Endangered category if the causal factors continue operating; 4. Rare, taxa with small populations that are not at present Endangered or Vulnerable but are at risk, and 5, Taxa known to be extinct, endangered, vulnerable or rare but where is not enough information which of the four above categories is most appropriate. There remain doubts whether all threatened species are on the list and whether they are in the correct category. The approach of the present paper was to use a practical though incomplete criterion of classification as a guide for studying the occurrence of polyprenols in plants. The fact of establishing the *ex situ* collection of endangered plants in the Botanical Garden – Centre for Biological Diversity Conservation could fulfil this aim. In classifying plants the system of five categories was abandoned and the term „endangered" was used instead for simplicity. We also used the term „accompanying species" for the non-endangered taxa which are typical for a given place.

We might have expected from the results of the search for polyprenols in plants of various ecological status some indications with respect of the factors responsible for the accumulation of polyprenols and also we wanted to gain general information on the phytochemical taxonomy of Polish flora in its most vulnerable part. The polyprenols are characteristic botanochemicals that so far have no special practical use except of serving as specific biochemicals in various areas of biochemistry. Examining natural plant resources (Mirek *et al.*,1995) especially the endangered species for the natural abundance of polyprenols seemed therefore justified. The previous screenings for plant polyprenols were performed mainly on leaves of shrubs and trees (cf. Swiezewska *et al.*,1994). In the present paper the majority of tests for polyprenols were done on leaves of herbaceous plants as these plants are mainly represented in the „Red List of Plants".

The studied plants, over 100 species, represent only a part of the whole collection of rare and endangered plants maintained by three co-authors (W.G.,A.G. and R.M) at the Botanical Garden – Centre for Biological Diversity Conservation. It consists of the open air collection and of a smaller part in the greenhouse. The whole list of rare and endangered plants cultivated in the above Botanical Garden was almost twice as long, but in the present study only those species were examined in which the old, mature green leaves could be collected. The list of studied endangered plants is not therefore complete as it does not include those species in case of which sampling aged leaves in the period September-October was not possible. It can however be considered as representative set of rare and endangered species.

In order to have some insight in the feature „rare and endangered" we have studied a group of plants classified as „accompanying" in the given habitats in Poland. The definition of the group of „accompanying species" reflected the actual knowledge concerning the Polish flora and was already used for grouping plant species in the living collection that was established in the respective Botanical Garden. The set of accompanying plants consisted of 26 species.

The structure of plant polyprenols is shown in Fig. 1. The polyisoprenoid alcohols presented there are of poly-*cis* type as shown in case of several molecular species (Chojnacki *et al.*, 1987).

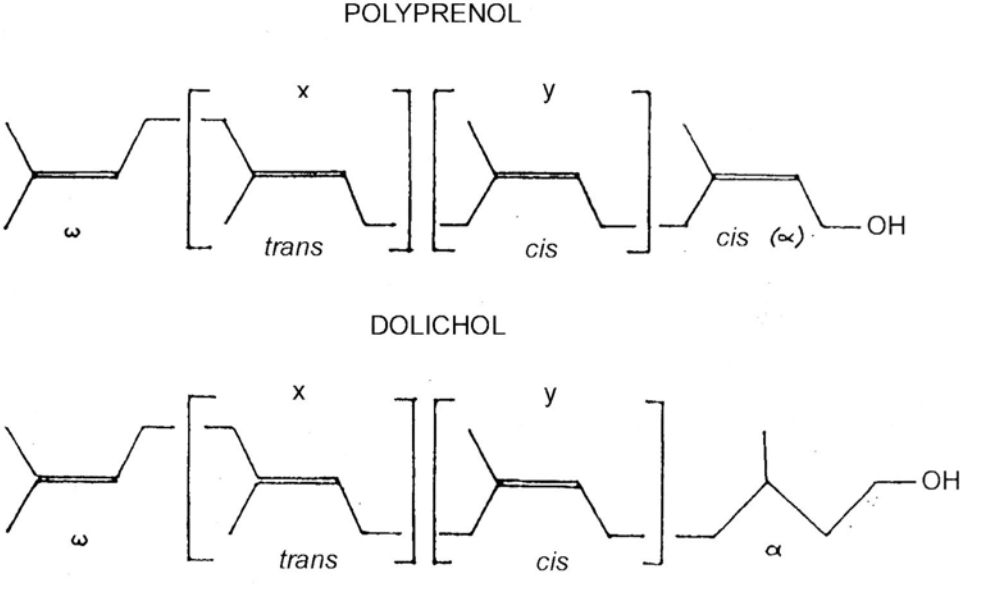


**Fig. 1.** Comparison of structure of plant polyprenols with mammalian dolichols

## MATERIALS AND METHODS

**Plant material and preparation of lipid extract.** All plants studied were from the living collection in the Botanical Garden – Centre of Biological Diversity of the Polish Academy of Sciences in Powsin. The green, old leaves were collected in the period 15 September – 15 October 1999 and 400 mg samples of fresh material were homogenized with 4 ml of acetone:hexane, 1:1,v/v mixture in an UltraTurrax homogenizer. The homogenates after standing at room temperature for 1–2 days were filtered, the extracts were evaporated to dryness in a stream of nitrogen and the lipid residues were dissolved in 0.1 ml of chloroform:methanol,2:1, v/v and stored for chromatographic examination.

Thin layer chromatography, high pressure liquid chromatography and other procedures.

Thin layer chromatography of 5–10 microliters of chloroform:methanol (2:1,v/v) solution of plant lipids was performed on Kiesel Gel plates in solvent A (ethyl acetate:toluene, 1:19,v/v) and in solvent B (toluene:hexane,1:2,v/v) and on RP-18 plates with concentrating zone in solvent C (acetone). All plates, solvents and materials for column chromatography were from Merck (Darmstadt, Germany). Spots of isoprenoid lipids were detected with iodine vapours. Standard substances, prenol-9,-10,. etc. up to prenol-25 were from Collection of Polyprenols at the Institute of Biochemistry and Biophysics in Warsaw, Poland. Polyprenyl acetates (with dominating prenologues composed of 17 and 18 isoprene units) were isolated from leaves of *Ginkgo biloba* (Ibata *et al.*, 1984). The semiquantitative assay of polyprenols was performed according to Wellburn and Hemming (1966) by comparing the size and intensity of chromatographic spots with those of known amounts of standard substances.

Strong alkaline hydrolysis of lipid extracts (Stone *et al.*,1967), preparative separation of polyprenol fraction on Kiesel gel column and high pressure liquid chromatography of polyprenol mixtures on an Hypersil ODS 3 /um reversed-phase column (Knauer, Germany) were performed as described previously (Wanke *et al.*, 1998) using Waters HPLC apparatus with gradient system and UV detector set at 210 nm.

## RESULTS

In Table 1 are listed the results of screening for the presence of polyprenols in 101 plant species classified as endangered. In only ca.40 % of them the presence of polyprenols could be detected. In most of them polyprenols were present as carboxylic esters. In most of them the dominating polyprenols were composed of 14–19 isoprene units. Especially high amounts of polyprenols were found in species belonging to *Graminae* family.

In Fig. 2 are shown the most characteristic types of polyprenols pattern in leaves of the studied group of endangered plants. The presence of both single (A,B and C) and multiple families (D and E) of polyprenols were observed.

In Table 2 are listed the results of screening 26 plant species classified as accompanying the endangered ones in the same habitats. In this group also the dominating size of polyprenol molecules was the chain length of 14–19 isoprene units.

The polyprenols in the studied plants occurred mainly as acetic acid esters. Only in few cases we could observe large amounts of free polyprenols. In several plants one could detect distinct amounts of hydrophobic substances (rl, rubber-like substances) that moved to the front on adsorption chromatography on Kiesel Gel and remained on the start on reversed-phase plates. This behaviour is characteristic for very long chain polymers composed of over 50 isoprene units (Skoczylas *et al.*,1994).

## DISCUSSION

The set of plants checked for the presence of long-chain polyprenols represents several systematic groups of mainly herbaceous plants. In most of them polyprenols were not detectable or occurred only in low amounts, so that they could not be considered as accumulated secondary metabolites. Only in few cases their amount was of the order 0.5–2.0%. These were four studied species of *Graminae* and 1–2 plants belonging to *Apiaceae*, *Boraginaceae*, *Ericaceae*, *Lamiaceae* and *Lythraceae*. The accumulation of large amounts of polyprenols observed

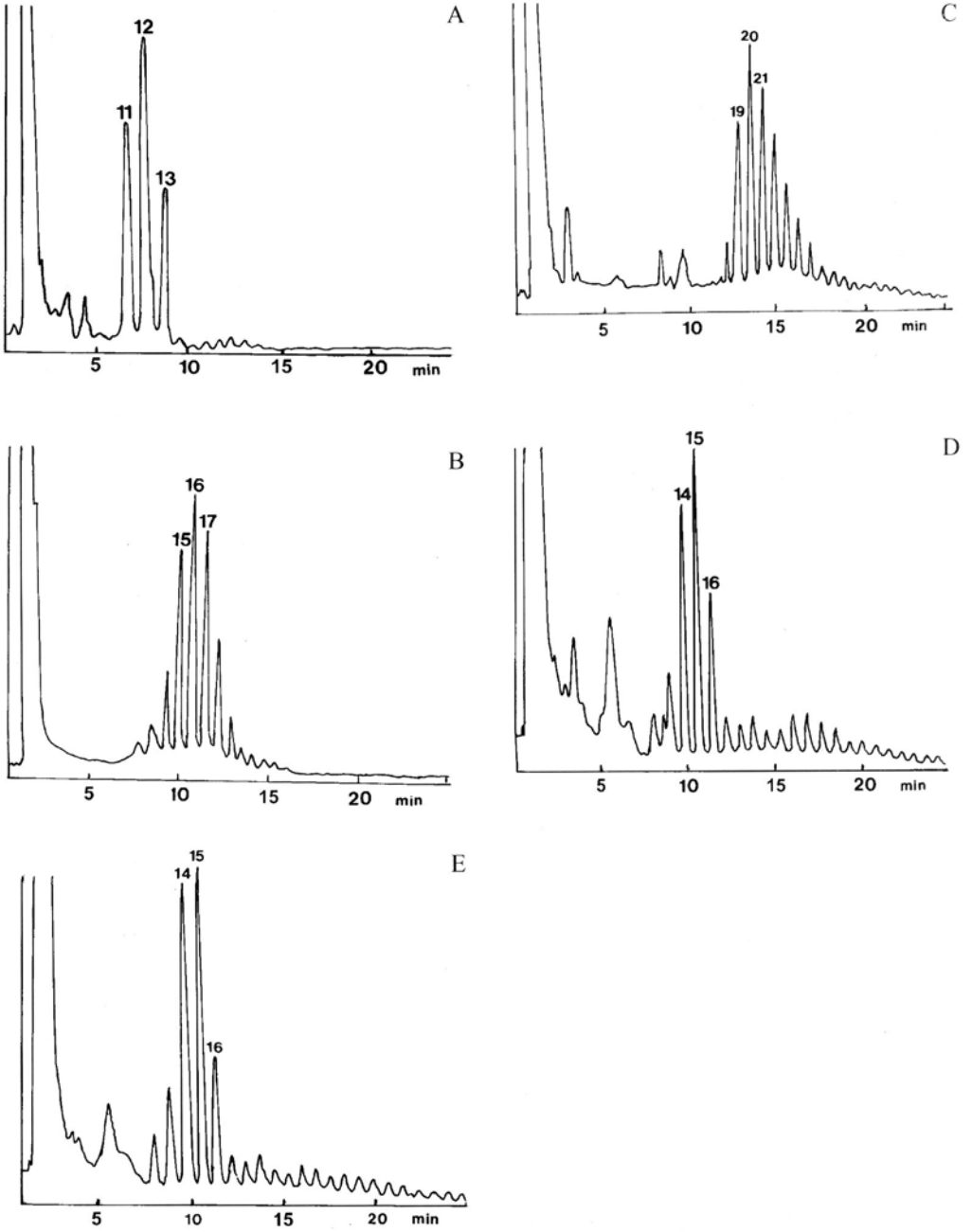


**Fig. 2.** HPLC records of polyprenol mixtures in leaves of some rare and endangered plant species (A) Lithospermum purpurocaeruleum; (B) Rhododendron luteum; (C) Lythrum virgatum; (D) Melica ciliata; (E) Poa supina
Chromatography was performed using Waters apparatus equipped with a gradient system and UV detector set at 210 nm. The numbers over chromatographic peaks mark the position of a given polyprenol (11, prenol-11; 12, prenol-12, etc.) and show the number of isoprene units in the molecule

**Tab. 1**. Results of screening endangered plants for the presence of long-chain polyprenols in leaves

| Name of plant | | Polyprenols | |
|---|---|---|---|
| Family | Species | Appr. amount (% of fresh weight) | Chain length (no of isoprene residues) |
| *Apiaceae* | *Angelica archangelica* L.. | 0.05-2.00 e,f * | 12,16-18 rl * |
| *Apocynaceae* | *Vinca minor* | 0.02-0.50 e | 18-20 |
| *Araliaceae* | *Hedera helix* L. | - | - |
| *Aristolochiaceae* | *Asarum europeum* L. | - | - |
| *Aspleniaceae* | *Phyllitis scolopendium* (L.) Newman<br>*Polypodium vulgare* L. | 0.05-0.20 e,f<br>- | 8,9<br>- |
| *Asteraceae* | *Achillea nobilis* L.<br>*Achillea stricta* Schleich.<br>*Arnica montana* L.<br>*Carlina acaulis* L.<br>*Carlina onopardifiolia* Besser<br>*Centaurea kotschyana* Heuff. ex W.D.J.Koch<br>*Cirsium decussatum* Janka<br>*Helichrysum arenarium* (L.) Moench<br>*Leontopodium alpinum* Cass.<br>*Saussurea alpina* (L.) D.C. | 0.01-0.05 e<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- | 11,14 rl<br>- rl<br>- rl<br>-<br>-<br>-<br>-<br>-<br>- rl<br>- |
| *Athyriaceae* | *Matteuccia struthipteris* (L.) Tod. | - | - |
| *Boraginaceae* | *Lithospermum purpurocae-ruleum* L. | 0.50-2.00 e,f | 11,12 |
| *Brassicaceae* | *Cochlearia polonica* E. Fröhl.<br>*Erisymum pieninicum* (Zapal.) Pawl.<br>*Lunaria rediviva* L. | -<br>0.01-0.05 e<br>0.20-0.50 e,f | -<br>15,16<br>10,11 rl |
| *Campanulaceae* | *Adenophora liliifolia* (L.) Besser<br>*Campanula scheuchzeri* Vill.<br>*Phyteuma orbiculare* L. | 0.01-0.05 e<br>0.01-0.05 e<br>0.01-0.05 e | 15,16<br>15,16 rl<br>15,16 |
| *Caprifoliaceae* | *Lonicera periclymenum* L. | - | - |
| *Caryophyllaceae* | *Dianthus collinus* Waldst. et Kit.<br>*Dianthus compactus* Kit.<br>*Dianthus glacialis* Haenke ex Jacq.<br>*Dianthus plumarius* subsp. praecox (Kit.) Pawl.<br>*Dianthus superbus* L.<br>*Dianthus nitidus* Waldst. et Kit.<br>*Gypsophila paniculata* L. | 0.01-0.05 e<br>0.01-0.05 e<br>0.01-0.05 e<br>-<br>-<br>-<br>0.01-0.05 e,f | 10, 15,16<br>10, 15,16<br>10, 16<br>-<br>-<br>-<br>11,16,17 |
| *Crassulaceae* | *Sempervivum montanum* L. | - | - |
| *Eleagnaceae* | *Hippophae rhamnoides* .L. | 0.01-0.05 f | 14,15 |

cont.

Table 1 cont.

| | | | |
|---|---|---|---|
| *Ericaceae* | *Arctostaphylos uvaursi* (L.) Spreng. | - | - |
| | *Ledum palustre* L. | - | - |
| | *Rhododendron luteum* Sweet | 0.50-2.00 e | 17-20 |
| *Euphorbiaceae* | *Euphorbia epithymoides* L. | 0.01-0.05 f | 14 |
| *Fabaceae* | *Astragalus penduliflorus* Lam. | 0.01-0.05 e | 15,16 rl |
| | *Ononis spinosa* L. | - | - |
| *Graminae* | *Calamagrostis epigeios* (L.) Roth | 0.50-2.00 e | 17,18 rl |
| | *Elymus arenarius* L. | 0.50-2.00 e | 17,18 rl |
| | *Melica ciliata* L. | 0.50-2.00 e | 14,15,18,19 rl |
| | *Stipa capillata* L. | 0.50-2.00 e | 17,18 rl |
| *Iridaceae* | *Gladiolus imbricatus* L. | 0.20-0.50 e | 15,16 |
| | *Iris aphylla* L. | - | - |
| *Lamiaceae* | *Dracocephalum ruyschiana* L. | - | - |
| | *Prunella laciniata* (L.) L. | 0.50-2.00 e | 15,16 rl |
| *Liliaceae* | *Allium sibiricum* L. | - | - |
| | *Anthericum liliago* L. | - | - |
| | *Convallaria majalis* L. | - | - |
| | *Lilium martagon* L. | 0.05-0.20 e | 17,18 |
| | *Veratrum lobelianum* Bernh. | - | - |
| | *Veratrum nigrum* L. | 0.20-0.50 f | 9 |
| *Linaceae* | *Linum austriacum* L. | - | - |
| | *Linum flavum* L. | 0.01-0.05 e,f | 17,18 rl |
| *Lycopodiaceae* | *Diphasiastrum complanatum* (L.) Holub | - | - |
| | *Lycopodium clavatum* L. | - | - |
| *Lythraceae* | *Lythrum virgatum* L. | 0.50-2.00 e | 19,20,21 |
| *Osmundaceae* | *Osmunda regalis* L. | - | - |
| *Papaveraceae* | *Papaver burseri* Crantz | - | - rl |
| *Pinaceae* | *Pinus cembra* L. | 0.50-2.00 e | 15,16,17 |
| *Plantaginaceae* | *Plantago coronopus* L. | - | - |
| | *Plantago maritima* L. | - | - |
| *Polemoniaceae* | *Polemonium coeruleum* L. | - | - |
| *Primulaceae* | *Cortusa matthioli* L. | - | - |
| | *Primula auricula* L. | - | - |
| | *Primula elatior* L. Hill | - | - |
| | *Soldanella carpatica* Vierh. | 0.50-2.00 e,f | 14,15,16 rl |
| *Pyrolaceae* | *Chimaphila umbellata* (L.) W.P.C.Barton | - | - |
| *Ranunculaceae* | *Aconiceta malasitum* | - | - |
| | *Aconitum lasiocarpum* (Rchb.) Gayer | - | - |
| | *Aconitum vulparia* Rchb. | - | - |
| | *Adonis vernalis* L. | - | - |
| | *Anemone sylvestris* L. | - | - |
| | *Aquilegia vulgaris* L. | - | - |

cont.

Table 1 cont.

| | | | |
|---|---|---|---|
| *Ranunculaceae* | *Cimicifuga europea* Schipcz. | - | - |
| | *Clematis recta* L. | - | - |
| | *Pulsatilla alba* Rchb. | - | - |
| | *Pulsatilla slavica* G.Reus. | 0.01-0.05 e | 19-22 rl |
| | *Trollius europaeus* L.s.s. | - | - |
| *Rhamnaceae* | *Frangula alnus* Mill. | 0.01-0.05 e | 15,16 |
| Rosaceae | *Aruncus sylvetris* Kostel | 0.50-2.00 e | 18,19 |
| | *Potentilla sterilis* (L.) Garcke | - | - |
| | *Sibbaldia procubens* L. | 0.05-0.20 e | 18-19 |
| | *Sorbus intermedia* (Ehr.) Pers. | 0.50-2.00 e | 19-22 |
| *Rubiaceae* | *Galium odoratum* (L.) Scop. | - | - |
| *Rutaceae* | *Dictamnus albus* L. | - | - |
| *Scrophulariaceae* | *Digitalis grandiflora* Mill. | - | - |
| | *Digitalis purpurea* L. | - | - |
| | *Verbascum chaixii* subsp. *austriacum* (Schott ex Roem.et Schult.) Hayek | - | - |
| *Staphylleaceae* | *Staphyllea pinnata* L. | 0.05-0.20 e | 18-20 |
| *Thymelaeaceae* | *Daphne cneorum* L. | - | - |
| | *Daphne mezereum* L. | - | - rl |

*e, esterified alcohols
f, free alcohols
rl, rubber-like substance, not migrating on RP-18 plate in acetone

in the present studies in conifers and in *Rosaceae* family has been described before (Swiezewska *et al.*,1994).The interesting fact found in the present research is that the group of plants representing a large proportion of endangered and rare species in Poland contains polyprenols of molecular size of 15-20 isoprene residues. This type of polyprenols seems now to be the most common in the plant kingdom. The previous reports have limited their occurrence to gymnosperms and to *Rosaceae* family. In several reports from this and other laboratories many plant species were known to accumulate rather polyprenols composed of 11 and 12 isoprene residues.

When undertaking the present studies we wanted to learn on whether the factor determining the vulnerability of a given plant species may bear on the type or content of polyisoprenoid alcohols. These studies might have shown whether there is a possibility that unique, special types of polyisoprenoid alcohols are in danger of disappearing in the course of elimination some rare and endangered plant species. This was not the case. While in the course of these studies new types of polyprenols pattern were detected, it was found that they were not limited to the group of species of the „Red List"; similar patterns were observed in plants classified as „accompanying species". The taxonomic background in these two groups was however not identical.

## ACKNOWLEDGEMENTS

The kind cooperation of Professors, Wirginia Janiszowska and Zdzisław Wojciechowski of the University of Warsaw is greatly appreciated. We acknowledge technical assistance of Ms Jozefina Hertel for completing and editorial of our results.

**Tab. 2.** Results of screening plants accompanying the rare and endangered ones for the presence of polyprenols in leaves

| Name of plant | | Polyprenols | |
|---|---|---|---|
| Family | Species | Appr. amount (% per fresh weight) | Chain length (no of isoprene residues) |
| *Asteraceae* | *Arabis alpina* L. | - | - |
| | *Biscutella laevigata* L. | 0.01-0.05 e | 15,17 |
| | *Centaurea jacea* L. | - | - |
| | *Centaurea mollis* Waldst.et Kit. | - | - |
| | *Hieracium aurantiacum* L. | 0.01-0.05 e | 15,16 |
| | *Hieracium bupleuroides* C.C.Gmel. | - | - |
| | *Telekia speciosa* (Schreb.) Baumg. | - | - |
| *Apiaceae* | *Astrantia major* L. | - | - |
| | *Eryngium campestre* L. | 0.01-0.05 | 14-17 |
| | *Falcaria vulgaris* Bernh. | - | - |
| *Boraginaceae* | *Cerinthe minor* L. | - | - |
| *Caryophyllaceae* | *Arenaria graminifolia* Schrad. | - | - |
| | *Cerastium lanatum* Lam. | - | - |
| | *Gypsophila repens* L. | - | - |
| | *Stellaria holostea* L. | 0.01-0.05 e | 18-20 |
| *Cistaceae* | *Helianthemum nummularium* (L.) Mill. | - | - |
| *Dipsaceae* | *Scabiosa lucida* Vill. | - | - |
| *Fabaceae* | *Lathyrus heterophyllus* L. | 0.05-0.20 e,f | 17-20 |
| *Graminae* | *Poa supina* Schrad. | 0.05-0.20 e,f | 14,15,18,19 |
| *Juncaceae* | *Juncus trifidus* L. | - | - |
| *Lamiaceae* | *Prunella grandiflora* (L.) Scholler | - | - rl |
| *Polygonaceae* | *Polygonum viviparum* L. | - | - |
| *Rosaceae* | *Dryas octopetala* L. | 0.01-0.05 e | 17,18 |
| | *Filipendula vulgaris* Moench | 0.01-0.05 e | 22-30 |
| | *Fragaria viridis* Duchesne | - | - |
| | *Potentilla aurea* L. | 0.05-0.20 e | 18,19-30 |
| *Salicaceae* | *Salix alpina* Scop. | - | - |

e, esterified alcohols
f, free alcohols
rl, rubber –like substance, not migrating on RP-18 plate in acetone

## STRESZCZENIE

W badaniach nad występowaniem długołańcuchowych poliprenoli u ponad stu gatunków roślin rzadkich i zagrożonych występujących w Polsce nie stwierdzono przypadków szczególnie intensywnego nagromadzania tych substancji w liściach. Jedynie u kilku z nich wykazano obecność poliprenoli zbudowanych z 10, 11 i 12 reszt izoprenowych. U niektórych gatunków z rodziny *Graminae* (np. *Melica ciliata*) stwierdzono nagromadzanie w liściach mieszanin poliprenoli zbudowanych z 14, 15 i 16 reszt izoprenowych. Poliprenole te występują w formie estrów karboksylowych. Ich ilość może dochodzić do 2% suchej masy liścia.Porównanie grupy roślin rzadkich i zagrożonych z grupą gatunków towarzyszących z tych samych siedlisk pod względem występowania długołańcuchowych poliprenoli w liściach nie wykazało znaczących różnic między nimi.


## REFERENCES


**Chojnacki T., Swiezewska E & Vogtman T.** 1987. Polyprenols from plants - structural analogues of mammalian dolichols. *Chem.Scripta* 27, 209-214.

**Ibata K.,Kageyu A.,Takigawa T.,Okada M.,Nishida T.,Mizuno M.& Tanaka Y.** 1984. Polyprenols from conifers: multiplicity in chain length distribution. *Phytochemistry* 23, 2517-2521.

**Mirek Z.,Piekos-Mirek H.,Zajac A.& Zajac M.** 1995. Vascular Plants of Poland. A Checklist. Polish Botanical Studies; Guidebook Series. No.15, Ed.office: W.Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Krakow, Poland.

**Skoczylas E., Swiezewska E., Chojnacki T. & Tanaka Y.** 1994. Long-chain rubber-like polyisoprenoid alcohols in leaves of *Lumnitzera racemosa*. *Plant Physiol.Biochem.* 32, 825-829.

**Stone K.J., Wellburn A.R., Heming F.W. & Pennock J.** 1967. The characterization of ficaprenol-10,-11, and 12 from leaves of *Ficus elastica* (decorative rubber plant). *Biochem.J.* 102, 325-330.

**Swiezewska E., SasakW., Mankowski T., Jankowski W., Vogtman T., Krajewska I., Hertel J., Skoczylas E. & Chojnacki T.** (1994) The search for plant polyprenols. *Acta Biochim.Polon.* 41, 211-260.

**Wanke M., Chojnacki T. & Swiezewska E.** 1998. The diversity of polyprenol pattern in leaves of fruit trees belonging to *Rosaceae* and *Cornaceae*. *Acta Biochim.Polon.* 45, 811-818.

**Wellburn A.R. & Hemming F.W.** 1966. The occurrence and seasonal distribution of higher isoprenoid alcohols in the plant kingdom. *Phytochemistry* 5, 969-975.

**Zarzycki K.,Wojewoda W. & Heinrich Z.**, eds. 1992. List of threatened plants in Poland (2nd ed.) Polish Academy of Sciences, W. Szafer Institute of Botany, Cracow, pp. 87-98.