IDENTIFICATION OF BIOACTIVE COMPONENTS OF ESSENTIAL OILS IN *HERACLEUM SOSNOWSKYI* AND *HERACLEUM MANTEGAZZIANUM* (APIACEAE)

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Abstract – The contents and composition of essential oils obtained from *Heracleum sosnowskyi* and *Heracleum mantegazzianum* (Apiaceae) were examined. Essential oils were investigated by the GC/MS method. The composition of hogweeds' essential oils was examined in order to determine toxic compounds that could have a direct influence on the health of people and animals living in close proximity to both species. It was found that the essential oils, except for derivatives of coumarins, contain numerous toxic compounds, e.g. isobutyl isobutyrate, isoamyl butyrate, hexyl hexanoate, 1-hexadecanol etc. No significant differences were found in the chemical composition of the examined seed samples of *Heracleum*, which confirms the suggestions that the species can be closely related.

Key words: Heracleum sosnowskyi, Heracleum mantegazzianum, oil composition, GC-MS, Poland

INTRODUCTION

Distribution and biology

The genus *Heracleum* (Apiaceae) consists of ca. 60 species, which occur mostly in the temperate zone of Eurasia. In Poland, until the middle of the 20th century only one species, *Heracleum sphondylium,* grew in the wild. New species were introduced from the Caucasus to Europe for decorative purposes or as fodder plants. Sosnowski's hogweed *Heracleum sosnowskyi* Manden. and the giant hogweed *Heracleum mantegazzianum* Sommier et Levier are two species from the Pubescentia section and are now widely spread in Poland. Although there is genetic evidence that they are separate species (Jahodová et al., 2007), both of them look very alike, so their morphological features became questionable. Formerly considered as important, attributes such as plant size, leaf blade shape, stem color and fruit proportions do not seem to be very reliable and sometimes they even vary between individuals at one site. This has led to several misunderstandings, and as a result the state of invasive hogweeds in Poland remains to be examined. Only its distribution map is available, where two species are considered as one (Zając and Zając, 2001).

Both species are close genetic relatives, having almost identical biology. They are biennial or perennial plants, flowering and fruiting only once per lifetime (Vavilov et al., 1976) There are four stages of plant development, which are small, middle and large vegetative forms, and reproductive forms (Hüls et al., 2007). The flowering plant's size is significant. The leaf rosette, which consists of 15 leaves in depending on the stage of development, is usually 2-3 m wide. The shape of the leaf blades is described as a major morphological feature that is different in both spe-

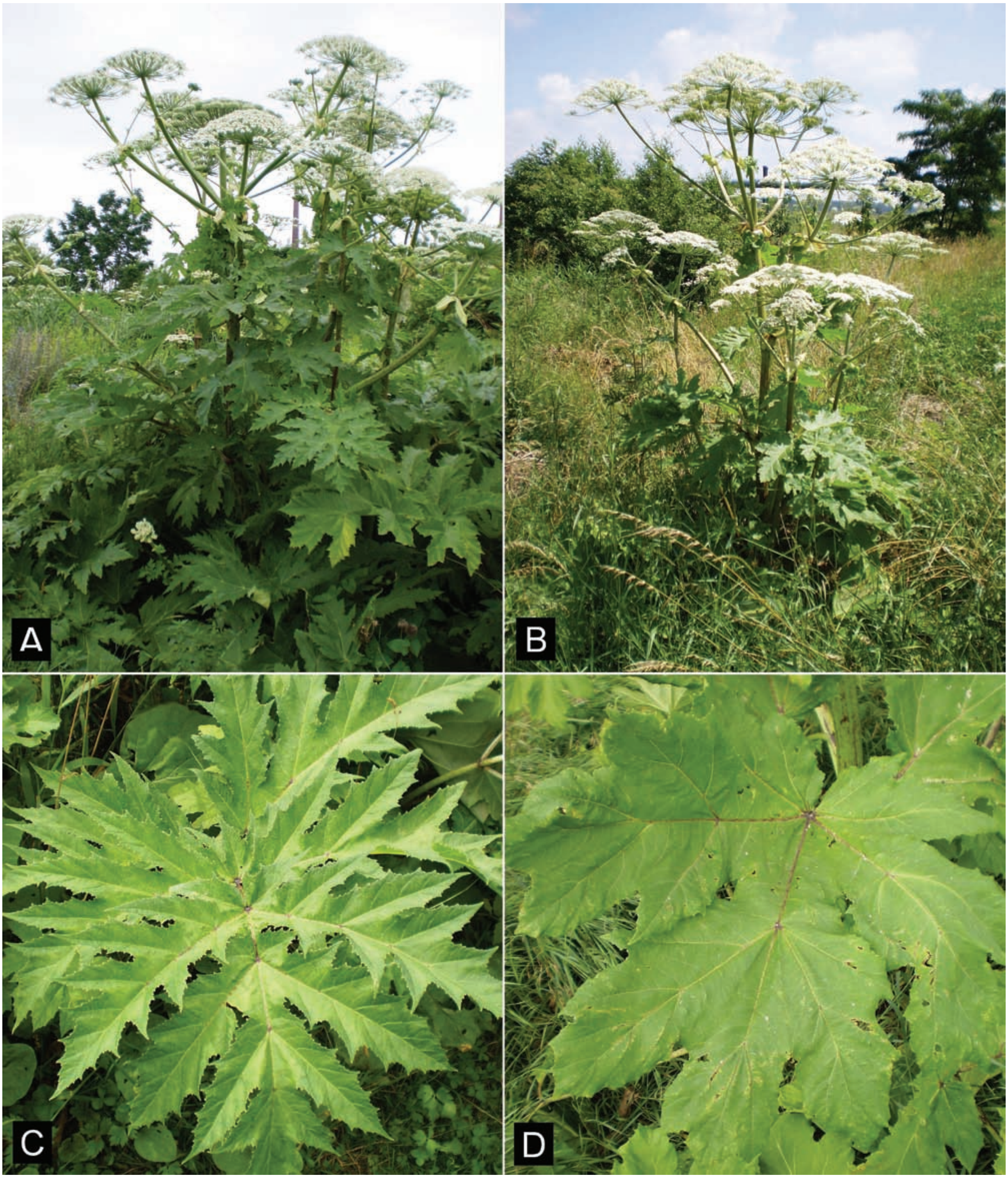

Figure 1. Plant profile and the shape of leaf blade; A, C – *Heracleum mantegazzianum*, B, D – *H. sosnowskyi*

cies (Fig. 1). The inflorescence-bearing stem is 3-5 m tall, depending on habitat conditions and species, but generally *Heracleum sosnowskyi* is considered to grow somewhat smaller. The reproduction cycle of hogweeds comes only in a generative way. Its flowers attract many pollinators from different groups, such as Coleoptera, Diptera, Hemiptera and Hymenoptera (Grace and Nelson, 1981). Self-pollination was also observed, which means that a single plant has a potential to build a large and dense population. If a plant is mown or damaged before bearing fruit it will regenerate intensively many times, even after the closing of its lifecycle. Under natural conditions, it dies after fructification (Tschiedel, 2005). The fruit of hogweeds consist of two mericarps, connected by a carpophore (Holub, 1997), which separate when ripened. Each of the mericarps contains one seed. The ripe fruit possesses a strong scent, which can be maintained over 10 years (Ćwikliński, 1973). An sverage-sized plant bears ca. 20,000 seeds, while 60-90% of them fall in a radius of 4 m from the parental plant. Among the seeds, 95% become concentrated in the upper (5 cm shallow) ground layer (Krinke et al., 2005). Research indicates that the seeds of *Heracleum sosnowskyi* are slightly more vigorous than the seeds of *H. mantegazzianum* (Moravcová et al., 2007).

Chemical compounds

Hogweeds produce a large amount of chemical compounds. The sap's main function is to protect from vertebrates, invertebrates, fungi, bacteria and viruses (Pyšek et al., 2007), being both repellent and toxic to them. It contains coumarin derivatives, which are responsible for its photodynamic effect. Contact with the sap leads to intense skin burning (Drever and Hunter, 1970). The skin reaction seems to increase with skin moisture (e.g. sweating) and warmth, but it is still dependant on the specimen's innate features. In most cases, the skin becomes reddened after 24 hours, and within 3 days shows the effects of intense burn. After a week, the skin darkens, and can remain like this for months, even years. However, it can be treated with topical steroids to minimize the effect (Mehta and Statham, 2007). Discolored spots can be oversensitive to ultraviolet light. Large doses of furanocoumarins present in the sap can cause cancer or fetus deformation (Nielsen et al., 2005). Sap extracts are also considered to have allelopathic features (Solymosi, 1994).

While the chemical defense systems of giant hogweed have been described (Hattendorf et al., 2007), only some information about Sosnowski's hogweed chemical components is available, mainly because European research has concentrated on the previous species. Some data about the essential oils' composition changing during the length of a season were published in 1963 (Kostecka-Mądalska and Bańkowski, 1963). From the beginning of plant history in Europe, it was believed that the most hazardous components of hogweeds are various coumarins, which are synthesized in abundance by both species. Coumarins, described as angelicin, bergapten, imperatorin, isobergapten, isopimpinellin, marmezin, osthole, (+)-oxypeucedanin, pangelin, pimpinellin, psoralen, sphondin, umbelliferone and methoxalen were mentioned even in the early research (Abyshev and Denisenko, 1973, Malikov and Saidkhodzahaev, 2004). It is also supposed, that a correlation exists between the furanocoumarin chemistry and habitat conditions (Berenbaum, 1981). To date, 17 different furanocoumarins have been isolated from various organs of the plant (Hattendorf et al., 2007). However, there was little interest in other, health-affecting components of invasive hogweed essential oils.

Data indicate that the invasive species of the genus *Heracleum* can be a serious threat to people living next to them due to their toxicity. Taking into account the expansive character of these taxa, one can expect the number of their populations in Europe and the health hazard for the people it involves to increase. It is therefore legitimate to determine the chemical composition of essential oils as well as their toxicity. Furthermore, of interest is whether the chemical composition of the essential oils in both studied species is similar or not. It is noteworthy as there are still controversies among taxonomists over the taxonomic status (the taxonomic position) of the two taxa.

MATERIALS AND METHODS

The seeds of both species were gathered in south-western Poland in July 2012. Seeds of *Heracleum mantegazzianum* were collected in Karpacz, from allotment specimens. *Heracleum sosnowskyi* seeds were collected in Siechnice, at a place of its experimental cultivation. In both cases, the sources of the seeds were primary (terminal) umbels.

The essential oils were collected from the seeds by using dichloromethane (GC Grade, Merck) and *n*-pentane (GC Grade, Merck) separately as eluents into glass vials.

One seed sample weighing 25.0 g was extracted with 50 ml of *n*-pentane in an airtight glass container with a teflon gasket. Another 25.0 g seed sample was extracted with 50 ml of dichloromethane. Both plants were treated in the same way. The samples were extracted for 15 min in an ultrasonic bath and then put aside for 24 h. Then both extracts were concentrated using a rotary vacuum evaporator to 1 ml volume.

The samples were analysed using a Perkin Elmer Gold TurboMass mass spectrometer with electron impact ionization EI and Perkin Elmer Autosysytem XL gas chromatograph equipped with an autosampler. The injector temperature was 320°C. A Elite 5MS column (5% phenyl polysiloxane) was used for the analyses (30 m long, inner diameter 0.25 mm, film thickness 0.25 μm, Perkin Elmer). The carrier gas was helium at a flow rate of 0.8 ml/min, maintained by electronic pneumatic control. The GC oven temperature was held for 5 min at 40°C, then increased by 12°C/min to 300°C and held for 5 min. The temperature of MS transfer line was 250°C and the ion source worked at 180°C. The mass spectra are taken at electron energy 70 eV, with a scanning speed of 1 scans per 0.1 s from m/z 20 to 400. The GC-MS data were processed using the TurboMass Software v.5.4.2. Component identification was carried out using the NIST 2.1.0, NBS and WILEY mass spectral database. Each analysis was performed in triplicate to assess the reproducibility of results.

RESULTS AND DISCUSSION

The major groups of compounds that appeared in the studied seed extracts of *H. mantegazzianum* and *H. sosnowskyi* were coumarins, furanocoumarins, hydrocarbons, alcohols, esters and aldehydes (Table 1). Examination of the extracts with *n*-pentane and dichloromethane using the GC-MS method did not reveal significant differences in the composition of the seed oil of *H. mantegazzianum* and *H. sosnowskyi*. Figs. 2 and 3 show chromatograms in dichloromethane for *H. mantegazzianum* and *H. sosnowskyi* respectively. The only difference that was observed in the chromatograms was the intensity of signals. The higher signal intensity was observed in the analysis of oil extract of *H. sosnowskyi*. This could indicate a higher content of the studied compounds in the seeds of this plant.

Both hogweed species belong to Apiaceae, one of several plant families known to cause phytophotodermatitis. In most cases, the visual effects are linear, inflammation, erythematous lesions or macular, streaky hyperpigmentations (Mehta and Statham, 2007). These usually result from contact with plants stem and leaves, but allergic people can receive burns at a distance of up to 20 m from the plants. Some authors suggest that the active features of the sap of *Heracleum sosnowskyi* fruit are connected with the presence of esters, rather than furanocoumarins (Sefidkon et al., 2004, Burgieł et al., 2008). The conducted investigations seem to confirm this as apart from coumarin derivatives, a large number of esters were also found in the essential oils of the plant.

The third species from the Pubescentia section is *Heracleum persicum*. Its major constituents were hexyl butyrate (22.5% and 35.5%), octyl acetate (19% and 27%) and hexyl isobutyrate (9.1% and 3.2%) in ripe and unripe seeds, respectively. The oil of *H. persicum* fruits contained about 95% of aliphatic esters. In addition, the isolation and identification of flavonoids and furanocoumarins have also been reported (Sefidkon et al., 2004). The analysis of the chemical composition of *Heracleum sosnowskyi* and *H. mantegazzianum* showed an approximate set of chemical

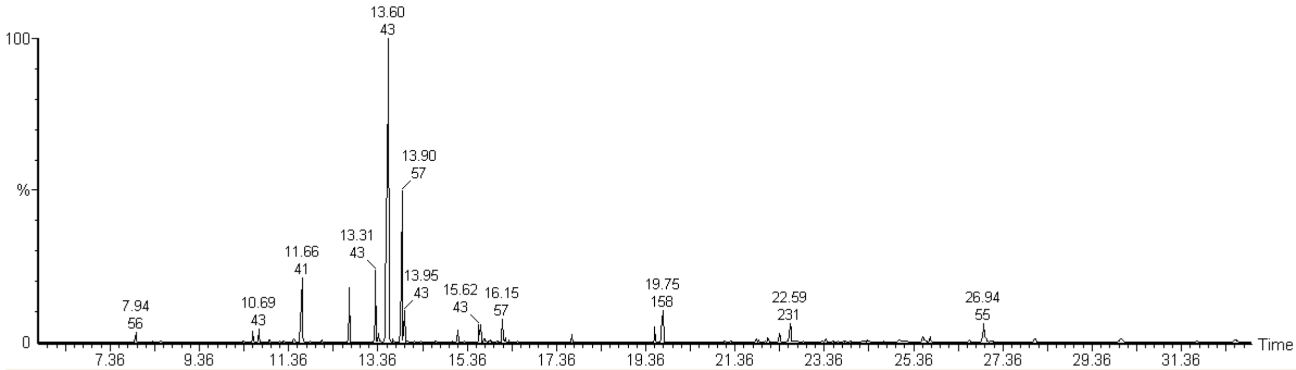

Fig. 2. Total Ion Current (TIC) chromatograms in dichloromethane for *Heracleum mantegazzianum* essential oils

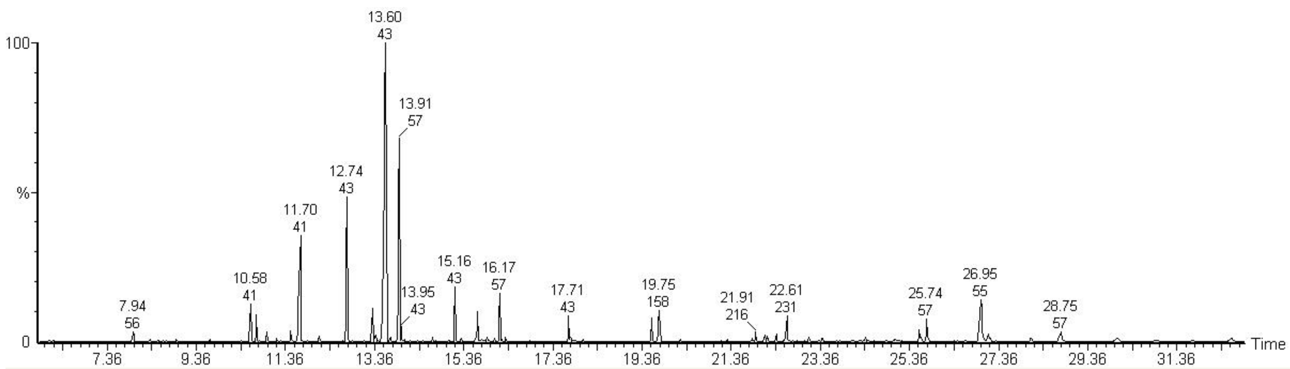

Fig. 3. Total Ion Current (TIC) chromatograms in dichloromethane for *Heracleum sosnowskyi* essential oils

substances in essentials oils of both species, which proves their close relation in terms of chemotaxonomy.

Malikov and Saidkhodzhaev (2004) described coumarins common to both hogweed species, these being angelicin, bergapten, isobergapten, isopimpinellin, methoxalen, osthole, pimpinellin, sphondin and umbelliferone. The coumarins, occurring only in *H. mantegazzianum* were imperatorin and psoralen, while in *H. sosnowskyi* they were isoimperatorin, marmesin, (+)-oxypeucedanin and pangelin. Berenbaum, however, has detected isoimperatorin also in the seeds of *H. mantegazzianum*, stating that angelicin and methoxalen occur only in its leaves (Berenbaum, 1981). Our research confirmed the presence of pimpinellin, isopimpinellin, psoralen (in both Hogweed species), bergapten and methoxalen (in seeds), but strangely, no other coumarins were identified (Table 1). Various lists of furanocoumarins and their placement in different organs may be proof that habitat conditions play a more significant role in the composition of coumarins than expected. It may also emphasize the variability of the genus *Heracleum*. Furanocoumarins are typical secondary substances in that they are restricted in distribution among plants. Reported in eight families, they occur with diversity and regularity only in the Apiaceae and Rutaceae (Berenbaum, 1981). Probably the most interesting component is psoralen. Its photosensitive properties are so strong; Mehta and Statham (2007) compared its effects to non-accidental injury and self-harm. Psoralen is used in medicine as a photosensitizer, especially in psoriasis treatment, although it is interesting that Autier found that psoralen acts as carcinogenic ausative agent in melanoma (Autier et al., 1997).

Table 1. Quality composition of essential oil from seeds of *Heracleum sosnowskyi* and *Heracleum mantegazzianum*.

Compounds present in seeds of both species	
Caprylaldehyde	$C_8H_{16}O$
Hexyl acetate	$C_8H_{16}O_2$
Isovaleric acid, isopropyl ester	$C_8H_{16}O_2$
[1–3] Isobutyl isobutyrate	$C_8H_{16}O_2$
[1, 2] 1-Octanol	$C_8H_{18}O$
[1–3] 1-Nonyne	C_9H_{16}
[1–3] Isoamyl butyrate	$C_9H_{18}O_2$
[1–3] Butyl 2-methylobutanoate	$C_9H_{18}O_2$
[1–3] Gamma-terpinene	$C_{10}H_{16}$
2,6-Octadiene, 2,7-dimethyl-	$C_{10}H_{18}$
[1–3] 2-Decen-1-ol	$C_{10}H_{20}O$
Butanoic acid, hexyl ester	$C_{10}H_{20}O_2$
Hexyl isobutyrate	$C_{10}H_{20}O_2$
Isobergapten	$C_{11}H_6O_3$
Psoralen	$C_{11}H_6O_3$
Hexyl 2-methylobutanoate	$C_{11}H_{22}O_2$
[1, 2] Hexyl isovalerianate	$C_{11}H_{22}O_2$
Propanoic acid, octyl ester	$C_{11}H_{22}O_2$
[2] Bergapten	$C_{12}H_8O_4$
[2, 4, 5] Methoxsalen	$C_{12}H_8O_4$
[1–3] Hexyl hexanoate	$C_{12}H_{24}O_2$
Octyl isobutyrate	$C_{12}H_{24}O_2$
[2, 3] Isopimpinellin	$C_{13}H_{10}O_5$
[2–4] Pimpinellin	$C_{13}H_{10}O_5$
Acetic acid, dodecyl ester	$C_{14}H_{28}O_2$
[2] 1-Tetradecanol	$C_{14}H_{30}O$
1-Decanol, 10-tetrahydro-2H-Pyran-2-yl	$C_{15}H_{30}O_3$
Octanoic acid, octyl ester	$C_{16}H_{32}O_2$
[1–3] 1-Hexadecanol	$C_{16}H_{34}O$
1,1-Bis(octyloxy)octane	$C_{24}H_{50}O_2$

Compounds present only in seeds of *Heracleum mantegazzianum*	
[3, 4] 4-Hexen-1-ol, acetate	$C_8H_{14}O_2$
Hexyl 3-methyl-2-butenoate	$C_{11}H_{20}O_2$
Octyl butyrate	$C_{12}H_{24}O_2$
Octyl valerate	$C_{13}H_{26}O_2$
Octadecanoic acid	$C_{18}H_{36}O_2$
1-Tetracosanol	$C_{24}H_{50}O$
Compounds present only in seeds of *Heracleum sosnowskyi*	
Acetic acid, octyl ester	$C_{10}H_{20}O_2$
Butanoic acid, 3-Methyl-, hexyl ester	$C_{11}H_{22}O_2$
1,11-Dodecadiene	$C_{12}H_{22}$

Hazardous components are marked gray; Type of hazard: [1] irritation to eyes, [2] irritation to skin. [3] irritation to respiratory system. [4] poisonous, harmful if swallowed, [5] genetic damage, may cause cancer

The compounds contained in both hogweed species' essential oils may pose a risk to the eyes, cause skin and respiratory system irritation, dizziness, breathing difficulties and nausea (Table 1). It is possible that the observed effect of their activity is a synergetic, i.e. the result of the combined activity of the two main groups of toxic compounds.

The obtained results confirm that the studied species are very closely related. Based on the results it cannot, however, be determined whether *H. sosnowskyi* and *H. mantegazzianum* are separate species or merely subspecies or forms. In addition, it cannot be decided whether the morphological differences between them are an indication of species divergence or merely the effect of phenotypic plasticity. The chemotaxonomic analysis is not as univocal here as the genetic analysis (Jahodová et al., 2007).

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