REVIEW ARTICLE

# Phytochemical, pharmacological and clinical studies of *Petasites hybridus* (L.) P. Gaertn., B. Mey. & Scherb. A review

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S u m m a r y

Preparations from rhizomes of *Petasites hybridus* (L.) Gaertn., B. Mey. & Scherb. (common butterbur) have a long history of use in folk medicine in treatment of several diseases as anti-inflammatory and spasmolytic drugs. Extracts from this species are of interest to researchers in the field of phytopharmacology due to their biologically active compounds, particularly two eremophilane sesquiterpenes (petasin and isopetasin), which are contained not only in rhizomes and roots, but also in leaves. Moreover, *P. hybridus*

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contains pyrrolizidine alkaloids, which showed hepatotoxic, carcinogenic and mutagenic properties. Hence, special extracts devoid of alkaloids obtained by sub- and super-critic carbon dioxide extraction were used in the preclinical, clinical studies and phytotherapy. Our review aims to provide a literature survey of pharmacological as well as clinical trials of *P. hybridus*, carried out in 2000–2013. Also several studies of other species used in non-European countries have been included. Besides, the botanical description of *Petasites* genus and phytochemical characteristic of *P. hybridus* and toxicological studies of pyrrolizidine alkaloids as well as chemical profile of patented commercial extracts from rhizomes, roots and leaves of this species used in European phytotherapy have been performed. In this review, attention has also been paid to the promising and potential application of special extracts of *P. hybridus* not only in the prevention of migraine, treatment of allergic rhinitis symptoms, asthma and hypertension, but also in prevention and slowing the progression of neurodegenerative diseases developing with the inflammatory process in the CNS as a new therapeutic strategy. In fact, there is already an evidence of promising properties of *P. hybridus* extracts and sesquiterpens – decrease in the prostaglandins and leukotrienes release, inhibition of COX-1 and COX-2 activity, as well as antagonism of L-type voltage-gated calcium channels. In order to explain the new mechanisms of action of *P. hybridus* extracts in the CNS and their future application in phytotherapy of diseases with neuroinflammatory process, further studies should be performed.

**Key words:** *butterbur, extracts, sesquiterpenes, pyrrolizidine alkaloids, pharmacological studies, clinical trials, migraine, allergic rhinitis, safety, toxicology, phytochemistry*

## BOTANICAL DESCRIPTION OF *PETASITES* GENUS

A taxonomic survey of *Petasites* Mill. (*Asteraceae*) shows 18 species of this genus [1]. In addition, a new species – *P. anapetrovianus* Kit Tan, Ziel., Vladimirov & Stevanović was recently described in Greece [2]. Butterbur taxa have a broad distribution in the Northern hemisphere, from North America through Europe, North Africa to Eastern Asia. In Europe, there eight native species are growing and two (*P. japonicus* and *P. fragrans*) have been introduced [1]. In Poland, four *Petasites* taxa occur; they occupy wetland habitats: banks of rivers, streams, springfens as well as moist forests [3, 4]. The most common species in Poland is *P. hybridus* (L.) P. Gaertn., B. Mey. & Scherb. (= *P. officinalis* Moench), which is widespread from Pomerania to upper subalpine zone of Sudetes and Carpathians. Further species in the incidence is *P. albus* (L.) Gaertn. which has been found in numerous mountain localities and less – from Silesia, Lesser Poland and Lublin Upland as well as Pomerania. Interesting distribution pattern is observed in *P. spurius* (Retz.) Rchb. This species grows mainly on sandy banks of large rivers (the Vistula, the Bug River, the Oder, the Warta) and the Baltic Sea. The last native taxon of butterbur is *P. kablikianus* Tausch ex Bercht., which occurs frequently in Carpathians and probably in only one stand in the Polish side

of Giant Mts. [3-8].

Species belonging to *Petasites* genus are perennial herbs with thick rhizomes and usually large leaves developing after flowering. Flowers are collected in heads and they form panicle-like or racemose secondary inflorescences. Butterbur taxa differ in flower color, size, shape and hairiness of leaves, ribbing of petioles, size and shape of scale leaves on the flowering stems, etc. [1, 3, 4, 9]. It is a taxonomically hard group of plants due to the conservative floral structure and the high variability of leaves. In addition, these species form hybrids. For this reason, there are various classifications of *Petasites* genus, and for example in North America one to ten species are distinguished [10]. Butterbur is sometimes confused with coltsfoot. Also, there is a contamination of *Tussilago farfara* raw material. However, both taxa are relatively easy to distinguish, and they differ in flowers as well as in size and shape of leaves. There is also significant anatomical difference: in the coltsfoot, vascular bundles in leaf petioles are arranged semicircular, while for butterbur – evenly over entire area of cross-section [11].

The main medicinal plant in *Petasites* genus used in European phytotherapy is *P. hybridus* [5, 12-19]. Most phytochemical and pharmacological studies are based on this species. In Far East (China, Japan and Korea), much attention is paid to the three other species: *P. japonicus* [21-26], *P. formosanus* [27- 30] and *P. tetawakianus* [31] because some compounds showed potent neuroprotective effects in various *in vitro* models [21, 23-26, 31].

## ACTIVE COMPOUNDS OF *PETASITES HYBRIDUS*

### Sesquiterpenes

Main biologically active compounds present in rhizomes and leaves of *P. hybridus* were classified as sesquiterpene esters of petasin and furanopetasin (tab. 1) [32-37]. Novotny et al. [32] were the first to assume that exist two chemovars of this species (petasin and furanopetasin chemotype). Currently, only petasin chemotype with main compound petasin is considered as pharmaceutically useful [35]. There were isolated more than 20 sesquiterpene esters of eremophilane type [38, 39] and six furanoeremophilanes [33]. The sesquiterpene (petasine chemotype) content depends on plant parts (tab. 1, 2), time of harvest, and geographical origin of specimens [39, 40], whereas furanoeremophilanes were observed only in rhizomes [28]. The results of HPLC analysis showed that among others, petasin, isopetasin, neopetasin, S-petasin, iso-S-petasin, neo-S-petasin are main components in rhizomes of petasine chemotype of common butterbur [39], while furanopetasin is a main compound in second chemotype of this species [33]. Wildi et al. [34] determined that the mean petasin content of various populations of *P. hybridus* from Switzerland ranged from 7.4 to 15.3 mg/g dry weight in rhizomes and from 3.3 to 11.4 mg/g dry weight in leaves.

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Chemical constituents in rhizomes and roots of *Petasites hybridus*

| Group | Compounds | Bibliography |
|---|---|---|
| **Furanopetasin chemotype** | | |
| sesquiterpenes | 9-hydroxy-furanoeremophilane (30-37%), furanopetasin (16-21%), 2-methyl-thioucryloyl-furanopetasol (6-10%), furanoeremophilane, 2-senecioyl-furanopetasol, 2-tigloyl-furanopetasol | 19, 21, 22, 23, 33, 36, 37 |
| **Petasin chemotype** | | |
| petasol derivatives | petasin (9.37 mg/g for dried rhizomes, 7.0 mg/g for roots), S-petasin, 3-methyl-crotonyl-petasol, methacryloyl-petasol, (E)-3-(methylthio)-acryloyl-petasol, 3-desoxy-13-(angeloyloxy)-petasol | 19, 38, 40, 46, 47 |
| iso-petasol derivatives | iso-petasin, iso-S-petasin, 3-desoxy-isopetazol, 3-desoxy-13-(angeloyloxy)-isopetasol, isobutyryl-isopetasol, 3-methyl-crotonyl-isopetasol, methacryloyl-isopetasol, (E)-3-(methylthio)-acryloyl-isopetasol | 19, 38, 39, 40 |
| neo-petasol derivatives | neo-petasin (2.3 mg/g for dried rhizomes, 2.46 mg/g for roots), neo-S-petasin, 3-desoxy-neoptasol 3-desoxy-13-(angeloyloxy)-neopetasol, 3-methyl-crotonyl-neopetasol, isobutyryl-neopetasol, (E)-3-(methylthio)-acryloyl-neopetasol | 19, 38, 39, 46, 47 |
| benzofuran derivative | 1-(6-hydroxy-2-isopropenyl-1-benzofuran-5-yl)-1-ethanone | 48 |
| **Furanopetasin an petasin chemotypes** | | |
| pyrrolizidine alkaloids | senecionine, integerrimine, seneciphylline, senkirkine, 7-angelolretronecine, 9-angelolretronecine | tussilagine, senecionine-N-oxide, integerrimine-N-oxide, 7-angelolretronecine-N-oxide, 9-angelolretronecine-N-oxide | 19, 34, 37, 46, 47 |

| Group | Compounds | | Bibliography |
|---|---|---|---|
| essential oil | α-alaskene,<br>albene,<br>4,5-di-*epi*-aristolochene,<br>*cis*-α-bergamotene,<br>β-bisabolene,<br>*Z*-γ-bisabolene,<br>*trans*-α-bisabolene,<br>(-)-eremophilene,<br>furanoeremophilane,<br>fukinanolide<br>α-humulene,<br>γ-humulene,<br>(-)-α-isocomene | β-isocomene,<br>α-longipinene,<br>1-nonene,<br>modhephene,<br>petasitene,<br>pethybrene,<br>α-santalene,<br>*epi*-β-santalene,<br>silphinene,<br>silphiperfol-7-ene,<br>α*H*-silphiperfola-5-ene,<br>1-undecene, | 44 |

T a b l e   2 .

Chemical constituents in leaves of *Petasites hybridus*

| Group | Compounds | Bibliography |
|---|---|---|
| furanopetasin chemotype | 9-oxo-furanoeremophilane<br>9-oxo-furanopetasin | 33 |
| petasin chemotype | petasin (2.77 mg/g of dried plant material),<br>neopetasin (1.88 mg/g),<br>isopetasin,<br>S-petasin,<br>iso-S-petasin,<br>neo-S-petasin | 34, 40 |
| flavonoids | isoquercitrin, astragalin, quercetin | 45, 47, 49 |
| pyrrolizidine alkaloids | seneciphylline, seneciphylline-N-oxide,<br>senecionine, integerrimine, senkirkine, | 47, 49 |
| others | volatile oil (26 compounds),<br>tannins,<br>mucilage,<br>triterpenoid saponins | 47, 49 |

## Pyrrolizidine alkaloids

The presence of different pyrrolizidine alkaloids (PA) was reported in various species of *Petasites* genus [34, 37]. These alkaloids contain two five-membered rings with one shared nitrogen atom at position 4. This amino alcohol (necine) base is being esterified with one or more necic acids to form monoesters or diesters (non-macrocyclic or macrocyclic) at the C7,9 hydroxyls [19, 41]. Four different types of necine base can be found in PAs: heliotridine- and retronecine-types (enantiomers at C7), platynecine-type and otonecine-type [42]. In underground

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plant parts of *P. hybridus*, more than 10 of PAs were detected (tab. 1). The predominant pyrrolizidine alkaloids in common butterbur are senecionine and integerrimine, which account for approximately 90% of total PAs content [43]. The analysis of concentration showed varying levels of PAs in this species, higher in rhizomes (4.8–89.9 $\mu$g/g dry weight) than in leaves (0.02–1.50 $\mu$g/g dry weight) [34]. The level of PAs varies depending on the site of plant growth. Furthermore, the alkaloid content in different plant parts can change by means of reallocation as a respond to herbivory attack on a plant [42]. It was observed that alkaloid amount was independent from sesquiterpene chemotype and they are mainly concentrated in young, fast growing parts of rhizomes. Furthermore, distribution of alkaloids in *P. hybridus* is similar to *Tussilago farfara* [35].

## Other constituents

*P. hybridus* contains active compounds of essential oil [44] (tab. 1). Moreover, flavonoids (isoquercitrin, astragalin, quercetin) were also identified in leaves [45].

## BIOLOGICAL ACTIVITY OF EXTRACTS AND COMPOUNDS OF *PETASITES*

*Petasites* is a medicinal plant with a long history of use in respiratory [13], gastrointestinal and urogenital diseases [12], and it is also traditionally used for the treatment of hay fever and migraine [17]. In recent years there have been numerous studies concerning the mechanism of antinociceptive, anti-inflammatory activities and relaxant action of selected compounds contained in extract of *Petasites* species, such as petasin, S-petasin and iso-petasin, which are main sesquiterpenes occurred not only in rhizomes and aerial parts of *Petasites hybridus*, but also in *Petasites formosanus* [27, 29, 30] and *P. japonicus* [21].

## Impact on prostaglandins and cyclooxygenases in microglial cells

Inflammation of microglial cells serves as an important model for the investigation of potential therapeutic compounds to slow the progression of neuronal cell death in neurological disorders. In neuroinflammation, microglia becomes activated and release various cytotoxic mediators, e.i. prostaglandins E2 [50] and leukotrienes [51]. Moreover, prostaglandins might play a key role not only in the etiopathogenesis of neuroinflammatory and neurodegenerative diseases [52], but also may be one of important *final* products involved in the generation of migraine attacks [53-55]. Therefore, searching for new chemical compounds, which may inhibit prostaglandin biosynthesis pathways, can be an interesting pharmacological strategy for the prevention of neurological diseases. However, only one study for extract from common butterbur was so far carried out in this area.

Fiebich et al. [56] investigated the effects of lipophilic extracts from rhizomes of *P. hybridus*, petasin and iso-petasin on the formation and release of prostaglandin $E_2$ ($PGE_2$) and also the activity and expression of cyclooxygenases (COX-1 and COX-2) in rat primary microglial cells. Results showed that all extracts exhibited strong inhibitory effect on COX-2 but little inhibitory activity on COX-1 and pure petasin and iso-petasin did not inhibit these enzymes. Moreover, it was observed that extracts dose-dependently inhibited LPS-induced $PGE_2$ release in cell lines. The data suggest that special lipophilic extracts are selective inhibitors of COX-2-mediated $PGE_2$ release. This mechanism of action in microglia may be considered as anti-neuroinflammatory activity of extracts of *P. hybridus*, and can explain their effectiveness in the prevention and treatment of migraine. Additionally, it was shown that petasin and isopetasin are not key factors responsible for $PGE_2$ release in microglia [56]. Thus, it seems that therapeutic application of special extracts is better than using selective pure sesquiterpenes. Observations of this study may be related to the possibilities of forward-looking application of the butterbur extracts in prevention and treatment of diseases associated with chronic neuroinflammation (i.e. multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and migraine) [57-62].

## Impact on leukotriene synthesis in monocytes and granulocytes

Leukotrienes belong to a family of products of the 5-lipoxygenase pathway of arachidonic acid metabolism and they have a multitude of biologic actions and have been suggested as factors in numerous pathological processes [63].

Previous studies [64] showed that extracts from *P. hybridus* as well as isopetasin and oxopetasan esters inhibited peptido-leukotriene biosynthesis in isolated peritoneal macrophages. These results have shown that *P. hybridus* may be taken into consideration in the prevention of inflammation processes. Subsequent studies [65] have confirmed that common butterbur extract (Ze339) and its isolated active sesquiterpene ester (petasin) inhibited both leukotriene synthesis in eosinophils and neutrophils. Moreover, it was observed that Ze339 and petasin may block earlier signaling events initiated by G protein-coupled receptors in granulocytes. It was concluded that Ze339 was effective as a synthetic 5-lipoxygenase inhibitor zileuton [65]. On the basis of these results it can be mentioned that the application of extract of *P. hybriduscan* can become an effective antileukotriene herbal product in leukotriene-mediated inflammatory diseases (i.e. asthma, allergic rhinitis, atopic dermatitis, chronic obstructive pulmonary disease, rheumatoid arthritis, leukaemias, cardiovascular disease, cerebrovascular disease and Alzheimer's disease) [63, 66-69]. Although, it seems that more biological and pharmacological studies are needed in order to explain the mechanism of action of *P. hybridus* extract and its clinical efficacy in anti-leukotriene therapy.

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Recently, only one study [28] was performed in order to investigate inhibitory effects of S-petasin (from *P. formosanus*) on phosphodiesterase (PDE) 1–5, and on ovalbumin-induced airway hyperresponsiveness in a murine model of allergic asthma. It was demonstrated that *S*-petasin inhibited activities of two cAMP-phosphodiesterases (PDE3 and PDE4) with 50% inhibitory concentrations ($IC_{50}$) of 25.5, and 17.5 $\mu$M, respectively. Moreover, *S*-petasin suppressed lymphocytes, neutrophils, eosinophils, and levels of cytokines, including interleukin (IL)-2, IL-4 and IL-5, tumor necrosis factor (TNF)-$\alpha$ and interferon (IFN)-$\gamma$ in bronchoalveolar lavage fluid of mice. Results of this study may partially explain mechanism of action of S-petasin in asthma.

## Impact on calcium channels

According to IUPHAR [70], there are several members of calcium channel family, mainly voltage-gated calcium channels (VGCCs: L and T type), calcium-activated potassium channels, CatSper and two-pore channels. In general, VGCCs mediate calcium influx in response to membrane depolarization and regulate intracellular processes such as contraction, secretion, neurotransmission, and gene expression in many different cell types and development as well as cell survival and death [71, 72]. Their dysfunction may lead i.e. to epilepsy, hypertension, migraine, inflammatory and neuropathic pain [73-75].

Wu et al. [20] showed that S-petasin (from *P. formosanus*) can interact directly with L-type $Ca^{2+}$ channels. Moreover, this compound had a little effect on voltage-dependent $Na^+$ current. In other study [76], it was investigated whether *P. hybridus* extract (free of pyrrolizidine alkaloids), petasin, neopetasin, isopetasin, S-petasin, and iso-S-petasin (50 $\mu$M) comprise the main constituents of the extract that inhibit currents through presynaptic VGCCs (expressed in *Xenopus laevis* oocytes). In this study it was demonstrated that S-petasin, iso-S-petasin are Ca(v)2.1 channel inhibitors, preferentially acting as use-dependent channel blockers. The Ca(v)2.1-inhibitory properties of these petasins may contribute to migraine-prophylactic properties described for extracts of *P. hybridus*.

Recently, Wang et al. [77] examined the cytosolic $Ca^{2+}$ regulatory mechanism involved in the vasorelaxation induced by petasin (from *P. formosanus*) at a concentration 0.01–100 $\mu$mol/l. In this study, cultured vascular smooth muscle cells, aortic rings from Sprague-Dawley rats, spontaneously hypertensive rats and normotensive Wistar-Kyoto rats were used. The results showed that petasin attenuated the $Ca^{2+}$-induced a contraction in aortic rings in a concentration-dependent manner, and inhibited VGCC activity in cultured cells. Observations exerted that direct $Ca^{2+}$ antagonism of L-type VDCC in vascular smooth muscle may account, at least in part, for petasin-induced vasorelaxation and petasin may have hypotensive effect and therapeutic potential in treatment of hypertension.

Other studies [27, 78] were performed in the rat heart *in vivo* and *in vitro* in order to explain effect of S-petasin (from *P. formosanus*). Experiments showed

that S-petasin inhibited the L-type $Ca^{2+}$ current concentration-dependently, and induced negative chronotropic and inotropic effects, although did not block properties of dihydropyridine binding sites of L-type $Ca^{2+}$ channel and did not produce the activation of the muscarinic receptor.

Previous *in vitro* and *in vivo* studies have shown that not only petasin and S-petasin, but also iso-S-petasin attenuated $Ca^{2+}$-induced vasoconstriction in a concentration-dependent manner in isolated rat thoracic aorta and directly inhibit the L-type voltage-dependent $Ca^{2+}$ channel activity. The results of these studies showed that iso-S-petasin also may exert hypotensive action [79]. Similar results were obtained by Esberg et al. [30] who observed that iso-S-petasin showed direct depressant action on ventricular contraction. Apart from petasins, also eremophilanlactones (from roots of *P. hybridus*) were investigated in isolated aortas and mesenteric arteries (from Sprague-Dawley rats) [80]. This study showed that eremophilanlactones had a vasodilatory effects.

## TOXICITY OF PYRROLIZIDINE ALKALOIDS IN *PETASITES*

A toxicologically important group of compounds in extracts of *Petasites* species are pyrrolizidine alkaloids (PAs) and their N-oxide derivatives. PAs are secondary metabolites in the plants providing the defence mechanism against herbivores, and they are known for their hepatotoxic as well as cardiotoxic, pneumotoxic and nephrotoxic properties. These compounds have been shown to cause cancer in animals, and they are potentially mutagenic and carcinogenic in humans [19, 42].

### Mechanism of action

The toxicity of PAs is determined primarily by the presence of 1,2-unsaturated bond, therefore platynecine-type PAs with saturated necine base are considered nontoxic. The ester part also influences PA toxicity; macrocyclic esters are the most toxic and monoesters are the least toxic [42]. Pyrrolizidine alkaloids require metabolic activation to exert their toxic effects. The biotransformation of Pas, mainly by liver cytochrome P-450, concerns the reactions of hydroxylation or oxidative N-demethylation followed by dehydratation, depending on the type of necine base, and produces highly electrophilic pyrrolic esters which can bind to vital cell constituents, e.g. DNA and proteins, resulting in liver damage [19, 42]. Due to the high reactivity, most PAs metabolites react in the site of their formation, but they can also migrate to adjacent sinusoids and cause veno-occlusive disease as a result of endothelial cell injury and haematological disturbances, or even further to lungs and heart which may result in the damage of these organs [42, 81].

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## General toxicity

Acute toxicity study of special *P. hybridus* extract in Wistar rats revealed oral $LD_{50}$ value of $\geq 2500$ mg/kg body weight and intraperitoneal $LD_{50}$ value of approximately 1000 mg/kg body weight. These doses are 833–1250-fold and 333–500-fold higher than the recommended human doses of common butterbur extract, respectively [82].

In humans, pyrrolizidine alkaloid poisoning may be described as acute, subacute and chronic. Typical manifestations of hepatotoxicity in acute PA poisoning are: haemorrhagic necrosis, hepatomegaly and ascites, in sub-acute: blockade of hepatic veins resulting in veno-occlusive disease and in chronic poisoning liver failure due to necrosis, fibrosis and cirrhosis [19]. Males are more susceptible to PAs induced toxicity than females, children are the most vulnerable [19, 81].

The hepatic lesions caused by veno-occlusive disease leading to death were reported in an infant, whose mother had been consuming large amounts of PAs containing herbal tea during pregnancy [43].

## Mutagenicity/genotoxicity/carcinogenicity

The mutagenicity of PAs is related to their primary metabolic activation and formation of pyrrolic esters. In laboratory tests, senecionine and seneciphylline caused primary DNA damages such as DNA adducts formation, DNA cross-linking and unscheduled DNA synthesis, but there were negative in DNA strand breakage tests. Integerrimine caused chromosome damages in micronucleus assay (*in vivo* polychromatic erythrocytes of mouse bone marrow test) and induced chromosomal aberration in mouse bone marrow. Seneciphylline and senkirkine induced sister chromatid exchange in *in vitro* V79 cells with primary chick embryo hepatocyte activation. In bacterial assays, senecionine and seneciphylline were negative or only weakly positive in *Salmonella typhimurium* TA100 test with S9 metabolic activation, while in *Drosophila* assays (wing spot test and sex-linked recessive lethal test) senecionine, seneciphylline and senkirkine were positive [81]. Moreover, the carcinogenicity of *Petasites japonicus* were investigated by Hirono et al. [83] in rats fed with a diet containing young flower stalks. Hemangioendothelial sarcoma of the liver, hepatocellular adenoma and carcinoma were reported after 480 days, while no tumors were observed in the livers of control animals.

## Legal regulations

The content of undesirable toxic compounds (pyrrolizidine alkaloids) in butterbur extracts restricts their medicinal use. Based on human toxicity studies, WHO [17] estimated the dose of PAs of 10 $\mu$g/kg body weight as potentially toxic

and leading to the development of veno-occlusive disease [42]. After a risk assessment, German Federal Health Bureau restricted oral exposure to pyrrolizidine alkaloids or their N-oxides in herbal preparations to $1.0\,\mu g$/day with a limit of use of six weeks per year or to $0.1\,\mu g$/day with no further restrictions of intake [19, 37].

## CLINICAL TRIALS OF SPECIAL EXTRACTS

Over last 10 years, results of 20 clinical trials performed to the assess efficacy and safety of standardized extracts from rhizomes (Petadolex®, Petaforce®) and leaves of *P. hybridus* (Tesalin®) were published: in patients suffering from migraine (6 studies), with allergic rhinitis (10, including skin prick tests), allergic skin disease (1) mild or moderate asthma (2), and somatoform disorders (1) (tab. 3). Most of these studies were randomized, double-blind, placebo-controlled trials, but few had also other design (open postmarketing surveillance study; short-term, pharmaco-clinical trial; prospective, non-randomized, open trial; cross-over study). Patients enrolled to clinical research were at age of 6–90. In order to evaluate the antiallergenic properties and efficacy in the reduction of migraine attacks of extracts from *P. hybridus*, the studies included 786 patients with allergic rhinitis and 733 patients with migraine. Other investigations involved 182 patients with somatoform disorders and 96 patients with asthma. Most of the trials in migraine, allergic rhinitis and asthma have used butterbur extracts in dosages ranging from 25 to 75 mg twice daily for 2–16 weeks. Analysis of results of clinical trials showed that Petadolex reduced headache frequency and might be superior to *placebo* in the prophylaxis of migraine in children, adolescents and adults. Moreover, it was demonstrated that monotherapy with use of Ze339 extract was an effective treatment for intermittent allergic rhinitis symptoms. The results of one study observed no therapeutic differences between fexofenadine and plant extract. Furthermore, extract Ze339 and cetirizine were similarly effective with regard to global improvement scores on the clinical global impression scale in patients with seasonal allergic rhinitis. It should be noted that results of two clinical studies extracts of *P. hybridus* did not appear to have an antihistaminic effect in skin prick tests with codeine, histamine, methacholine and allergens. Thus, extracts may not be effective in allergic skin disease. Moreover, one study demonstrated no significant clinical efficacy of extract Ze339, as compared with *placebo* group.

Patented standardized special lipophilic plant extract, Petadolex® (Weber & Weber), used for the prevention of migraine, is obtained by high-pressure liquid carbon dioxide extraction of the *P. hybridus* rhizomes (concentrated at a ratio of 28–44:1) standardized to 15% petasin and isopetasin with pyrrolizidine alkaloids (PAs) reduced to <0.08 ppm in the final product [15, 84]. Other $CO_2$ extract from the leaves of *P. hybridus* (Ze339; Tesalin®, Zeller Medical AG, Switzerland) is standardized to 8.0 mg of total petasine per tablet [85]. Moreover, extract of Ze339 contains 20.3% of petasins (petasin, neo-petasin, iso-petasin, S-petasin,

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**Table 3.**

Summary of clinical trials results of special extracts from rhizomes and leaves of *Petasites hybridus*

| Plant products | Population | Treatment scheme/doses (Study design) | Effects/ conclussions | Ref. |
|---|---|---|---|---|
| | | Prophylaxis of migraine | | |
| Petadolex | primary school children with migraine, n=58 ages 8– 12 years | prospective, randomized, partly double–blind, placebo–controlled, parallel–group trial; Petadolex – 50 mg daily (children aged 8–9 years) x 12 weeks, 50 mg x 2 times daily (children aged 10–12 years) for 8 weeks | reduction of headache frequency, effectiveness superior to placebo | 91 |
| Petadolex | children and adolescents with severe migraines, n=108 age: 6–17 years | multicenter, prospective, open–label randomized, placebo–controlled trials; Petadolex – 25 mg x 2 times daily (children aged 8–9 years), 50 mg x 2 times daily (children aged 10–17 years) for 16 weeks | reduction of frequency of migraine attack | 92 |
| Petadolex | patients with migraine, n=60 age: 18–60 years | randomised, placebo–controlled, parallel–group study; Petadolex – 50 mg x 2 times daily x 12 weeks | reduction of number, duration, and the intensity of migraine attacks | 84 |
| Petadolex | patients with migraine n = 245 age: 1 –65 years | randomised, placebo–controlled, parallel–group study; Petadolex – 50 mg and 75 mg x 16 weeks | reduction of migraine attack frequency, effectiveness: 75 mg > 50 mg | 89 |
| Petadolex | patients with migraine with and without aura, n = 202 age: 19– 65 years | multicenter, randomized, double–blind, placebo–controlled clinical trial for migraine prophylaxis; Petadolex – 50 mg and 75 mg x 2 times daily x 12 weeks | dose–dependent effects: daily dose of 150 mg – effective in reducing the frequency of migraine attacks, the number of migraine days per month, and headache intensity, daily dose of 100 mg – not significant effect compared to placebo | 93 |
| Petadolex | patients with migraine, n = 60 mean age: 28.7 years | randomized, group–parallel, placebo–controlled, double–blind clinical study; Petadolex - 50 mg x 2 times per day x 12 weeks | reduction of the number, duration and intensity of migraines attacks and the mean number of accompanying symptoms (e.g., nausea, vomiting) | 94, 95 |

| Plant products | Population | Treatment scheme/doses (Study design) | Effects/conclussions | Ref. |
|---|---|---|---|---|
| | | **Treatment of allergic diseases** | | |
| Ze339 | patients with seasonal allergic rhinitis, n= 580 age: 6– 90 years | open postmarketing surveillance study on basis of randomized, double–blind, prospective, controlled trials; Ze339 –2 tablets (2x8mg) x 2 weeks additional antiallergic medication (n=251) | improvements in symptoms of allergic rhinitis, no statistically significant differences between monotherapy with using Ze339 and combined therapy including Ze339 and other antiallergic drugs | 85 |
| Ze339 | patients with respiratory allergy (n=8) age: 28–59 years and healthy volunteers (n=10) age: 20– 63 years | randomized, double–blind, placebo–controlled study; skin reactions were assessed 90 minutes after a double dose of ZE 339 (2x8 mg) or acrivastine (8 mg) | no antihistaminic effect in skin prick tests with codeine, histamine, methacholine, and an inhalant allergen | 96 |
| Ze339 | patients with intermittent allergic rhinitis, n=330 age: $\geq$ 18 years | prospective, randomized, double–blind, placebo–controlled, parallel group comparison study; Ze339 – 1 tablet x 3 times daily for 2 weeks, Fexofenadine (Telfast 180) – 1 tablet x 1 daily for 2 weeks | effective treatment and no differences between Ze339 and fexofenadine | 97 |
| Ze339 | patients with intermittent allergic rhinitis, n=186 age: $\geq$ 18 years | multicenter, prospective, randomized, double–blind, parallel group comparison study; Ze339 – 1 tablet (8 mg) x 3 times daily x 2 weeks or 1 tablet x 2 times daily x 2 weeks | improvements in symptoms of allergic rhinitis | 90 |
| Ze339 | patients with intermittent allergic rhinitis, n = 35 age: n.i. | double–blind, placebo–controlled, cross–over study; Ze339 –50 mg x 2 times daily x 2 weeks | no significant clinical efficacy of Ze339 vs placebo | 99 |
| Petaforce | patients with perennial allergic rhinitis, n = 60 age: 31–64 years | randomized, double–blind, cross–over study; Petaforce – 50 mg x 2 times daily x 1 week, Fexofenadine – 180 mg once daily x 1 week | improvements in symptoms of perennial allergic rhinitis | 87 |
| Ze339 | patients with allergic rhinitis, n = 6 age: n.i. | placebo–controlled study; Ze339 – 3 tablets (3x8 mg) daily or 2 tablets (2x8 mg) daily for 2 weeks | effective treatment for seasonal allergic rhinitis symptoms (rhinorrhea, sneezing, itchy nose/ eyes, nasal congestion) | 86 |

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| Plant products | Population | Treatment scheme/doses (Study design) | Effects/ conclussions | Ref. |
|---|---|---|---|---|
| Petaforce | patients with grass-pollen-sensitized seasonal allergic rhinitis, n = 20 age: 19–61 years | randomized, double-blind, placebo-controlled, cross-over study; Petaforce – 50 mg x 2 times daily x 2 weeks | protection against nasal responsiveness during the grass pollen season in sensitized patients | 100 |
| Ze339 | patients with allergic rhinitis, n = 6 age: 19–42 years | open clinical trial, double-blind study; Ze339 – two tablets (2x 8 mg) x 3 times daily x 1 week | improvements in symptoms of allergic rhinitis by decreasing levels of nasal inflammatory mediators | 101 |
| Ze339 | patients with seasonal allergic rhinitis (hay fever), n = 125 age: >18 years | randomized, double blind, parallel group comparison study; Ze339 –1 tablet x 4 times daily x 2 weeks, Cetirizine (10 mg) (n=64) –1 tablet in the evening x 2 weeks | improvements in symptoms of allergic rhinitis similarly as cetirizine | 98 |
| **Other diseases** | | | | |
| Ze185 | patients with somatoform disorders (F45.0, F45.1) n = 182 age: n.i. | short-term, pharmaco-clinical trial; Ze 185 = 4-combination versus 3-combination without butterbur and placebo; 1 tablet x 3 times daily for 2 weeks | effectiveness: 4-combination > 3-combination > placebo | 88 |
| Petadolex | patients with mild or moderate asthma, n = 80 (64 adults, 16 children/adolescents) age: 6–85 years | prospective, nonrandomized, open trial; Petadolex – 50 mg x 3 times daily (adults), or 50–150 mg daily (children) x 16 weeks | reduction of severity of various asthma-related parameters, improvement in lung function | 102 |
| Butterbur (n.i.) | atopic patients, n = n.i. age: n.i. | randomized, double-blind, cross-over study; Butterbur – 50 mg x 2 times daily fexofenadine –180 mg once daily montelukast – 10 mg once daily for 1 week | no antihistaminic effect in skin prick tests with histamine and allergens | 103 |
| Petaforce | atopic asthmatic patients receiving inhaled corticosteroids, n = 16 mean age: 45 | randomized, double–blind, placebo–controlled, cross–over study; Petaforce –25 mg x 2 times daily for 1 week | support of therapy in atopic asthmatic patients maintained on inhaled corticosteroids by anti-inflammatory activity | 87 |

**Abbreviations:** n.i. – no information

neo-S-petasin, iso-S-petasin), 40.2% of fatty acids, 7% of aroma components, 1.2% of steroids/phytosterols [86]. This product was investigated in patients with seasonal allergic rhinitis. Moreover, in three clinical trials there were included patients with perennial allergic rhinitis, grass-pollen-sensitized seasonal allergic rhinitis and in atopic asthma. In these patients Petaforce® (Bioforce Ltd, Irvine, UK) [87] was used, which is carbon dioxide standardized extract from the rhizomes of *P. hybridus* and contains 7.5 mg of petasin and isopetasin per tablet. Another plant preparation, named Ze185, is composed of herbal extracts from butterbur root, valerian root, passionflower herb, lemon balm leaf. This herbal product has been registered since the mid-1990s in Switzerland [88].

These standardized extracts were well-tolerated in patients and no serious adverse effects were reported. Moreover, extracts did not exhibit the sedative effects often associated with antihistaminics. According to Brown [15], side effects are rare with the application of the PA-free special extract and have consisted primarily of mild gastrointestinal symptoms (nausea, burping, stomach pain) with rare reports of vomiting, diarrhea, and skin rash. There is a need to inform that patients should be cautioned against consuming any part of the *Petasites* plants in any form other than the specific products prepared commercially, in which the toxic alkaloids have been removed [89].

On the basis of the clinical results it can be stated that Petadolex® should be considered as an alternative treatment for prophylaxis of migraine. Moreover, according to Käufeler et al. [85] and Schapowal et al. [90], Ze339 extract may be used an innovative, reliable, and well-tolerated treatment for patients with intermittent allergic rhinitis, particularly those for whom the undesired effects of antihistaminics should be avoided.

## CONCLUSIONS

At present, it is known that petasin and furanopetasin chemotypes of *Petasites hybridus* exist. However, the activity of furanoeremophilane sesquiterpenes was not well studied in comparison with petasin chemotype. In aerial part of this plant, flavonoids and essential oil were also identified, which pharmacological activity have not yet been studied. Moreover, *P. hybridus* contains pyrrolizidine alkaloids. Their concentration is higher in the rhizomes than in leaves. Therefore, it is considered that leaves of this species may be more favorable for medicinal use. In clinical trials, only special extracts of common butterbur were used, from which the toxic alkaloids have been removed (the content lower than 0.1 ppm). The utilization of technological methods using the carbon dioxide at sub-and super-critical fluid extractions is very expensive and difficult. Therefore, there is a need to develop the alternative methods for the production of safe extracts of *P. hybridus*. In addition, using of plant *in vitro* cultures may allow to obtain a new regenerated plants without alkaloids. Moreover, in pharmacological studies only

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few mechanisms of pharmacological action have been well documented. These extracts showed inhibitory effect on COX-2 and COX-1, inhibited leukotriene synthesis and release of prostaglandin $E_2$, petasin exerted direct $Ca^{2+}$ antagonism of L-type voltage-gated calcium channels in vascular smooth muscle causing vasorelaxation. The effectiveness of these mechanisms was confirmed in few clinical trials, which showed that special extracts of *P. hybridus* were effective in treatment for intermittent allergic rhinitis symptoms and in prevention of migraine. However, it seems that long-term clinical trials are needed to confirm these effects. Further studies are now required to assess the potential role for plant extracts from common butterbur in patients with asthma, somatoform disorders, and hypertension. These standardized extracts were well-tolerated in patients and no serious adverse effects were reported. There is a need to inform that patients should use in the phytotherapy only high-quality standardized products with very low concentration of the toxic alkaloids. On the basis of these results, it may be mentioned the application of *P. hybridus* extract as effective herbal product in prostaglandin and leukotriene-mediated inflammatory diseases.

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BADANIA FITOCHEMICZNE, FARMAKOLOGICZNE I KLINICZNE NAD *PETASITES HYBRIDUS* (L.) P. GAERTN., B. MEY. & SCHERB. PRZEGLĄD

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Streszczenie

Przetwory z kłączy lepiężnika różowego (*Petasites hybridus* (L.) P. Gaertn., B. Mey. & Scherb.) mają długą historię stosowania w medycynie ludowej jako środki o działaniu przeciwzapalnym i spazmolitycznym w leczeniu różnych chorób. Ekstrakty uzyskane z omawianego gatunku są interesujące z punktu widzenia badań fitofarmakologicznych ze względu na obecność licznych związków biologicznie czynnych, głównie seskwiterpenów z grupy eremofilanów: petazyny i izopetazyny. Wspomniane substancje występują nie tylko w kłączach i korzeniach, ale także w liściach lepiężnika różowego. Ponadto, *P. hybridus* zawiera alkaloidy pirolizydynowe, które wykazują właściwości hepatotoksyczne, karcinogenne oraz mutagenne. Dlatego w badaniach przedklinicznych i klinicznych oraz fitoterapii są stosowane ekstrakty specjalne pozbawione tych związków, które otrzymuje się metodami ekstrakcji dwutlenkiem węgla w stanie podkrytycznym lub nadkrytycznym. Celem niniejszej pracy był przegląd danych bibliograficznych, z zakresu badań farmakologicznych, toksykologicznych oraz klinicznych lepiężnika różowego, przeprowadzonych w latach 2000–2013. Uwzględniono również kilka prac dotyczących gatunków stosowanych poza Europą. Dokonano opisu botanicznego rodzaju *Petasites* oraz charakterystyki fitochemicznej *P. hybridus*, a także przedstawiono profil chemiczny opatentowanych komercyjnych ekstraktów z kłączy, korzeni i liści lepiężnika różowego, stosowanych w europejskiej fitoterapii. W pracy przeglądowej zwrócono uwagę na możliwość zastosowania specjalnych ekstraktów z *P. hybridus* nie tylko w prewencji migreny, leczeniu objawów kataru alergicznego, astmy czy nadciśnienia, lecz także w zapobieganiu lub spowalnianiu postępu chorób neurodegeneracyjnych, przebiegających ze stanem zapalnym w OUN. Istnieją już dowody na obiecujące właściwości seskwiterpenów i ekstraktów z lepiężnika różowego – zmniejszanie uwalniania prostaglandyn i leukotrienów, hamowanie aktywności COX-1 i COX-2 oraz antagonizm wobec kanałów wapniowych bramkowanych napięciem typu L. Konieczne jest jednak przeprowadzenie dalszych badań farmakologicznych ekstraktów *P. hybridus*, celem wyjaśnienia nowych mechanizmów działania w OUN oraz ich przyszłego zastosowania w fitoterapii chorób ośrodkowego układu nerwowego przebiegających z procesem zapalnym.

**Słowa kluczowe:** *Petasites hybridus, ekstrakty, seskwiterpeny, alkaloidy pirolizydynowe, badania farmakologiczne, badania kliniczne, migrena, katar sienny, bezpieczeństwo, toksykologia, fitochemia*