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Article

Wild Harvesting vs. Cultivation: Total Petasin Content in *Petasites hybridus* Rhizome Extracts Determines Spasmolytic Effects

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Abstract: The use of herbal medicines containing *Petasites hybridus* extracts has a long history in the treatment of various ailments. The observed effects are primarily due to pharmacologically active compounds such as petasin, isopetasin, and neopetasin. In evidence-based phytotherapy, extracts from leaves and rhizomes are applied for different indications. While leaf extracts are administered to treat allergic rhinitis symptoms, rhizome extracts are utilized among others in the management of gastrointestinal spasms and migraines. The quality and source of plants are critical for producing authorized herbal medicinal products. Although the preparation of *P. hybridus* leaf extracts from cultivated plant material is already established, the rhizomes used for preparing extracts are still derived from commercial wild collections. However, switching to cultivation is desirable to ensure consistent quality and availability. For regulatory purposes, comparative pharmacological studies are needed to assess the bioactivity of plant material from different sources. Therefore, this study analyzed rhizome extracts from wild harvesting and cultivation for their petasin composition (i.e., isopetasin, neopetasin, petasin) and spasmolytic effects on Ca^{2+}-dependent precontracted guinea pig ileum ex vivo. The results confirm petasins as active compounds of *P. hybridus* rhizome extracts. Moreover, they demonstrate that the total content of petasins determines the spasmolytic effects, regardless of the individual composition of the different petasins. No significant differences in efficacy were found between cultivated and wild-collected rhizomes, demonstrating that cultivated material is a reliable, consistent, and sustainable alternative for *P. hybridus* rhizome extract production.

Keywords: *petasites hybridus*; petasin; isopetasin; neopetasin; test anxiety; stress; Ze 185; cultivation; wild harvesting; butterbur; spasmolysis

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1. Introduction

In Switzerland, the fixed herbal extract combination Ze 185 containing valerian roots (*Valeriana officinalis* L.), lemon balm leaves (*Melissa officinalis* L.), passionflower herbs (*Passiflora incarnata* L.), and butterbur rhizomes (*Petasites hybridus* L. (*G. Gaertn., B. Mey., & Scherb*)) is indicated for the treatment of nervousness, tension and restlessness, or test anxiety [1]. These complaints can manifest themselves in psychological and somatoform symptoms, such as cramp-like gastrointestinal complaints, increased irritability, occasional difficulty falling asleep, and sleeping through the night. While *V. officinalis*, *P. incarnata*, and *M. officinalis* exert sedative and anxiolytic effects [2], *P. hybridus* rather targets somatoform symptoms via spasmolytic effects [3]. The efficacy of Ze 185 in relieving these symptoms

has been demonstrated in several clinical trials [4–9]. Previously, a randomized, placebo-controlled study with 182 patients showed that the Ze 185 formulation containing *P. hybridus* was significantly more effective than both the *P. hybridus*-free version and placebo in improving depression and anxiety [7]. This highlights the essential role of *P. hybridus* extract in the formulation.

P. hybridus (Asteraceae) contains various secondary metabolites, including the sesquiterpene esters petasin, neopetasin, and isopetasin, collectively referred to as petasins, as well as essential oils, flavonoids, bitter substances, mucilage, and pyrrolizidine alkaloids (PAs) [10,11]. The petasins in *P. hybridus* partially undergo isomerization, where petasin may rearrange into the thermodynamically more stable isomer, isopetasin.

In folk medicine, *P. hybridus* extracts are used to treat spastic pain, dysmenorrhea, cough, wound healing, or for migraine prophylaxis [3,12,13]. Modern therapeutic uses of *P. hybridus* are largely based on standardized extracts that have undergone rigorous testing to ensure safety and efficacy. For example, *P. hybridus* leaf extract (Ze 339) is used to treat the symptoms of allergic rhinitis with comparable efficacy to conventional antihistamines [14–16]. The leaves are sourced from cultivated plants, and the extracts are generated via CO_2 extraction to achieve a high yield of lipophilic compounds, particularly the petasins [17]. The film-coated tablets containing Ze 339 are standardized to 8 mg petasins/tablet. In the context of allergic rhinitis, it has been demonstrated that petasin and isopetasin inhibit leukotriene and histamine release, thereby reducing acute allergic responses and signs of inflammation [18–20].

Besides these new findings, *P. hybridus* retains its traditional characteristic to alleviate muscle spasms. The initial investigation into the spasmolytic properties of *P. hybridus* on the intestinal tract was conducted and demonstrated in guinea pig ileum by Karl Bucher in 1951 [21]. Later investigations also revealed spasmolytic effects of petasins on isolated guinea pig trachea and rat aorta [22,23]. However, the precise mechanism by which *P. hybridus* extracts exert antispasmodic effects remains to be elucidated. It is hypothesized that petasins could reduce intestinal smooth muscle contraction by modulating the availability of intracellular Ca^{2+} [22,24]. This may explain the beneficial effects of *P. hybridus* extracts on gastrointestinal wellbeing, as intestinal cramps can derive from dysregulated intestinal smooth musculature. Although the petasins appear to be of crucial importance for the spasmolytic effects, it remains unclear whether the total petasin content or the composition of individual petasins is critical for the total effect. Therefore, the present study investigated the antispasmodic effects of purified petasin and different *P. hybridus* rhizome extracts in an ex vivo model of Ca^{2+}-dependent precontracted guinea pig ileum. The objective was to evaluate the effect of extracts with varying petasin compositions from wild harvesting and cultivation, all standardized to a total petasin content of between 3% and 3.5%, while acknowledging that the composition of the individual petasins differed between the extracts. Demonstrating the equivalence of the spasmolytic activity of rhizome extracts from cultivated and wild-harvested plants would provide significant advantages for the preparation of *P. hybridus* rhizome extracts. Switching the procurement of plants to cultivation enables enhanced sustainability, improved quality control, and a reduced risk of environmental overharvesting.

2. Materials and Methods

2.1. Extract Preparation

P. hybridus rhizomes from wild harvesting or cultivation were milled and macerated in 90% (*m*/*m*) ethanol. The crude extract was concentrated under a vacuum and heat, followed by the removal of herbal solids through phase separation. To reduce pyrrolizidine alkaloids (PAs), the ethanolic phase underwent a dedicated purification process involving a strongly acidic, gel-type, polymer-based resin. Following this step, the extract was adjusted

to a pH range of 4–6. The resulting soft extract was blended with a pre-calculated amount of excipients (microcrystalline cellulose, colloidal anhydrous silica) required for granulation and standardized to a defined petasin content. The extract was then dried using a fluid bed dryer and tested for water content, toxic metals, residual solvents, microbiological quality, pesticides, mycotoxins, and PAs. Acceptance criteria complied with the European Pharmacopeia (Ph. Eur.). The drug-to-extract ratio (DER) ranged from 7 to 14:1. Dry extracts from cultivated *P. hybridus* rhizomes were stored for 8 months prior to analysis, either at room temperature (stored) or at −20 °C (fresh). Wild-collected plants were harvested in Albania and Serbia and cultivated plants grown in Poland. Internal storage numbers for voucher specimens are 150128, 162348/0, V1802_01, and V2408.

2.2. Gas Chromatography

Quantitative gas chromatography (GC) was performed using the Agilent 6890 GC system with a flame ionization detector (FID) to determine the amount of neopetasin, petasin, and isopetasin in *P. hybridus* rhizome extracts. Extracts were dissolved in diisopropyl ether (ultrasonic bath, 30 °C), filtered, and separated via a DB-1 GC column (length: 25 m, inner diameter: 0.32 mm, film thickness: 0.52 µm) (Agilent Technologies, Santa Clara, CA, USA). Detector frequency recording rate: 20 Hz. Petasins were quantified using α-Santonin (Sigma-Aldrich, St. Louis, MO, US) as the internal standard substance.

2.3. Animals

Male Dunkin–Hartley albino guinea pigs (Charles River Laboratories supplied by Janvier Labs, 53940 Le Genest-Saint-Isle, France), 269–300 g body weight range, were stabilized for at least 5 days after delivery in macrolon cages (2 per cage) on wood litter with nesting material and gnawing material, with free access to food and water at the Porsolt animal facility. Animals were exposed to an artificial lighting cycle (12 h) between 7:00 and 19:00 in a controlled ambient temperature of 22 ± 2 °C, and relative humidity between 30 and 70%. A total of 20 animals were required for this study.

2.4. Tissue Preparation

Guinea pigs were deprived of food overnight before the test and euthanized by a blow to the head followed by exsanguination. The whole ileum was removed and the 10 cm section closest to the caecum was discarded. The lumen was rinsed with warmed and oxygenated Kreb's solution (NaCl: 112 mM, KCl: 5 mM, $CaCl_2$ ($2H_2O$): 2.5 mM, $MgSO_2$ ($7H_2O$): 1.4 mM, $NaHCO_3$: 25 mM, KH_2PO_4: 1 mM, glucose: 11.5 mM) without any distension of the organ. Segments of approximately 2 cm were then immediately suspended in a 20 mL organ bath filled with Krebs's solution maintained at 37.0 ± 0.5 °C and gassed with a mixture of 95% O_2 and 5% CO_2.

2.5. Contractile Activity Measurement

Contraction experiments in the guinea pig ileum have been performed since the 1950s [21], and the protocol has been adapted to the specific requirements of this study as follows. Isolated ileum segments were equilibrated in a test bath containing 20 mL Kreb's solution for 60 min at an optimum resting tension of 1 g. The preparations were precontracted with 1 µM histamine (Sigma-Aldrich), 1 µM carbachol (Sigma-Aldrich), or 40 mM KCl (Sigma-Aldrich) until a stable tonic contraction was reached. Five cumulative concentrations (indicated in the figure legends) of petasin (Max Zeller Söhne AG, Romanshorn, Switzerland), *P. hybridus* rhizome extract from wild harvesting or cultivation (Max Zeller Söhne AG), or vehicle control (DMSO) were added into the bath until a relaxation steady state was reached. The tensions developed by the ileum preparations were measured using an isometric force transducer and the signals were analyzed using specialized software

(IOX version 1.554, EMKA Technologies, Paris, France) to calculate the dose–response curves and the corresponding IC_{50} values.

2.6. Ethics

The study has been conducted in compliance with Animal Health regulations, in particular Council Directive No. 2010/63/UE of 22 September 2010, on the protection of animals used for scientific purposes and French decree No. 2013-118 of 1 February 2013, on the protection of animals; in accordance with the Porsolt facility accreditation for experimentation (E 53 1031, renewed on 19 April 2016); and in accordance with the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) of which the accreditation was granted in June 2012 and renewed in 2015. Furthermore, Porsolt has an in-house ethics program, which covers animal care and use within the facility. The present study has been approved by Porsolt's Ethical Committee.

2.7. Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (v. 10.2.2; La Jolla, San Diego, CA, USA). Where appropriate, the data were analysed by an unpaired students *t*-test. Normal distribution of the data was tested using the D'Agostino and Pearson test, Anderson–Darling test, Shapiro–Wilk test, and Kolmogorov–Smirnov test. Data were considered normally distributed only if all tests were passed ($p > 0.05$). Further details are indicated in the respective figure legends.

3. Results

3.1. P. hybridus Rhizome Extract Exerts Dose-Dependent Antispasmodic Effects

Initially, antispasmodic effects of *P. hybridus* extract have been assessed in an established ex vivo model using guinea pig ileum. Therefore, ileum segments from Dunkin–Hartley albino guinea pigs were prepared and the tension developed by the segments under different conditions was measured with an isometric force transducer (Figure 1A). Prior to the actual measurement, the extract was analyzed for its petasin content via gas chromatography with a flame ionization detector using santonin as the internal standard (Figure 1B,C). The *P. hybridus* rhizome extract contained varying quantities of the sesquiterpene esters neopetasin, petasin, and isopetasin (Figure 1C). The three petasins are structural isomers, differing only in their C7–C11 bond (Figure 1B). The total content of petasins in the extract was determined to be 3.5% (Figure 1C).

In order to investigate the relaxing effects of *P. hybridus* rhizome extract, it was first necessary to precontract the ileum ex vivo. The addition of histamine (1 µM), KCl (40 mM), and carbachol (1 µM), a cholinomimetic drug, resulted in a stable precontraction of the organ segments. In accordance with previous reports, it was observed that the application of purified petasin resulted in a dose-dependent relaxation, reaching the maximum effect between 3 and 10 µg/mL purified petasin (Figure 2A–C). Similarly, the application of *P. hybridus* rhizome extract resulted in a substantial relaxation of ileum segments for all three precontraction methods, with the greatest effects observed between 30 µg/mL and 100 µg/mL extract (Figure 2A–C). These results confirm antispasmodic effects of petasin but also of *P. hybridus* rhizome extract.

3.2. Petasin Composition Varies in P. hybridus Rhizome Extracts Derived from Wild Harvesting and Cultivation

After confirming the antispasmodic effect of *P. hybridus* rhizome extract, *P. hybridus* plants from different sources were investigated. The previously tested *P. hybridus* rhizome extract was obtained from wild-harvested plants. However, controlled cultivation allows for the assurance of consistent availability of raw materials and compliance with quality

standards. Thus, it was of interest to compare the composition of neopetasin, petasin, and isopetasin in extracts from wild harvesting and cultivation. It is known that the storage of dry extracts leads to the conversion of petasin to isopetasin. In order to include this variable in the ongoing investigations, an analysis was conducted on both fresh (stored for 8 months at −20 °C) and stored (stored for 8 months at room temperature) material from cultivated *P. hybridus*. The extracts were adjusted for better comparison to approximately 3% total petasin content (Figure 3A). Neopetasin, petasin, and isopetasin were detectable in all three extracts, but the respective amounts varied considerably (Figure 3B, Supplementary Figure S1A–C). As expected, the extract from wild harvesting and cultivation (fresh) contained more neopetasin and petasin, compared to the extract from stored cultivation material, which appeared to contain more isopetasin (Figure 3B).

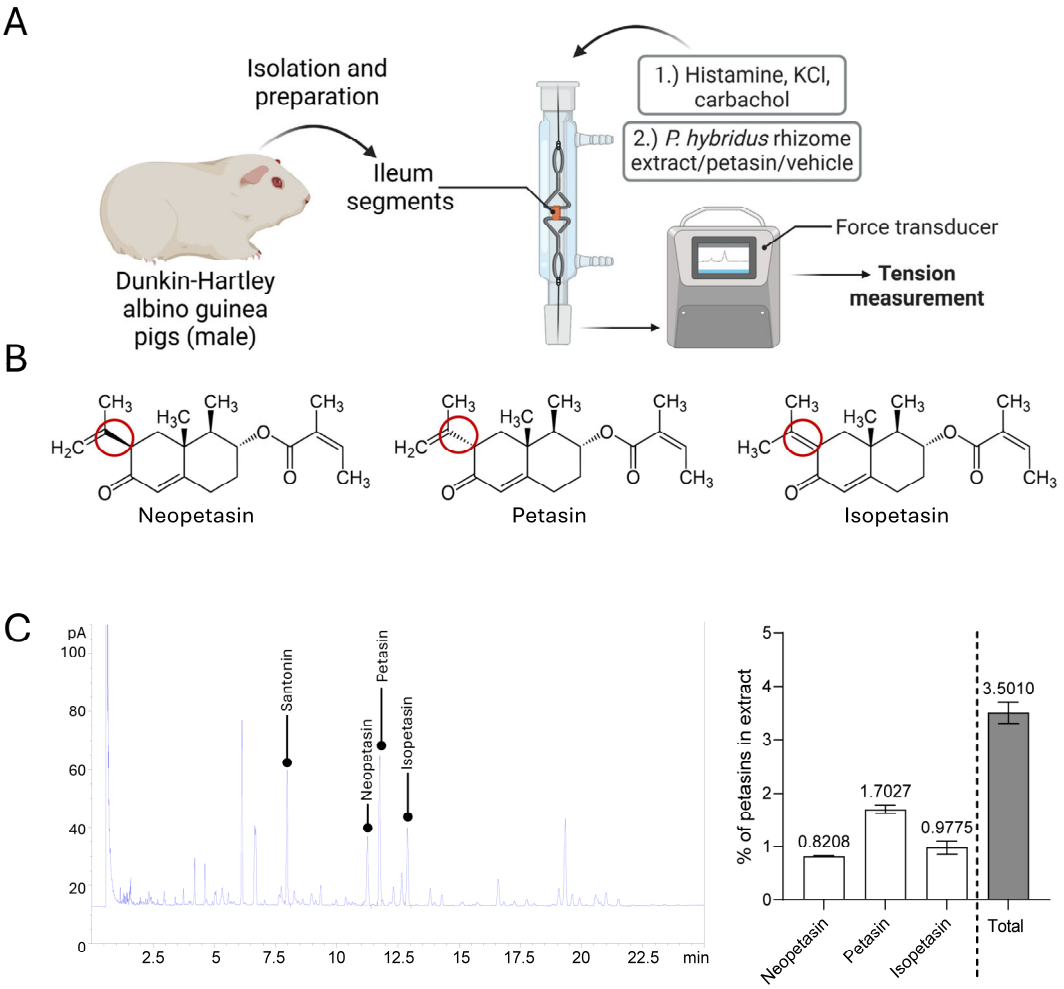

Figure 1. Experimental setup and extract analysis. (**A**) Schematic overview of the experimental setup. The figure was created with BioRender.com. (**B**) Structural formulae of the sesquiterpene esters neopetasin, petasin, and isopetasin. Circles indicate structural differences. (**C**) Representative spectra of gas chromatography analysis and quantitative analysis of neopetasin, petasin, isopetasin, and total petasins of *P. hybridus* rhizome extract. Santonin served as an internal standard. Bars show mean ± SD (n = 3 analyses). Numbers indicate mean values.

3.3. Spasmolytic Effects of P. hybridus Rhizome Extracts Derived from Wild Harvesting and Cultivation Are Comparable

Finally, to investigate the functional impact of the differing petasin compositions in extracts from wild harvesting and cultivation, their antispasmodic effects on precontracted guinea pig ileum ex vivo was compared as described before (Figure 1A). To encompass

the greatest possible range and in accordance with the 3R principle (reduce, refine, replace) for animal studies, only the two extracts that exhibited the most notable differences in petasin composition were compared: wild harvesting versus cultivation (stored). No statistically significant differences between the two extracts were found with regard to the dose-dependent relaxation of the precontracted ileum, irrespective of whether the precontraction was induced by histamine, KCl, or carbachol (Figure 4A–C). Furthermore, calculated IC_{50} [µg/mL] values did not differ significantly (Figure 4A–C). This indicates that extracts derived from cultivated plants exhibit a comparable relaxing effect on ileum contraction as extracts from wild harvesting.

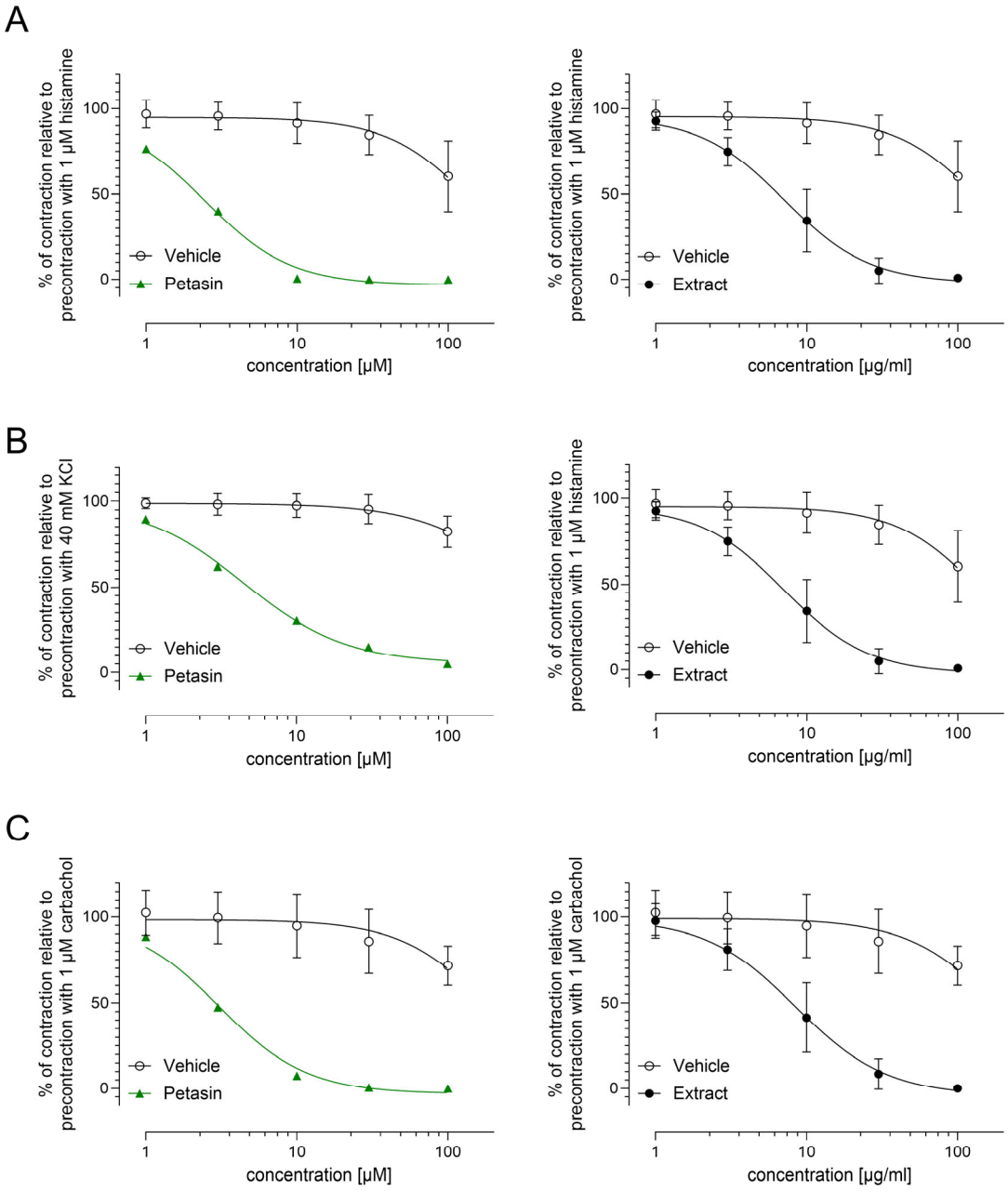

Figure 2. *P. hybridus* rhizome extract exerts antispasmodic effects dose-dependently. (**A**–**C**) Isolated guinea pig ileums were precontracted with (**A**) 1 µM histamine, (**B**) 40 mM KCl, or (**C**) 1 µM carbachol and five cumulative concentrations of *P. hybridus* rhizome extract (1 µg/mL–100 µg/mL), petasin (0.32 µg/mL–32 µg/mL), or vehicle control were added. Relaxation was detected by an isometric force transducer and is shown relative to the precontraction. Dots show means ± SD of n = 6 (*P. hybridus* rhizome extract, vehicle) and triangles show means of n = 2 (petasin).

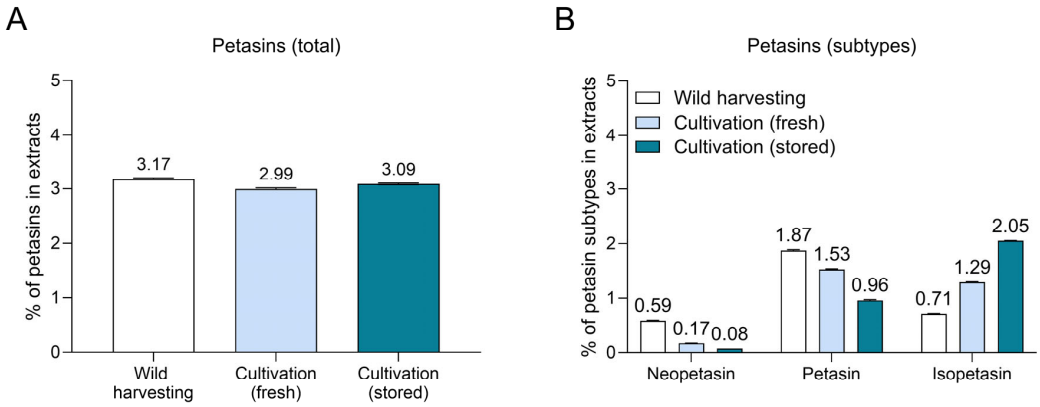

Figure 3. Petasin composition varies in *P. hybridus* rhizome extracts derived from wild harvesting and cultivation. (**A**,**B**) Quantitative analysis from gas chromatography analysis of (**A**) total petasins and (**B**) petasin subtypes (neopetasin, petasin, isopetasin) in different *P. hybridus* rhizome extracts from wild harvesting and cultivation (fresh and stored). Bars show means ± SD (n = 3 analyses). Numbers indicate mean values.

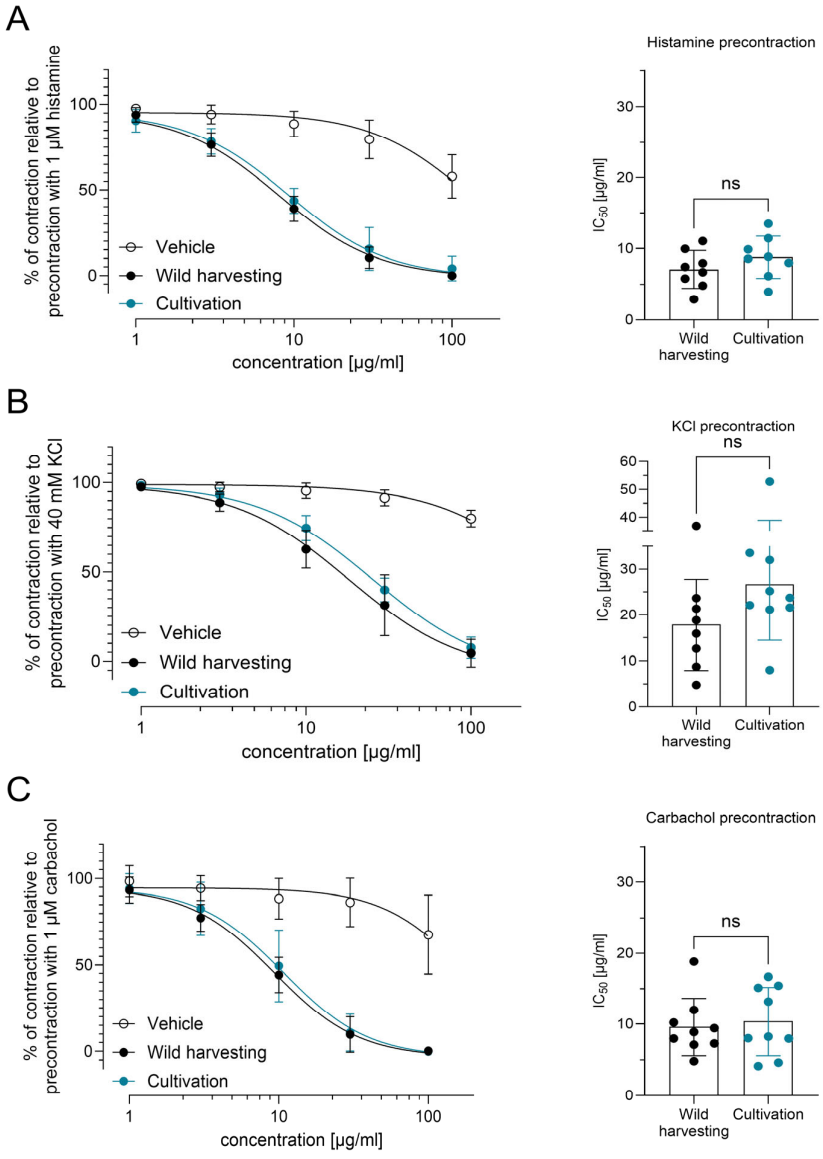

Figure 4. Spasmolytic effects of *P. hybridus* rhizome extracts derived from wild harvesting and cultivation (stored) are similar. (**A**–**C**) Isolated guinea pig ileums were precontracted with (**A**) 1 µM

histamine, (**B**) 40 mM KCl, or (**C**) 1 µM carbachol and five cumulative concentrations of *P. hybridus* rhizome extracts from wild harvesting or cultivation or vehicle control were added (1 µg/mL–100 µg/mL). Relaxation was detected by an isometric force transducer and is shown relative to precontraction. Dots show means ± SD of n = 8. IC_{50} [µg/mL] values have been calculated from each individual dose response curve. Bars show means ± SD of n = 8. Statistical analyses were performed using unpaired students *t*-test, ns = not significant.

4. Discussion

P. hybridus is a perennial plant that has been valued in folk medicine for centuries. Traditionally, it has been used to alleviate symptoms such as spastic pain, dysmenorrhea, coughs, and to promote wound healing [3]. These therapeutic applications are largely attributed to bioactive sesquiterpenes, particularly neopetasin, petasin, and isopetasin [12]. In evidence-based phytotherapy *P. hybridus* extracts are, for example, used in combination preparations such as Ze 185, which is a fixed herbal formulation comprising extracts from valerian root (*Valeriana officinalis*), passionflower herb (*Passiflora incarnata*), lemon balm leaf (*Melissa officinalis*), and butterbur rhizome (*Petasites hybridus*). This combination is registered in its current composition in Switzerland since the 1990s and is utilized primarily as a non-sedating alternative for patients seeking relief from stress-related symptoms, test anxiety, and mild psychological disturbances [1]. In order to guarantee the long-term supply of medicinal plants, procurement of *P. hybridus* rhizomes in particular needs to be reconsidered. Analyses have shown that the concentration of the different sesquiterpenes (petasin, isopetasin, and neopetasin) can vary depending on the source (wild harvesting, cultivation) of *P. hybridus* rhizomes. It is, therefore, crucial to demonstrate the efficacy of extracts from different sources to enable a shift from wild collection to more sustainable cultivated plants in supply chains.

The spasmolytic effects of *P. hybridus* rhizome extracts were confirmed in this study using precontracted guinea pig ileum segments. The application of histamine, KCl, or carbachol induced stable contractions, which were significantly relaxed in a dose-dependent manner by both purified petasin and *P. hybridus* rhizome extracts. Purified petasin exerts the strongest relaxant effect on the guinea pig ileum at a concentration of about 3 µg/mL (10 µM), depending on the method of precontraction. This concentration corresponds approximately to the total content of petasins in 100 µg/mL extract and, thus, to the extract concentration with the maximum effect. These findings align with the hypothesis that total petasin content, rather than the specific composition of individual petasins, determines the spasmolytic efficacy of *P. hybridus* extracts. It is noteworthy that the composition of petasins in *P. hybridus* rhizome extracts can vary depending on factors such as storage and source material. The conversion of petasin to isopetasin during storage alters the relative proportions of the three petasins without changing the total petasin content. Despite these compositional changes, no significant differences in spasmolytic effects were observed between extracts derived from wild-harvested or cultivated plants, or between fresh and stored materials. This indicates that the relaxing effects of *P. hybridus* extracts are determined by the total petasin content rather than the specific ratios of neopetasin, petasin, and isopetasin. The precise mechanism of action of petasins in smooth muscle relaxation remains to be fully elucidated. However, studies suggest that petasins may influence intracellular calcium concentrations by inhibiting L-type voltage-dependent calcium channels (VDCC) [13,22,23,25,26]. This mechanism, previously demonstrated in guinea pig trachea and rat aorta models, provides a plausible explanation for the observed relaxation of Ca^{2+}-dependent precontracted tissues. Further investigations into this mechanism are warranted to provide greater clarity. It is worth mentioning that the gas chromatography spectra demonstrate variations in the extract matrix of the tested extracts. However, these differences do not appear to have any substantial pharmacological implications with regard

to spasmolysis. This is in line with previous analyses of *P. hybridus* leaf extracts, which differed substantially in their extract matrix composition, but their inhibitory effect on leukotriene synthesis was solely determined by the petasin content [27].

Beyond its spasmolytic effects, *P. hybridus* or petasins exhibit a range of additional pharmacological activities, emphasizing its potential for broader therapeutic applications. For example, the *P. hybridus* leaf extract Ze 339 is effective in managing allergic rhinitis symptoms by combining anti-allergic and anti-inflammatory effects. This is achieved primarily by inhibiting the release of inflammatory mediators, altering the production of pro-inflammatory cytokines and inhibiting the 5-lipoxygenase (5-LOX) pathway, thereby decreasing leukotriene synthesis [18–20]. Clinical studies have demonstrated that *P. hybridus* extracts provide comparable efficacy to conventional antihistamines [14,15,28]. Unlike some antihistamines, which can cause sedation, *P. hybridus* extracts are less likely to induce drowsiness, offering a non-sedating alternative for individuals seeking symptom relief [16]. Another common use of *P. hybridus* is in migraine prophylaxis, where petasin and isopetasin inhibit transient receptor potential (TRP) channels, thereby reducing nociception and neurogenic dural vasodilation [29,30]. Preclinical research has also highlighted antiviral properties [31,32], anti-adipogenic effects [33], and demonstrated selective antitumor potential in colon carcinoma models [34,35]. These findings could promote the development of new areas of clinical application for *P. hybridus* in the future.

There have been isolated reports of liver damage associated (other extracts) with the use of *P. hybridus* [13]. The risk of liver damage is attributable to PAs, and it is, therefore, critical to only take medicines that comply with the strict limits of PAs. The benefit–risk assessment of the period safety update report for products containing Ze 185 concludes that Ze 185 is safe and well tolerated for the treatment of stress-related somatoform disorders. This is based on the very good safety profile, which is reflected in the patient exposure since market authorization and post-marketing data sources.

5. Conclusions

In conclusion, this study confirms the substantial spasmolytic effects of *P. hybridus* rhizome extracts on guinea pig ileum ex vivo, demonstrating that these effects are primarily determined by total petasin content. It further confirms the scientific rationale for the use of *P. hybridus* rhizome extract in the treatment of somatoform disorders and advances the understanding of its pharmacological properties. The results also indicate that extracts derived from cultivated plants are comparable in efficacy to those from wild harvesting, underscoring the feasibility of replacing wild-collected material with cultivated sources. These findings are not only important for extracts from *P. hybridus*, but also for extracts from other medicinal plants that are also wild collected. In general, future studies should investigate the comparability of extracts from other plants from wild collection and cultivation with a view to gradually moving towards controlled cultivation of medicinal plants. Cultivation not only ensures a sustainable and ethical supply of plant material but also enhances the consistency and quality of medicinal products.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/scipharm93020015/s1, Figure S1: Petasin content in *P. hybridus* rhizome extracts derived from wild harvesting and cultivation (fresh) is comparable.

Author Contributions: Conceptualization, C.H., J.D. and G.B.; investigation, C.H. and J.D.; visualization, V.M.M., V.B. and C.H.; writing—original draft, V.M.M.; writing—review and editing: V.B., C.H., J.D. and G.B., supervision, C.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All animal experiments have been conducted in compliance with Animal Health regulations, in particular as follows: Council Directive No. 2010/63/UE of 22 September 2010, on the protection of animals used for scientific purposes and French decree No. 2013-118 of 1 February 2013, on the protection of animals; in accordance with the Porsolt facility accreditation for experimentation (E 53 1031, renewed on 19 April 2016); and in accordance with the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) of which the accreditation was granted in June 2012 and renewed in 2015.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: Christiane Halbsguth, Verena M. Merk, Veronika Butterweck, Juergen Drewe, and Georg Boonen are employed at Max Zeller Söhne AG, CH-8590 Romanshorn, Switzerland.

Abbreviations

The following abbreviations are used in this manuscript:

5-LOX	5-lipoxygenase
P. hybridus	Petasites hybridus
FID	Flame ionization detector
GC	Gas chromatography
PA	Pyrrolizidine alkaloids
TRPs	Transient receptor potential channels
VDCC	Voltage-dependent Ca2+ channels

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