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Original research paper

Total phenolic content, flavonoid content and antioxidant potential of *Petasites hybridus* and related species from Croatia and considerations regarding their pharmaceutical significance

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ABSTRACT

Extracts obtained from common butterbur (*Petasites hybridus*), standardized to petasins, are existing pharmaceutical options for allergic rhinitis (leaves) and migraine (rhizomes) treatment and/or prevention. In this study, total phenolic content, flavonoid content and antioxidant potential of ten samples of Croatian *Petasites* species (four *P. hybridus*, four *P. albus*, one *P. kablikianus*, and one *P. paradoxus*) obtained by ultrasound-assisted extraction of leaves were compared. The total phenolic content (Folin-Ciocalteu assay) of methanolic leaf extracts ranged from 4.43 ± 0.09 to 10.76 ± 0.60 mg gallic acid equivalent g^{-1} dry weight (mg GAE g^{-1} DW) for *P. hybridus* and from 6.66 ± 0.43 to 19.92 ± 2.90 mg GAE g^{-1} DW for *P. albus* samples, while those of *P. kablikianus* and *P. paradoxus* were equal to 7.56 ± 0.17 mg GAE g^{-1} DW and 10.22 ± 0.46 mg GAE g^{-1} DW, respectively. Flavonoid content ($AlCl_3$ assay) varied between 2.51 ± 0.10 and 4.03 ± 0.08 mg quercetin equivalent g^{-1} dry weight (mg QE g^{-1} DW) for *P. hybridus* and between 2.21 ± 0.09 and 5.22 ± 0.02 mg QE g^{-1} DW for *P. albus* samples, while those of *P. kablikianus* and *P. paradoxus* were equal to 5.59 ± 0.05 mg QE g^{-1} DW and 5.50 ± 0.09 mg QE g^{-1} DW, respectively. Antioxidant potential was in high correlation with total phenolic content ($r = 0.93$, $p < 0.001$). Due to expected contribution of plant polyphenols and flavonoids to the activity of butterbur extracts and their observed great variabilities, determining the content of these compounds may be of interest to the pharmaceutical industry.

Keywords: *Petasites*, Asteraceae, phytotherapy, green synthesis, polyphenols, DPPH assay

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INTRODUCTION

Petasites Mill. (butterbur) species are large-leaved rhizomatous perennial herbs from the daisy (Asteraceae) family, which are distributed all over Europe, Asia and North America. Their

name comes from the Greek word *petasos*, which refers to a large hat commonly worn in Ancient Greece. Out of 19 species that are accepted today (1), the widespread *Petasites hybridus* G.Gaertn., B.Mey. & Scherb. (common butterbur) is pharmaceutically and phytotherapeutically the most important (2). In recent years, the use of its extracts as green media for generating magnetic organometallic nanocomposites that are used as catalysts in one-pot multicomponent reactions (MCRs) is becoming increasingly popular, *e.g.*, for the synthesis of compounds that could reduce organic pollutants or that could be used in synthesis of new pharmaceuticals (3, 4). Flavonoids and phenolics present in the *P. hybridus* rhizome extract were suggested as the main compounds responsible for the reduction of metal ions to nano zero-valent metallic particles (3). Modern pharmacotherapy also recognizes the use of *P. hybridus* rhizome extract (Petadolex®) for the prevention of migraine attacks (5), while *P. hybridus* leaf extract (Tesalin® – Ze 339) is clinically approved as a herbal medicine for the treatment of symptoms of seasonal allergic rhinitis such as rhinorrhea, sneezing, and nasal congestion (1). In fact, out of twenty-nine randomized controlled trials which evaluated the use of single medicinal plants for allergic rhinitis among adults and children, the greatest number of studies included *P. hybridus* (6).

Rare and idiosyncratic cases of herb-induced liver injury caused by the rhizome extract Petadolex®, often confounded by hepatotoxic co-medications, were reported (N = 48 cases over a period of > 30 years and an estimated 2.6 million patient month exposure), while clinically relevant liver function abnormalities were not observed in clinical trials with migraine patients (5). On the other hand, there have been no reports of serious adverse drug reactions with the butterbur leaf extract so far (6). This was recently acknowledged by the Swiss health authority as Tesalin® (Ze 339) was switched from prescription to the nonprescription status. It was concluded that *P. hybridus* leaf extract Ze 339 may be regarded as safe if used in the clinically recommended dose regime (7). Also, results from a recent study that evaluated *in vivo* single and repeated oral dose toxicity and *in vitro* genotoxicity of *P. japonicus* (Siebold & Zucc.) Maxim. leaves, suggested that they may be safe for human consumption (8). The clinically

approved butterbur extracts mentioned above are standardized to petasins and are declared as PA-free, *i.e.*, free of hepatotoxic and carcinogenic pyrrolizidine alkaloids (PAs). Due to their initially lower contents of PAs, leaves may be a more suitable source of petasins (9), the pharmacologically active ingredients at least partially responsible for the anti-inflammatory effects of butterbur extracts (5). Various populations of *P. hybridus* were shown to vary considerably in petasin content both in their rhizomes (7.4 to 15.3 mg g^{-1} dry weight (DW)) and leaves (3.3 to 11.4 mg g^{-1} DW), while even greater differences were observed between rhizomes (4.8 – 89.9 µg g^{-1} DW) and leaves (0.02–1.50 µg g^{-1} DW) in the content of PAs (9). Besides between different organs, PA content may vary considerably within and between populations, while seasonal variations seem to be of minor importance (10). Similarly, great variabilities in essential oil constituents were observed for different plant parts and populations of *P. hybridus* and *P. albus* (L.) Gaertn. from Croatia (11). Recently, also the content of total phenolic compounds, antioxidant and antimicrobial activity were reported for extracts obtained by ultrasound-assisted extraction (UAE) of different plant parts of *P. hybridus* from Turkey (12). However, little is known about the possible variabilities of phenolics and flavonoids between different populations of *P. hybridus* and related species.

Besides *P. hybridus*, which has been recognized as one of the most important Central European medicinal herbs used from classical antiquity to modern and contemporary era (13), other species from the genus *Petasites* such as *P. japonicus*, *P. tricholobus* Franch., *P. formosanus* Kitam., and *P. frigidus* (L.) Fries have been used worldwide both as food and traditional medicines (1, 14, 15). In Bosnia and Herzegovina, ointments prepared from leaves of wild *P. hybridus* and *P. albus* are used for rheumatism (16). Ethnomedicinal use of the same two species was recently reported from Serbia (17). The aim of this study was to evaluate the total phenolic and flavonoid contents of *P. hybridus*, *P. albus*, *P. kablikianus* Bercht., and *P. paradoxus* (Retz.) Baumg. together with their antioxidant potential. Samples of the former two species were collected from four different locations in Croatia, two of which were shared by

both species (Medvednica, Fužine). To our knowledge, this is the first study that compared the phytochemical content and antioxidant activity of several *Petasites* species, and the first such study that compared the contents of polyphenols and flavonoids in different populations of *P. hybridus* and *P. albus*.

EXPERIMENTAL

Plant material

Leaves of four different *Petasites* species were collected during the flowering period from ten wild populations in Croatia: *Petasites hybridus* (Mount Medvednica, Fužine, Mount Ivanščica, Zagreb - Maksimir), *P. albus* (Mount Medvednica, Fužine, Mount Risnjak, Northern Velebit), *P. kablikianus* (Plitvice Lakes), and *P. paradoxus* (Baške Oštarije). Samples were authenticated by Prof. Kroata Hazler Pilepić and voucher specimens deposited at the herbarium of the Department of Pharmaceutical Botany, University of Zagreb Faculty of Pharmacy and Biochemistry.

Extract preparation

After air-drying at room temperature, the leaves were initially cut into smaller pieces and then ground to powder using an electric mill. Ultrasound-assisted extraction (UAE) was performed twice by adding 5 mL of methanol to 0.5 g of powdered plant material in the duration of 2 × 30 minutes. After filtering, the two extracts were combined and made up to the mark with methanol in a 10 mL flask.

Chemical reagents and standards

Folin-Ciocalteu's reagent and sodium carbonate decahydrate were purchased from Kemika (Croatia). Aluminum chloride hexahydrate was obtained from Sigma-Aldrich (USA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Fluka (Switzerland). Standard compounds gallic acid,

quercetin and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were purchased from Merck Schuchardt (Germany), Sigma-Aldrich (USA) and Acros Organics (Belgium), respectively. All chemicals used were of analytical grade.

Evaluation of total phenolic and flavonoid content

Total phenolic content was evaluated using the Folin-Ciocalteu's reagent, similarly as previously described (18). In brief, 0.25 mL of extract was mixed with 1.25 mL of Folin-Ciocalteu's reagent (10 % (v/v)). After 5 min, 1 mL sodium carbonate decahydrate (7.5 g 100 mL^{-1}) was added. After 60 min of incubation, the absorbance was read at 765 nm. The results are expressed as mg gallic acid equivalent g^{-1} dry weight (mg GAE g^{-1} DW).

Flavonoid content was evaluated using the previously described aluminum chloride method (18). In brief, 1 mL of extract was mixed with 1 mL $AlCl_3 \times 6\ H_2O$ (2 g 100 mL^{-1}). After 15 min, absorbance was measured at 415 nm. The results are expressed as mg quercetin equivalent g^{-1} dry weight (mg QE g^{-1} DW).

DPPH assay

Antioxidant potential was evaluated based on the DPPH radical scavenging activity as previously described (18). To 2 mL of methanolic DPPH solution, adjusted to initial absorbance of 0.70 ± 0.02, 10 µL of extract was added. After 30 min incubation, the decrease in absorption of the radical was measured at 517 nm. The results are expressed as mg Trolox equivalent g^{-1} dry weight (mg TE g^{-1} DW).

Statistical analysis

All measurements were performed in triplicate. The results are expressed as means ± standard deviations (SD). Correlations between measured parameters were assessed using Pearson's correlation coefficient (r) with the significance level, a, set at 0.05. Statistical analysis was performed in GraphPad Prism 9.0. (GraphPad Software, San Diego, USA).

RESULTS AND DISCUSSION

In this study, four European *Petasites* species were harvested from ten wild populations in Croatia in order to evaluate their (poly)phenolic content, flavonoid content and antioxidant potential: *P. hybridus* (Medvednica, Fužine, Ivanščica, Zagreb - Maksimir), *P. albus* (Medvednica, Fužine, Risnjak, Northern Velebit), *P. kablikianus* (Plitvice Lakes), and *P. paradoxus* (Baške Oštarije).

Total phenolic content

The total phenolic content of collected samples was assessed using the Folin-Ciocalteu assay, one of the most commonly used methods for the determination of (poly)phenolic compounds in plant-based foods and beverages (19). The assay is based on a single electron-transfer (SET) in which the antioxidant species acts as the electron donor and the Folin−Ciocalteu's reagent acts as the oxidant, causing a change in color from yellow to blue, directly proportional to the reducing activity of the phenolic compounds. This is frequently displayed as gallic acid equivalents (GAE) (20).

In the present study, the total phenolic content of *P. hybridus* samples ranged from 4.43 ± 0.09 to 10.76 ± 0.60 mg GAE g^{-1} DW, while for *P. albus* samples it varied between 6.66 ± 0.43 and 19.92 ± 2.90 mg GAE g^{-1} DW (Fig. 1). Furthermore, the total phenolic contents of the remaining two species were within the ranges observed for *P. hybridus* and *P. albus*, 7.56 ± 0.17 mg GAE g^{-1} DW in *P. kablikianus* and 10.22 ± 0.46 mg GAE g^{-1} DW *P. paradoxus*. In a recent study from Turkey, the content of total phenolic compounds of *P. hybridus* leaf extract, under optimal conditions, was found to be 3.78 μg GAE mg^{-1} extract (12). Total phenolic acid content of extracts obtained from *P. japonicus* leaves and stalks, assessed from peak areas of the UPLC-DAD chromatogram, was 16.76 ± 0.42 mg g^{-1} DW. The major phenolic acid was 3,5-di-*O*-caffeoylquinic acid followed by 5-*O*-caffeoylquinic acid and fukinolic acid, while kaempferol 3-*O*-(6"-*O*-acetyl) glucoside, quercetin 3-*O*-(6"-*O*-acetyl) glucoside, astragalin,

and kaempferol 3-*O*-rutinoside (nicotiflorin) were the most represented flavonoids (21). The presence of caffeoylquinic and feruloylquinic acid derivatives was reported in *P. hybridus* leaves as well (22).

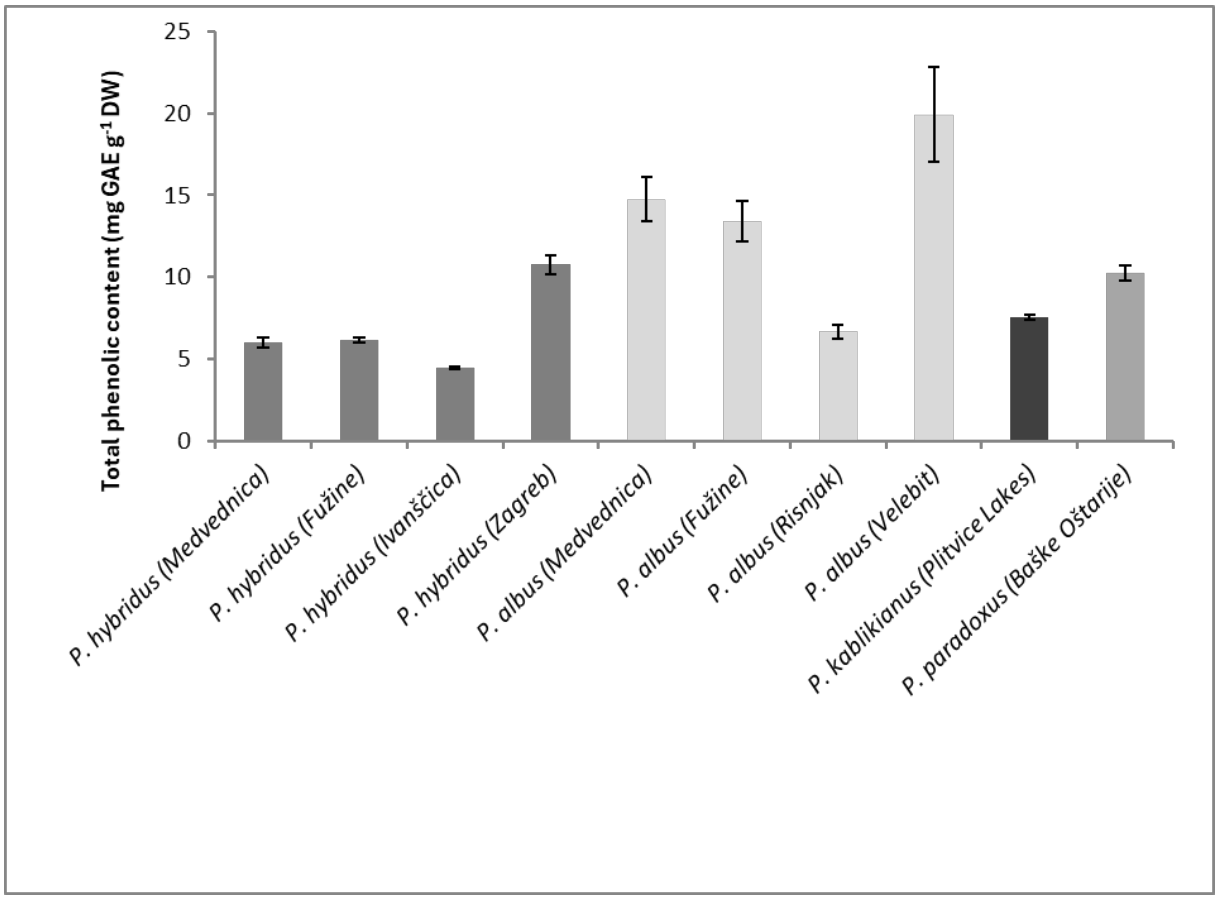

Fig. 1. Total phenolic content of ten investigated *Petasites* samples (averages ± SD, $n = 3$), GAE – gallic acid equivalent, DW – dry weight.

Flavonoid content

Flavonoid content in this study was assessed based on a spectrophotometric aluminum chloride chelation method, one of the most commonly used methods for the so-called total flavonoid determination, using the procedure without $NaNO_2$ (23), which may only be used for the estimation of the contents of certain classes of flavonoids, *i.e.*, flavones and flavonols (24). Flavonols such as quercetin and kaempferol and/or their glycosides were previously reported for

192 *Petasites* species (21, 25). In the assay, flavonols form complexes with Al(III) with C-3 and C-
193 5 hydroxy groups and with the dihydroxy groups in B ring (23).

194 The highest flavonoid contents were recorded for *P. kablikianus* and *P. paradoxus*, 5.59 ±
195 0.05 mg QE g^{-1} DW and 5.50 ± 0.09 mg QE g^{-1} DW, respectively (Fig. 2). For *P. hybridus*, the
196 same varied between 2.51 ± 0.10 and 4.03 ± 0.08 mg QE g^{-1} DW, while for *P. albus*, a wider
197 range of flavonoid contents was recorded (from 2.21 ± 0.09 to 5.22 ± 0.02 mg QE g^{-1} DW).
198 Not a lot is known about the flavonoids from investigated *Petasites* species. For *P. hybridus*,
199 quercetin, quercitrin and rutin were reported (1). Flavone glycosides quercetin-3-*O*-*β*-glucopy-
200 ranoside and kaempferol-3-*O*-*β*-glucopyranoside and their rhamnosylated derivatives (rutin,
201 nicotiflorin) were isolated from *P. tricholobus* (25). Recently, a more detailed list of flavonoid
202 compounds was given for 80 % ethanolic extract obtained by Soxhlet extraction from *P. japon-*
203 *icus* leaves and stalks, which was based on ultra performance liquid chromatography coupled
204 with diode array detector, quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS)
205 characterization. The total flavonoid content of the same extract was estimated to be 10.65 ±
206 1.25 mg g^{-1} DW (21).

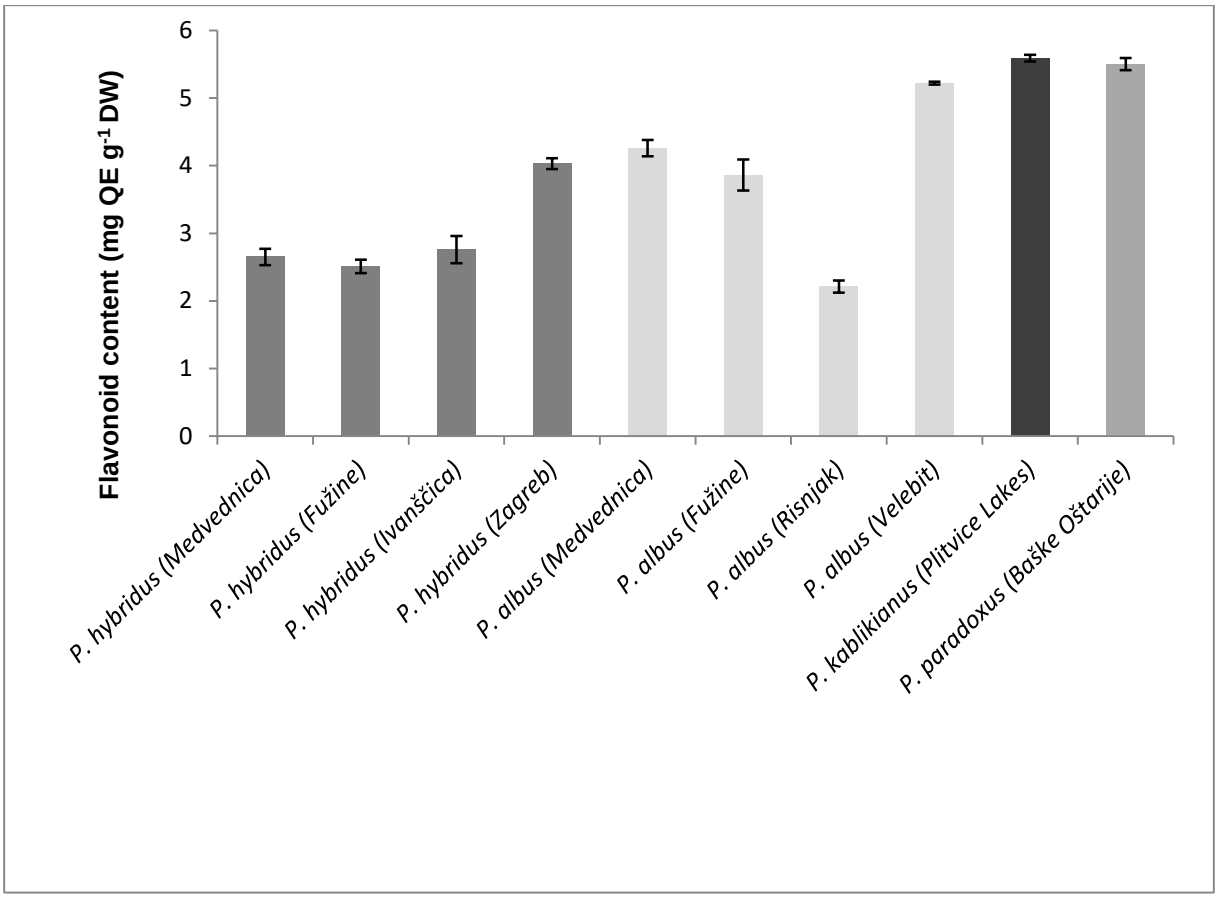

Fig. 2. Flavonoid content of ten investigated *Petasites* samples (averages ± SD, n = 3), QE – quercetin equivalent, DW – dry weight.

Antioxidant potential

Antioxidant potential was evaluated using the DPPH radical scavenging assay, the most known and commonly used method to determine antioxidant ability of food and pharmaceutical ingredients. The DPPH assay is based on spectrophotometric measurements of the capacity of antioxidants to scavenge DPPH radicals. The method is popular due to its ease of use, sensitivity, reproducibility, and execution speed, and can be readily used without the need for free radical preparation prior to performing the test (26). The reaction mechanism is primarily based on the electron transfer (ET), which can be further subdivided into single electron transfer followed by a proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET) (27).

Also, a marginal reaction pathway in the assay is hydrogen atom transfer (HAT), *i.e.*, reduction of the single electron of the nitrogen atom in DPPH radical (intense deep violet color) to the corresponding hydrazine DPPH-H (pale yellow color) by taking a hydrogen atom from the antioxidants (H-donors) (28). A standard antioxidant Trolox may be used as a reference standard and the results of the assay may be expressed as Trolox equivalents (TE) (26).

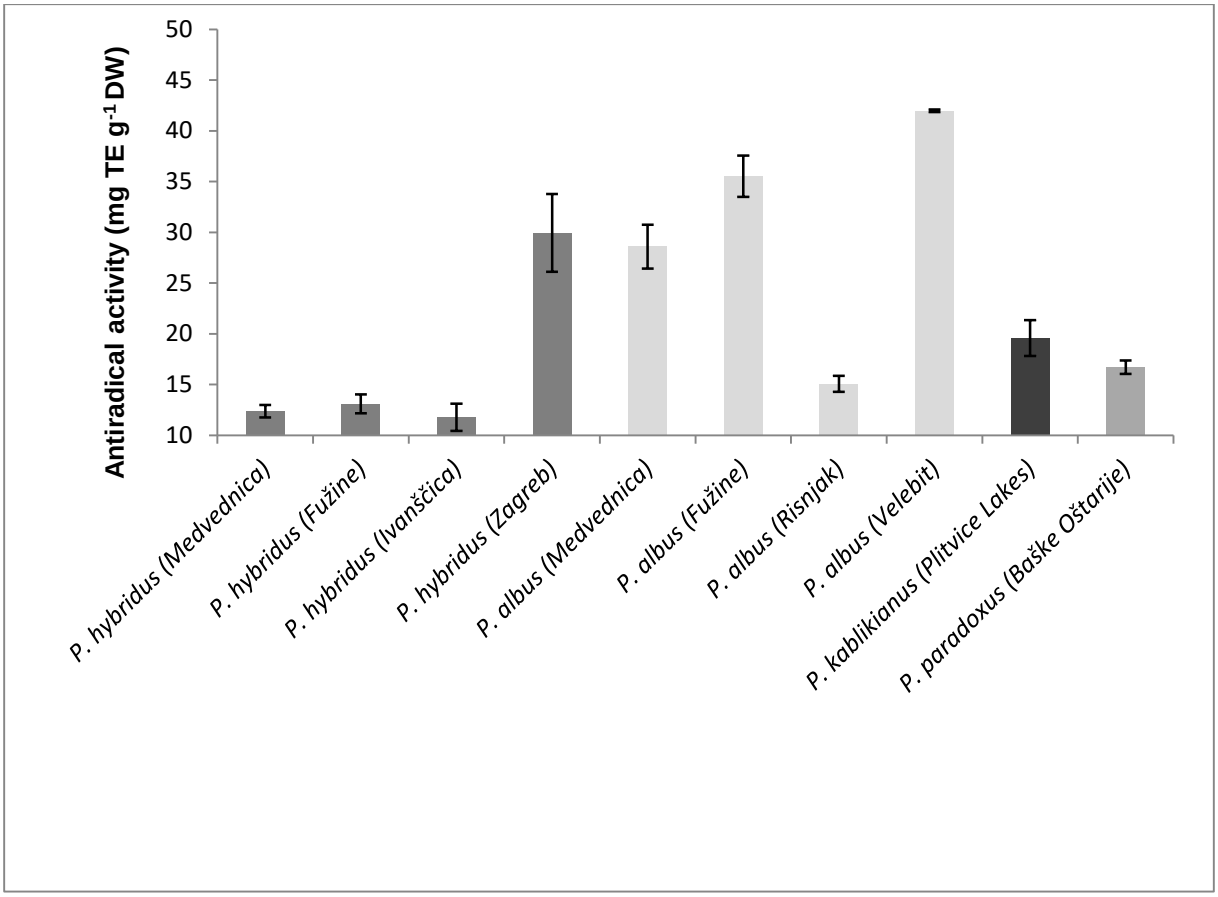

Fig. 3. DPPH radical scavenging activity of ten investigated *Petasites* samples (averages ± SD, $n = 3$), TE – Trolox equivalent, DW – dry weight.

The DPPH radical scavenging activity found in this study ranged from 11.78 ± 1.34 to 29.94 ± 3.83 mg TE g^{-1} DW in *P. hybridus* samples and from 15.07 ± 0.80 to 41.98 ± 0.12 mg TE

234 g^{-1} DW in *P. albus* samples, while it was within the same ranges for the remaining two species,
235 *P. kablikianus* (19.59 ± 1.77 mg TE g^{-1} DW) and *P. paradoxus* (16.72 ± 0.66 mg TE gv^{1} DW)
236 (Fig. 3). The antioxidant activity estimated by the DPPH assay of *P. hybridus* ethanolic leaf
237 extract obtained under optimized conditions was found to be 2.27 µg TE mg^{-1} extract (12). In
238 the present study, the DPPH radical scavenging activity was found to be in high correlation
239 with the total phenolic content ($r = 0.93$, $p < 0.001$, Table I). This may be explained by the fact
240 that both assays are based on SET reactions (20, 27).

241 *Table I. Pearson's correlation coefficients (*r*) between the measured parameters (total phe-*
242 *nolic content, flavonoid content, and DPPH radical scavenging activity) of ten* Petasites *sam-*
243 *ples (one significant correlation is marked in bold)*

244

Measured parameters	Total phenolic content	Flavonoid content
Flavonoid content	0.59 ($p = 0.071$)	1
DPPH radical scavenging activity	**0.93** ($p < 0.001$)	0.52 ($p = 0.125$)

Until today, most of the investigations of antioxidant activity of *Petasites* species were done on *P. japonicus* and the DPPH assay was the most frequently used *in vitro* method (14). Caffeic acid derivatives such as 5-*O*-caffeoylquinic acid, fukinolic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid present in methanolic extracts of leaves and roots, were some of the compounds responsible for the antioxidant activity with 3,5-di-*O*-caffeoylquinic acid having the greatest radical scavenging capacity in leaf (23.09 %) and root extracts (26.47 %). On the other hand, flavone glycoside quercetin-3-*O*-(6''-acetyl)-*β*-glucopyranoside, which was present only in the leaf extract, showed weak activity, while no activity was observed for kaempferol-3-*O*-(6''-acetyl)-*β*-glucopyranoside (29). This is consistent with our results, according to which the antiradical activity of investigated *Petasites* species was not significantly correlated with flavonoid content. Indeed, the aforementioned caffeoylquinic acid derivatives were, likewise, reported to be present in *P. hybridus* leaves together with 5-*O*-feruloylquinic acid, 1-*O*-caffeoyl-3-*O*-feruloylquinic acid, and 1-*O*-caffeoyl-4-*O*-feruloylquinic acid. It is interesting to notice that other well-known medicinal plants such as *Achillea millefolium* L. (yarrow) and *Cynara scolymus* L. (artichoke) also possessed most of the aforementioned hydroxycinnamic acid derivatives (22). Fukinolic acid is yet another phenolic compound, which was recognized as the major antioxidant constituent in ethanolic extracts of *P. japonicus* flower buds (30). However, to our knowledge, its presence in other *Petasites* species has not been reported so far (31). On the other hand, in investigated plants, other non-phenolic antioxidants may be present such as the benzofuran derivative euparin found in essential oils of *P. albus* (aerial parts), which was observed to possess moderate antioxidant activity according to the DPPH radical scavenging assay (32).

Evaluation of antioxidant activity of extracts prepared from leaves, stems and roots of *P. japonicus* based on various methods, including DPPH, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$), and superoxide anion radical scavenging assay, and the ferric reducing ability of plasma (FRAP), showed that the antioxidant activity of leaf extract is

superior to those of other extracts (33). Similarly, essential oils obtained from leaves of *P. hybridus* subsp. *ochroleucus* exhibited greater antioxidant activity in the DPPH assay in comparison to essential oils obtained from rhizomes of the same plant (34).

Variabilities in specialized metabolites content within and between species

As observed in this and in some of our previous studies, the contents of specialized (secondary) metabolites may not only differ between different species of the same genus (35), but they may also vary considerably between different populations of the same species (18, 36). For this reason, to gain a better insight into the richness of biologically active compounds of a certain species, it is beneficial to include, when possible, more than one population of the species of interest in the study. This is certainly more challenging in terms of sample collection and is not always possible, *e.g.*, when the species of interest are growing in single and/or hard-to-reach locations. Generally, investigated *Petasites* species are known to inhabit moist, damp and shady areas, and are often covering large surfaces on riverbanks, near lakes and streams thanks to their creeping underground stems (rhizomes) (1, 37). Common butterbur (*P. hybridus*) and white butterbur (*P. albus*) are widespread in Europe and in Croatia (1, 38). On the other hand, in Croatia, *P. kablikianus* (glabrous butterbur) is found only in the Plitvice Lakes area (38). In fact, it is the dominant fast-decomposing species growing on the tufa barriers, whose formation is promoted by leaf litter breakdown (and *vice versa*) (39). *Petasites paradoxus* (Alpine butterbur), on the other hand, as its names suggests, may be found in mountainous regions (37, 38).

In this study, *P. kablikianus* and *P. paradoxus* were each collected from a single location, while four different populations of *P. hybridus* and *P. albus* were investigated due to the better distribution and accessibility of these species. Conveniently, two of the four harvesting locations were shared by the same species, which enabled a more reliable comparison between them, bearing in mind that they had been growing in equal environmental conditions. In our

296 previous studies, contents of secondary metabolites in leaf extracts were observed to be corre-
297 lated with monthly precipitation amounts and mean monthly temperature (11, 18). Based on the
298 comparison of the results obtained for the samples harvested from the shared locations
299 (Medvednica and Fužine), it could be observed that *P. albus* contained more (poly)phenolic
300 compounds and more flavonoids than *P. hybridus*. The total phenolic content of these popula-
301 tions of *P. albus* was also higher than those of *P. kablikianus* and *P. paradoxus*. On the other
302 hand, the latter two species contained the most flavonoids. These results make the three species
303 of *Petasites* potentially interesting in terms of their possible exploitation as sources of natural
304 antioxidants and/or utilization as media in green synthesis of nanoparticles.

305 *Considerations regarding the use of* Petasites *species in green synthesis*

306 Green synthesis is a subdivision of green chemistry, which aims to develop safer and more
307 sustainable chemical products and procedures. The fundamental green chemistry principles that
308 are applied in green synthesis include environmental pollution mitigation, renewable feedstock
309 usage, usage of non-toxic (or safer) solvents/auxiliaries, derivatives usage minimization, and
310 waste prevention or reduction (40). Green synthesis of nanoparticles from biomass and waste,
311 as an eco-friendly, biocompatible, and cost-effective approach for use in medicine, agriculture,
312 environmental remediation, and other fields, is believed to allow up to 30 % reduction in energy
313 consumption, up to 40 % cost savings, and up to 50 % production increase and could therefore,
314 contribute to a more sustainable future (41). Compared to other green synthesis methods of
315 nanoparticles, plant-mediated synthesis is the most efficient (40).

316 Lately, the number of papers suggesting the use of *P. hybridus* rhizomes in green synthesis
317 is on the rise (3, 4). The root extract of this plant is considered a renewable, mild, and safe
318 reducing agent and effective stabilizer (4). Moreover, utilization of *P. hybridus* leaf extract has
319 also been demonstrated for the same purpose (42). However, caution should be taken consider-

ing that *Petasites* species are natural sources of highly hepatotoxic and carcinogenic pyrrolizidine alkaloids (PAs), especially in their underground parts (9, 43). Although the right mechanism by which butterbur preparations might cause liver injury is not known, it is suggested that liver related adverse effects of commercially available butterbur products were likely connected to PAs contamination or mislabelling of the products (44). With that in mind, although the use of these extracts may be possibly advantageous, it may also be potentially harmful, even when low levels of PAs are present.

Leaves, which were used for *Petasites* extract preparation in this study, could potentially serve as a better and more renewable source of biologically active compounds (*e.g.*, those acting as reducing agents in the synthesis of nanoparticles/nanomaterials) in comparison to roots/rhizomes, considering their expected higher content of reducing substances/antioxidants (33) and significantly lower PAs content (9, 43). Leaves of other species such as *Eucalyptus* sp., *Thymus vulgaris* L., and *Ginkgo biloba* L. have been successfully used to synthesize nanoparticles. However, the main research focus should be put on materials that are not limited by seasonal and geographical availability (45).

Utilization of leaves from *Petasites* species could be potentially interesting due to the worldwide distribution of these species and their large size (1). The results of our study, although based on simple spectrophotometric reactions and a relatively small number of samples, indicate that *P. albus*, *P. kablikianus* and *P. paradoxus* may exhibit similar, if not better, antioxidant/reducing properties to those of *P. hybridus*. Considering the need to minimize the risk from exposure to PAs, it would be interesting to compare pyrrolizidine alkaloid contents of *P. hybridus* and related species that may be locally available in future studies. Also, since information on the phytochemical composition/constituents, especially those including phenolic acids, flavonoids, and other polyphenols of *P. albus*, *P. kablikianus* and *P. paradoxus* as well as most other *Petasites* species are lacking or are relatively modest in the case of *P. hybridus*, it

345 would be interesting to evaluate those as well, having in mind their potential antioxidant (re-
346 ducing) properties important for green synthesis.

347

Considerations regarding the use of standardized extracts of Petasites hybridus *leaves*

Up to now, five randomized controlled trials evaluated the use of *P. hybridus* standardized leaf extract (Tesalin – Ze 339) for allergic rhinitis among adults and children and, to the best of our knowledge, the extract has been used without reported serious side effects (6, 46). Also, in a randomized, placebo-controlled trial, a fixed herbal drug combination composed of four plant extracts including *P. hybridus* leaf extract (Ze 185) was recorded to be efficacious and safe in short-term treatment of patients with somatoform disorders (47) and the same preparation was observed to reduce self-reported anxiety response to stress in healthy men (48). The mentioned extract Ze 339 is obtained by supercritical CO_2 fluid extraction (SFE-CO_2) and is standardized to 8 mg petasins (petasin, isopetasin, and neopetasin) as active substances (49). Phenolic acids and flavonoids have not been considered as important biologically active compounds present in this extract.

Together with ultrasound-assisted extraction (UAE), pressurized liquid extraction, and microwave-assisted extraction, SFE-CO_2, is a green chemistry method used for extraction and isolation of bioactive compounds from plants-based materials. It is considered as one of the best techniques for obtaining flavonoids, essential oils, and other natural chemical components from natural plant materials (50). Flower extracts obtained by SFE-CO_2, which have shown anti-inflammatory activity, were often characterized by flavonoids (*e.g.*, quercetin, kaempferol, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rutinoside) and phenolic acids as their major products (51). Similarly, a recent study on the flavonoid yield and profile of *Ziziphus jujuba* leaves indicated that, compared with conventional Soxhlet extraction and UAE, SFE-CO_2 with ethanol as a cosolvent may provide an extract with significantly increased flavonoid yield, antioxidant activity and antiproliferative activity (52). The flavonoids compounds identified in the study were kaempferol and quercetin glycosides including some that may be found in *Petasites* species such as rutin and quercetin-3-*O*-glucoside.

It has been shown that the extract Ze 339 is five times as active as purified petasin indicating that other constituents present in the plant material (extract matrix) may influence the biopharmaceutical properties of active ingredients. However, variations of fatty acids (17.1 – 27.2 %), crude oil and fat (17.7 – 44.2 %), sterols (3.0 – 4.9 %) and essential oils (1.3 – 10.5 %) observed in a quantitative analysis of twelve extract batches did not result in significant differences in inhibition of leukotriene synthesis (49). Polyphenolic compounds are important plant specialized metabolites, whose use may provide health promoting effects due to their diverse biological activities including, but not limited to antioxidant, anti-inflammatory, and antiallergic activity, making them interesting for industries such as food and pharmaceutical (53). It is possible that some of these compounds also contribute to the observed beneficial activities of butterbur and its preparations. Recently, *P. japonicus* leaf extract, which was standardized to 3,4-dicaffeoylquinic acid (0.02 μg g^{-1}), 3,5-dicaffeoylquinic acid (0.15 μg g^{-1}), and 4,5-dicaffeoylquinic acid (0.43 μg g^{-1}), showed neuroprotective activity against amyloid beta 25-35 protein fragment ($A\beta_{25–35}$) plaque neurotoxicity *in vitro* and *in vivo* (54). The results of our study indicate that antioxidant activity could be connected to the total phenolic content of these extracts. Amounts of these compounds in *Petasites* samples harvested from different locations may vary significantly, just as those of other biologically active substances, which in turn could influence the overall activity of the extracts. Therefore, it may be interesting to analyze the contents of these compounds in the original plant material used for marketing as well as in different batches of marketed products and evaluate their possible contribution to the biological activities of pharmaceutical interest.

CONCLUSIONS

It is known that plant species and, consequentially, the herbal preparations obtained from them may vary considerably in the amounts of their chemical constituents such as specialized

(secondary) metabolites, which are mostly associated with their diverse biological activities and consequent effects on human health. Besides comparing, for the first time, the total phenolic content and flavonoid content of four different species of the genus *Petasites*, the results of our study also give insights into the possible variations of these compounds in the two species used in European folk medicine, *P. hybridus* and *P. albus*. The former species is especially pharmaceutically important as it is contained in herbal medicines and dietary supplements in the form of standardized leaf or rhizome extracts. Our evaluation was done on extracts prepared from leaves as a more ecologically sustainable source of *Petasites* bioactive compounds that, from a health perspective, may also be more appropriate considering their initially lower pyrrolizidine alkaloid content. Antioxidant activity of prepared extracts observed in this study was in high correlation with their total phenolic content. Considering that the biological effects of polyphenols and flavonoids, which are potentially beneficial to health, could probably be added to the effects of the bioactive sesquiterpenes (petasins) to which the marketed *Petasites* products are standardized, and considering their possible variabilities observed in this study, it could also be interesting to investigate these compounds in more detail in future studies.

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Authors contributions. – Conceptualization and sample collection, M.F. and K.H.P.; investigation and analysis, M.F. and K.V.; writing, original draft preparation, M.F.; writing, review and editing, M.F., K.V., S.J. and Ž.M.; funding acquisition, Ž.M.; supervision, K.H.P. and Ž.M. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Ł. Kulinowski, S. V. Luca, M. Minceva and K. Skalicka-Woźniak, A review on the ethnobotany, phytochemistry, pharmacology and toxicology of butterbur species (*Petasites* L.), *J. Ethnopharmacol.* **293** (2022) Article ID 115263 (24 pages); https://doi.org/10.1016/j.jep.2022.115263

2. M. Ożarowski, J. Przystanowicz and A. Adamczak, Phytochemical, pharmacological and clinical studies of *Petasites hybridus* (L.) P. Gaertn., B. Mey. & Scherb. A review, *Herba Pol.* **59**(4) (2013) 108–128; https://doi.org/10.2478/hepo-2013-0028

3. M. Avarsaji, Z. Hossaini, A. Varasteh Moradi, H. R. Jalilian and R. Z. Mehrabian, Synthesis and biological activity investigation of new oxazolopyrimidoazepine derivatives: Application of Ag/Fe_3O_4/TiO_2/CuO@MWCNTs MNCs in the reduction of organic pollutants, *Polycycl. Aromat. Comp.* **43**(7) (2023) 5996–6017; https://doi.org/10.1080/10406638.2022.2110903

4. Z. Hossaini, N. Tabarsaei, S. Khandan, P. Valipour and M. Ghorchibeigi, ZnO/Ag/Fe_3O_4 nanoparticles supported on carbon nanotubes employing *Petasites hybridus* rhizome water extract: A novel organometallic nanocatalyst for the synthesis of new naphthyridines, *Appl. Organomet. Chem.* **35**(3) (2021) Article ID e6114 (15 pages); https://doi.org/10.1002/aoc.6114

5. J. Borlak, H.-C. Diener, J. Kleeberg-Hartmann, K. Messlinger and S. Silberstein, *Petasites* for migraine prevention: New data on mode of action, pharmacology and safety. A narrative review, *Front. Neurol.* **13** (2022) Article ID 864689 (16 pages); https://doi.org/10.3389/fneur.2022.864689

6. X. Y. Lim, M. S. Lau, N. A. Zolkifli, U. R. Sastu Zakaria, N. S. Mohd Rahim, N. M. Lai and T. Y. C. Tan, Medicinal plants for allergic rhinitis: A systematic review and meta-analysis,

PLoS One **19**(4) (2024) Article ID e0297839 (32 pages); https://doi.org/10.1371/journal.pone.0297839

7. K. Forsch, V. Schöning, G. M. Assmann, C. Moser, B. Siewert, V. Butterweck and J. Drewe, *In vitro* hepatotoxicity of *Petasites hybridus* extract (Ze 339) depends on the concentration, the cytochrome activity of the cell system, and the species used, *Phytother. Res.* **34**(1) (2020) 184–192; https://doi.org/10.1002/ptr.6516

8. S. Park, J. Lim, K. T. Lee, M. S. Oh and D. S. Jang, Single and repeated oral dose toxicity and genotoxicity of the leaves of butterbur, *Foods* **10**(8) (2021) Article ID 1963 (13 pages); https://doi.org/10.3390/foods10081963

9. E. Wildi, T. Langer, W. Schaffner and K. Berger Büter, Quantitative analysis of petasin and pyrrolizidine alkaloids in leaves and rhizomes of *in situ* grown *Petasites hybridus* plants, *Planta Med.* **64**(3) (1998) 264–267; https://doi.org/10.1055/s-2006-957422

10. R. Chizzola, B. Ozelsberger and T. Langer, Variability in chemical constituents in *Petasites hybridus* from Austria, *Biochem. Syst. Ecol.* **28**(5) (2000) 421–432; https://doi.org/10.1016/S0305-1978(99)00077-0

11. M. Friščić, I. Jerković, Z. Marijanović, S. Dragović, K. Hazler Pilepić and Ž. Maleš, Essential oil composition of different plant parts from Croatian *Petasites albus* (L.) Gaertn. and *Petasites hybridus* (L.) G. Gaertn., B. Mey. & Scherb. (Asteraceae), *Chem. Biodiversity* **16**(3) (2019) Article ID e1800531 (13 pages); https://doi.org/10.1002/cbdv.201800531

12. M. Gündüz, Ş. Karabiyikli Çiçek and S. Topuz, Extraction and optimization of phenolic compounds from butterbur plant (*Petasites hybridus*) by ultrasound-assisted extraction and determination of antioxidant and antimicrobial activity of butterbur extracts, *J. Appl. Res. Med. Aromat. Plants* **35** (2023) Article ID 100491; https://doi.org/10.1016/j.jarmap.2023.100491

13. M. Dal Cero, R. Saller, M. Leonti and C. S. Weckerle, Trends of medicinal plant use over the last 2000 years in Central Europe, *Plants* **12**(1) (2023) 135 (17 pages); https://doi.org/10.3390/plants12010135
14. M. Hiemori-Kondo, Antioxidant compounds of *Petasites japonicus* and their preventive effects in chronic diseases: a review, *J. Clin. Biochem. Nutr*. **67**(1) (2020) 10–18; https://doi.org/10.3164/jcbn.20-58
15. Native American Ethnobotany DB (2003); http://naeb.brit.org/uses/search/?string=petasites+frigidus; last access date July 7, 2024
16. B. Šarić-Kundalić, C. Dobeš, V. Klatte-Asselmeyer and J. Saukel, Ethnobotanical study on medicinal use of wild and cultivated plants in middle, south and west Bosnia and Herzegovina, *J. Ethnopharmacol*. **131**(1) (2010) 33–55; https://doi.org/10.1016/j.jep.2010.05.061
17. P. Janaćković, M. Gavrilović, M. Miletić, M. Radulović, S. Kolašinac and Z. Dajić Stevanović, Small regions as key sources of traditional knowledge: a quantitative ethnobotanical survey in the central Balkans, *J. Ethnobiol. Ethnomed*. **18**(1) (2022) Article ID 70 (68 pages); https://doi.org/10.1186/s13002-022-00566-0
18. M. Friščić, S. Maslo, R. Garić, Ž. Maleš and K. Hazler Pilepić, Comparative analysis of specialized metabolites and antioxidant capacity *in vitro* of different natural populations of *Globularia* spp. *Acta Bot. Croat*. **77**(1) (2018) 1–9; https://doi.org/10.1515/botcro-2017-0017
19. I. Dominguez-López, M. Pérez and R. M. Lamuela-Raventós, Total (poly)phenol analysis by the Folin-Ciocalteu assay as an anti-inflammatory biomarker in biological samples, *Crit. Rev. Food Sci. Nutr.* (2023) 1–7; https://doi.org/10.1080/10408398.2023.2220031
20. M. Pérez, I. Dominguez-López and R. M. Lamuela-Raventós, The chemistry behind the Folin-Ciocalteu method for the estimation of (poly)phenol content in food: Total phenolic

intake in a Mediterranean dietary pattern, *J. Agric. Food Chem*. **71**(46) (2023) 17543−17553; https://doi.org/10.1021/acs.jafc.3c04022

21. E. M. Ahn, G. Asamenew, H. W. Kim, S. H. Lee, S.-M. Yoo, S.-M. Cho, Y.-S. Cha and M.-S. Kang, Anti-obesity effects of *Petasites japonicus* (meowi) ethanol extract on RAW 264.7 macrophages and 3T3-L1 adipocytes and its characterization of polyphenolic compounds, *Nutrients* **12**(5) (2020) Article ID 1261 (16 pages); https://doi.org/10.3390/nu12051261
22. R. Jaiswal, J. Kiprotich and N. Kuhnert, Determination of the hydroxycinnamate profile of 12 members of the Asteraceae family, *Phytochemistry* **72**(8) (2011) 781–790; https://doi.org/10.1016/j.phytochem.2011.02.027
23. A. Pękal and K. Pyrzynska, Evaluation of aluminium complexation reaction for flavonoid content assay, *Food Anal. Methods* **7** (2014) 1776–1782; https://doi.org/10.1007/s12161-014-9814-x
24. D. Mammen, *Chemical Perspective and Drawbacks in Flavonoid Estimation Assays*, in *Frontiers in Natural Product Chemistry*, Vol. 10 (Ed. Atta-ur-Rahman), Bentham Science Publishers Pte. Ltd, Singapore 2022, pp. 189–228.
25. Y. Zhang, F. Guo, P. Zeng, Q. Jia, Y. Li, W. Zhu and K. Chen, [Phenolic components from *Petasites tricholobus*], *Zhongguo Zhongyao Zazhi* **37**(12) (2012) 1782–1787; https://doi.org/10.4268/cjcmm20121219
26. İ Gulcin and S. H. Alwasel, DPPH radical scavenging assay, *Processes* **11**(8) (2023) Article ID 2248 (20 pages); https://doi.org/10.3390/pr11082248
27. M. Platzer, S. Kiese, T. Herfellner, U. Schweiggert-Weisz, O. Miesbauer and P. Eisner, Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays, *Molecules* **26**(5) (2021) Article ID 1244 (17 pages); https://doi.org/10.3390/molecules26051244

28. Y. Zhong and F. Shahidi, *Methods for the Assessment of Antioxidant Activity in Foods*, in *Handbook of Antioxidants for Food Preservation* (Ed. F. Shahidi), Woodhead Publishing Ltd, Cambridge 2015, pp. 287–333.

29. S. M. Kim, S. W. Kang, S.-J. Jeon, Y.-J. Jung, C. Y. Kim, C. H. Pan and B.-H. Um, Rapid identification and evaluation of antioxidant compounds from extracts of *Petasites japonicus* by hyphenated-HPLC techniques, *Biomed. Chromatogr*. **26**(2) (2012) 199–207; https://doi.org/10.1002/bmc.1646

30. M. Hiemori-Kondo and M. Nii, *In vitro* and *in vivo* evaluation of antioxidant activity of *Petasites japonicus* Maxim. flower buds extracts, *Biosci. Biotechnol. Biochem*. **84**(3) (2020) 621–632; https://doi.org/10.1080/09168451.2019.1691913

31. A. Jahn and M. Petersen, Fukinolic acid and cimicifugic acids: a review, *Phytochem. Rev.* **21** (2022) 1247–1271; https://doi.org/10.1007/s11101-021-09781-1

32. M. Mohammadi, M. Yousefi, Z. Habibi and D. Dastan, Chemical composition and antioxidant activity of the essential oil of aerial parts of *Petasites albus* from Iran: a good natural source of euparin, *Nat. Prod. Res*. **26**(4) (2012) 291–297; https://doi.org/10.1080/14786410903374819

33. J. Y. Choi, K. T. Desta, V. V. G. Saralamma, S. J. Lee, S. J. Lee, S. M. Kim, A. Paramanantham, H. J. Lee, Y.-H. Kim, H.-C. Shin, J.-H. Shim, M. Warda, A. Hacımüftüoğlu, J. H. Jeong, S. C. Shin, G.-S. Kim and A. M. Abd El-Aty, LC–MS/MS characterization, anti-inflammatory effects and antioxidant activities of polyphenols from different tissues of Korean *Petasites japonicus* (Meowi), *Biomed. Chromatogr*. **31**(12) (2017) Article ID e4033 (9 pages); https://doi.org/10.1002/bmc.4033

34. T. Mihajilov-Krstev, B. Jovanović, B. Zlatković, J. Matejić, J. Vitorović, V. Cvetković, B. Ilić, Lj. Đorđević, N. Joković, D. Miladinović, T. Jakšić, N. Stanković, V. Stankov Jovanović and N. Bernstein, Phytochemistry, toxicology and therapeutic value of *Petasites*

547 *hybridus* subsp. *ochroleucus* (common butterbur) from the Balkans, *Plants* **9**(6) (2020) Ar-
548 ticle ID 700 (15 pages); https://doi.org/10.3390/plants9060700
549 35. M. Friščić, M. Štibrić Baglama, M. Milović, K. Hazler Pilepić and Ž. Maleš, Content of
550 bioactive constituents and antioxidant potential of *Galium* L. species, *Croat. Chem. Acta*
551 **91**(3) (2018) 411–417; https://doi.org/10.5562/cca3379
552 36. T. Marić, M. Friščić, Z. Marijanović, Ž. Maleš and I. Jerković, Comparison of volatile or-
553 ganic compounds of *Sideritis romana* L. and *Sideritis montana* L. from Croatia, *Molecules*
554 **26**(19) (2021) 5968 (20 pages); https://doi.org/10.3390/molecules26195968
555 37. T. Nikolić. *Flora Croatica – vaskularna flora Republike Hrvatske*, Vol. 2, Alfa, Zagreb
556 2020, pp. 551–552.
557 38. T. Nikolić (ed.) (2005-onwards): Flora Croatica Database, University of Zagreb, Faculty of
558 Science, Department of Botany and Botanical Garden, Zagreb; http://hirc.botanic.hr/fcd;
559 last access date July 7, 2024
560 39. M. Miliša, A. Belančić, R. Matoničkin Kepčija, M. Sertić-Perić, A. Ostojić and I. Habdija,
561 Calcite deposition in karst waters is promoted by leaf litter breakdown and *vice versa*, *Ann.*
562 *Limnol. – Int. J. Lim.* **46** (2010) 225–232; https://doi.org/10.1051/limn/2010023
563 40. N. S. Alsaiari, F. M. Alzahrani, A. Amari, H. Osman, H. N. Harharah, N. Elboughdiri and
564 M. A. Tahoon, Plant and microbial approaches as green methods for the synthesis of nano-
565 materials: Synthesis, applications, and future perspectives, *Molecules* **28**(1) (2023) Article
566 ID 463 (38 pages); https://doi.org/10.3390/molecules28010463
567 41. A. I. Osman, Y. Zhang, M. Farghali, A. K. Rashwan, A. S. Eltaweil, E. M. Abd El-Monaem,
568 I. M. A. Mohamed, M. M. Badr, I. Ihara, D. W. Rooney, P.-S. Yap, Synthesis of green
569 nanoparticles for energy, biomedical, environmental, agricultural, and food applications: A
570 review, *Environ. Chem. Lett.* **22** (2024) 841–887; https://doi.org/10.1007/s10311-023-
571 01682-3

42. M. A. Khalilzadeh, S. Tajik, H. Beitollahi and R. A. Venditti, Green synthesis of magnetic nanocomposite with iron oxide deposited on cellulose nanocrystals with copper (Fe_3O_4@CNC/Cu): Investigation of catalytic activity for the development of a venlafaxine electrochemical sensor, *Ind. Eng. Chem. Res.* **59**(10) (2020) 4219−4228; https://doi.org/10.1021/acs.iecr.9b06214

43. B. Debrunner and B. Meier, *Petasites hybridus*: A tool for interdisciplinary research in phytotherapy, *Pharm. Acta Helv*. **72**(6) (1998) 359−380; https://doi.org/10.1016/S0031-6865(97)00027-7

44. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Butterbur, updated 2019 Feb 18; https://www.ncbi.nlm.nih.gov/books/NBK547997/; last access date July 7, 2024

45. S. Ying, Z. Guan, P. C. Ofoegbu, P. Clubb, C. Rico, F. He and J. Hong, Green synthesis of nanoparticles: Current developments and limitations, *Environ. Technol. Innov*. **26** (2022) 102336 (20 pages); https://doi.org/10.1016/j.eti.2022.102336

46. M. Blosa, J. Uricher, S. Nebel, C. Zahner, V. Butterweck and J. Drewe, Treatment of early allergic and late inflammatory symptoms of allergic rhinitis with *Petasites hybridus* leaf extract (Ze 339): Results of a noninterventional observational study in Switzerland, *Pharmaceuticals* **14**(3) (2021) Article ID 180 (13 pages); https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7996175/

47. J. Melzer, E. Schrader, A. Brattström, R. Schellenberg and R. Saller, Fixed herbal drug combination with and without butterbur (Ze 185) for the treatment of patients with somatoform disorders: Randomized, placebo-controlled pharmaco-clinical trial, *Phytother. Res.* **23**(9) (2009) 1303–1308; https://doi.org/10.1002/ptr.2771

48. S. Meier, M. Haschke, C. Zahner, E. Kruttschnitt, J. Drewe, E. Liakoni, F. Hammann and J. Gaab, Effects of a fixed herbal drug combination (Ze 185) to an experimental acute stress

setting in healthy men – An explorative randomized placebo-controlled double-blind study, *Phytomedicine* **39** (2018) 85–92; https://doi.org/10.1016/j.phymed.2017.12.005

49. U. Kodjadjiku, B. Nägele, C. Halbsguth and V. Butterweck, Extract matrix composition does not affect *in vitro* leukotriene inhibitory effects of the *Petasites hybridus* extract Ze 339, *Fitoterapia* **153** (2021) Article ID 104986 (6 pages); https://doi.org/10.1016/j.fitote.2021.104986
50. P. A. Uwineza and A. Waśkiewicz, Recent advances in supercritical fluid extraction of natural bioactive compounds from natural plant materials, *Molecules* **25**(17) (2020) Article ID 3847 (23 pages); https://doi.org/10.3390/molecules25173847
51. L. López-Hortas, P. Rodríguez, B. Díaz-Reinoso, M. C. Gaspar, H. C. de Sousa, M. E. M. Braga and H. Domínguez, Supercritical fluid extraction as a suitable technology to recover bioactive compounds from flowers, *J. Supercrit. Fluids* **188** (2022) Article ID 105652 (21 pages); https://doi.org/10.1016/j.supflu.2022.105652
52. L. Song, P. Liu, Y. Yan, Y. Huang, B. Bai, X. Hou and L. Zhang, Supercritical CO_2 fluid extraction of flavonoid compounds from Xinjiang jujube (*Ziziphus jujuba* Mill.) leaves and associated biological activities and flavonoid compositions, *Ind. Crop. Prod.* **139** (2019) Article ID 111508 (8 pages); https://doi.org/10.1016/j.indcrop.2019.111508
53. N. B. Rathod, N. Elabed, S. Punia, F. Ozogul, S.-K. Kim and J. M. Rocha, Recent developments in polyphenol applications on human health: A review with current knowledge, *Plants* **12**(6) (2023) Article ID 1217 (30 pages); https://doi.org/10.3390/plants12061217
54. N. Kim, J. G. Choi, S. Park, J. K. Lee and M. S. Oh, Butterbur leaves attenuate memory impairment and neuronal cell damage in amyloid beta-induced Alzheimer's disease models, *Int. J. Mol. Sci.* **19**(6) (2018) Article ID 1644 (15 pages); https://doi.org/10.3390/ijms19061644