Antioxidant compounds of *Petasites japonicus* and their preventive effects in chronic diseases: a review

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*Petasites japonicus* (P. japonicus) is a plant of the Asteraceae family that is native to Japan. Sesquiterpenoids, lignans, and flavonoids are components of *P. japonicus*. Regarding the biological activity of *P. japonicus*, its anti-allergic effect has been researched extensively using IgE antigen-stimulated degranulation of RBL-2H3 cells or passive cutaneous anaphylaxis reaction in experimental animal models. The study of the antioxidant activity of *P. japonicus* was initiated approximately 15 years ago using *in vitro* assays. In addition, its *in vivo* effect has also been examined in animal models with induced oxidative injury. Moreover, recently, many types of antioxidant compounds have been rapidly identified using the liquid chromatography–mass spectrometry technique. The number of reports on the other functions of this plant, such as its neuroprotective and anti-inflammatory effects, has been increasing. In this review, I summarized the studies of functional foods derived from *P. japonicus*, which may provide a basis for the development of potential functional foods. Finally, I discuss the future research avenues in this field.

**Key Words:** *Petasites japonicus*, antioxidant activity, anti-allergy, neuroprotection, metabolic improvement

Antioxidant Compounds and *in vitro* Antioxidant Activity of *P. japonicus*

The antioxidant activity of the extracts from different tissues of *P. japonicus* was examined in various *in vitro* systems, such as the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and ferrie-reducing ability of plasma (FRAP) assays. Moreover, its antioxidant compounds were identified using a combination of an antioxidant assay with high-performance liquid chromatography (HPLC), liquid chromatography–tandem mass spectrometry (LC–MS/MS), and NMR techniques (Table 1). Matsuura et al. screened for antioxidative compounds in the flower buds of *P. japonicus* subsp. giganteus Kitam using the HPLC–DPPH method, and identified caffeic acid and several quercetin glucosides by HPLC coupled to a diode array detector, as well as 1H-NMR and flash desorption mass spectrometry analyses. In *P. formosanus*, petasiformin A was identified as a phenylpropanol sulfonic acid with DPPH radical scavenging activity. In *P. japonicus*, petasignolide A is purified a new furofuran lignan with antioxidant activity. Kim et al. purified and isolated kaempferol as the active compounds of the stems of *P. japonicus*. The antioxidant activity of the active compound was examined by DPPH radical scavenging assay, thiobarbituric acid-reactive substance (TBARS) assay in the linoleic acid model system, and lipoxygenase inhibition assay. Moreover, several varieties of plants grown in Japan

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Furthermore, *P. japonicus* subsp. giganteus Kitam, a subspecies of *P. japonicus*, is cultivated in the northern area of the Kanto region; its leaves are very large and extend upward. Rawan-buki grows naturally in Hokkaido and is a kind of *P. japonicus* subsp. giganteus Kitam.

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*Petasites japonicus* (P. japonicus) is a plant of the Asteraceae family. Its roots and stems have been used for the treatment or the prophylaxis of migraine and tension headache as a traditional Chinese medicine in Japan and Korea. Sesquiterpenoids, lignans, and flavonoids are components of *P. japonicus*. Regarding the biological activity of *P. japonicus*, its anti-allergic effect has been researched extensively using IgE antigen-stimulated degranulation of RBL-2H3 cells or passive cutaneous anaphylaxis reaction in experimental animal models. The study of the antioxidant activity of *P. japonicus* was initiated approximately 15 years ago using *in vitro* assays. In addition, its *in vivo* effect has also been examined in animal models with induced oxidative injury. Moreover, recently, many types of antioxidant compounds have been rapidly identified using the liquid chromatography–mass spectrometry technique. The number of reports on the other functions of this plant, such as its neuroprotective and anti-inflammatory effects, has been increasing. In this review, I summarized the studies of functional foods derived from *P. japonicus*, which may provide a basis for the development of potential functional foods. Finally, I discuss the future research avenues in this field.

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Table 1. Analysis and identification of antioxidant compounds in *P. japonicus*

| Assay                                      | Compound (Source, part, and fraction)                                                                                                                                  | Author Ref.                                                                 |
|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| HPLC–DPPH                                  | Quercetin 3-O-β-D-glucoside, quercetin 3-O-β-D-6″-O-acetylglucoside, rutin, caffeic acid (70% ethanol extraction of *P. japonicus* subsp. gigantea Kitam. flower bud) | Matsuura et al. (2002)                                                                                                        |
| DPPH radical scavenging assay              | Petasformin A (leaves of *P. formosanus* KITAMURA)                                                                                                                  | Lin et al. (2004)                                                                                                              |
| DPPH radical scavenging assay              | Petaslinigoid A [n-butanol fraction of the methanolic extract of *P. japonicus* (Sieb. et Zucc.) Maxim. leaves]                                                          | Min et al. (2005)                                                                                                              |
| Scavenging superoxide anion, NO, DPPH, radical scavenging, Raw 264.7 | Chlorogenic acid, fukinolic acid, 3,5-dicaffeoyl quinic acid, and 3,4,5-tricaffeoyl quinic acid (leaves of *P. japonicus* Fr. Schmidt) | Watanabe et al. (2007)                                                                                                        |
| DPPH radical scavenging assay              | Kaempferol ( *P. japonicus* stem)                                                                                                                                 | Kim et al. (2008)                                                                                                              |
| HPLC system with post-column online antioxidant detection based on ABTS⁺ radical scavenging activity | 5-Caffeoylquinic acid, fukinolic acid, 3,5-di-O-cafeoylquinic acid, quercetin-3-O-(6″-acetyl)-β-D-glucopyranoside, 4,5-di-O-cafeoylquinic acid, and kaempferol-3-O-(6″-acetyl)-β-D-glucopyranoside (methanol extract of *P. japonicus* leaves and roots) | Kim et al. (2012)                                                                                                              |
| Aldose reductase inhibition on rat lenses   | Kaempferol-3-O-(6″-acetyl)-β-D-glucoside, quercetin-3-O-(6″-acetyl)-β-D-glucoside, kaempferol-3-O-β-D-glucoside, quercetin-3-O-(6″-acetyl)-β-D-glucoside (methanol extract of *P. japonicus* leaves) | Lee et al. (2015)                                                                                                              |
| Scavenging activity against superoxide anion radical, LLC-PK1 cells | Ethyl acetate extract of *P. japonicus* (high polyphenol and flavonoid content)                                                                                       | Kim et al. (2016)                                                                                                              |
| DPPH scavenging activity, ABTS⁺ scavenging activity, superoxide radical scavenging activity, FRAP assays, RAW 264.7 | 3,5-Dihydroxy-7,3′,4′,5′-tetramethoxy flavanone hydroxy feruloyl glucose, dicaffeoylquinic acid, naringenin hexoside, luteolin-7-O-[6″-dihydroxypropyl]-glucosyl-8-C-pentosyl-glucoside, liquiritin, 3,4-di-O-cafeoylquinic acid, 1,3-O-dicaffeoylquinic acid hexoside, kaempferol-3-O-acetylglucoside, chrysoseryl-methyl ether (Korean *P. japonicus* leaves, stems, and roots) | Choi et al. (2017)                                                                                                              |
| HPLC–DPPH, ORAC                            | 3-O-Caffeoylquinic acid, fukinolic acid, 3,4-di-O-cafeoylquinic acid, 3,5-di-O-cafeoylquinic acid, and 4,5-di-O-cafeoylquinic acid (80% ethanol extract of *P. japonicus* (Sieb. et Zucc.) Maxim. flower bud) | Hiemori-Kondo et al. (2020)                                                                                                     |

HPLC–DPPH, high performance liquid chromatography-1,1-diphenyl-2-picrylhydrazyl; NO, nitric oxide; TBARS, thiobarbituric acid-reactive substance; ABTS⁺, 2-2′-azino-bis(3-ethylbenothiazoline-6-sulfonic acid); FRAP, ferric-reducing ability of plasma; ORAC, oxygen radical absorbance capacity.

compounds such as caffeoylquinic acids and its isomer, quercetin, kaempferol glycosides, and fukinolic acid in the leaves and roots were identified. Among them, 3,5-di-O-cafeoylquinic acid exhibited the greatest radical-scavenging capacity, as assessed using an HPLC system with post-column online antioxidant detection based on 2-2′-azino-bis(3-ethylbenothiazoline-6-sulfonic acid) (ABTS⁺) radical-scavenging activity. (10) Lee et al. (27) identified four flavonoids in *P. japonicus* leaves and reported that quercetin-3-O-β-D-glucoside, which was extracted among these flavonoids, showed the highest aldose reductase inhibitory activity on rat lens and was a potent agent against diabetic complications.

With the advancement of analyses and compound identification based on LC–MS/MS, antioxidant compounds have been identified rapidly using on-line HPLC–DPPH or on-line ABTS⁺. Choi et al. (29) analyzed 10 components, including catechin, di-cafeoylquinic acid isomers, and naringenin, luteolin, liquiritin, kaempferol, and chrysoseryl derivatives and examined the antioxidant activity of extracts from the roots, stems, and leaves of Korean *P. japonicus* (Meowi) using DPPH, ABTS⁺, superoxide radical scavenging activities, and FRAP assays. Moreover, those authors also reported the anti-inflammatory effects of these compounds. We evaluated the antioxidant activity of an 80% ethanol extract of the flower buds of *P. japonicus* using oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging activity. The ORAC values were attributed to H-ORAC; therefore, the trends in the results of the DPPH radical scavenging assay were consistent with those of the ORAC assay. Moreover, the antioxidative compounds that were determined using HPLC–DPPH methods and identified and quantified using LC–MS/MS included six antioxidant active compounds: caffeic acid, 3-O-

caffeoylquinic acid [3-O-cafeoylquinic acid (chlorogenic acid)], fukinolic acid, and three di-cafeoylquinic acids (3,4-di-O-cafeoylquinic acid, 3,5-di-O-cafeoylquinic acid, and 4,5-di-O-cafeoylquinic acid). Fukinolic acid and 3,4-di-O-cafeoylquinic acid are major active compounds based on their activity and abundance. (30) Conversely, Watanabe et al. (23) reported that DPPH was epigallocatechin-3-O-gallate<fukinolic acid>chlorogenic acid and that the order of potency of the scavenging hydroxyl radical was epigallocatechin-3-O-gallate<fukinolic acid>gallic acid based on a mouse macrophage Raw 264.7 cell assay.

As mentioned above, the representative antioxidant components are caffeic acid, di-cafeoylquinic acid, fukinolic acid, and quercetin glycosides. The difference in their composition seems to depend on the tissue, the method of extraction, and the assay. Caffeic acid, caffeoylquinic acid, and quercetin glycosides are widely distributed in the plant kingdom, while fukinolic acid is specific to *P. japonicus*. The structures of fukinolic acid and fukic acid in *P. japonicus* were reported by Sakamura et al. (31) in 1973, which yield enzymatic browning substances by oxidation. Black cohosh (*Actaea racemose*) is used as an herb in America and Europe and is a member of the Ranunculaceae family that contains caffeic acid and fukinolic acid, which is a derivative of caffeic acid. (31) *Cimicifuga heracleifolia* is also closely related to the genus *Actaea*. These plants contain fukinolic acid and cimicifugic acids, which are caffeic acid derivatives with documented antioxidant activities. (31)

Furthermore, the antioxidant activities of *P. japonicus* were examined using an *in vitro* assay with the cell lines Raw 264.7 and HCT-116, a human colorectal carcinoma cell line. Nitric oxide (NO) production was inhibited by fukinolic acid, as a main
Mg/ml), and Angelica gigas extracts of assays to evaluate the antioxidant activity of the flower bud with kainic acid have been reported in mouse brain based on or the butanol extract from the leaves of are performed (Table 2). Antioxidative effects of petaslignolide A in addition, antioxidant activities of the methanol extract of acetate fraction of epithelial cell line of renal origin, it was shown that the ethyl Moreover, based on an assay that used LLC-PK1 cells, an related factor 2 (Nrf2) signaling pathway.

Table 2. In vivo antioxidant activity of P. japonicus and its derived compounds

| Animal model                  | Effect and mechanism                                                                 | Source, part (fraction), and compounds                  | Author                          | Ref. |
|-------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------|--------------------------------|------|
| Kainic acid-challenged mice    | Restore TBARS values and cytosolic GSH levels in the brain                            | P. japonicus butanol extract (400 mg/kg) gavage for 4 days | Oh et al. (2005)                | (34) |
| Kainic acid-challenged mice    | Restore TBARS values and cytosolic GSH levels in the brain                            | Petaslignolide A in P. japonicus (Sieb. et Zucc.) Maxim. leaves (40 mg/kg for 4 days) | Cui et al. (2005)                | (35) |
| Kainic acid-treated mice       | Antioxidant and antiseizure activities                                               | Petaslignolide A in P. japonicus (Sieb. et Zucc.) Maxim. leaves (50 mg/kg for 4 days) | Min et al. (2005)                | (4)  |
| Alcohol-treated Sprague- Dawley rats | Suppression in the decrease in AST activity, suppression or increase in the hepatic activities of catalase and GSH-Px, and in GSH levels | Methanol extract of P. japonicus (1.0 g/kg)             | Park (2007)                     | (37) |
| CCl4-induced lipid peroxidation, hepatotoxicity in rats | Increase in anti-lipid peroxidative effects and decrease in the levels of GOT, GPT, ALP, BUN, and cholesterol | The butanal fraction from the methanol extract of butterbur (P. japonicus Max.) leaves (0.1% or 0.3% for 1 week and on day 7) | Park et al. (2010)               | (38) |
| Monosodium L-glutamate-treated ICR mice | Improvement in plasma lipid profiles and decrease in oxidative stress by the upregulation of hepatic antioxidant enzymes | P. japonicus leaves and its acetone extract (5% leaf powder for 4 weeks) | Han et al. (2012)                | (39) |
| F344/DuCrj rats                | Increased liver weight, increased TBARS and glutathione levels in the serum and liver, and hepatic GR and GST activities | P. japonicus (Sieb. et Zucc.) Maxim. flower bud (80% ethanol extract) (8 g of powder base/kg or 1% for 16 weeks) | Hiemori-Kondo et al. (2020)      | (30) |
| Iron-induced oxidative ICR mice, plasma TBARS of C57BL/6 mice fed with a high-fat diet | Suppression in plasma TBARS production in ICR mice, plasma TBARS, and decrease in triglyceride concentrations in C57BL/6 mice | P. japonicus (Sieb. et Zucc.) Maxim. flower bud (80% ethanol extract) (8 g of powder base/kg or 1% for 16 weeks) | Hiemori-Kondo et al. (2020)      | (30) |

TBARS, thiobarbituric acid-reactive substance; GSH, glutathione; AST, aspartate aminotransferase; GSH-Px, glutathione peroxidase; CCl4, carbon tetrachloride; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; GR, glutathione reductase; GST, glutathione S-transferase.

phenolic constituent in P. japonicus. Moreover, the polyphenolic extracts of leaves and roots exhibited anti-inflammatory effects by inducing the levels of the lipopolysaccharide-activated cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) proteins. Conversely, its higher cytotoxic activity (IC50<25.0 Mg/ml) against HCT-116 cells compared with that of Angelica gigas (34.75 Mg/ml), Erythronium japonicum (44.06 Mg/ml), and Aster scaber (54.87 Mg/ml) has been shown. Moreover, based on an assay that used LLC-PK1 cells, an epithelial cell line of renal origin, it was shown that the ethyl acetate fraction of P. japonicus exhibited a high antioxidant activity via the upregulation of heme oxygenase 1 and thioredoxin reductases through the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway.

In vivo Antioxidant Activity of P. japonicus

With regard to oxidative stress in vivo, several examinations are performed (Table 2). Antioxidative effects of petaslignolide A or the butanol extract from the leaves of P. japonicus challenged with kainic acid have been reported in mouse brain based on TBARS value. Furthermore, improvement in seizure in kainic acid-treated mice by petaslignolide A has also been reported. In addition, antioxidant activities of the methanol extract of P. japonicus Max. have been demonstrated in monosodium L-glutamate-challenged mice. We performed two types of in vivo assays to evaluate the antioxidant activity of the flower bud extracts of P. japonicus. An animal model of Fe-nitrilotriacetate induced acute oxidative injury and mice fed with normal or high-fat diets were used as models of chronic disorders. The administration of these extracts at a concentration of 1% to C57BL/6 mice fed with high-fat diets for 16 weeks significantly decreased TBARS and triglyceride concentrations in the plasma of the mice, with no toxic symptoms. The effect of a methanol extract of P. japonicus on hepatotoxicity in rats induced by alcohol or carbon tetrachloride was also examined. The extract revealed protective effect and anti-lipid peroxidative effects in liver by decrease in glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase, which is increased in the case of cardiovascular and biliary tract diseases. Cholesterol increased on liver cirrhosis and blood urea nitrogen directed post in liver function also decreased.

In contrast, Han et al. have reported an increase in hepatic TBARS values and glutathione reductase and glutathione S-transferase activities and hepatic cytochrome mRNA expression following diets with 5% acetone extract of P. japonicus leaf powder, as revealed by the presence of pyrrolizidine alkaloids. Therefore, considering that a high amount of antioxidants were required to suppress the acute reaction, the amount of the toxic compound present in the P. japonicus flower bud extracts should be considered.

Anti-Allergic Effect of P. japonicus

The anti-allergic effect of P. japonicus is well known at the research (Table 3). Regarding the former, RBL-2H3 cells from rats with basophilic leukemia with high-affinity IgE receptors are often used. The degranulation of IgE-antigen-stimulated RBL-2H3 cells leads to the release of β-hexosaminidase, similar to that observed for histamine and leukotriene. Therefore, β-hexosaminidase or its cytokine are measured and the inhibitory effect is examined. Yoshikawa et al. reported the degranulation inhibitory effect by fukinoside A from P. japonicus. Shimoda et al. examined the inhibitory effects of an aqueous ethanol extract of...
the aerial parts of Japanese P. japonicus and screened for active compounds. Several compounds, such as fukinones, caffeic acid, and di-cafeoylquinic acids, were identified as inhibitors. In vivo, the inhibitory effect of P. japonicus extracts on allergic reactions was examined using a passive cutaneous anaphylaxis (PCA) reaction on experimental guinea pig, rats, or mice. 

An ovalbumin-induced asthma model was also used to examine the anti-allergic effect of this plant. Recently, eremophilane lactone, a novel family of sesquiterpene compound, were isolated from the aerial parts of Japanese P. japonicus. It has been reported that petasitesin A and cimicifugic acid D inhibit the production of both prostaglandin E2 and NO, and fluorescence change of Ca²⁺, via the high affinity IgE receptor.

### Table 3. Anti-allergic effect

| Assay | Effect and mechanism | Source and compounds | Author | Ref. |
|-------|----------------------|---------------------|--------|-----|
| Guinea pig PCA | Antihistaminic and anti-allergic activities | 6β-hydroxyeremophilenoide and 6β,8-dihydroxyeremophilenoide from the rhizomes of P. japonicus Maxim. var. giganteus Hort. | Tobinaga et al. (1983) | (40) |
| RBL-2H3 mast cells | Inhibition of β-hexosaminidase release; degranulation | Fukinose A from P. japonicus Maxim. | Yoshikawa et al. (2006) | (41) |
| IgE-sensitized RBL-2H3 cells, rat PCA reaction, a guinea pig trachea strip | Inhibition of β-hexosaminidase release (leukotriene C4/D4/E4 synthesis and TNF-α production) and PCA reaction and suppression of smooth muscle constriction induced by histamine and leukotriene D4 | 70% Ethanol extract from aerial parts of Japanese butterbur, (+)-fukinone, caffeic acid, 2β-hydroxyfukinone, chlorogenic acid, fukinolic acid, 4,5-dicafeoylquinic acid, 3,5-dicafeoylquinic acid, 4,5-dicafeoylquinic acid methyl ester, and dotorioside II | Shimoda et al. (2006) | (42) |
| IgE-sensitized RBL-2H3 cells, mouse PCA reaction | Inhibition of IgE antigen-stimulated degranulation, TNF-α and IL-4 cytokine expression and transcription factor NF-κB, IgE-antigen-induced PCA reactions | Ethyl acetate extract from fermented P. japonicus leaves | Bae et al. (2009) | (43) |
| RBL-2H3 mast cells, peritoneal macrophages, ovalbumin-induced asthma model | Inhibition of degranulation, gene inductions of iNOS synthase and COX-2 | Bakkenolide B from P. japonicus (Sieb. et Zucc.) Maxim. leaves | Lee et al. (2013) | (44) |
| RBL-2H3 mast cells, C6 glioma cells, ovalbumin-induced asthma model | Suppression of β-hexosaminidase and fluorescence change of Ca²⁺, inhibition of iNOS induction, NO production, and accumulations of eosinophils, macrophages, and lymphocytes | Petatewalide B from P. japonicus (Sieb. et Zucc.) Maxim. leaves | Choi et al. (2016) | (45) |
| RBL-2H3 mast cells | Inhibition of degranulation activated via high affinity IgE receptor, FcεR1 | 6β-Angeloyloxy-3β,8-dihydroxyeremophil-7(11)-en-12,8β-olide | Qian et al. (2016) | (46) |
| RAW264.7 macrophages, docking studies | Inhibition of the production of both PGE2 and NO, expressions of iNOS and COX-2, and high affinity with iNOS and COX-2 | Bold water extract of the leaves of P. japonicus, petasitesin A and cimicifugic acid D | Lee et al. (2019) | (47) |

PCA, passive cutaneous anaphylaxis; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; NO, nitric oxide; PGE2, prostaglandin E2.

### Neuroprotection by P. japonicus

Neuroprotective and anti-inflammatory activities are examined using in vitro assays with cell lines such as PC12 or B103 (Table 4). The neuroprotective effects of petasignolide A isolated from P. japonicus leaves and of crude butanol extracts of P. japonicus leaves treated with kainic acid have been reported in the mouse brain. Moreover, the ethanol fraction and quercetin and kaempferol 3-O-(6"-acetyl)-β-glucopyranoside on β-secretase 1 (BACE1) production in B103 cells showed the presence of inhibitory activity and reducing the extracellular secretion of amyloid β (Aβ). Many patients with Alzheimer’s disease (AD) have deposition of Aβ in cortical blood vessels, leading to cerebral amyloid angiopathy. Aβ is directly responsible for the free radical production and lipid peroxidation, leading to apoptosis and cellular death. BACE1 is a key enzyme in the production of Aβ because of the deposition of the Aβ-peptide after proteolytic processing of the amyloid precursor protein by BACE1 and γ-secretase during the progression of AD. Therefore, BACE1 is a prime target for therapeutic intervention in AD. In addition, the...
suppression of reactive oxygen species (ROS) and the subsequent recovery of apoptotic cell death by the inhibition of Aβ-induced apoptotic cellular damage, ROS generation, and caspase-3 activity by kaempferol 3-O-(6''-acetyl)-β-glucopyranoside were reported. Song et al. also showed neuroprotective effects on HT22 glutamate-induced oxidative stress cells by the regulation of the expression levels of Bcl-2, Bid, AIF, and MAPK. Kaempferol also showed neuroprotective effects against neuronal cell death of five sesquiterpenes. Ten sesquiterpenes isolated from the whole P. japonicus plant. Wang et al. (2013) reported that high-fat diet induced oxidative stress in PC12 cells have been reported. Recently, protein aggregation has been described as the principal component of numerous protein misfolding pathologies termed proteinopathies, such as AD, Parkinson’s disease, prion diseases, and AA amyloidosis with treatment needs. An automated real-time microtiter-scale high-throughput screening system for amyloid aggregation inhibitors using quantum-dot nanoprobes that can simultaneously screen multiple samples was developed and P. japonicus was assessed. However, subsp. giganteus seemed to have low inhibitory effects. Based on this information, petasin is thought to be a representative candidate for the regulation of obesity. However, the attenuation memory impairment and neuronal cell damage in Aβ-induced AD model using P. japonicus leaves was also demonstrated. The protective effects of sesquiterpenoids against neuronal cell death and its promoting effects on neurite outgrowth from PC12 cells have been reported. Recently, protein aggregation has been described as the principal component of numerous protein misfolding pathologies termed proteinopathies, such as AD, Parkinson’s disease, prion diseases, and AA amyloidosis with treatment needs. An automated real-time microtiter-scale high-throughput screening system for amyloid aggregation inhibitors using quantum-dot nanoprobes that can simultaneously screen multiple samples was developed and P. japonicus was assessed. However, subsp. giganteus seemed to have low inhibitory effects. On the other hand, the anti-neuroinflammatory effects of petatewalide B on lipopolysaccharide-stimulated microglia and its mechanism underlying AMPK-activated protein kinase (AMPK)/Nrf2-signaling pathway have been reported.

Metabolic Improvement by *P. japonicus*

There are few reports of anti-obesic and anti-adipogenic activities (Table 5). Han et al. reported that high-fat diet containing 3% chikusetsusaponins isolated from *P. japonicus* rhizomes significantly increased the fecal content and triacylglycerol level in rats at day 3. In addition, orally administered chikusetsusaponins also exhibited inhibition in the elevation of the plasma triacylglycerol and the pancreatic lipase activity, delaying the intestinal absorption of dietary fat. Lee et al. (2012) demonstrated the inhibitory activity of pancreatic lipase in leaf and stem in vitro. Watanabe et al. have reported that the administration of diets comprising *P. japonicus* ethanol extracts resulted in a decrease in weight gain, visceral fat accumulation, plasma cholesterol, and glucose concentrations in mice fed with a high-fat diets. Its energy expenditure is reported to be upregulated by flavonoids, such as quercetin. The mechanism consists in the suppression of preadipocyte differentiation/three adipogenic transcription factors, the peroxisome proliferator-activated receptor (PPAR) γ, the CCAAT enhancer-binding protein, and the sterol regulatory element-binding protein 1C, with a decrease in body weight, gain and accumulation of visceral fat tissue, and amelioration of the plasma cholesterol concentration. Adachi et al. (2013) reported that petasin modulates glucose metabolism and activates AMPK through the inhibition of mitochondrial respiration. Moreover, S-petasin isolated from *P. japonicus* extracts yielded reduction of glucose uptake and inhibition of triglyceride accumulation by inhibiting the PPAR-γ signaling pathway in the 3T3-L1 cell line. These results indicate that S-petasin has anti-adipogenic activity. Based on this information, petasin is thought to be a representative candidate for the regulation of obesity. However, the mechanism underlying the improvement of metabolic syndrome and obesity is limited by the uptake of glucose and the activation of AMPK. Moreover, S-petasin is the only active compound identified as anti-obesic in *P. japonicus*. Nevertheless, it has been reported that caffeic acid and chlorogenic acid increase body weight, lipid metabolism, and obesity-related hormone levels in mice fed with high-fat diets. Because many compounds occur in *P. japonicus*, as shown in Table 1, the identification of the active compounds and their mechanisms of action in this species warrants further investigation.
tion of other mechanisms and active compounds are needed for the management of metabolic syndrome.

### Anti-Cancer Effect of P. japonicus

Reports on the anti-cancer effects of this plant are scarce (Table 6). Picrasin B isolated from *Picrasma quassioides* inhibited tumor growth and showed antitumor activity against P-388 lymphocyte leukemia cells. In addition, fukinolide isolated from *P. japonicus* showed antitumor activity; however, it was not as strong as that observed by picrasin B. Petasiphenol, a polyphenol from *P. japonicus*, inhibited DNA polymerase activity. Furthermore, the comparison of hepatic TBARS values after diets including a 5% acetone extract of *P. japonicus* flower buds for 15 weeks with those of untreated mice revealed an absence of differences; moreover, a toxic effect was not detected. Several types of pyrrolizidine alkaloids have been identified that are mainly found in plant families such as Asteraceae, Aabaceae, and Oraginaceae. Pyrrolizidine alkaloids are toxic and can cause liver damage and cancer. Therefore, the intake of such extracts may be considered safe for humans. However, because some adverse effects of the absorption of pyrrolizidine alkaloid

### Possible Adverse Effects of P. japonicus and Attention to Pyrrolizidine Alkaloids

As described above, Han et al.(39) reported an increase in hepatic TBARS values after diets including a 5% acetone extract of *P. japonicus* leaf powder. As revealed by the presence of pyrrolizidine alkaloids, Pyrrolizidine alkaloids are toxic and can cause liver damage and cancer. Several types of pyrrolizidine alkaloids have been identified that are mainly found in plant families such as Asteraceae, Aabaceae, and Oraginaceae. Pyrrolizidine alkaloids in *P. japonicus* comprise mainly petasin, neopetasitenine, and senkirukin, while mass signals corresponding to them were not detected. Furthermore, the comparison of the liver and kidney weights of C57BL/6 mice administrated 1% acetone extract of *P. japonicus* flower buds for 15 weeks with those of nontreated mice revealed an absence of differences; moreover, a toxic effect was not observed. However, because the concentrations of pyrrolizidine alkaloids are not sufficient for causing acute poisoning in most cases. Therefore, the intake of such extracts may be considered safe for humans. However, because some adverse effects of the absorption of pyrrolizidine alkaloid...
have been reported, as described above, attention must be paid to the use of large amounts of the extract at once individually, particularly for patients with diseases, pregnant women, or children. Conversely, the concentrations of pyrrolizidine alkaloids can be decreased by boiling and simmering the plant in tap water. Therefore, the reduction of the concentrations of pyrrolizidine alkaloids is recommended before the consumption of the stems or flower buds of *P. japonicus*.

**Conclusion**

In this review, I described the potential pharmacological efficacy of *P. japonicus* extracts or its isolated compounds, such as polyphenols and sesquiterpenes. It can also be a useful bioresource in the production of functional ingredients. However, the bioactive compounds of this plant have not been explored in detail *in vivo*, except for the antioxidant activity of petaslinole A in the brain, usefulness of petatewalide B in anti-asthma, and activities of petasin and chikusetsusaponins in improvement of the metabolism of fat and glucose. *In vivo* examinations were primarily performed using plant powder or crude extracts. Therefore, it is important to identify and purify active compounds for its functional utilization. In particular, it would be interesting to elucidate the *in vivo* effects of bioactive compounds that exist only in *P. japonicus*.

Studies focusing on neuroprotective and anti-inflammatory functions have been increasing, indicating increased concern toward anti-aging to prolong healthy life expectancy. Some mechanisms underlying neuroprotection have been elucidated and toward anti-aging to prolong healthy life expectancy. Some functions have been increasing, indicating increased concern using plant powder or crude extracts. Therefore, it is important to elucidate their bioactivities. Moreover, we must also consider the concerning pyrrolizidine alkaloid is recommended before the consumption of *P. japonicus*.

**Conflict of Interest**

No potential conflicts of interest were disclosed.

**References**

1. Naya K, Takagi I. The structure of petasinit: a new sesquiterpene from *petasites japonicus* maxim. *Tetrahedron Lett* 1968; 9: 629–632.
2. Abe N, Onoda R, Shirahta K, Kato T, Woods MC, Kitahara Y. The structure of bakkenolide-A. *Tetrahedron Lett* 1968; 9: 369–373.
3. Sakamura S, Yoshihara T, Toyoda K. The constituents of *Petasites japonicus*: structures of fukic acid and fukinic acid. *Agric Biol Chem* 1973; 37: 1915–1921.
4. Min BS, Cui HS, Lee HK, Sok DE, Kim MR. A new fuurofuran lignan with antioxidant and antiseizure activities from the leaves of *Petasites japonicus*. *Arch Pharm Res* 2005; 28: 1023–1026.
5. Matsuura H, Amano M, Kawabata J, Mizutani J. Isolation and measurement of quercetin glucosides in flower buds of Japanese butterbur (*Petasites japonicus* subsp. *gigantea* Kitam.). *Biosci Biotechnol Biochem* 2002; 66: 1571–1575.
6. Kim SM, Kang SW, Jeon JS, et al. Rapid identification and evaluation of antioxidant compounds from extracts of *Petasites japonicus* by hplc-tandem mass spectrometry techniques. *Biomed Chromatogr* 2012; 26: 199–207.
7. Diener HC, Rahlfis VW, Danesch U. The first placebo-controlled trial of a special butterbur root extract for the prevention of migraine: reanalysis of efficacy criteria. *Eur Neurol* 2004; 51: 89–97.
8. Lipton RB, Gibbel H, Einhülpel KM, Wilks K, Mauskop A. *Petasites hybridus* root (butterbur) is an effective preventive treatment for migraine. *Neurology* 2004; 63: 2240–2244.
9. Orr SL. The evidence for the role of nutraceuticals in the management of pediatric migraine: a review. *Curr Pain Headache Rep* 2018; 22: 37.
japonicus (Sieb. et Zucc.) Fr. Schmidt) cultivar for fukinoto, “AWAHARUKA”. Bulletin of Tokushima Agriculture, Forestry and Fisheries Technology Support Center 2014; 1: 1–6. (in Japanese)

19 Shibata H, Shimizu S. Three chemovars of Petasites japonicus Maxim. Agric Biol Chem 1978; 42: 1427–1428.

20 Takagi H. Japanese butterbur, Fuki. In: Konishi K, Iwahori S, Kitagawa H, editors. Three chemovars of Petasites japonicus Maxim. Agric Biol Chem 1978; 42: 1427–1428.

21 Heo BG, Park YS, Chon SU, Lee SY, Cho JY, Gorinstein S. Antioxidant activity and cytotoxicity of methanol extracts from aerial parts of Korean salad plants. Biofactors 2007; 30: 79–89.

22 Hwang KA, Hwang YJ, Park DS, Kim J, Om AS. In vitro investigation of antioxidant and anti-apoptotic activities of Korean wild edible vegetable extracts and their correlation with apoptotic gene expression in HepG2 cells. Food Chem 2011; 125: 483–487.

23 Masuda T, Inouchi T, Fujimoto A, et al. Radical scavenging activity of spring mountain herbs in the Shikoku mountain area and identification of antiradical constituents by simple HPLC detection and LC–MS methods. Biosci Biotechnol Biochem 2012; 76: 705–711.

24 Lin CH, Li CY, Wu TS. A novel phenylpropenoyl sulfonic acid and a new chlorophyll from the leaves of Petasites formosanus Kitamura. Chem Pharm Bull (Tokyo) 2004; 52: 1151–1152.

25 Watanabe S, Hashimoto K, Tazaki H, et al. Radical scavenging activity and inhibition of macrophage NO production by fukinolic acid, a main phenolic constituent in Japanese butterbur (Petasites japonicus), Food Sci Technol Res 2007; 13: 366–371.

26 Kim MY, Yi HJ, Hwang YY, Song KS, Jun MR. Isolation and identification of antioxidant substances from the stems of butterbur (Petasites japonicus). J Korean Soc Food Sci Nutr 2008; 37: 979–984. (in Korean)

27 Lee DG, Lee KH, Park KW, et al. Isolation and identification of flavonoids with aldose reductase inhibitory activity from Petasites japonicus Maxim. Biol Med Chromatogr 2017; 31: e4033.

28 Kim JH, Lee J, Lee S, Cho EJ. Ethyl acetate fraction from Petasites japonicus attenuates oxidative stress through regulation of nuclear factor E2-related factor-2 signal pathway in LLC-PK1 cells. Korean J Pharmacogn 2016; 47: 55–61. (in Korean)

29 Choi JY, Desta KT, Saralamma VVG, et al. LC–MS/MS characterization, in vivo evaluation of antioxidant activity of Petasites japonicus Maxim. Phytochem Anal 2014; 25: 298–304. (in Korean)

30 Hiemori-Kondo M, Nii M. Korean J Environ Health 2017; 52: e4033.

31 Choi YW, Lee KP, Kim JM, et al. Petawaiwale B, a novel compound from Petasites japonicus with anti-allergic activity. J Ethnopharmacol 2016; 178: 17–24.

32 Qian F, Guo G, Li Y, Kulkla M. A novel erenmopholine lactone inhibits FcεRI-dependent release of pro-inflammatory mediators: structure-dependent bioactivity. Inflamm Res 2016; 65: 303–311.

33 Lee JS, Jeong M, Park S, et al. Chemical constituents of the leaves of butterbur (Petasites japonicus) and their anti-inflammatory effects. Biomolecules 2019; 9: 806.

34 Kagataki S, Tsunoda T, Moriyama T. Two cases of oral allergy syndrome to “Fukinoto”. Jpn J Dermatol 2006; 116: 331–334. (in Japanese).

35 Tanaka A, Miyaki A, Omokada S, Takata M. Four cases of allergy to the flower stalk of butterbur. Jpn J Clin Dermatol 2010, 64: 743–746. (in Japanese).

36 Kikuchi R, Hanada M, Akasaka T. A case of anaphylactic shock to the flower stalk of butterbur. Jpn J Clin Dermatol 2014; 68: 395–397. (in Japanese).

37 Yagami T. Allergies to cross-reactive plant proteins. Latex-fruit syndrome is comparable with pollen-food allergy syndrome. Int Arch Allergy Immunol 2002; 128: 271–279.

38 Sode DE, Oh SH, Kim YB, Kang HG, Kim MR. Neuroprotection by extract of Petasites japonicus leaves, a traditional vegetable, against oxidative stress in brain of mice challenged with kaenic acid. Eur J Nutr 2006; 45: 61–69.

39 Song KS, Choi SH, Hur JM, et al. Inhibitory effects of flavonoids isolated from leaves of Petasites japonicus on β-secretase (BACE1). Food Sci Biotechnol 2008; 17: 1165–1170.

40 Song KS, Jeong WS, Jun M. Inhibition of β-amyloid peptide-induced neurotoxicity by kaempferol 3-O-(6″-acetyl)-β-glucopyranoside from butterbur (Petasites japonicus) leaves in B103 cells. Food Sci Biotechnol 2012; 21: 845–851.

41 Wang S, Jin DQ, Xie C, et al. Isolation, characterization, and neuroprotective activities of sesquiterpenes from Petasites japonicus. Food Chem 2013; 141: 2075–2082.

42 Yang EJ, Kim GS, Jun M, Song KS. Kaempferol attenuates the glutamate-induced oxidative stress in mouse-derived hippocampal neuronal HT22 cells. Food Funct 2014; 5: 1395–1402.

43 Xu J, Ji F, Cao X, et al. Sesquiterpenoids from an edible plant Petasites japonicus and their promoting effects on neurite outgrowth. J Funct Foods 2016; 22: 291–299.

44 Okada M, Okada Y. Potential properties of plant sprout extracts on amyloid β. Biochem Res Int 2016; 2016: 9347468.

45 Kim N, Choi JG, Park S, Lee JK, Oh MS. Butterbur leaves attenuate memory impairment and neuronal cell damage in amyloid beta-induced Alzheimer’s disease models. Int J Mol Sci 2018; 19: 1644.

46 Park SY, Choi MH, Li M, Li K, Park G, Choi YW. AMPK/Nr2a signaling is involved in the anti-neuroinflammatory action of Petawaiwale B from Petasites japonicus against lipopolysaccharides in microglia. Immunopharmacol Immunotoxicol 2018; 40: 232–241.

47 Sasaki R, Tanaka R, Ando Y, et al. An automated microtitre-scale high-throughput screening system (MSHTS) for real-time monitoring of protein stress in male rats. Biosci Biotechnol Biochem 2012; 76: 2026–2031.

48 Tobinaga S, Takeuchi N, Kasama T, Yamashita J, Aida Y, Kaneko Y. Anti-histaminic and anti-allergic principles of Petasites japonicus Maxim. Chem Pharm Bull (Tokyo) 1983; 31: 745–748.

49 Yoshikawa M, Morikawa T, Tanaka J, Shimoda H. Medicinal foodstuffs. XXIII. Novel sesquiterpene glycoside sulfate, fukinosite A, with antiallergic activity from Japanese butterbur (Petasites japonicus). Heterocycles 2006; 68: 2335–2342.

50 Shimoda H, Tanaka J, Yamada E, Morikawa T, Kasajima N, Yoshikawa M. Anti type I allergic property of Japanese butterbur extract and its mast cell degranulation inhibitory ingredients. J Agric Food Chem 2006; 54: 2915–2920.
aggregation using quantum-dot nanoprobes. Sci Rep 2019; 9: 2587.

64 Han LK, Zheng YN, Yoshikawa M, Okada H, Kimura Y. Anti-obesity effects of chikusetsu-sapaponins isolated from Panax japonicus rhizomes. BMC Complement Altern Med 2005; 5: 9.

65 Watanabe T, Hata K, Hiwatashi K, Hori K, Suzuki N, Itoh H. Suppression of murine preadipocyte differentiation and reduction of visceral fat accumulation by a Petasites japonicus ethanol extract in mice fed a high-fat diet. Biosci Biotechnol Biochem 2010; 74: 499–503.

66 Lee YM, Kim YS, Lee Y, et al. Inhibitory activities of pancreatic lipase and phosphodiesterase from Korean medicinal plant extracts. Phytother Res 2012; 26: 778–782.

67 Adachi Y, Kanbayashi Y, Harata I, et al. Petasin activates AMP-activated protein kinase and modulates glucose metabolism. J Nat Prod 2014; 77: 1262–1269.

68 Guo L, Li K, Cui ZW, Son BG, Choi YW. S-Petasin isolated from Petasites japonicus exerts anti-adipogenic activity in the 3T3-L1 cell line by inhibiting PPAR-γ pathway signaling. Food Funct 2019; 10: 4396–4406.

69 Hossain MK, Dayem AA, Han J, et al. Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids. Int J Mol Sci 2016; 17: 569.

70 Cho AS, Jeon SM, Kim MJ, et al. Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. Food Chem Toxicol 2010; 48: 937–943.

71 Nanaditsu S, Segawa M, Okano M, Kondo K, Aratani T. Effects of four chemicals isolated from Picrasma quassioides and Petasites japonicus on P-388 lymphocytic leukemia cells in vitro. La Kromosomo II 1985; 38: 1179–1188.

72 Matsubara K, Mori M, Mizushima Y. Petasiphenol which inhibits DNA polymerase λ activity is an inhibitor of in vitro angiogenesis. Oncol Rep 2004; 11: 447–451.

73 Kim HJ, Park SY, Lee HM, Seo DI, Kim YM. Antiproliferative effect of the methanol extract from the roots of Petasites japonicus on Hep3B hepatocellular carcinoma cells in vitro and in vivo. Exp Ther Med 2015; 9: 1791–1796.

74 Hwang YJ, Wi HR, Kim HR, Park KW, Hwang KA. Induction of apoptosis in cervical carcinoma HeLa cells by Petasites japonicus ethanol extracts. Food Sci Biotechnol 2015; 24: 665–672.

75 Hirono I, Mori H, Yamada K, Hirata Y, Haga M. Carcinogenic activity of petasitenine, a new pyrrolizidine alkaloid isolated from Petasites japonicus Maxim. J Natl Cancer Inst 1977; 58: 1155–1157.

76 Hirono I, Haga M, Fujii M, et al. Induction of hepatic tumors in rats by senkirkine and symphytine. J Natl Cancer Inst 1979; 63: 469–472.

77 Chen T, Mei N, Fu PP. Genotoxicity of pyrrolizidine alkaloids. J Appl Toxicol 2010; 30: 183–196.

78 LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012. https://www.ncbi.nlm.nih.gov/books/NBK547997/. Accessed 20 March 2020.

79 Din L, Lui F. Butterbur. Treasure Island (FL): StatPearls Publishing, 2020. https://www.ncbi.nlm.nih.gov/books/NBK537160/. Accessed 20 March 2020.

80 Survey of the content of pyrrolidine alkaloids in the domestic butterbur. Ministry of Agriculture Forestry and Fisheries. (in Japanese) https://www.maff.go.jp/j/press/syouan/nouan/180831.html Accessed 20 March 2020.