# Phytochemicals and Biological Activities of Pueraria Flower: A Review

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**ABSTRACT**

Pueraria lobata (Kudzu root) has been well documented as a food and also an herbal plant for its ability to alleviate hangover, diarrhea and cardiovascular diseases. However, the flower of Pueraria lobata has been attracted attention only in recent decades. Bioactive phytochemicals such as isoflavones, saponins, essential oils and other components have been isolated and identified in Pueraria flower extracts (PFE). Both *in vivo* and *in vitro* research have indicated the health promoting effects of Pueraria flower including hepatoprotective property, estrogenic effects, antioxidant activity, anti-inflammation activity and other pharmacological activities. In this review, we have summarized the chemical compositions and pharmacological actions of Pueraria flower and updated knowledge with recent progress.

Key words: Pueraria Flower, Isoflavone, Saponin, Phytochemicals, Biological Activities

## 1. Introduction

Dietary plants are important sources of nutraceuticals and support 70-80% of the population as a primary and non-conventional medicine worldwide (Chan, 2003). Growing evidence from epidemiological and case-control studies indicate that the reduced risk of chronic diseases is tightly associated with the intake of phytochemicals originating from dietary plants (Li et al., 2020; Wen et al., 2020; Wu et al., 2019). Indeed, dietary interventions including raw plant materials and nutraceuticals have been identified to prevent various diseases such as obesity, diabetes, cardiovascular diseases, Alzheimer's disease, and cancers (Li et al., 2019; Sheng et al., 2019; Wang et al., 2014; Wang et al., 2020; Wirngo et al., 2016; Zhang et al., 2018).

Pueraria lobata belongs to *Leguminosae* family, and it is one of the earliest medicinal plants used in traditional Chinese medicine. The components and pharmacological activities of the root of Pueraria lobata have been extensively studied (Keung and Vallee, 1998; Wong et al., 2011; Zhang et al., 2017; Zhou et al., 2014). There are more than 70 phytochemicals identified in the root of Pueraria lobate (Kudzu root). Among these compounds, isoflavonoids and triterpenoids are the major constituents. Thus, compounds-oriented tactics lead to Kudzu root as an effective medicinal intervention for diabetes, cardiovascular diseases and imbalance in endocrine systems (Wong et al., 2011).

As the flower-based herb from Pueraria lobata, Pueraria flower (Puerariae Flos) has attracted increasing attention due to its bioactivities in hypoglycemia, hypolipidemia, and weight loss. Assessment of phytochemicals indicates that isoflavonoid and essential oils are the chief components in Pueraria flower (Lertpatipanpong et al., 2020; Wang et al., 2013; Yu et al., 2011). On the basis of its promising development potential, we summarized the up to date knowledge regarding phytochemicals and pharmacological activities of Pueraria flower. In particular, key issues involving the relationship between active ingredients and molecular mechanisms are highlighted in this review.

## 2. Methods

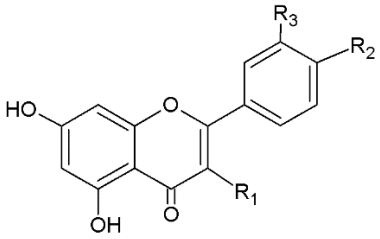
The current review considered the literature published prior to September 2020 on phytochemistry, pharmacology and toxicity of extracts isolated from Pueraria flower. All the available information on Pueraria flower was collected via electronic search such as PubMed, Google Scholar, and Web of Science. The literature was searched from the databases using the keywords “Pueraria flower” with no exact time limit (all fields) as well as various books that were accessed for information that were directly related to the present contribution. Information of all related books, full-text articles and conference notes written in English and Chinese were also very reliable.

## 3. Phytochemicals

### 3.1 Flavonoids

Chemical structures of flavonoids from Pueraria flower are summarized in Table 1. The flowers of Pueraria flower were extracted with MeOH. Identification by high performance liquid chromatography (HPLC) and other methods showed that there were three major flavonoids, apigenin (0.0047 mg/g), nicotiflorin (0.034 mg/g), and apigenin 4′-O-β-D-glucoside (0.016 mg/g) from the dry powder of Pueraria flower (Ding et al., 2013). In addition, rutin and luteolin were analyzed using ultra-performance liquid chromatography coupled with quadrupole/time-of-flight mass spectrometry (UPLC-QTOF/MS), their contents were 0.09mg/g and 0.04-0.07 mg/g, respectively (Lu et al., 2013).

Table 1. Major flavonoids in Pueraria flower




| No. | compounds | $R_1$ | $R_2$ | $R_3$ | Reference |
|---|---|---|---|---|---|
| 1 | Rutin | glc-rha | OH | OH | Ding et al., 2013; Lertpatipanpong et al., 2020 |
| 2 | Luteolin | H | OH | OH | Ding et al., 2013 |
| 3 | Apigenin | H | OH | H | Lu et al., 2013 Lertpatipanpong et al., 2020 |
| 4 | Nicotiflorin | O-glc-(6→1)-xyl | OH | H | Lu et al., 2013 |
| 5 | Apigenin 4'-O-β-D-glucoside | O-glc | H | H | Lu et al., 2013 |

### 3.2 Isoflavones

Isoflavones are generally considered as the major bioactive compounds in Pueraria flower. Isoflavones from Pueraria flower are usually thought to be chemoprotective and also serve as an alternative therapy for female hormonal disorders including ovarian cancer and menopausal symptoms (Han et al., 2018; Tousen et al., 2019; Yang et al., 2012). Until now, more than 30 isoflavones have been quantified in Pueraria flower (Table 2) and some of their structures are well elucidated (Tong et al., 2018). A summary of the current findings are presented below.

Ultrafiltration with liquid chromatography and mass spectrometry (UF-LC-MS) coupled with high-speed counter-current chromatography（HSCCC）are the fundamental tools for rapidly screening and isolating isoflavones from Pueraria flower. Tectoridin and kakkalide were identified as the main isoflavones in Pueraria flower, followed by puerarin, genistin, and tectorigenin which are valuable as α-glucosidase and lactate dehydrogenase (LDH) inhibitors and are effective in drug design for preventing and treating diabetes mellitus and stroke (Wu et al., 2018).

Zhang et al.(2012) used HPLC combined with 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assays to access the antioxidant activity of isoflavones identified in Pueraria flower. In this research, it was found that the antioxidant activity of extracts from Pueraria flower was strongly dependent on the solvent. Solvents with different polarities were used to further fractionate crude ethanolic extract of Pueraria flower. The ethyl acetate fraction showed more potent capacity to scavenge DPPH radical than petroleum ether or n-BuOH fractions (Zhang et al., 2012).

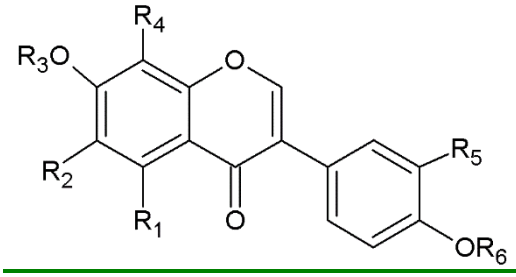
In addition, Lu et al.(2013) determined a total of 25 isoflavones by using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry and mass spectrometry (UPLC -QTOF/MS). According to these authors, kakkalide and irisolidone were abundant in Pueraria flower (10.3–17.7 mg/g kakkalide, and 2.76–4.95 mg/g irisolidone) (Lu et al., 2013). Interestingly, when the estrogenic activity of kakkalide and its metabolite irisolidone were investigated, the results showed that irisolidone had a better estrogenic effect than kakkalide (Shin et al., 2006). Moreover, kakkalide was isolated as a potent 3-hydroxy-3- methylglutaryl (HMG)-CoA reductase (HCR) inhibitor down-regulating the biosynthesis of triacylglycerols and cholesterol (Min and Kim, 2007).

Yuan et al.(2009) found 11 isoflavones from Pueraria flower with therapeutic

potential for alcoholism. Among them, two new isoflavones, 6-hydroxybiochanin A-6,7-di-O-β-D-glucopyranoside and 6-hydroxygenistein-7-O-β-D-glucopyranoside, were isolated from the ethanolic extract of the flowers and their structures elucidated by ultraviolet (UV), infrared spectroscopy (IR), high resolution mass spectrum (HR-MS), and 1D and 2D nuclear magnetic resonance (NMR) spectroscopic methods. The release of lipopolysaccharide (LPS)-induced nitric oxide, using primary cultured rat cortical microglia, was tested for all these 11 isoflavones. Tectorigenin, genistein and irisolidone were stronger in inhibiting nitric oxide release activity than gehuain, tectoridin, tectorigenin-7-O-β-D-xylosyl-(1→6)-β-D-glucopyranoside and 6-hydroxygenistein-6,7-di-O-β-D-glucopyranoside. However, there was little inhibitory activity for 6-hydroxybiochanin A-6,7-di-O-β-D-glucopyranoside, 6-hydroxygenistein-7-O-β-D-glucopyranoside, 6-hydroxygenistein-7-O-β-D-glucopyranoside genistin and 6-hydroxygenistein-7-O-β-D-glucopyranoside kakkalide. As far as the structure–activity relationship is concerned, the glycosylation at the C-7 hydroxyl group reduced the inhibitory activity of microglial activation. The methoxylation of 4′-hydroxyl group of 7-glycosylated isoflavones reduced the inhibitory activity, while the methoxy group at the 6-position enhanced the activity (Yuan et al., 2009).

Additionally, prolonged storage of Pueraria flower led to chemical transformation of some compounds. For example, tectoridin in Pueraria flower could be methylated and transform into kakkalide (Kim et al., 2003). Thus, tectoridin is a prodrug of tectorigenin.

Table 2. Major isoflavones in Pueraria flower




| No. | Compounds | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | Analysis | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Kakkalide | OH | OMe | Glc-Xyl | H | H | Me | IR, UV | Kubo et al., 1975 |
| 2 | 3′-Hydroxypuerarin | OH | H | H | Glc | OH | H | UFLC-MS | Li et al., 2017 |
| 3 | Puerarin | H | H | H | Glc | H | H | UFLC-MS | Li et al., 2017 |
| 4 | Puerarinxyloside | H | H | Xyl | Glc | H | H | UFLC-MS | Li et al., 2017 |
| 5 | Tectoridin | OH | OMe | Glc | H | H | H | UFLC-MS | Li et al., 2017 |
| 6 | Tectorigenin | OH | O-$CH_3$ | H | H | H | H | UFLC-MS | Li et al., 2017 |
| 7 | Ononin | OH | OH | Glc | H | H | H | UV, MS, TLC | Kurihara and Kikuchi, 1976 |
| 8 | Puerarin-4'-*O*-β-D-glucopyranoside | H | H | H | Glc | H | Glc | HPLC-MS/MS | Zhang et al., 2012 |
| 9 | Glycitin | H | OMe | Glc | H | H | H | UPLC-QTOF/MS | Lu et al., 2013 |
| 10 | Tectorigenin-7-*O*-β-D-xylosyl-(1→6)-β-D-glucopyranoside | OH | OMe | Glc-Xly | H | H | H | HPLC-MS/MS | Wang et al., 2013 |
| 11 | Genistein-8-*C*-β-D-glucopyranoside | OH | H | H | Glc | H | H | HPLC-MS/MS | Zhang et al., 2012 |
| 12 | Irisolidone-7-*O*-β-D-glucopyranpsyl-(1→6)-β-D-glucopyranoside | OH | OMe | Glc-Glc | H | H | Me | HPLC-MS/MS | Zhang et al., 2012 |
| 13 | Biochanin A-7-*O*-β-D-glucopyr | OH | H | Glc | H | H | Me | HPLC-MS/MS | Zhang et al., 2012 |

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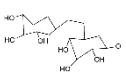
| | anoside | | | | | | | | |
| 14 | Daidzein | H | H | Glc | H | H | H | IR, NMR | Kurihara and Kiruchi, 1973 |
| 15 | 3'-methoxydaidzin | H | H | Glc | H | OMe | H | HPLC-MS/MS | Zhang et al., 2012 |
| 16 | Irisolidone | OH | OMe | H | H | H | Me | IR, TLC | Kurihara and Kiruchi, 1973 |
| 17 | Formononetin | H | H | H | H | H | Me | UV, MS | Kurihara and Kiruchi, 1973 |
| 18 | 6-Hydroxygenistein-6,7-di-*O*-glucoside | OH | OGlc | Glc | H | H | H | UPLC-QTOF/MS | Lu et al., 2013 |
| 19 | Tectorigenin-7-*O*-xylosylglucoside | OH | OMe | Glc-Xyl | H | H | H | UPLC-QTOF/MS | Lu et al., 2013 |
| 21 | 6-Hydroxybiochanin A-6,7-di-*O*-glucoside | OH | OGlc | Glc | H | Me | H | UPLC-QTOF/MS | Lu et al., 2013 |
| 22 | Gehuain | H | OMe | Glc-Xyl | H | Me | H | UPLC-QTOF/MS | Lu et al., 2013 |
| 23 | Glycitein | H | OMe | H | H | H | H | UPLC-QTOF/MS | Lu et al., 2013 |
| 24 | Genistein | OH | H | H | H | H | H | IR, NMR | Kurihara and Kiruchi, 1973 |
| 25 | Biochanin A | OH | H | H | H | Me | H | IR, TLC | Kurihara and Kiruchi, 1973 |
| 26 | Tectorigenin-7-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] | OH | OMe | Glc-Xyl | H | H | H | HPLC-ESI-Q/TOF-MS | Ma et al., 2019 |
| 27 | Genistein-7-glucoside | OH | H | Glc | H | H | H | HPLC-ESI-Q/TOF-MS | Wang et al., 2013 |
| 28 | 3'-Hydroxytectorigenin-7-*O*-β-D-xylosyl-(1→6)-β-D-glucop | OH | OMe |  | H | OH | H | HR-ESI-MS and NMR | Wang et al., 2013 |

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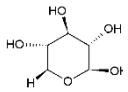
|---|---|---|---|---|---|---|---|---|---|
| | yranoside | | | | | | | | |
| 29 | Sissotorin | OH | H | Glc | H | H | Me | UV, MS, TLC | Kurihara and Kikuchi, 1976 |
| 30 | Quercetin | OH | H | H | H | OH | H | IR, NMR | Kurihara and Kikuchi, 1973 |
| 31 | Genistein | OH | H |  | H | H | H | UV, MS, TLC | Kurihara and Kiruchi, 1973 |
| 32 | Wistin | H | OMe | O-glc | H | H | OMe | HPLC | Ding et al., 2013 |

### 3.3 Saponins

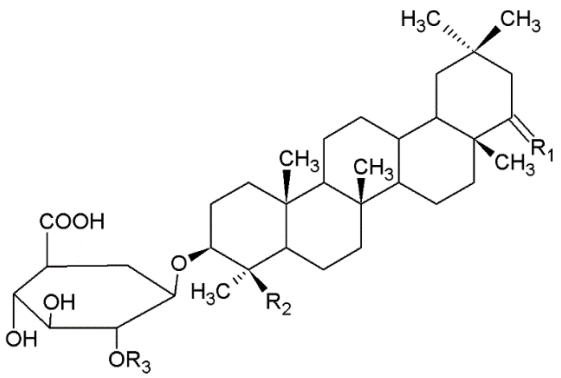
Saponins are a large family of amphiphilic glycosides of steroids and triterpenes found in plants and some marine organisms (Yang et al., 2014). Naturally occurring saponins constitute a structurally diverse class of glycosides that are composed of one or more sugar moieties and aglycones linked via glycosidic bonds (Sahu and Achari, 2001). The amounts of total saponins varied from 0.43 to 2.00% according to analysis of Pueraria flower collected from 30 different areas (Niiho et al., 2010). Based on the chemical structure of saponins, a number of saponins identified from Pueraria flower are listed in Table 3.

Lu et al.(2013) simultaneously quantified 12 saponins by UPLC -QTOF/MS analysis. The total content of the saponins was 23.2–60.6 mg/g (Lu et al., 2013). The major saponin compositions are kaikasaponin III (1.26 ~ 15.2 mg/g) and soyasaponin I (2.65~19.1 mg/g). The contents of saponins secondary to kaikasaponin III were kaikasaponin II (1.63~5.74 mg/g) and kakkasaponin I (5.08~12.3 mg/g). The rest of saponins identified included soyasaponin IV and baptisiasaponin I, phaseoside IV,

astragaloside VIII, kaikasaponin I, azukisaponin I, kakkasaponin II, and kakkasaponin III, which were detected at a much lower amounts compared to those mentioned above.

The health promoting activities of saponin compositions found in Pueraria flower are reported in the literature. Kaikasaponin III was found to possess hypoglycemic and hypolipidemic effects in the streptozotocin (STZ)-induced diabetic rat (Choi et al., 2004). Soyasaponin I and kaikasaponin III from Pueraria flower have been reported to inhibit testosterone 5α-reductase and to promote hair growth (Murata et al., 2012).

Table 3. Major saponins in Pueraria flower (Lu et al., 2013)




| No. | Compounds | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| 1 | AstragalosideⅧ | H, OH | $CH_2OH$ | Xly-rha |
| 2 | Soyasaponin Ⅰ | H, OH | $CH_2OH$ | Gal-rha |
| 3 | SoyasaponinⅢ | H, β-OH | $CH_2OH$ | Gal |
| 4 | SoyasaponinⅣ | H, OH | $CH_2OH$ | Ara |
| 5 | KaikasaponinⅢ | H, OH | Me | Gla-rha |
| 6 | Kaikasaponin Ⅱ | H, OH | Me | Glc-rha |
| 7 | Kaikasaponin Ⅰ | H, OH | Me | Gal |
| 8 | Kakkasaponin Ⅰ | H, OH | Me | Ara-rha |

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|---|---|---|---|---|
| 9 | Azukisaponin Ⅰ | H, OH | Me | Glc |
| 10 | Baptisiasaponin Ⅰ | H, OH | Me | Xyl-rha |
| 11 | PhaseosideⅣ | O | Me | Gal-rha |
| 12 | Kakkasaponin Ⅱ | O | Me | Gal |
| 13 | KakkasaponinⅢ | O | Me | Xyl-rha |
| 14 | Sophoradiol monoglucuronide | O | Me | Rha-gal |

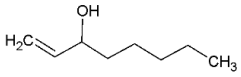
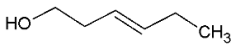
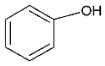
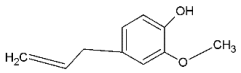
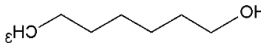
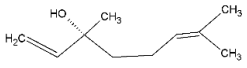
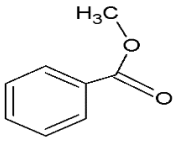
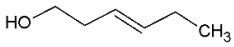
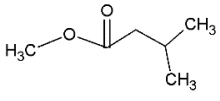
### 3.4 Essential oils (EOs)

EOs are characterized by volatile and semi-volatile compounds with lower molecular weight (Song et al., 2019). Generally, EOs are secondary plant metabolites containing complex mixtures of volatile organic compounds (Aziz et al., 2018) such as terpenes and their oxygenated derivatives, and some aromatic and aliphatic compounds (Abad et al., 2012).

Kurihara and Kikuchi (1973) identified 13 essential oil components extracted from Pueraria flower. A total of 2.9 g essential oils were obtained, including 2.4 g neutral oil and 0.5 g acid oil. The neutral oils included 1-octen-3-ol, cis-3-hexene-1-ol, benzyl alcohol, eugenol, isoamyl alcohol, octyl alcohol, phenethyl alcohol, l-linalool; and acid oil included methyl benzoate, methyl propionate, methyl isovalerate, and methyl caproate (Table 4).

In addition, at least 12 more compounds from Pueraria flower essential oils were identified by gas chromatography-mass spectrometry (GC-MS). These were nonanal, camphor, terpinyl acetate, trans-caryophyllene, thujopsene, humulene, 2.6-bis (1-dimethylethyl)-4-methyl-phenol, hexahydrofarnesy lacetone, methyl palmitate, dibutyl terephthalate, hexadecanoic acid, n-docosane. Among them, hexahydrofarnesy lacetone was the richest compound with a content of 15.9% and was considered as a spice (Wang et al., 2002).

Table 4. Major essential oils in Pueraria flower

| Compounds | Chemical structure | Analysis | Molecular weight | References |
|---|---|---|---|---|
| 1-Octen-3-ol |  | IR, GLC | 128.21200 | Kurihara and Kikuchi, 1973 |
| Leaf Alcohol |  | IR, GLC | 100.15900 | Kurihara and Kikuchi, 1973 |
| Benzyl Alcohol |  | IR, GLC | 108.14 | Kurihara and Kikuchi, 1973 |
| Eugenol |  | IR, GLC | 164.2 | Kurihara and Kikuchi, 1973 |
| Isoamyl Alcohol |  | IR, GLC | 88.1481 | Kurihara and Kikuchi, 1973 |
| Octyl Alcohol |  | IR, GLC | 130.2279 | Kurihara and Kikuchi, 1973 |
| l-Linalool |  | IR, GLC | 154.25 | Kurihara and Kikuchi, 1973 |
| Methyl Benzoate |  | IR, GLC | 136.15 | Kurihara and Kikuchi, 1973 |
| Methyl Propionate |  | IR, GLC | 100.159 | Kurihara and Kikuchi, 1973 |
| Methyl Isovalerate |  | IR, GLC | 116.16 | Kurihara and Kikuchi, |

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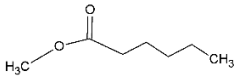
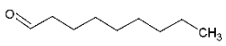
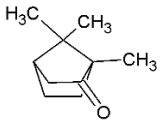
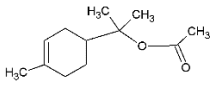
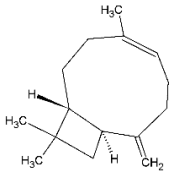
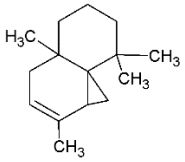
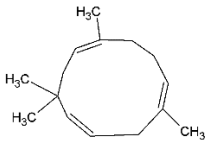
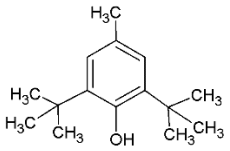
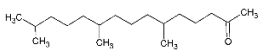
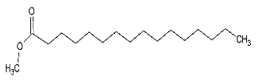
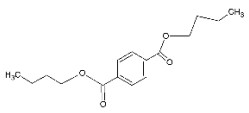
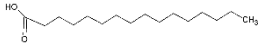
| Methyl Caproate |  | IR, GLC | 130.18 | 1973 Kurihara and Kikuchi, 1973 |
| Nonanal |  | GC-MS | 142.24 | Kurihara and Kikuchi, 1973 |
| Camphor |  | GC-MS | 152.23 | Wang et al., 2002 |
| Terpinyl acetate |  | GC-MS | 196.29 | Wang et al., 2002 |
| Trans-caryophyllene |  | GC-MS | 204.35 | Wang et al., 2002 |
| Thujopsene |  | GC-MS | 204.35 | Wang et al., 2002 |
| Humulene |  | GC-MS | 204.35 | Wang et al., 2002 |
| 2.6-Bis(1-dimethylethyl)-4-methyl-phenol |  | GC-MS | 220.35 | Wang et al., 2002 |
| Hexahydrofarnesyl acetone |  | GC-MS | 268.48 | Wang et al., 2002 |
| Methyl palmitate |  | GC-MS | 270.45 | Wang et al., 2002 |
| Dibutyl terephthalate |  | GC-MS | 278.34 | Wang et al., 2002 |
| Hexadecanoic acid |  | GC-MS | 256.42 | Wang et al., 2002 |
| n-Docosane | $CH_3(CH_2)_{20}CH_3$ | GC-MS | 310.60 | Wang et al., 2002 |

## 3.5 Other bioactive compounds

Apart from isoflavones, saponins and essential oils, some other compounds were identified in Pueraria Flower. β-sitosterol, and β-sitosterol-3*O*-β-D-glucoside were isolated from the methanolic extract of the Pueraria flower.(Kurihara and Kikuchi, 1976) Additionally, 10 compounds were isolated from Pueraria flower including 3,5-di-tert-butyl-4-hydroxybenzaldehyde, palmitic acid, 1-octadecene, octadecanoic acid, eicosanoic acid, squalene, (E)-23-ethylcholesta-5,22-dien-3β-ol, 24-methylenecycloartanol, β-amyrin, lupenone. Among them, β-amyrin was present atthe highest percentage of 19.7%, followed by palmitiic acid (6.8%), lupenone (5.6%), and 24-methylenecycloartanol (4.0%) (Kim et al., 2015).

## 4. Pharmacological activities

Both *in vivo* and *in vitro* research suggest that Pueraria flower has a wide range of biological activities including hepatoprotective and estrogenic effects, as well as antioxidant, and anti-inflammation activities. The current findings regarding the main bioactive components of Pueraria flower and their underlying action mechanisms are summarized in this contribution (Table 5).

### 4.1 Hepatoprotective Effect

Akin to other herbal medicines, extracts or compounds from Pueraria flower possess protective effects and therapeutic properties against liver diseases. Among the bioactives, isoflavones obtained from Pueraria flower have been well documented to be active against liver dysfunction and damage caused by liver diseases (Miltonprabu et al., 2017). Tectoridin, one of the main isoflavones in Pueraria flower, showed significant protective effect on hepatic steatosis induced by ethanol through the modulation of PPARα pathway and protection of mitochondrial injury. The hepatoprotective effect may be connected with inhibiting the increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and triacylglycerol (TG), adjusting the levels of mitochondrial permeability transition (MPT) and

transmembrane potential (Δψm) (Xiong et al., 2010). Furthermore, tectoridin is an inhibitor of β-glucuronidase and treatment with tectoridin attenuated the increase of β-glucuronidase in blood caused by liver damage. In addition, the hepatoprotective effect of tectoridin could be linked to the metabolism with intestinal bacteria (Lee et al., 2005). Intraperitoneal tectorigenin injection protected mice from $CCl_4$-induced liver injury by inhibiting the increase of ALT, AST and lactic acid dehydrogenase levels by 22.4, 44.4 and 58.7%, respectively (Lee et al., 2003).

**4.2 Estrogenic Effects**

Estrogenic effects are associated with a variety of physio- or pathological effects, in addition to regulating female reproduction and secondary sex characteristics. Abnormal estrogen is closely associated with broad spectrum of diseases. Pueraria flower is rich in isoflavones, which is the most well-known subgroup of phytoestrogens and plays protective roles against abnormal estrogenic effects (Ososki and Kennelly, 2003; Wang et al., 2020). Therefore, it is not surprising that Pueraria flowers possess estrogenic effects. The estrogenic effect of Pueraria flower has been confirmed by many researchers. Park et al. (2009) found that Pueraria flower could cause significant reversal of stress-induced deficits in learning and memory on a spatial memory task, and also increased choline acetyltransferase (ChAT) immunoreactivities in ovariectomized (OVX) mice. Besides, tectorigenin has estrogenic effect through attenuating the levels of RANKL, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and enhancing the levels of estrogen, estrogen receptor (ER)-β, 5-HT1A, 5-HT2A, and tryptophan hydroxylase (Han et al., 2018). In addition, Shin et al. (2006) found that kakkalide was metabolized to irisolidone and tectoridin, which were further metabolized to tectorigenin by human intestinal microflora. Interestingly, kakkalide and tectoridin showed less potent

estrogenic effect than their metabolites (Shin et al., 2006).

In addition, Pueraria flower extracts displayed anti-endometriotic effects. Pueraria flower extracts suppressed the adhesion of human endometriotic11Z and 12Z cells to human mesothelial Met5A cells through targeting extracellular signal regulated kinase (ERK)1/2 pathway to inhibit matrix metalloproteinase (MMP)-2 and MMP-9 in endometriotic cells (Kim et al., 2017).

### 4.3 Antioxidant activity

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and the antioxidants to scavenge the ROS (Nocella et al., 2019). ROS consist of radical and non-radical oxygen-based molecules, such as hydroxyl radical (•OH), hydrogen peroxide ($H_2O_2$), singlet oxygen ($^1O_2$), and superoxide ($O_2$•-) (Yang et al., 2019). To investigate the correlation between the phytochemicals in Pueraria flower and their antioxidant capacity, measurement of antioxidant capacity of isoflavones with different structures was performed *in vitro*. The results so obtained for antioxidant activity from high to low was tectorigenin sodium sulfonate>tectorigenin>tectoridin, demonstrating that appropriate chemical modifications could greatly improve the biological activities of the naturally occurring products (Han et al., 2012). Eighteen antioxidants were screened and identified from Pueraria flowers by DPPH spiking HPLC-MS/MS (Zhang et al., 2012).

Pueraria flower extracts were beneficial in improving the antioxidative function in ethanol-treated rats by modulating redox enzymes such as Cu/Zn SOD, CAT and GSH-Px(Lee et al., 2001). In addition, tectorigenin prevented $MPP^+$-induced human neuroblastoma SH-SY5Y cells damage due to its potent antioxidant activity. The addition of tectorigenin blocked $MPP^+$-induced ROS formation and NADPH oxidase

(NOX) expression to protect the antioxidant enzyme activities from MPP wreckage (Gong et al., 2017). Notably, by means of targeting oxidative stress, tectorigenin and kaikasaponin III were reported to alleviate the streptozotocin-induced toxicity and to contribute to hypoglycemic and hypolipidemic effects (Lee et al., 2000). Besides, puerarin, one of components of Pueraria flower, showed antioxidant effect by regulating the expression of Nrf2 pathway and antioxidant enzymes dextran sulfate sodium-induced colitis mice model.(Jeon et al., 2020).

**4.4 Anti-inflammatory activity**

Increasing evidence proved that the bioactive extracts of various natural plants including Pueraria flower display a variety of pharmacological effects on acute and chronic inflammatory diseases (Lowry, 1993; Arulselvan et al., 2016). Research from a rat model suggested that methanol extracts of Pueraria flower prevented osteoarthritis by inhibiting the pro-inflammatory mediators iNOS, MMP-9 and MMP-3 in the knee tissues (Sun et al., 2019).

Tectorigenin attenuated endothelial dysfunction associated with insulin resistance through inhibiting ROS-related inflammation and facilitating insulin IRS-1/PI3K/Akt/eNOS signaling pathway (Qi et al., 2013). Another isoflavone, kakkalide ameliorated insulin resistance in human umbilical vein endothelial cells induced by palmitate via inhibiting ROS-associated inflammatory tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) production and facilitating insulin PI3K/Akt/eNOS signal pathway (Zhang et al., 2013). Furthermore, observation from LPS-stimulated peritoneal macrophages suggested that kakkalide and irisolidone down-regulated TNF-α, interleukin-1 beta (IL-1β) and cyclooxygenase-2 (COX-2) via the NF-κB pathway (Min et al., 2011). In addition, kakkalide and irisolidone alleviated the inflamed gut by inhibiting TLR4-NF-κB signaling pathway and

reversing the transition of M1 into M2 macrophage polarization ( Jang et al., 2019; Park et al., 2019).

**4.5 Other biological effects**

In addition to above activities, extracts or compounds from Pueraria flower are reported to have anti-cancer, anti-allergic, and antimicrobial activities.

As for the anti-cancer activity, tectorigenin was found to enhance paclitaxel cytotoxicity against ovarian carcinoma cells involved in the activation of apoptotic caspases and regulation of the NF-κB and Akt pathways (Yang et al., 2012). Additionally, tectorigenin also exhibited antiproliferative activity against human leukemia HL-60 cells and this activity may be based upon the induction of differentiation and apoptosis (Lee et al., 2001).

Allergic diseases such as asthma and atopic dermatitis are based on IgE-mediated pharmacologic processes of a variety of cell populations such as mast cell and basophils (Park et al., 2004). Orally administered tectoridin can be transformed, by intestinal bacteria, into the more active agent tectorigenin which potently inhibited the passive cutaneous anaphylaxis reaction. In vitro experiments suggest that tectorigenin inhibited the release of β-hexosaminidase from RBL-2H3 cells induced by IgE (Stevens and Austen, 1989). Tectorigenin is also considered as inhibitors for expression of IgE receptor (FcεRI), the key molecule triggering the allergic reactions, on human mast cells (Tamura et al., 2010). These findings indicate that tectorigenin has potential to be an antiallergic agent.

Akin to the Pueraria root, PFE has traditionally been used as an anti-amnesic medicine for treatment of alcoholic intoxication. Data from the observation of passive avoidance behavior in mice supported that aqueous extract of Pueraria flower improved the scopolamine-induced memory impairment (Yamazaki et al., 2005).

Table 5.The main bioactives of Pueraria flower.

| Compounds | Bioactivities | Experimental models | Effects | References |
|---|---|---|---|---|
| Tectoridin | Hepatoprotective Effect | Ethanol-induced mice | ↓ALT,AST,TG,MDA MPT, Δψm levels.↑PPARα,MCAD,CYP 4A10/14,DGAT,GPAT | Xiong et al., 2010 |
| Kakkalide, irislidone | hypolipidemic effect | Trition WR1339-induced mice | ↓HCR activity,TC,TG level | Min and Kim, 2007 |
| Kakkalide, irislidone | anti-inflammation | TNBS-induced mice | ↓NF-κB activation, M1 macrophage polarization marker expression. ↑gut Proteobacteria population | Jang et al., 2019 |
| Kaikasaponin ,Tectorigenin | Antimutagenic,anti-lipid peroxidative effect | Bromobenzene induced rats | ↓Salmonella typhymurium TA100,$AFB_1$,MDA | Park et al., 2002 |
| Tectorigenin | Hepatoprotective Effect | $CCl_4$-induced mice | ↓ALT,AST,LDH,MDA, calcium levels, ↑GSH,GST activity. | Lee et al., 2003 |
| Tectorigenin | antioxidant | $H_2O_2$-induced HUVECs | ↓ LDH, MDA levels. Bax and Cleaved Caspase-3. ↑Bcl-2 expression, SOD and GSH-Px. | Chen et al., 2021 |
| Tectorigenin, Tectoridin | antioxidant | In vitro | ↓hydroxy radical, Superoxide anion radical,DPPH radical,Lipid peroxidation levels | Han et al., 2012 |
| Kaikasaponin III | antidiabetic | Streptozotocin-induced rat | ↓lipid peroxide,hydroxy radical levels, ↑SOD, TF,Phase Ⅰ, Phase Ⅱ enzymes | Choi et al., 2004 |

| | | | | |
|---|---|---|---|---|
| Tectorigenin, genistein | Antileukemia | HL-60cells | activities ↓Bcl-2, EGF-receptor expression | Lee et al., 2001 |
| Irisolidone, kakkalide | Anti-gastric injury | ethanol-induced mice | ↓TNFα, IL-8,IFNγ,COX-2expression,NF-κB activation | Kang et al., 2016 |
| Tectorigenin | anti-inflammation | Palmitate-stimulated HUVECs | ↓ROS production, Δψm, IKKβ/NF-κB and JNK activation, TNF-α,IL-6 expression,IRS-1serine/tyrosine phoshorylation,NO production,ET-1,VCAM-1 expression | Qi et al., 2013 Zhang et al., 2013 |
| Kakkalide, irisolide | anti-inflammation | Carrageenan-Induced | ↓TNFα,IL-β,$PGE_2$,COX-2 expreesion, NF-κB activation. | Min et al., 2011 |
| Tectorigenin | antioxidant | MPP+-induced SH-SY5Y cell | ↓cell cytotoxicity and poptosis, Bax/Bcl-2,ROS,NOX, antioxidant enzyme expression, | Min et al., 2011 |
| Irisolidone | Hepatoprotective effect | tert-Butyl Hyperoxide(t-BHP) -Induced mice | ↓cell cytotoxicity, ALT,AST. | Lee et al., 2005 |
| Irisolidone | antibacterial activity | Helicobacter pylori | ↓H+/K+ ATPase | Bae et al., 2001 |
| Kaikasaponin Ⅲ, Tectorigenin | Hypoglycemic, hypolipid effect,antioxidant | Streptozotocin-Lnduced rats | ↓glucose,body weight,LDL,VLDL cholesterol,DPPH,XOD,superoxide anion,lipid peroxidation.↑HDL cholesterol | Lee et al., 2000 |
| Puerarin | Antioxidant, anti-inflammation | DSS-induced mice | ↓ myeloperoxidase (MPO) activity, | Jeon et al., 2020 |

| | | | | |
|---|---|---|---|---|
| Tectoridin | Estrogenic effects | ovariectomy-induced mice | NF-κB, pro-inflammatory mediators, Nrf2 ↑ tight junctions ↓ osteoclastogenesis. Trap, Ctsk, ATP60, DC-Stamp, c-Fos, and NFATc1, NF-κB | Wang et al., 2020 |

## 5. Toxicity

Although very few reports are available so far, the toxicity of PFE or compounds from PFE is still needed. Takano et al.(Takano et al., 2013) performed oral toxicological studies of PFE and their results provided a fundamental reference for further development and clinical translation of functional food based on Pueraria flower. In their acute toxicity study with 14 days observation, no death or abnormalities were observed and the estimated oral LD50 of PFE was higher than 5 g/kg body weight. Likewise, subchronic toxicity study using Sprague-Dawley rats for 90 days showed no apparent toxicological issues. Thus, the corresponding human equivalent dose of PFE for low toxicity was estimated to be 5.0% in the diet.

## 5 Conclusion

This review provides a comprehensive review on bioactive compounds derived from Pueraria flower. The main baioctive classes of compounds present included isoflavones, saponins, and flavonoids, among others. Although Pueraria flower as a dietary source is broadly used in traditional medicine, toxicological assessments, pharmacokinetics, and the metabolites of phytochemicals needs to be further investigated.

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