Progress in Chemical Compositions and Pharmacological Activities of FlosPopuli

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Abstract FlosPopuli is a dry male inflorescence of *Populus tomentosa*, *Populus canadensis*. Some researches show that FlosPopuli contains rich flavonoids and phenolic glycosides, which have antibacterial, anti-inflammatory, anti-oxidant and anti-viral effects. In order to systematically understand the chemical compositions and biological activities of FlosPopuli and lay the foundation for subsequent research, this article reviews the two above aspects. Fifty-three kinds of flavonoids, phenol glycosides and others were isolated from FlosPopuli; moreover, their antibacterial, anti-inflammatory and hypolipidemic effects were investigated.

Keywords FlosPopuli, flavonoids, antibacterial, chemical components, activities

Introduction

FlosPopuli is a dry male inflorescence of *Populus tomentosa* (*P. tomentosa*), *Populus canadensis* (*P. canadensis*) or several plants of the same genus, which is widely distributed in Liaoning, Shanxi, Hebei and Gansu provinces of China.^[1,2] It is bitter and slightly cold, and taken to clear away heat and toxins, and eliminate dampness and reduce diarrhea. FlosPopuli is mostly used as a folk medicine for bacterial dysentery, diarrhea of young animals, etc. It is also applied for treating urticaria and itching of the skin. FlosPopuli is easy to obtain, low in price and yet high in medicinal value. Because of its widespread distribution, the research on active ingredients was carried out as far back as in 1976. Some studies showed that the main antibacterial components of FlosPopuli were flavonoids, polysaccharides, glycosides, phytosterols, terpenes, amino acids, tannins, and volatile oils.^[3] This article reviews the research papers on FlosPopuli from 1976 and lays the foundation for subsequent research.

Chemical Constituents

A total of 53 flavonoids, glycosides, phytosterols, esters and other compounds were isolated from the dried male inflorescence of FlosPopuli.

Flavonoids

Flavonoids are the main antibacterial active ingredients in FlosPopuli. Chrysoeriol (1), apigenin (2), luteolin (3), dillenetin (4), kaempferol (5), quercetin (6), myricetin (7), rhamnzin (8), pinocembrin (9), naringenin (10), isosakuranetin (11), eriodictyol (12), taxifolin (13), dihydromyricetin (14), pinostrobin (15), galangin-3-methoxy (16), chrysin (17), puerctin-3,7-dimethoxy (18), rhamnetin (19) and kaempferol-3-methoxy (20) were isolated. Among them, compounds 1, 4, 7, 8, 10–14 and 18 were obtained from this plant for the first time (Figure 1).^[4–9]

In recent years, the following phenol glycosides have been isolated from FlosPopuli: apigenin-7-O- β -D-glucopyranoside (21), luteolin-7-O- β -D-glucopyranoside (22), diosmetin-7-O- β -

Figure 1 Flavonoids isolated from FlosPopuli.

D-glucopyranoside (23), kaempferide-7-O- β -D-glucopyranoside (24), 5-hydroxy-flavanone-7-O- β -D-glucopyranoside (25), kaempferol-3-O-[(2-O- α -L-rhamnopyranosyl-4-O- β -D-glucopyranosyl)- β -D-glucopyranoside] (26), apigenin-7-O-(-6"-O-p-coumaroyl)- β -D-glucopyranoside (27), syringin (28), salicin (29), benzylalcohol-O- β -D-glucopyranoside (30), tremulacin (31), salireposide (32), 2-hydroxycyclohexyl-6'-O-p-coumaroyl- β -D-glucopyranoside (33), Daucosterol (34), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6'-O-acetyl)- α -rhamnosyl-(1 $\rightarrow$ 2)- β -D-glucoside (35), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O- α -rhamnosyl(1 $\rightarrow$ 2)- β -D-glucoside (36), (2S)-5,7-dihydroxyflavanone-7-O- β -D-glucopyranoside (37), apigenin-7-O- β -D-(-6"-pcoumaroyl)-glucoside (38), chrysoeriol-7-O- β -D-glucopyranoside (54), 2-acetyl-1,3-diacaffeoylglycerol (55). Among them, compounds 21, 23, 25, 28, 30 and 33 were obtained from this plant for the first time (Figure 2).^[6–8,10,27]

Figure 2 Polysaccharides and glycosides isolated from FlosPopuli.

Figure 3 Other compounds isolated from FlosPopuli.

Other compounds

Li *et al.*^[10] mentioned that phytosterols and triterpenes were isolated from FlosPopuli: γ -sitosterol (**39**), β -sitosterol (**40**), ursolic acid (**41**), betulonic acid (**42**). Four lipids were isolated: methylisoferylate (**43**), *p*-coumaric acid glucoside (**44**), tremuloidine (**45**), and 4'-hydroxy-1'-O-(β -D-glucoside)-benzyl-2-hydroxy-benzoate (**46**). Three phenylpropanoids: coumaric acid (**47**), protocatechuic acid (**48**), caffeic acid (**49**) and other compounds xanthopurpurin (**50**), catechol (**51**), 3,3',4,4'-tetrahydroxybiphenyl (**52**), 2-acetyl-1,3-dicaffeoylglycerol (**53**) were isolated. Among them, compounds **41**, **42**, **46**, **48**, **51**—**53** were obtained from this plant for the first time (Figure 3).^[4-9]

In addition, Zhao *et al.*^[13] used a pre-column derivative

RP-HPLC to identify 21 amino acids from FlosPopuli. The total amount of free amino acids was 1630.47 mg/100 g, and the total amount of amino acids after hydrolysis was 16386.74 mg/100 g. He *et al.*^[12] extracted volatile components from *P. tomentosa* by using distillation extraction method, and isolated and identified 35 volatile components by GC/MS/DS.^[11]

Pharmacological Activities

FlosPopuli in the early days was used to treat animal diarrhea, with the function of clearing heat and detoxifying, removing dampness and stopping dysentery. As early as recorded in the "Compendium of Materia Medica" that: "Rinse stewed FlosPopuli to relieve the toothache, take it to detoxify the arsenic, burn it to ash and then mix with oil to heal empyrosis."

Elimination of free radicals

Li *et al.*^[14] measured the elimination effect of male inflorescence extract of *P. tomentosa* on active oxidative free radicals of O_2^- , $\cdot OH$, H_2O_2 by chemiluminescence and colorimetry. The results showed that the flavonoid extracts from male inflorescence of FlosPopuli had an obvious elimination effect on free radicals of O_2^- , $\cdot OH$ and H_2O_2 *in vitro*. The experimental results showed that the IC_{50} for $\cdot O_2^-$ radical was 0.217 mg/mL. As the flavonoid concentration of FlosPopuli male inflorescence increased, the O_2^- clearance rate continued to increase. The IC_{50} for the $\cdot OH$ radical was 0.606 mg/mL, and there was also a dose-effect relationship between its concentration and clearance, which is equivalent to 0.100 mg/mL of quercetin and it could significantly inhibit DNA damage caused by $\cdot OH$. When examining the scavenging rate of H_2O_2 free radicals, it was found that the inflorescence flavones of *P. tomentosa* can obviously inhibit the chemiluminescence of the system, and the luminescence value decreased significantly with the increasing of flavonoid concentration, and its IC_{50} was 0.061 mg/mL. The results showed that the extract from male inflorescence of *P. tomentosa* had a good scavenging effect on H_2O_2 free radicals, or it could compete for the energy of H_2O_2 free radicals or both of them.^[10,23]

Tang *et al.*^[15] further investigated the antioxidant capacity of FlosPopuli alcohol extracts and found that alcohol extracts had a remarkable scavenging effect on DPPH free radicals. As the concentration increased, the scavenging capacity of alcohol extract from free radical in FlosPopuli also increased significantly, showing a positive correlation. The concentration of IC_{50} was $36.40 \pm 0.62 \mu g/mL$, which was equivalent to the IC_{50} of vitamin C. At the same time, it had strong reducing ability, and the ED_{50} was $206.32 \pm 1.61 \mu g/mL$.

Antibacterial effect

Zhou *et al.*^[18] disclosed that the ultrasonic extract of FlosPopuli had a strong inhibitory effect on *E.coli* and Salmonella *in vitro* by MIC assay. The results showed that the MIC of *E.coli* and Salmonella were 16–64 mg/mL and 64–128 mg/mL, respectively. The antibacterial effect on *E.coli* was better than Salmonella. Fang *et al.*^[28] compared MIC values of ethyl acetate and *n*-butanol extracts of FlosPopuli extracts with other types of Chinese herbal medicines against *E.coli* and Salmonella. They found that the antibacterial effect of FlosPopuli was better than that of Atractylodeslobata, Houttuynia cordata, Honeysuckle, Cork, Isatis root, Dandelion and Forsythia. The main components possessing antibacterial activities might be flavonoids and phenolic glycosides.

By measuring the inhibitory effect of FlosPopuli on pathogenic pig-derived *E.coli*, Wang *et al.*^[19] obtained MIC of 0.0039 g/mL and MBC of 0.03125 g/mL, which indicated that it had an obvious inhibitory effect on *E.coli* from pigs, and also on *E.coli* from chickens and rabbits.

In order to observe the effect of FlosPopuli on chicken vibrios hepatitis, Chen *et al.*^[20] dissolved the extracts of FlosPopuli into water, and three different concentrations were administered once per day for three consecutive days, with cefotaxime sodium as the control group. It was found that the cure rate of water dilutions of 100 g/500 kg for Vibrio hepatitis in chicken was more than 80%.^[17-21,25-26]

In vitro cytotoxicity

Hou *et al.*^[8] reported that the flavonoids luteolin, quercetin, pinocembrin, and xenophytin extracted from FlosPopuli had significant inhibitory effects *on vitro* cells, of which luteolin and quercetin exhibited significant inhibitory effects on human liver cancer cell line HepG2, human hepatoma cell line Hela, and

human normal hepatocyte line L02. The IC_{50} of luteolin on the three cell lines was $5.79 \pm 2.55 \mu g$, and allopurinol had significant inhibitory effects on human hepatoma cell line HepG2 with IC_{50} of $13.50 \pm 2.59 \mu g/mL$ and $9.13 \pm 2.56 \mu g/mL$.

Anti-inflammatory effect

Hou *et al.*^[8] studied the anti-inflammatory activity of FlosPopuli extracts by MTT method and found that tremulacin and 2-acetyl-1,3-dicaffeoylglycerol extracted from FlosPopuli had inhibitory effects on TNF- α , IL-6, IL-1 β and other related inflammatory factors. Among them, the anti-inflammatory effect of tremulacin may be related to the inhibition of the expression of related inflammatory cytokines TNF- α , IL-6 and IL-1 β , and the anti-inflammatory effect of 2-acetyl-1,3-dicaffeoylglycerol might be related to the down-regulation of TNF- α , IL-6, IL-1 β and other related inflammatory cytokines in cells, but the underlying mechanism of their anti-inflammatory effects remain to be discovered.

Hypolipidemic effect

Tang *et al.*^[15] treated hyperlipidemic mice (induced by a high-fat) with FlosPopuli extract by enema. They found that compared with the control group, the levels of TC, TG, and LDL-C in both the prevention group and the treatment group were significantly lower, while the levels of HDL-C were significantly increased. This suggested that the extract of FlosPopuli had a hypolipidemic effect.^[26]

Antidiarrheal effect

Xu *et al.*^[22] experimentally determined the effect of water extract of FlosPopuli (FPAE) on castor oil-induced diarrhea in mice. They found that FPAE (100–500 mg/kg, p.o.) had dose-dependent and significantly protective effects against castor oil diarrhea in mice. Delayed onset of diarrhea induced by castor oil, reducing the frequency and severity of diarrhea, total stool, wet stool number, wet weight, and so on, were improved compared to control mice. In addition, FPAE significantly reduced the normal intestinal push and transport, compared to berberine hydrochloride, but lower than morphine. They also found that FPAE did not show any acute toxicity. When the concentration was lower than 5 g/kg, FPAE did not cause any death or change the normal behavior of animals.

Impact on immune function

Li *et al.*^[24] compared the effects of water extracts and alcohol extracts of FlosPopuli on the immune function of mice. They disclosed that both water and alcohol extracts could significantly improve the spleen and thymus index, phagocytic capacity and lymphocytes of normal mice. Proliferative capacity, delayed allergic levels, cellular and humoral immune functions and the effects of alcohol extracts were significantly higher than those of water extracts, which might be related to the higher content of flavonoids, polysaccharides and other immune substances in alcohol extracts. However, the effect of different components in different extracts on immune function needs to be further explored.

Conclusion and Perspective

In summary, FlosPopuli is widely distributed in China, which is cheap and easy to obtain. With the efforts done by researchers, the chemical components have been isolated including flavonoids, polysaccharides and glycosides, sterols and triterpenes, phenylpropanoids and other types, as well as various amino acids and volatile components. FlosPopuli extract has also been employed in a variety of pharmacological activities, including antioxidant, antibacterial, cytotoxic, anti-inflammatory and hypolipidemic effects. Nevertheless, the research on the mechanism of its biological activities is very

limited, which greatly hinders its application. Therefore, it is necessary for us to further study the chemical compositions, pharmacological activities and mechanism of action of FlosPopuli for better utilization of the resource.

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Conflict of Interest

The authors declare no conflict of interest.

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