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Investigation on Flos Trollii: Constituents and bioactivities

YUAN Ming¹, WANG Ru-Feng¹*, WU Xiu-Wen¹, AN Yan-Nan¹, YANG Xiu-Wei²

¹ School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, China;
² State Key Laboratory of Natural and Biomimetic Drugs and Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

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[ABSTRACT] Flos Trollii, the flowers of Trollius chinensis Bunge, has been widely used in Chinese and Mongolian medicine for its efficacy of heat-clearing and detoxification. This drug has both medicinal and edible applications, and has led to various pharmacognosy, natural product chemistry, and pharmacology studies. As a result, its chemical constituents and bioactivities have been well-characterized in recent years. Nevertheless, a couple of critical issues, such as the major effective components, are still unresolved. The present review summarizes research progress on this drug regarding the constituents and bioactivities based on investigations in these laboratories and the results reported in recent publications. In addition, the pending issues are discussed and constructive suggestions for further investigation are proposed.

[KEY WORDS] Flos Trollii; Trollius chinensis; Constituents; Bioactivities

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1 Introduction

Flos Trollii is the dried flowers of Trollius chinensis Bunge (Ranunculaceae), which is a perennial herb, widely distributed in northern China [1-2]. It has been used as both a medicinal and edible material for the development of a drug and as a tea by the Chinese people [3-5]. As a drug, Flos Trollii has been employed to treat respiratory infections, tonsillitis, pharyngitis and bronchitis in Chinese and Mongolian medicine due to its efficacy of heat-clearing and detoxification [6-14]. In recent years, this drug has drawn even more attention from researchers and physicians for its antiviral and antibacterial effects. For example, it was selected as one of the compatible drugs in a compound prescription for the prevention of Severe Acute Respiratory Syndromes (SARS) when this disease broke out in China in 2003. Many investigations regarding efficacy have also been conducted for the purpose of better utilization of this wonderful drug. In the present review, the research results on the constituents and bioactivities of Flos Trollii on the basis of our research and the results reported in recent publications are summarized. Additionally, the unsettled issues, for example, the major effective constituents and the further investigations required are discussed.

2 Constituents

It is well-known that Flos Trollii contains flavonoids, organic acids, and alkaloids [15-16]. These compounds constitute the majority of chemical substances of the flowers, and are always the principal investigational targets of researchers interested in pharmaceutical sciences, bromatology, and nutrition. Beyond that, other groups of compounds such as phytosterols and terpenoids were also reported.

2.1 Flavonoids

Flavonoids are the most abundant compounds in Flos Trollii, and they account for approximately 16% of the total dried weight of these flowers [17-27].

2.1.1 Flavone C-glycosides

The overwhelming majority of flavonoids found in Flos Trollii are flavone C-glycosides which are characterized by a flavone skeleton linked with one or more sugar moieties through a carbon-carbon bond. Interestingly, among the flavone C-glycosides isolated from this drug, these sugar moieties always specifically connect to C-8 of the flavones, and C-5 and C-7 of the flavones are usually oxygenated.

Vitexin and orientin are the two most important flavone C-glycosides, and they and their derivatives comprise almost two-thirds of the total flavonoids [28-34].
2.1.2 Others

Other flavonoids other than flavone C-glycosides were also found in Flos Trollii, including aglycones of flavonol, flavone and flavonone, as well as their O-glycosides. Compared with C-glycosides, these compounds, for example, quercetin, apigenin, luteolin, salvigenin [37], acacetin, pectolinarigenin [38], cirsimaritin, and naringenin (Fig. 1) and their derivatives only account for a small portion of the flavonoids. Nevertheless, some of these compounds, such as apigenin and luteolin, might be derived from the parent compounds of flavone skeleton, among others, is a characteristic of these two compounds (Fig. 1) and their derivatives. In contrast, the compounds with a methoxy at C-7 constitute another subgroup of flavone C-glycosides which include isosvertisin and isosvertiajaponin [35], as well as their derivatives.

Acylated flavone C-glycosides were recently isolated by our research group and others [36]. These compounds, such as trollisin I, II and III, are usually acylated at the sugar moieties with a carboxyl group of various acids, including veratic acid, 2-methylbutanoic acid, and so on.

Fig. 1 Major flavonoids isolated from Flos Trollii
the C-glycosides, vitexin and orientin, in vivo. Strangely, the aglycones of isoswertisin and isoswertiajaponin, i.e., 5-hydroxy-7-methoxy-4'-hydroxyflavone and 5-hydroxy-7-methoxy-3', 4'-dihydroxyflavone are not known from this drug [39-47].

### 2.2 Organic acids

Organic acids constitute the second most abundant group of compounds in Flos Trollii, and they mainly include phenolic acids and fatty acids [48-52].

#### 2.2.1 Phenolic acids

Almost all of the phenolic acids found in this drug are derivatives of benzoic acid (Fig. 2). These compounds are further classified into two groups, one group includes veratric acid [53], benzoic acid [37], methyl veratrerase [38], globeflowery acid, trollioside [35, 45, 54] and 4-(β-D-glucopyranosyloxy)-3-(3-methyl-2-butenyl)benzolic acid [49] are usually without a free hydroxyl group due to methylation and glycosidation. Another group, including vanillic acid, protocatechuic acid, 4-hydroxybenzonic acid, methyl p-hydroxybenzoate [38], proglobeflowery acid [35, 55] and methyl 3, 4-dihydroxy- benzate [43] are compounds with a free hydroxyl group. It was deduced that some phenolic acids, for example, proglobeflowery acid and globeflowery acid may be interconvertible. This was partially established by a hydrolysis experiment on trollioside (Fig. 3) in this laboratory, which demonstrated that trollioside could be hydrolyzed into globeflowery acid and glucose under acid conditions, and could also be hydrolyzed into proglobeflowery acid and glucose by cellulase [35].

#### 2.2.2 Fatty acids

Thirty fatty acids including twenty three saturated and nine unsaturated fatty acids were detected in Flos Trollii by GC-MS analysis [56]. This group of compounds in this drug was considered as nutritious because the unsaturated acids, including oleic acid and linoleic acid, account for about 30% of the total fatty acids.

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![Fig. 2 Phenolic acids and an alkaloid isolated from Flos Trollii](https://example.com/fig2.png)

**Fig. 2** Phenolic acids and an alkaloid isolated from Flos Trollii

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### 2.3 Alkaloids

Trolline is an isoquinoline-type alkaloid (Fig. 2), newly found in Flos Trollii in this laboratory. Although alkaloids were previously shown to exist in this drug through physico-chemical identification, it was isolated and structurally elucidated [57-58]. Because alkaloids usually exhibit useful bioactivities, thus the isolation of trolline is important for the pharmaceutical investigation of this drug. Subsequently, additional alkaloids have been detected in this drug by high performance liquid chromatography [59].

### 2.4 Others

Other types of compounds, including ceramides [60], phenols [43], terpenoids [43, 61], coumarin [38], and steroids [35, 62] were also reported to be present. Among these compounds, some were isolated as chemical entities and some were detected by GC-MS.

### 3 Bioactivities

Flos Trollii was first recorded in an ancient Chinese monograph of materia medica compiled in the Qing dynasty named Supplement to Compendium of Materia Medica as a drug for disorders of the oral cavity, throat, teeth, eyes, and ears [63-69]. From this time, it has been used by Chinese people to treat infectious diseases, such as respiratory infections, tonsillitis, pharyngitis, and bronchitis. Accordingly, the pharmacological investigations of this drug have primarily focused on its effects against pathogenic microbes, including viruses and bacteria. In addition, antineoplastic and anti-oxidative activities related to this drug and its components were also investigated.

#### 3.1 Antiviral

Both the crude extract of Flos Trollii, and the compounds thereof, exhibit significant inhibitory effects against a variety of viruses, e.g., adenovirus, ECHO virus, poliovirus, coxsackievirus, influenza, and parainfluenza virus.

It was reported that the aqueous extract of Flos Trollii exhibited potent inhibitory effect against Cox B3 with an IC₅₀ value of 0.318 mg·mL⁻¹, and appreciable inhibitory effect against the Cox A24 variant [70]. In an anti-influenza virus test which was conducted using chicken embryos as the subject, the ethanol extract of Flos Trollii at a concentration of
25–200 g·L\(^{-1}\) could directly kill influenza virus A PR8 \textit{in vitro} \cite{71}. Another investigation showed that the 60% ethanol extract and the total flavonoids exhibited weak antiviral activity against para-influenza virus type 3 (Para 3) with IC\(_{50}\) values of 11.7 and 20.8 \(\mu\text{g·mL}^{-1}\), and SI values of 32.1 and 16.0, respectively. Proglobeflowery acid also showed slight antiviral activity against Para 3 with an IC\(_{50}\) value of 184.2 \(\mu\text{g·mL}^{-1}\), and a SI value of 4.0 \cite{55}. 2’-O-(2’-Methylbutanoyl) isoswertisin was found to be moderately active \textit{in vitro} against influenza virus A with an IC\(_{50}\) value of 74.3 \(\mu\text{g·mL}^{-1}\), and a SI value of 7.17 \cite{56}. The only alkaloid isolated so far, trolleyline exhibited moderate activity against influenza virus A with an IC\(_{50}\) value of 56.8 \(\mu\text{g·mL}^{-1}\), and a SI value of 4.81 \cite{57}.

The structure-activity relationships of the flavonoids were studied, and the results indicated that the concurrent existence of a 3-hydroxy or 3’-hydroxy, a 4’-hydroxy or 2’-hydroxy, and a 4’-hydroxy was related to their antiviral activity. \cite{72} Some scholars considered a 3-methoxy and a 5-hydroxy as the superior groups in terms of antiviral activity. For example, the main component orientin has hydroxyl groups at C’-3, C’-4, and C-5, and vitexin has hydroxyl groups at C’-4 and C-5.

3.2 Antibacterial

Bacteria are considered to be the pathogen of many infectious diseases, thus a number of antibacterial tests have been carried out on Flos Trollii and its constituents.

Both aqueous and ethanolic extracts of Flos Trollii have inhibitory effects against a broad spectrum of bacteria, including both Gram-positive and Gram-negative bacteria such as \textit{Staphylococcus aureus}, \textit{Streptococcus pneumoniae}, \textit{Streptococcus hemolyticus}, \textit{Neisseria catarrhal}, \textit{Pseudomonas aeruginosa}, and \textit{Bacillus dysenteriae}. \cite{73}.

All three kinds of compounds, namely, alkaloids, flavonoids, and organic acids in Flos Trollii exhibited antibacterial effects. It is noteworthy that the alkaloid trolleyline showed significant inhibitory effects against both Gram-positive and Gram-negative bacteria with MIC values of 32, 128, and 128 mg·L\(^{-1}\) toward \textit{Staphylococcus aureus}, \textit{Klebsiella pneumoniae} and \textit{Streptococcus pneumoniae}, respectively \cite{57}. It is more potent than the well-known antibacterial agent berberine (MIC toward \textit{Staphylococcus aureus} is about 100 mg·L\(^{-1}\)). As the major constituents, flavonoids also play an important role in the antibacterial effects of Flos Trollii. Total flavonoids, orientin, and vitexin demonstrated antibacterial activities against \textit{Staphylococcus aureus}, \textit{Staphylococcus epidermidis}, \textit{Bacillus pyocyaneus}, \textit{Escherichia coli} and \textit{Shigella dysenteriae}. \cite{74-77}. Among the individual flavonoids, orientin showed the most potent inhibitory effects against both \textit{Staphylococcus aureus} and \textit{Staphylococcus epidermidis} with MIC values of 25 mg·L\(^{-1}\). Total flavonoids and vitexin exhibited potent to moderate effects against those two bacteria with MIC values of 50 mg·L\(^{-1}\) and 100 mg·L\(^{-1}\) toward \textit{Staphylococcus aureus}, and 25 mg·L\(^{-1}\) toward \textit{Staphylococcus epidermidis}. Another flavonoid, cirsimartinit, was also reported to be active against both Gram-positive and Gram-negative bacteria, including \textit{Staphylococcus aureus} and \textit{Escherichia coli} \cite{78}. As for the phenolic acids, proglobeflowery acid, veratic acid, and trolloside showed moderate effects against both \textit{Staphylococcus aureus} and \textit{Staphylococcus epidermidis} \cite{75, 76}.

3.3 Anticancer

Total flavonoids isolated from Flos Trollii at a concentration of 0.793–12.688 g·L\(^{-1}\) had apparent inhibitory effects on proliferation of K562, HeLa, Ec-109, NCI-H446, and MCF-7 cells cultured \textit{in vitro}. \cite{79, 80}. They could keep the cell cycle at the G0/G1 phase, affect the expression of TERT, and reduce the telomerase activity. \cite{80}. They could also inhibit the growth of A549 cells in a dose-dependent manner, and induce cell apoptosis by increasing the expression of tumor suppressor gene p53 and decreasing the expression of oncogene Bcl-2 \cite{81}. Also, a MTT test carried out in this laboratory demonstrated that the alkaloid trolline at the concentration of 1–100 mg·L\(^{-1}\) showed very weak cytotoxic activity against HeLa cells \textit{in vitro} in a dose-dependent manner with inhibitory rates of 21.5%–54.18%.

3.4 Anti-oxidative

The flavonoids from Flos Trollii could efficiently eliminate the free-radical of DPPH with an IC\(_{50}\) value of 112 mg·L\(^{-1}\) and had efficient anti-oxidative effect on lard. When the concentration of flavonoids was 0.5% in lard, the anti-oxidative effect was as good as BHT. In addition, the flavonoids exhibited a synergistic effect with VC and BHT \cite{17, 82}. The aqueous alcoholic extract of Flos Trollii could effectively scavenge superoxide anion, hydroxyl radical, lipid-derived radicals, and singlet oxygen with EC\(_{50}\) values of 46, 5.64, 5.19, and 3.97 mg·mL\(^{-1}\), respectively. The radical scavenging activities of the aqueous alcoholic extract are higher than those of ascorbic acid and DPPH \cite{83}. Orientin and vitexin also had an effective inhibition on superoxide anion, hydroxyl radical, and DPPH with IC\(_{50}\) values of 5.02, 6.08, and 4.03 \(\mu\text{g·mL}^{-1}\) and 5.65, 7.68, and 4.74 \(\mu\text{g·mL}^{-1}\), respectively \cite{84}.

4 Discussion and Conclusions

Flos Trollii is a widely-used medicinal and edible plant material which has been demonstrated to be useful for the treatment and prevention of upper respiratory diseases due to its characteristic active compounds. To date, most of the compounds of this drug have been characterized, and their
bioactivities have been revealed. However, the major effective components and the individual contribution degree of these three compound classes have not yet been elucidated. Most researchers consider the flavonoids as the major effective components because they are the most abundant ones, and the principal components, orientin and vitexin, exhibit both antiviral and antibacterial activities in vitro. Nevertheless, others have argued that flavonoids are not the major effective compounds, or at least not the only effective compounds, because the bioactivity of Flos Trollii is not proportional to the content of the flavonoids in various samples, which may imply that other kinds of compounds might be responsible, or synergistic with flavonoids, for the therapeutic effects. Moreover, flavonoids are poor in solubility, which results in inadequate absorption by the human body and subsequently leads to less-than-anticipated effectiveness in vivo. Hence, a strategy in vivo, e.g., sero-pharmacochemical or sero-pharmacological investigation, in combination with pharmacokinetics, should be employed to investigate the actual effective components of Flos Trollii in order to determine the material basis for the efficacy of this drug.

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金莲花的化学成分及生物活性研究进展

袁 铭 1, 王如峰 1*, 吴秀稳 1, 安燕南 1, 杨秀伟 2

1 北京中医药大学中药学院, 北京, 100102
2 北京大学药学院, 北京, 100191

【摘 要】目的: 金莲花由于其清热解毒作用在中药及蒙药中被广泛使用。作为一种药食两用药物，金莲花已经引起了药学家、药理学家、天然药物学家、营养学家等的广泛兴趣。其化学成分和生物活性研究发展较快，然而也存在一些问题，诸如有效成分的归属问题，这些问题限制了金莲花的进一步开发利用。本文结合本研究组对金莲花的化学成分和生物活性的研究成果，以及近几十年有关金莲花的研究报道，对金莲花的化学成分和生物活性研究进展进行了系统总结，并对存在的问题进行了讨论与展望。

【关键词】金莲花；化学成分；生物活性

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