Pharmacological Properties of Phillyrin

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Forsythia suspensa (Thunb.) Vahl (Oleaceae) is a traditional Chinese medicine first recorded in *Shennong Bencao Jing*, which was a book published ca. 2000 years ago documenting Chinese folk medicines. Forsythiae Fructus, the dried fruit of *F. suspensa*, is frequently used in China by physicians for heat clearing and detoxifying. Modern pharmacological studies showed that Forsythiae Fructus has antipyretic, anti-inflammatory, antiviral, antibacterial, as well as anti-tumor effects, and therefore, it is clinically used to treat fever, influenza, tumor, hypertension, and other diseases. As the main active components of this medicinal plant, more than 50 lignans have been isolated and characterized from various organs of *F. suspensa* (fruit, flower, leaf and root), with phillyrin ($C_{27}H_{34}O_{11}$) as the key compound. Phillyrin, a lignan glycoside, is the phytochemical marker for Forsythiae Fructus quality assessment in *Chinese Pharmacopoeia* 2020 edition, and it is stipulated that the content of phillyrin shall not be less than 0.15% when calculated as dry product.

phillyrin pharmacological properties anti-inflammatory effects

1. Biology

F. suspensa is a deciduous shrub that is sometimes cultivated in gardens as an ornamental plant due to its beautiful yellow flowers blossomed in spring. With its branches pendulous or spreading, the plant has yellow–brown or gray–brown branchlets, and the internodes are hollow. The leaves are simple, and the shape of the leaf blade is ovate to broadly ovate. The flowers are solitary or two to several in leaf axils. With scattered lenticels, the capsules of the plant are ovoid to long ellipsoid (**Figure 1**A). Except for South China, *F. suspensa* is widely distributed in Eastern Asia [1].

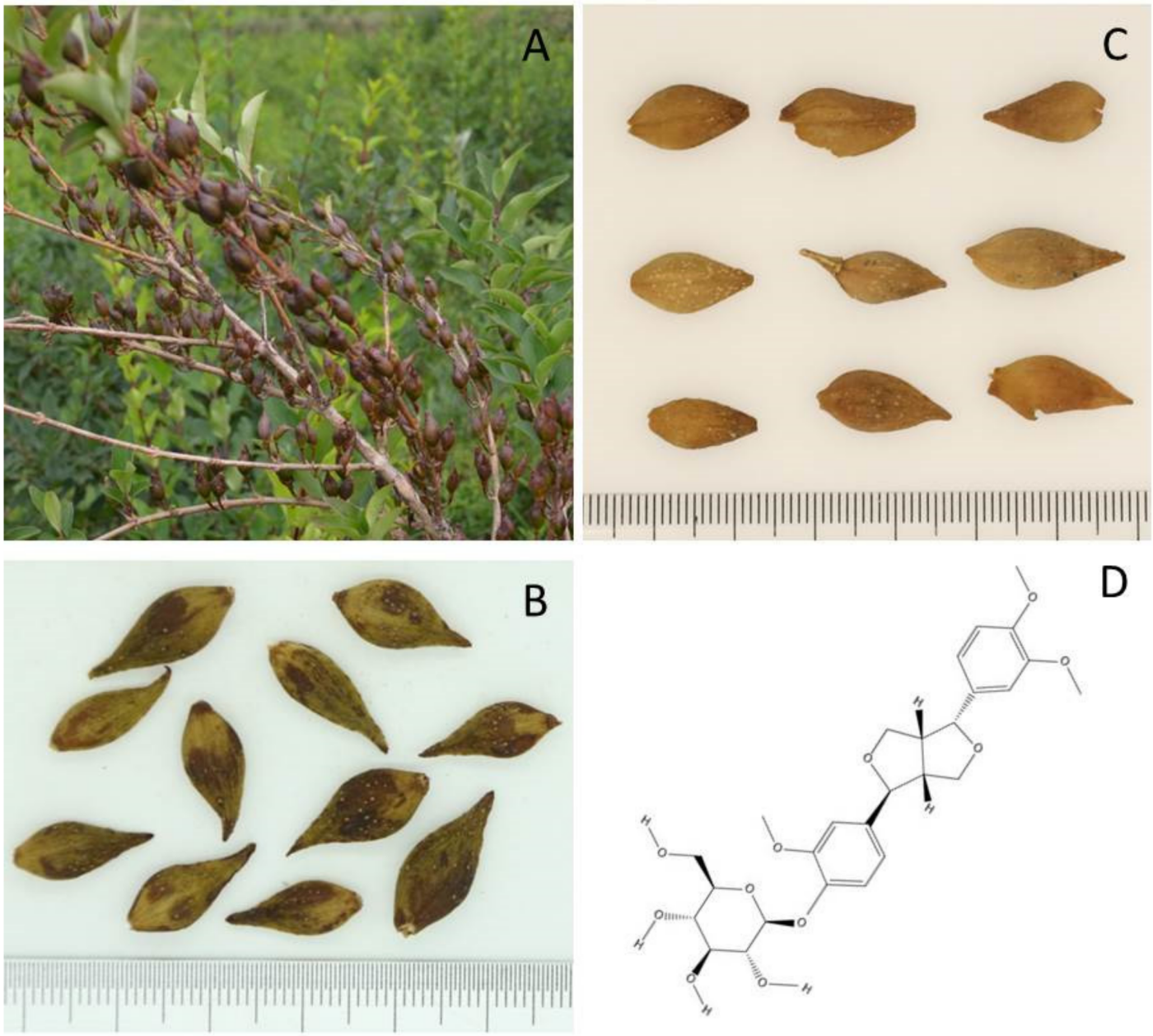

Figure 1. *F. suspensa* and phillyrin. (**A**) Fruits of *F. suspensa* grown in Lingchuan County, Shanxi Province, China; (**B**) Qing qiao; (**C**) Lao qiao; (**D**) Structure of phillyrin (created with ChemDraw 19.0).

As a crude drug recorded in *Chinese Pharmacopoeia*, the fruit of *F. suspensa* is usually categorized into two forms: unripe Forsythiae Fructus (Qing qiao) and ripe Forsythiae Fructus (Lao qiao) (**Figure 1**B,C). Using high-performance liquid chromatography (HPLC), Hu et al. found that the content of phillyrin in Qing qiao was significantly higher than that of Lao qiao from the same origin [2][3]. However, the content of phillyrin in the fruit is not as high as in other parts of the plant. Based on the comparison of reference substances and analysis of HPLC fingerprint for extracts from different parts of *F. suspensa* in the literature, Ma et al. demonstrated that Forsythoside A has maximum concentration in the fruits. Nevertheless, the content of phillyrin is much lower than that in the root and stem of *F. suspensa* [4]. When researchers turned their attention to the leaves, regarding the content of phillyrin, they reached the conclusion as follows: leaves (2.60%) > Qing qiao (0.91%) > Lao qiao (0.17%) [5]. To

sum up, in addition to the traditional application of Forsythiae Fructus as medicine, the effective utilization of *F. suspense* leaves, roots and stems is of great significance as far as phillyrin extraction is concerned. Because of its physicochemical property, the extraction of phillyrin from various parts of *F. suspensa* often involves different concentrations of methanol or ethanol in combination with optimal solid–liquid ratio, extraction temperature and time to achieve high extraction efficiency. In addition, flash extraction and ultrasonic or microwave-assisted extractions are also frequently reported [6]. The yield of phillyrin with the above-mentioned approaches is generally satisfactory.

The biosynthesis of lignans in plant utilizes the phenylpropanoid pathway. Such compounds can be divided into the following types according to their molecular architecture: aryltetralin, dibenzylbutyrolactone, arylnaphthalene, furofuran, tetrahydrofuran and dibenzylbutane. Phillyrin is a type of furofuran lignan that features phenolic dimers containing a 2, 3-dibenzylbutane structure (**Figure 1**D). Phillygenin, an aglycon of phillyrin, can be obtained by enzymolysis with cellulase or hydrolysis.

2. Pharmacological Properties of Phillyrin

2.1. Effects on Metabolic Disorders

2.1.1. Obesity

Obesity refers to an abnormal or excessive accumulation of fat, which is a well-known risk factor to diabetes, hypertension and other related metabolic diseases [7]. In vitro, phillyrin has shown an inhibiting effect on lipid accumulation induced by high glucose in the Hep G2 cell line [8]. It has also been proven in animal experiments that medium (53.2 mg/(kg m_b·d)) and high (159.6 mg/(kg m_b·d)) doses of phillyrin could significantly reduce the volume of adipocytes in adipose tissue in rats fed with high-fat diet (HFD) [9]. However, despite that there are many in vivo data supporting the anti-obese action of phillyrin, few studies in the literature have covered the underlying mechanisms. Xiao et al. speculated that phillyrin functioned as an AMPK activator, increasing the expression of PPARβ/δ and ANGPTL4 to promote fat metabolism and weight loss in HFD mice [10]. In addition, by using molecular docking technology, phillyrin was identified as an inhibitor of phosphodiesterase 3B (PDE3B). PDE is an enzyme that catalyzes the hydrolysis of cyclic adenosine monophosphate (cAMP). cAMP binds to the regulatory units of protein kinase A (PKA), allowing for lipolysis in adipocytes [11]. Thus, PDE3B might be a target for phillyrin-initiated weight loss in obesity [8]. Nevertheless, more efforts are needed for understanding the role of phillyrin better in the regulation of systemic metabolism.

2.1.2. Diabetic Nephropathy (DN)

Previous reports suggested some potential for phillyrin in the treatment of DN, which is one of the common chronic complications of diabetes and the main cause of death in diabetic patients. Firstly, phillyrin has been shown to increase insulin sensitivity by promoting glucose uptake in insulin-resistant 3T3-L1 adipocyte via activation of the PI3K/Akt signaling pathway [12]. Secondly, phillyrin was also reported to significantly improve the pathological changes in kidneys in DN animals. After treatment with phillyrin, glomerular volume, basement membrane

thickness and inflammatory cell infiltration were significantly reduced in diabetic rats, which was associated with a reduced expression of TGF-β_1 in renal tissue of DN rats [13]. By activating the PI3K/Akt/GSK-3β signaling pathway, phillyrin inhibited glycogen synthase kinase-3β (GSK-3β) activity, thereby suppressing the activation of caspase-3 and ultimately preventing the apoptosis of renal cells [14]. Therefore, phillyrin might be applied as a therapeutic target of DN.

2.2. Anti-Inflammatory Effects

As the “holy medicine for sore family”, the anti-inflammatory effects of Forsythiae Fructus are consistent with the traditional usage of reducing swelling and dispersing knots. Extracted from the dried fruit of Forsythiae Fructus, phillyrin is composed of two phenylpropanoid side chains linked to each other. The oxygen bridge on the structure of phillyrin is one of the groups that plays the crucial role in exerting antioxidant, antibacterial and anti-inflammatory effects [15].

Inflammation is a kind of body defensive response to ensure that harmful stimuli are removed and damaged tissue is repaired. When immune cells are under the action of inflammatory factors, certain soluble proteins or peptides with small molecular weight, which can transmit information between cells and have specific immune regulation function, are secreted by the body and can participate in or cause inflammatory reactions. These substances are called inflammatory factors, such as NO, TNF-α, IL-6, PGE2, IL-1, and so on [16]. Phillyrin changes the expression level of these inflammatory factors by influencing the inflammation-related signaling pathways .

2.3. Anti-Aging Effect

According to the free radical aging theory, the human body produces free radicals in the process of life activities, and the chain reaction of free radicals leads to the damage of the biofilm and the cross-linking of biological macromolecules. As a result, protein and nucleic acid molecules are damaged, so that biofilms fail to function and lipofuscin accumulates, leading to aging and death [17]. In a mouse model of aging induced by D-galactose, phillyrin significantly increased the activities of SOD, T-AOC and GSH-Px in the serum, liver and brain of aged mice, reducing the accumulation of MDA and the activity of MAO-B, and protecting the body from free radical damage [18].

2.4. Antiviral Effects

In modern pharmacology, the antiviral effects of phillyrin are basically consistent with the heat-clearing and detoxifying effect of Forsythiae Fructus recorded in ancient prescriptions. Phillyrin can inhibit the nuclear protein (NP) gene expression of influenza A virus after transfection so as to achieve the anti-“A flu” effect [19]. Other studies have shown that phillyrin can significantly prevent the replication of influenza A virus [20]. It is not only influenza A virus but also the devastating coronavirus (COVID-19), the cause of the global public health crisis in the past two years, that has brought phillyrin as an effective remedy into the field of scientific research again [21]. Based on a bioinformatics analysis, researchers identified 192 common core targets and 25 biological pathways for phillyrin in the treatment of SARS-CoV-2 and influenza virus co-infection. It is concluded that HIF-1, PI3K-AKT and RAS may

be the main signaling pathway of the antiviral effect of phillyrin, which provides a new idea for follow-up COVID-19 treatment.

2.5. Antibacterial Effects

Forsythiae Fructus holds certain inhibitory effects on bacteria and has a wide antibacterial spectrum. As one of its main active components, phillyrin can antagonize bacterial endotoxin [22]. In vitro experiments showed that phillyrin had the potential to inhibit the production of quorum sensing regulatory virulence factors such as pyocyanin, elastase, and rhamnolipid in *P. aeruginosa*. The results suggest that phillyrin can be used as an alternative of antibiotics against bacterial infection [23]. Phillyrin also shows therapeutic effects on *K. pneumonia*-induced diarrhea, pneumonia, and urinary tract infections. The addition of phillyrin can activate the STAT5/Foxp3 pathway in *Kp*-infected mice with pneumonia to promote Treg differentiation and to reduce the level of inflammatory factors. The imbalance of Th17/Treg cell ratio was alleviated for protecting *Kp*-infected pneumonia mice [24]. In the rat perianal abscess model, phillyrin could significantly reduce the number of *Escherichia coli* colonies on the wound surface, and the researchers proposed that the mechanism of action of phillyrin might be related to activation of the Janus kinase/Signal transducer and activator of transcription 3 (JAK2/STAT3) signaling pathway [25].

2.6. Hepatoprotective Effects

Chronic hepatitis is a long-term chronic liver injury and inflammation caused by a variety of pathogenic factors, and fibrosis is the most important pathological change in the development of chronic hepatitis. According to the results of animal experiments, phillyrin can ameliorate CCL4/ANIT-induced inflammation, fibrosis and injury in experimental animals. The mechanism may be related to the inhibition of the NF-κB signaling pathway and $TGF\beta_1/SMad_{2/3}$ signaling pathway, which was further verified in vitro in hepatic stellate cells [26]. In the liver of patients with alcoholic hepatitis, alcohol metabolite acetaldehyde can directly cause the apoptosis of hepatocytes and thus increase reactive oxidative species (ROS) levels. Studies have shown that phillyrin exhibited a hepatoprotective effect by reversing alcohol-induced apoptosis in the liver in vivo [27]. However, the molecular mechanisms of this protective effect remain elucidated.

2.7. Anti-Cancer Effects

Researchers also suggested that phillyrin has striking anti-tumor potential based on the links between tumor pathogenesis and proven pharmacological effects of phillyrin. For example, according to an experiment studying laryngeal squamous cell carcinoma (LSCC), phillyrin alone has almost no effect on the proliferation and apoptosis of HEp-2 cells, but it can significantly improve the autophagy level of HEp-2 cells. A growing number of studies have shown that various inflammatory factors lead to the occurrence and metastasis of tumors by participating in and changing the formation of the microenvironment [28]. Combined with the anti-inflammatory effect of phillyrin, this induction of autophagy in tumor cells may be related to the AMPK/mTOR/p70S6K pathway [29]. From another one of Lewis lung carcinoma, in vivo experiments in mice showed that phillyrin inhibited VEGF specifically expressed in lung cancer tissue, thereby inhibiting further tumor angiogenesis. In addition, hematoxylin and eosin (HE) section staining showed that a high dose of phillyrin could significantly decrease the volume and tissue

density of lung tumors. It is suggested that phillyrin may be a potential active ingredient for inhibiting the development of lung cancer, although the specific mechanism needs further research [30].

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