Journal of Biosciences and Medicines, 2026, 14(5), 300-312
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Recent Advances in Antiviral Research on *Platycladus orientalis* Leaves

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How to cite this paper: Zhang, C.Y. and Zheng, Y. (2026) Recent Advances in Antiviral Research on *Platycladus orientalis* Leaves. *Journal of Biosciences and Medicines*, **14**, 300-312.
https://doi.org/10.4236/jbm.2026.145021

Received: April 11, 2026
Accepted: May 19, 2026
Published: May 22, 2026

Abstract

Platycladus orientalis (L.) Franco leaves, a traditional Chinese medicine, have been traditionally used to cool blood, stop bleeding, resolve phlegm, and relieve cough. In recent years, their antiviral potential has gained increasing attention. This review comprehensively summarizes research advances on the antiviral effects of *P. orientalis* leaves, focusing on active components, mechanisms of action, and quality control. Flavonoids, terpenoids, and polysaccharides isolated from *P. orientalis* leaves exhibit notable antiviral activities. To date, robust experimental evidence supports anti-HBV activity, with demonstrated efficacy in HepG2.2.15 cells by reducing HBsAg, HBeAg, and HBV DNA. For other viruses such as HSV and SARS-CoV-2, the potential remains speculative, with suggestions arising from structural similarities to known antiviral compounds or the inclusion of *P. orientalis* in multi-herb formulations, none of which have been validated by direct viral testing. Additionally, HPLC fingerprinting combined with chemical pattern recognition has been established for quality control. Current research limitations include insufficient *in vivo* studies and a lack of systematic evaluation of monomeric compounds. Future directions should focus on activity-guided isolation, molecular mechanism elucidation, and clinical application development. This review provides a reference for further development of *P. orientalis* leaves as antiviral agents.

Keywords

Platycladus orientalis Leaves, Antiviral, Flavonoids, Hepatitis B Virus, Polysaccharides, Mechanism of Action

1. Introduction

Platycladus orientalis (L.) Franco leaves, first recorded in *Shennong's Classic of Materia Medica* as a superior-grade herb, are the dried branch tips and leaves of

the cypress family plant. According to traditional Chinese medicine theory, they are slightly cold in nature, bitter and astringent in taste, and act on the lung, liver, and spleen meridians. Their traditional uses include cooling blood to stop bleeding, resolving phlegm to relieve cough, and promoting hair growth. Clinically, they are used to treat hemoptysis, epistaxis, hematemesis, hematochezia, metrorrhagia, lung heat cough, and alopecia. Notably, *P. orientalis* leaves are not only a traditional Chinese medicinal material but also an approved health food ingredient by the National Medical Products Administration [1].

In recent years, the frequent emergence of viral infectious diseases has posed serious challenges to global public health. The outbreak of coronavirus disease 2019 (COVID-19) has highlighted the urgent need for antiviral drug development [2]. Traditional Chinese medicine has shown unique advantages in the prevention and treatment of viral diseases, and searching for highly effective, low-toxic antiviral active components from natural medicines has become an important research direction [3] [4]. As an abundant traditional Chinese medicine resource, *P. orientalis* leaves have gradually attracted scholarly attention for their antiviral activities. Modern research has revealed that *P. orientalis* leaves contain various chemical components, including flavonoids, terpenoids, volatile oils, and polysaccharides, some of which exhibit promising antiviral activities [1] [5].

This review comprehensively summarizes the research progress on the antiviral effects of *P. orientalis* leaves, analyzes their active components, mechanisms of action, quality control status, and discusses future research directions, aiming to provide a reference for further development and clinical application.

2. Chemical Constituents of *Platycladus orientalis* Leaves

2.1. Flavonoids

Flavonoids are one of the main active components of *P. orientalis* leaves and an important material basis for their antiviral effects. Various flavonoids have been isolated from the 80% ethanol extract using separation and purification techniques such as silica gel, MCI, polyamide, and preparative liquid chromatography. Identified compounds include myricitrin, 5,8,3',4'-tetrahydroxyflavone 7-O-β-D-xyloside, kaempferol-7-O-α-L-rhamnoside, kaempferol-3-O-β-D-xylopyranoside, kaempferol-3-O-β-D-glucoside, and quercetin-3-O-β-D-galactoside [1]. In addition, a new compound named platyclaside A (4-O-(1',3'-dihydroxypropyl-2'-)-dihydroconiferyl alcohol 9-O-β-D-glucoside) was also isolated from the leaves [1] [6].

Myricitrin, a flavonoid with high content in *P. orientalis* leaves, exhibits various biological activities, including antioxidant, anti-inflammatory, and α-glucosidase inhibitory effects. Quercitrin is the quality control indicator component specified in the 2020 edition of the Chinese Pharmacopoeia. Studies have shown that flavonoids such as myricitrin and quercitrin possess significant antioxidant and α-glucosidase inhibitory activities [1]. The chemical diversity of flavonoids in *P. orientalis* leaves has been systematically reviewed [7] [8].

2.2. Terpenoids and Volatile Components

Terpenoids are the main components of the volatile oil of *P. orientalis* leaves. Studies have analyzed the chemical constituents of the volatile oil by GC-MS, separating numerous peaks and identifying major components. A total of 42 compounds belonging to five classes (alkenes, alcohols, ketones, aldehydes, and esters) were identified from the petroleum ether extract, with pinene being the most abundant. In addition, isopimaradienol and pinusolide were isolated, with isopimaradienol being reported from *P. orientalis* leaves for the first time. Other terpenoids isolated from *P. orientalis* leaves include cryptojaponol, sugiol, and ferruginol, which have shown various biological activities [7] [8].

2.3. Polysaccharides

Polysaccharides represent another important class of active components. Studies have used hot water extraction, Savege method for protein removal, ethanol precipitation, and ion exchange column chromatography to isolate and purify polysaccharide fractions from *P. orientalis* leaves [5]. A related patent discloses a *P. orientalis* leaf polysaccharide with antiviral and immune-enhancing activities and its preparation method. This polysaccharide has uniform molecular weight, high purity, and good thermal stability, with a preparation process that is reasonable, simple, and suitable for industrialization [9]. Recent advances in polysaccharide research from *P. orientalis* leaves have been summarized [10].

Lin *et al.* (2016) systematically characterized the polysaccharide POP1. Physicochemical analysis showed that POP1 has a relative molecular mass of 8.10×10^3 Da and consists of rhamnose (5.74%), arabinose (12.58%), mannose (10.97%), glucose (64.96%), and galactose (6.55%). The main linkage types were determined by periodate oxidation, Smith degradation, methylation, and NMR analysis [11]. Studies have also shown that polysaccharides from *P. orientalis* leaves possess immunomodulatory activities. The isolation, structural characterization, and bioactivities of natural polysaccharides have been extensively reviewed [12].

3. Antiviral Effects of *Platycladus orientalis* Leaves

3.1. Anti-Hepatitis B Virus (HBV) Effects

Extracts of *P. orientalis* leaves show significant inhibitory effects against HBV. Studies have evaluated the anti-HBV activities of flavonoids and polysaccharides using different cell models. The results showed that flavonoids exert antiviral effects by inhibiting HBV DNA replication and reducing the expression levels of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) [5].

Lin *et al.* (2016) reported the anti-HBV activity of POP1 in detail. The half-maximal inhibitory concentration (IC_{50}) of POP1 against HBsAg was 1.33 mg/mL ± 0.12 mg/mL, against HBeAg was 1.67 mg/mL ± 0.13 mg/mL, and against HBV DNA replication was 0.80 mg/mL ± 0.03 mg/mL. These data indicate that *P. orientalis* leaf polysaccharide significantly inhibits HBV replication [11] (**Table 1**). The pol-

ysaccharide showed a dose-dependent inhibitory effect on HBV antigens and DNA replication, suggesting its potential as an anti-HBV agent.

Table 1. Summary of reported anti-HBV activity of *P. orientalis* leaf extracts.

Active Component	Experimental Model	Key Readouts (Method)	Effect Magnitude	Ref.
POP1 (Polysaccharide)	HepG2.2.15 Cells	HBsAg (ELISA)	IC_{50} = (1.33 ± 0.12) mg/mL	[11]
POP1	HepG2.2.15 Cells	HBeAg (ELISA)	IC_{50} = (1.67 ± 0.13) mg/mL	[11]
POP1	HepG2.2.15 Cells	HBV DNA (qPCR)	IC_{50} = (0.80 ± 0.03) mg/mL	[11]
Total Flavonoids	HepG2.2.15 Cells	HBsAg/HBeAg (ELISA)	Dose-Dependent Reduction	[5]

It has been speculated that the anti-HBV effect of *P. orientalis* leaves might involve modulation of host immune function. Flavonoid components have been observed to promote macrophage polarization, which could potentially enhance viral clearance. Similarly, polysaccharide components are thought to exert indirect antiviral effects through immune regulation [5]. Further support for the immunomodulatory properties of *P. orientalis* polysaccharides comes from studies demonstrating enhanced macrophage activity and cytokine secretion [9].

The relevant patent clearly states that *P. orientalis* leaf polysaccharide has good antiviral and immune-promoting effects and can be used as an adjuvant therapeutic agent for hepatitis B or as a functional health food with immune-boosting effects [9].

3.2. Antiviral Effects against Other Viruses—Indirect Clues Rather than Direct Evidence

To date, no experimental studies have directly demonstrated that *P. orientalis* leaf extracts inhibit herpes simplex virus (HSV), influenza virus, or respiratory syncytial virus. What exists instead are several indirect clues that suggest potential avenues for future research, none of which constitute direct evidence of antiviral efficacy.

Structural similarity as a starting point, not proof. Certain flavonoids present in *P. orientalis*, such as quercitrin and amentoflavone, share structural features with known anti-HSV compounds. While this observation is chemically interesting, structural similarity alone does not demonstrate antiviral activity and serves only as a hypothesis-generating clue.

Antimicrobial data do not predict antiviral effects. One study examining the biological effects of *P. orientalis* extracts against disease vectors (*Culex pipiens*) noted some antimicrobial properties [13]. However, antimicrobial activity (against bacteria or fungi) is mechanistically distinct from antiviral activity and cannot be extrapolated to predict efficacy against viruses.

Multi-herb formulations obscure the role of *P. orientalis*. A patented herbal spray containing *P. orientalis* leaves together with several other herbs (honeysuckle,

agastache, clove, and eupatorium) has been proposed for air and environmental disinfection [14]. Because multiple herbs are present, the specific contribution of *P. orientalis* to any potential antiviral effect cannot be determined from this formulation alone.

Vector studies are not human antiviral studies. Research has been conducted on the effects of *P. orientalis* extracts against disease-transmitting mosquitoes (*Culex pipiens*) [13]. While such studies have ecological or parasitological relevance, they do not provide evidence of antiviral activity against human viruses.

In summary, while anti-HBV activity is supported by direct experimental evidence from cell-based assays, claims regarding activity against HSV, influenza, or other respiratory viruses remain speculative. The observations described above should be viewed as indirect clues that generate hypotheses for future investigation, not as demonstrated antiviral efficacy.

3.3. Potential Anti-Coronavirus Effects—A Hypothesis without Experimental Support

The COVID-19 pandemic has renewed interest in traditional Chinese medicines with potential antiviral properties [2] [3]. It must be emphasized that no experimental study—whether *in vitro* or *in vivo*—has yet evaluated the anti-coronavirus effects of *P. orientalis* leaves. Direct evidence against SARS-CoV-2 or any other coronavirus is completely absent at this time.

Given that several other traditional Chinese medicines have demonstrated inhibitory effects against SARS-CoV-2 in preclinical studies [3] [4], *P. orientalis* leaves may be worth investigating as a candidate. However, any suggestion of anti-coronavirus activity remains entirely hypothetical at present. Readers should understand that this is a research gap to be filled, not a claim supported by current data.

4. Antiviral Mechanisms of Action

4.1. Direct Inhibition of Viral Replication—Evidence from HBV Studies

The polysaccharide POP1 has been shown to directly inhibit HBV DNA replication in HepG2.2.15 cells, with an IC_{50} of 0.80 mg/mL as measured by qPCR, and to reduce HBsAg/HBeAg secretion as measured by ELISA [11]. These findings demonstrate a direct effect on viral replication. However, it is important to note that none of the cited studies has directly measured polymerase activity, viral protein synthesis, or any specific molecular step in the viral life cycle. Claims about "polymerase inhibition" or "blockade of viral protein synthesis" are not supported by the available data and should be considered speculative. Overall, the available evidence indicates that *P. orientalis* extracts can interfere with the viral replication process within host cells, but the precise molecular target—whether HBV polymerase, pgRNA packaging, cccDNA formation, or another step—has yet to be identified [5] [11].

4.2. Immunomodulatory Effects—Observations and Hypotheses

Several studies have explored the immunomodulatory properties of *P. orientalis* components using macrophage polarization models in the absence of viral infection. These findings provide a basis for hypotheses about potential antiviral mechanisms, though direct evidence in the context of viral infection is still lacking.

Macrophage polarization and cytokine production: Using macrophage polarization models (without viral challenge), researchers have found that *P. orientalis* flavonoids and polysaccharides promote M1-type polarization, which is associated with enhanced phagocytic capacity and antigen-presenting ability [5]. Lin *et al*. (2016) further demonstrated that POP1 enhances macrophage secretion of NO, TNF-α, IL-6, and IL-12, and activates related mRNA expression [11]. These observations have led to the hypothesis that such immunomodulatory effects could contribute to antiviral defense, though this remains to be tested in virus-infected systems. Additional immunomodulatory activity of *P. orientalis* polysaccharides has been reported [9].

Interferon induction: It has been suggested that *P. orientalis* components might induce interferon production, which could enhance antiviral immunity. However, none of the cited studies has directly measured interferon levels (e.g., IFN-α, IFN-β, IFN-γ) or demonstrated interferon induction in response to *P. orientalis* treatment. This remains a hypothetical mechanism requiring experimental validation.

NK cell and cytotoxic T-cell activation: It has been suggested that flavonoid components may enhance the activity of natural killer (NK) cells and cytotoxic T lymphocytes [9]. It is critical to note that the cited reference [9] is a patent that does not provide primary experimental data—such as cytotoxicity assays, target cell killing assays, or flow cytometry-based lymphocyte activation measurements—to support these claims. No peer-reviewed studies have demonstrated NK cell or CTL activation by *P. orientalis* extracts. These suggestions should therefore be considered purely hypothetical at this stage.

4.3. Antioxidant Effects—A Potential Indirect Contribution

Viral infections are frequently accompanied by oxidative stress, which can facilitate viral replication and exacerbate tissue damage. The flavonoid compounds in *P. orientalis* leaves have demonstrated strong antioxidant activity in chemical assays. By scavenging reactive oxygen species (ROS) and reducing oxidative stress, these components could theoretically suppress viral propagation and aid recovery from viral infection. The antioxidant activity of *P. orientalis* flavonoids has been confirmed using DPPH and ABTS radical scavenging assays, with compounds such as myricitrin showing significant free radical scavenging capacity [1] [15]. Nevertheless, no study has directly linked this antioxidant activity to antiviral efficacy, and this remains an indirect mechanistic hypothesis.

5. Quality Control Studies

To ensure the quality control of *P. orientalis* leaf medicinal material, researchers

have established HPLC fingerprint analysis methods. Wang *et al.* (2023) established HPLC fingerprints of *P. orientalis* leaves from different origins. A total of 13 common peaks were found in 24 batches of samples. Compared with the reference fingerprint, the similarity of 23 batches (except one batch of *Juniperus chinensis* leaves) was above 0.92. The study identified four common peaks: myricitrin, quercitrin, isoquercitrin, and amentoflavone. Chemical pattern recognition methods such as cluster analysis, principal component analysis, and partial least squares-discriminant analysis can be used for quality control and authentication of *P. orientalis* leaves [16]. Principal component analysis showed that six principal components had a cumulative variance contribution rate of 88.412%, which could reflect most of the information of the original chromatographic peaks [16]. Additional quality control studies for *P. orientalis* formulations have also been reported [17].

A critical question for antiviral development is whether these marker compounds are functionally linked to antiviral activity or serve merely as chemical markers. Table 2 addresses this question.

Table 2. Functional relevance of quality control marker compounds.

Marker Compound	Detected Antiviral Activity in *P. orientalis*	Functional Role
Myricitrin	None reported	Chemical marker only
Quercitrin	None reported (antioxidant activity only)	Chemical marker only (Pharmacopoeia indicator)
Isoquercitrin	None reported	Chemical marker only
Amentoflavone	Anti-HBV activity reported in other plants; direct evidence from *P. orientalis* lacking [18]	Potential functional marker (needs validation)

Taken together, none of the four marker compounds has been directly validated for antiviral activity specifically in *P. orientalis*. Quercitrin, the Pharmacopoeia indicator, was selected based on chemical abundance rather than functional relevance. Amentoflavone holds promise as a potential functional marker, but validation in *P. orientalis* is required.

These findings have important implications for future quality control. Ideally, quality specifications should prioritize compounds with demonstrated antiviral activity. The current reliance on non-activity-linked chemical markers is sufficient for authentication but inadequate for ensuring batch-to-batch consistency of antiviral efficacy. Future efforts should identify active compounds, validate them as functional markers, and incorporate them into updated quality standards.

These methods provide a scientific basis for improving the quality standards of *P. orientalis* leaf medicinal material. The establishment of comprehensive quality evaluation systems is essential for the development of *P. orientalis* leaves as a reliable source of antiviral agents. The application of chromatographic fingerprinting combined with chemometrics for quality control of traditional Chinese medicine has been well established [19].

6. Other Pharmacological Activities

In addition to the antiviral effects discussed above, *P. orientalis* leaves exhibit a range of other pharmacological activities that may complement their therapeutic potential. Anti-inflammatory effects of *P. orientalis* flavonoids have been reported, which could help mitigate virus-induced inflammatory responses [5]. The anti-inflammatory biomarkers of *P. orientalis* have been further characterized using UPLC/MS/MS and network pharmacology [18]. The antioxidant properties of *P. orientalis* components [1] [15] may also contribute to tissue protection during viral infections. Furthermore, consistent with traditional use for hemostatic purposes, modern research has confirmed the ability of *P. orientalis* leaves to promote blood coagulation and shorten bleeding time [8]. These multifaceted activities suggest that *P. orientalis* leaves might serve as an adjunctive therapy for viral diseases complicated by inflammatory or hemorrhagic manifestations. The pharmacological effects of *P. orientalis* have been comprehensively reviewed [8]. For a broader context, research progress on antiviral mechanisms of traditional Chinese medicine in general has also been summarized [20], and the immunomodulatory effects of polysaccharides from traditional Chinese medicine have been reviewed [21].

7. Safety, Toxicity, and Pharmacokinetic Limitations

Despite the promising anti-HBV activity observed *in vitro*, no systematic safety, toxicity, or pharmacokinetic (PK) studies have been reported for *P. orientalis* leaf extracts or their pure compounds, which represents a major barrier to clinical translation. Regarding toxicity, acute or subacute toxicity studies in animals are currently absent; traditional use suggests low toxicity, but this has not been quantified using modern toxicological standards. In terms of pharmacokinetics, based on studies of other plant sources, the key flavonoids found in *P. orientalis* (quercitrin, myricitrin, amentoflavone) are known to have very low oral bioavailability (typically <5%) due to poor absorption and rapid glucuronidation, and no PK studies have been conducted specifically on *P. orientalis* compounds. Furthermore, potential herb-drug interactions mediated by cytochrome P450 (CYP) enzymes (e.g., CYP3A4, CYP2D6, CYP1A2) have not been evaluated for *P. orientalis* extracts. Finally, no Phase I or Phase II clinical trials have been conducted to date. These limitations must be addressed before any clinical application can be seriously considered. This section is intended to provide a balanced perspective on the translational challenges facing the development of *P. orientalis* leaves as antiviral agents.

8. Problems and Prospects

Although certain progress has been made in antiviral research on *P. orientalis* leaves, several challenges remain. Research on active components lacks sufficient depth; most work has focused on isolation and identification, while systematic antiviral activity screening and structure-activity relationship studies remain rel-

atively underdeveloped. Although flavonoids and polysaccharides have demonstrated some anti-HBV activity, evaluations of individual monomeric compounds are still not systematic or comprehensive [5]. Mechanistic studies also require strengthening, as existing work is largely preliminary and lacks in-depth molecular target analysis. While immunomodulatory mechanisms have been explored using macrophage polarization models, the complexity of virus-host interactions demands investigation from multiple perspectives and levels. Furthermore, *in vivo* and clinical research are insufficient. Most current studies rely on *in vitro* experiments without systematic *in vivo* pharmacodynamic evaluation, leaving the clinical value of *P. orientalis* leaves' antiviral effects to be further validated. The lack of standardized animal model studies limits the translation of *in vitro* findings to clinical applications. Quality control standards, though established, need further refinement to ensure batch-to-batch consistency for potential pharmaceutical development. As noted in Section 7, the complete absence of safety, toxicity, and pharmacokinetic data represents a critical barrier to clinical translation. Finally, in the context of the COVID-19 pandemic, the potential anti-coronavirus effects of *P. orientalis* leaves—a traditional Chinese medicine with recognized antiviral potential—have not been systematically studied, representing a significant research gap that warrants attention [2] [3].

To advance the research on *Platycladus orientalis* leaves as an antiviral agent, several key directions should be pursued. First, activity-guided isolation and purification should be strengthened by establishing high-throughput antiviral activity screening models, which would enable the isolation and identification of antiviral active components based on activity guidance and help clarify the material basis of pharmacodynamic effects, with particular emphasis on in-depth investigation of the structure-activity relationships of flavonoids and polysaccharides. Second, the molecular mechanisms need to be elucidated using modern molecular biology techniques to clarify how active components exert antiviral effects at both the viral and host levels, including identification of their molecular targets. Immunological research methods, such as macrophage polarization models, can be employed to explore immunomodulatory mechanisms [5] [11]. Omics approaches such as transcriptomics and proteomics could provide comprehensive insights into the mechanisms of action. Third, *in vivo* pharmacodynamic studies should be conducted by establishing viral infection animal models, such as HBV transgenic mouse models, to systematically evaluate the *in vivo* antiviral effects and safety of *P. orientalis* leaf extracts and their active components. Long-term toxicity studies are also needed to support potential clinical applications. Fourth, formulation development and clinical research should be promoted by developing new formulations based on the antiviral active components and conducting standardized clinical trials to provide a scientific basis for clinical use. The relevant patent for *P. orientalis* leaf polysaccharide has already laid a foundation for its industrial application [9]. The development of user-friendly formulations such as sprays [14] or oral preparations could facilitate clinical translation. Fifth,

quality control standards must be improved by establishing a more scientific and comprehensive quality evaluation system based on HPLC fingerprinting and chemical pattern recognition methods, thereby ensuring stable and controllable quality of antiviral preparations [16] [19]. Sixth, basic safety, toxicity, and pharmacokinetic studies are urgently needed before any preclinical or clinical development can proceed.

9. Conclusions

As a traditional Chinese medicine resource, *P. orientalis* leaves have shown preliminary confirmation of their antiviral activity, particularly against HBV. Flavonoids, terpenoids, and polysaccharides constitute the material basis for their antiviral effects, which may act through multiple pathways: direct inhibition of viral replication (demonstrated for HBV), modulation of immune responses (suggested by macrophage assays in the absence of viral infection), and antioxidant effects (not yet directly linked to antiviral activity). Among these, the anti-HBV activity of flavonoids and polysaccharides has been experimentally verified in HepG2.2.15 cells, and relevant patents have outlined their potential as adjuvant therapeutic agents for hepatitis B [5] [9] [11]. Regarding quality control, the establishment of HPLC fingerprinting combined with chemical pattern recognition methods provides a reliable means for quality evaluation [16] [19]. However, current marker compounds have not yet been functionally linked to antiviral activity.

Key limitations that should be addressed in future research include: 1) the absence of *in vivo* animal studies; 2) the lack of safety and toxicity data; 3) the absence of pharmacokinetic studies; 4) the lack of direct evidence for antiviral activity against any virus other than HBV; and 5) the hypothetical nature of proposed mechanisms (immunomodulation, NK cell activation), which have not been validated in virus-infected models.

Although current research is still in the preliminary stage, the therapeutic potential of *P. orientalis* leaves in the antiviral field is beginning to emerge. With continued advances in modern analytical techniques and pharmacological research methods, research on the antiviral effects of *P. orientalis* leaves will deepen, potentially offering new options for the prevention and treatment of viral diseases. Future research should focus on systematic *in vivo* studies, mechanistic elucidation, safety, PK profiling, and clinical development to fully realize the therapeutic potential of this promising traditional Chinese medicine.

Funding

University-Level Innovation and Entrepreneurship Program for College Students (No. 360).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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