

Research Progress of Yihuang Decoction and Prediction Analysis on Quality Markers

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Abstract

Background: Yihuang Decoction, a classical prescription found in Fu Qingzhu's *Obstetrics and Gynecology*, is formulated with fried *Dioscoreae Rhizoma*, fried *Euryales Semen*, wine-fried *Plantaginis Semen*, salt-fried *Phellodendri Chinensis Cortex* and *Ginkgo Semen*. Quality marker (Q-marker) plays a crucial role in promoting the standardization of the quality of traditional Chinese medicine (TCM). Hence, this study aimed to predict and analyze the Q-marker of Yihuang Decoction based on TCM Q-marker theory. **Methods:** A comprehensive review of relevant literature and the TCMSMP database was conducted to investigate the chemical constituents, pharmacological effects, and clinical applications of Yihuang Decoction. Furthermore, the study predicted the Q-marker of Yihuang Decoction by considering its effectiveness, specificity, measurability, transmissibility and traceability, and compatibility, as per the concept of Chinese medicine Q-marker. **Results:** Yihuang Decoction exhibits pharmacological effects such as anti-inflammation and immune modulation, anti-bacteria and anti-virus properties. Clinically, it is mostly used to treat inflammation of the female reproductive system, including vaginitis, cervicitis and pelvic inflammation. According to analysis and prediction, it was suggested that allantoin, diosgenin, gallic acid, α -tocopherol, bilobalide, ginkgolide A, ginkgolide B, berberine, phellodendrine, magnoflorine, geniposidic acid and verbascoside could serve as Q-markers of Yihuang Decoction. **Conclusion:** This review establishes a foundation for the research and development of Yihuang Decoction, and providing a crucial scientific reference for ensuring its comprehensive quality control and enhancing evaluation standards.



1 Introduction

Yihuang Decoction is described in Fu Qingzhu's *Obstetrics and Gynecology*, a monograph of gynecology written by Fu Shan in Qing Dynasty, and is composed of 50 g *Dioscoreae Rhizoma*, 50 g *Euryales Semen*, 10 g *Plantaginis Semen*, 5 g *Phellodendri Chinensis Cortex*, and 10 g *Ginkgo Semen*. This herbal combination is conventionally prepared by decocting in water. Yihuang Decoction has been included in the 100 list of Catalogue of Ancient Classical Formulas (The First Batch) issued by the State Administration of Traditional Chinese Medicine in 2018. In the decoction, *Dioscoreae Rhizoma* and *Euryales Semen* serve as principal herbs, addressing deficiency of Ren meridian and promoting urination. *Ginkgo Semen* serve as the ministerial herb, primarily promoting astringent and mitigating leukorrheal diseases, and concurrently eliminating dampness and heat [1,2]. *Plantaginis Semen* and *Phellodendri Chinensis Cortex* are adjuvants. *Plantaginis Semen*, with a bitter taste and cold properties, clears heat and dry dampnesses by acting on the kidney [3]. *Phellodendri Chinensis Cortex*, with a sweet taste and cold properties, can clear heat and promote diuresis [4]. Yihuang Decoction is primarily employed to treat kidney deficiency, damp-heat and leukorrheal diseases, which has the functions of reinforcing the kidney for stopping leucorrhea, clearing heat and expelling damp. Clinically, Yihuang Decoction is mainly used to treat female reproductive system inflammation as well as other diseases, including human papillomavirus (HPV) infection and urinary tract infection [5,6].

Quality marker (Q-marker) is a new concept about quality evaluation and quality control of traditional Chinese medicine proposed by Academician Liu Changxiao and other scholars in recent years [7]. Zhang Tiejun et al. have expounded the core theory and research method of Q-marker from five perspectives, including effectiveness, specificity,

measurability, transmissibility and traceability, and compatibility [8]. This study aims to summarize the chemical constituents, pharmacological effects and clinical application of Yihuang Decoction in recent years, and predict potential Q-markers for the formulation. The findings will provide a foundation for the quality evaluation and rational application of Yihuang Decoction.

2 Modern research of Yihuang Decoction

2.1 Chemical constituents

Dioscoreae Rhizoma is the rhizome of *Dioscorea opposita* Thunb., and contains various medicinal components such as polysaccharides, saponins, polyphenols, fatty acids, proteins, allantoin, etc [9]. Polysaccharides are the main active constituent known for its complex composition and structure. Xu Chunping et al. [10] employed GC/MS and fourier transform infrared spectrometer to characterize the structure of polysaccharides in *Dioscoreae Rhizoma*, and found that the main monosaccharides are glucose (84.5%), xylose (11.4%), galactose (2.3%) and arabinose (1.4%). Liu Lijie et al. [11] adopted HPLC and spectrophotometry to determine the contents of allantoin, dioscin and total polyphenols in the long *Dioscoreae Rhizoma* with fine hairs from different regions of Northeast China, and detected the content of active polysaccharides after water extraction and alcohol precipitation. The study found higher levels of these active components in *Dioscoreae Rhizoma* from Shenyang city compared to Qiqihar city and Changchun city. Based on the interactive application of various chromatographs as well as UV, IR, HRESIMS, NMR, and chemical analysis, Zhao Weixue et al. [12] isolated and analyzed 9 new and 11 known steroidal saponins from *Dioscoreae Rhizoma*, with diosgenin often used as a quality control index for *Dioscoreae Rhizoma* medicinal materials. *Dioscoreae Rhizoma* also contains polyphenols including catechin, quercetin and kaempferol, as well as essential

unsaturated fatty acids such as linoleic acid and linolenic acid [9]. Allantoin, as a derivative of uric acid, is another quality control indexes for *Dioscoreae Rhizoma* [13].

Euryales Semen refers to the dried ripe kernel of *Euryale ferox* Salisb., containing virous medicinal components, such as polysaccharides, polyphenols, lignans, tocopherols and cerebrosides, as well as nutritional ingredients including starches, proteins, amino acids and minerals. Guo Zhihui [14] analyzed polyphenolic components in the shell and kernel of *Euryales Semen* using HPLC-MS/MS, and detected 8 common active components, gallic acid, ferulic acid, rutin, caffeic acid, apigenin, carvacrol, kaempferol and quercetin. Wang Yu et al. [15] separated and analyzed the chemical components of *Euryales Semen* using preparative TLC and HPLC. Later, they identified 27 chemical compounds including α -tocopherol, polysorbate and β -sitosterol, and separated 12 chemical components (such as polysorbate, isopropyl ethynodiol and 2-methoxy-sorbitol) that can suppress α -amylase. Deng Qitong et al. [16] summarized the previous processing methods and modern research of *Euryales Semen*, confirming that α -tocopherol and gallic acid may serve as the quality control indexes for *Euryales Semen*.

Ginkgo Semen refers to the dried ripe kernel of *Ginkgo biloba* L., which mainly contains bilobol, terpene lactones, flavonoids, polysaccharides, alkaloids and other active components. Zhang Qunqun et al. [17] measured the contents of 4 terpene trilactones (bilobalide and ginkgolide A/B/C) and unveiled that the highest content of the four is 4 times that of the lowest. They accordingly suggested to use the content limit of these four terpene trilactones as quality standard, laying a foundation for research on *Ginkgo Semen* quality control. Shan Shujun [18] analyzed the components of *Ginkgo Semen* kernel with antioxidant activity and identified 16 phenylpropanoids and lignan

glycosides compounds using LC-Q-TOF-MS, providing a reference for harvesting, identifying and analyzing micromolecule with oxidative activity in *Ginkgo Semen*.

Plantaginis Semen is derived from the dried bark of *Phellodendron chinense* Schneid., containing alkaloids (including berberine, phellodendrine, magnoflorine and palmatine), flavonoids, phenolic acids, triterpenoids, phenylpropanoids and amides. Cui Xing et al. [19] dissected the chemical components of *Plantaginis Semen* using UPLC-Q-TOF MSE combined with UNIFI database, and found 15 chemical components including berberine, phellodendrine and obakulacton, which provides a vital basis for establishing quality control standard and fingerprint of *Plantaginis Semen*. Shi Min et al. [20] employed UPLC-Q-TOF-MS for rapid detection of chemical components in *Plantaginis Semen*, and obtained 35 chemical components, including alkaloids (19), phenolic acids (7), phenylpropanoids (5), limonins (3) and other compound (1), which can potentially be used as the quality control standards. Feng Yuan et al. [21] studied *Phellodendron amurense* and *Phellodendron chinense* through analyses of principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), and identified berberine, magnoflorine and palmatine as their difference markers to determine their quality control.

Phellodendri Chinensis Cortex, the dried ripe seed of *Plantago asiatica* L. containing iridoids, phenylethanoid glycosides, polysaccharides, flavones and alkaloids. Wang Liting [22] et al. analyzed 3 solvent extracts of *Phellodendri Chinensis Cortex* through UPLC-Q-Exactive Orbitrap-MS, and obtained 11 flavones, 6 phenylethanoid glycosides, 3 iridoids, and 3 triterpenoids. Sun Yujing et al. [23] used UPLC-Q-TOF/MSE to detect the chemical components of *Phellodendri Chinensis Cortex* at different

processing times and found that the contents of iridoids (such as geniposidic acid) and phenylethanoid glycosides (such as verbascoside) are reduced after processing. Gu Caimei et al. [24] studied crude *Phellodendri Chinensis* Cortex or stir-fried *Phellodendri Chinensis* Cortex with salt-water as well as PCA and OPLS-DA methods. They demonstrated that the contents of verbascosides and geniposidic acids are greatly changed in *Phellodendri Chinensis* Cortex after stir-frying with salt-water. These changes may be used as potential indicators to distinguish whether *Phellodendri Chinensis* Cortex is crude or subjected to stir-frying with salt-water.

2.2 Pharmacologic properties

2.2.1 Anti-inflammation and immune modulation

Yihuang Decoction regulates levels of inflammatory factors to decrease the number of inflammatory cells and thus modulate the immunity system. Yihuang Decoction can reduce IL-6, IL-17 and IL-12 levels, while elevating the levels of IL-2, IL-4 and INF- γ in vaginal secretions of patients. These changes help to ameliorate the vaginal environment of patients infected with high-risk cervical human papillomavirus [25,26]. Moreover, the clysis of Yihuang Decoction has been verified to shorten the time for leukocytes, neutrophils and C-reactive protein to return to normal levels, thereby alleviating damp-heat-toxin-type pelvic inflammation [27,28].

2.2.2 Anti-bacteria activities

Vaginal lactobacillus maintains the acidic vaginal environment and inhibits or kills foreign bacteria by producing hydrogen peroxide. Disruption of the local vaginal environment leads to a reduction or complete disappearance of lactobacillus, allowing excessive growth of other bacteria such as anaerobic bacteria, *Gardnerella*, and mycoplasma, resulting in vaginal dysbacteriosis [29]. The pH value in vagina can impact vaginal bacteria, and Yihuang Decoction can improve

vaginal pH value to mitigate vaginal dysbacteriosis in senile vaginitis [30-33].

2.2.3 Anti-virus effects

Hypersensitivity C-reactive protein (hs-CRP) reflects the body's immunity and inflammatory response, while TNF- α is implicated in the immune response and inflammatory factor expressions in patients infected with cervical virus. HPV virus can decrease immunity, thereby increasing levels of inflammatory factors such as TNF- α and hs-CRP. The combination of Yihuang Decoction and self-made Qingdu prescription can reduce the viral load of HPV patients and decrease the levels of TNF- α and hs-CRP [34]. Duan Qianni et al. [35] proved that modified Yihuang Decoction downregulates the expressions of viral proteins gB, gD, VP16, ICP5 and ICP4, improves host cell morphology, enhances host cell viability, and notably affects the adhesion and penetration stages of herpes simplex virus type 2 (HSV-2). In addition, Li Shi et al. [36] validated that Yihuang Decoction, combined with chitosan anti-bacteria membrane, interferes with HPV infection through modulating expressions of HPV encoded proteins HPV E6/E7.

2.2.4 Other functions

Yihuang Decoction also possesses the functions of regulating the spleen and stomach, alleviating diarrhea, promoting diuresis and providing neuroprotection. Chronic colitis and infantile diarrhea are caused by damp evil, weakness of the spleen and the stomach, rise and fall disorder of Qi and blood, and dysfunction in separating the clear Qi from the turbid Qi, with diarrhea as the main manifestation. Yihuang Decoction can warm kidney and strengthen spleen, elevate clear Qi, remove dampness, as well as astringe intestine to relieve diarrhea [37].

2.3 Clinical application

2.3.1 Inflammation of the female reproductive system

Inflammation of the female reproductive system is a prevalent gynecological condition, encompassing vaginitis, cervicitis and pelvic inflammation. These diseases belong to “leukorrheal diseases” in TCM, manifested by the apparent increase of leucorrhea, chromatic aberration, and pruritus vulvae or ulceration. However, Yihuang Decoction has shown significant efficacy in alleviating these symptoms.

Vaginitis: Currently, Yihuang Decoction has been widely applied to treat senile vaginitis and bacterial vaginitis, which are characterized by increased leucorrhea and foul smell, often companied by vaginal flora dysregulation [30,31,33,38-41]. Xu Zhenhui [30] distributed 102 elderly patients with vaginitis into control and study groups, where patients in control group were treated with Baofukang suppository and those in study group were treated with Baofukang suppository and modified Yihuang Decoction. The results showed that the cure rate, total effective rate, vaginal pH value, bacterial culture score, vaginal cleanliness and symptom score in study group are superior to those in control group.

Pelvic inflammation: In a study by Hao Furong [42], 80 patients with acute pelvic inflammation were divided into control (treated with conventional Western medicine) and treatment (treated with conventional Western medicine and Yihuang Decoction) groups. The data revealed higher cure rates and overall effectiveness in the treatment group compared to the control group. Kuang Xunhong et al. [27] investigated the clinical therapeutic efficacy of clysis of Yihuang Decoction for damp-heat-toxin-type pelvic inflammation, demonstrate that Yihuang Decoction, as adjuvant therapy, yielded better treatment outcomes. Also, Yihuang Decoction combined with antibiotics is superior to antibiotics

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alone in treating chronic pelvic inflammation [43].

Cervicitis: Modified Yihuang Decoction can be utilized in the late stage of chronic cervicitis treatment for cervical erosion [44]. Liu Shimei et al. [44] conducted a study in which 104 patients received microwave treatment as the control group and 102 patients received modified Yihuang Decoction as the treatment group. No statistically significant differences were found in the treatment efficacy between the two approaches for damp-heat-type cervical erosion; however, the vaginal fluid rates were increased in control group, while the treatment group has no complications.

In addition to the aforementioned diseases, Yihuang Decoction combined with or without other prescription can also be used to treat ovulatory bleeding, vulvovaginal candidiasis, female mycoplasma genitalium infection, etc. [45-47].

2.3.2 HPV infection

Clinically, the common symptoms of HPV infection include increased leucorrhea and vaginal pruritus, which are closely resemble the symptoms of the “leukorrheal diseases” in TCM. Several studies have confirmed that Yihuang Decoction can assist in the treatment of damp-heat type of cervical HPV infection [6]. Numerous studies have revealed that Yihuang Decoction can reduce HPV viral load and increase the infection negative conversion ratio, thereby potentiating the ability of recombinant human interferon to clear HPV virus and improving the body's antiviral capability [25,48,49]. Lin Lan et al. [6] found that Yihuang Decoction reduced the viral load of patients infected with HR-HPV.

3 Prediction and analysis of Q-marker

The compound Yihuang Decoction is composed of 5 TCM materials (Figure 1), which contains complex chemical components and can be influenced by place of origin, climate, processing methods, etc., resulting

in varied quality of TCM. At present, the research of Q-marker focus on single medicinal material, and there is a lack of studies elucidating the Q-markers of Yihuang Decoction as a compound formulation. This study predicted and summarized the Q-marker of Yihuang Decoction from the perspectives of quality

transmissibility and traceability, component specificity, component effectiveness, compound compatibility, and component measurability (Figure 2). These findings lay a foundation for quality control of Yihuang Decoction.

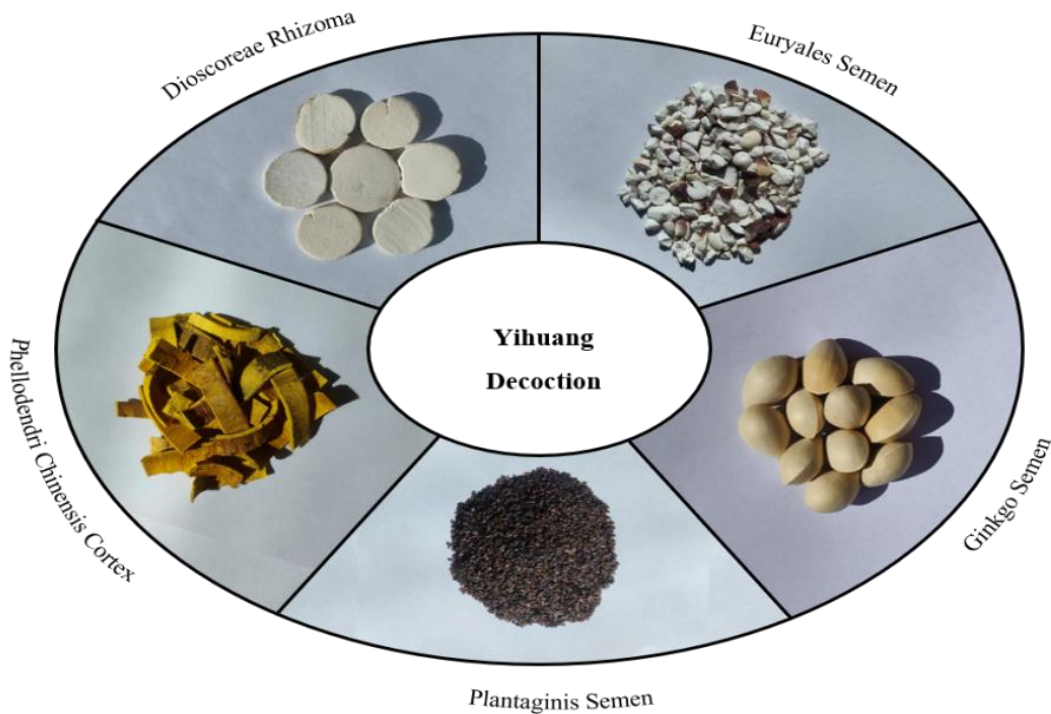


Figure 1 The medicinal composition of Yihuang Decoction.

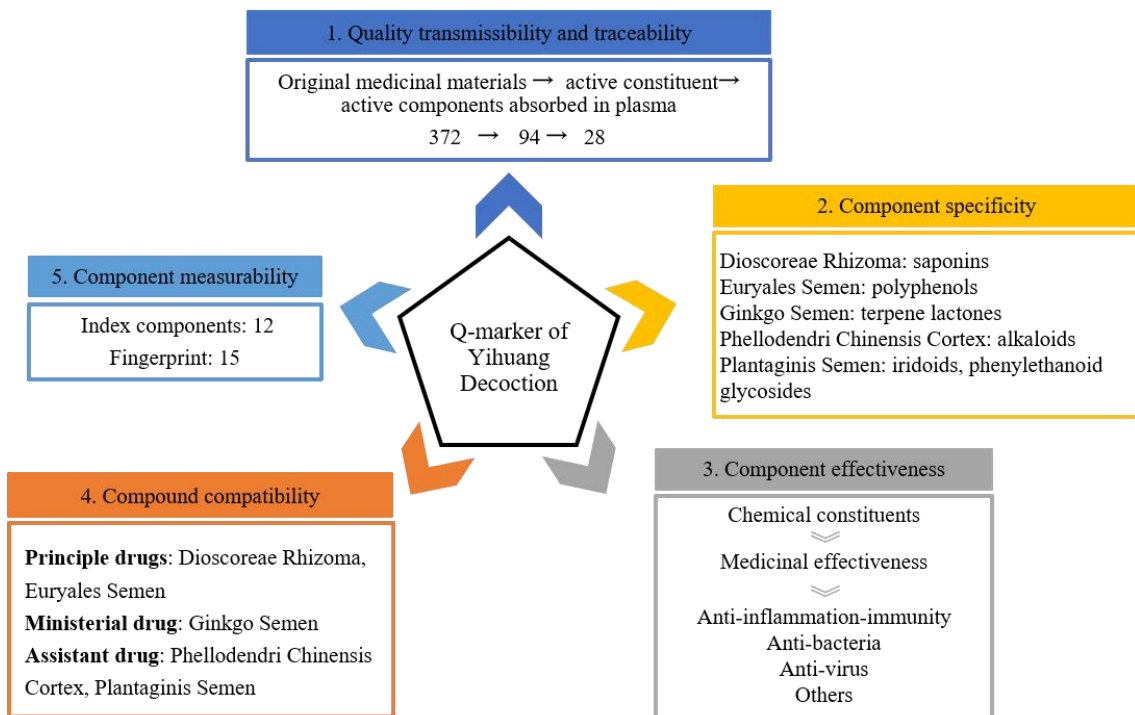


Figure 2 Research pathway of Q-marker of Yihuang Decoction based on “five principles”.

3.1 Predictive analysis on the Q-marker of Yihuang Decoction based on quality transmissibility and traceability

“Dioscoreae Rhizoma”, “Euryales Semen”, “Plantaginis Semen”, “Phellodendri Chinensis Cortex” and “Ginkgo Semen” as the keywords were searched in the TCMSP (<http://tcmssp.com/tcmssp.php>) database [50], and a total of 372 chemical components in Yihuang Decoction were retrieved, including 71 Dioscoreae Rhizoma, 26 Euryales Semen, 80 Ginkgo Semen, 140 Plantaginis Semen and 55 Phellodendri Chinensis Cortex. According to the oral bioavailability (OB) of the drug $\geq 30\%$ and drug likeness (DL) ≥ 0.14 , 94 active components were screened, containing 16 Dioscoreae Rhizoma (diosgenin, stigmasterol, Denudatin B, (-)-dihydroquercetin, etc.), 4 Euryales Semen (vitamin E, β -carotene, oleic acid, and ethyl isocyanate), 24 Ginkgo Semen (gibberellin, quinic acid, β -sitosterol, isorhamnetin, etc.), 41 Plantaginis Semen (berberine, coptisine, rutaecarpin, palmatine), and 9 Phellodendri Chinensis Cortex (quercetin, sitosterol, hispidulin, daucosterol, etc.). Bi Jiayao et al. [51] used acetonitrile-0.1% phosphoric acid aqueous solution as the mobile phase to determine the HPLC fingerprint of 15 batches of Yihuang Decoction at a wavelength of 230 nm. Later, 15 common peaks were detected, and 8 characteristic peaks information were confirmed, of which 6 were from Plantaginis Semen (chlorogenic acid, phellodendrine, magnoflorine, 5-O-feruloylquinic acids, 4-feruloylquinic acids, and berberine), 1 from Phellodendri Chinensis Cortex marinated with liquor (geniposidic acid), and 1 from stir-baked rhizoma dioscorea (allantoin). Zhou Jinxin et al. [52] confirmed 90 constituents of Yihuang Decoction through HPLC-Q-TOF-MS, including 23 alkaloids (berberine, palmatine, phellodendrine, jatrorrhizine, and tetrahydropalmatine), 14 organic acids, 3 phenylethanoid glycosides, 4 iridoid glycosides, 5 terpene lactones, 10 flavonoids, 8 nucleobases and

nucleosides, 12 amino acids and 11 other chemical compounds. Besides, they tested 8 representative chemical compounds (verbascoside, allantoin, berberine, 4-O-feruloylquinic acid, 5-O-feruloylquinic acid, gallic acid, geniposidic acid and phellodendrine) in the 10 batches of Yihuang Decoction samples using HPLC-DAD, and the results indicated that this method is fast, accurate, reliable, and easy to operate, which provides strong evidence for the quality control of Yihuang Decoction.

Compound formulation of TCM have complicated constituents that exert pharmacologic effects through absorption, distribution and metabolism in the body. Hence, selecting appropriate absorbed constituents and metabolites is pivotal to the research on quality control of compound formulation of TCM. Allantoin and diosgenin are the main constituents of Dioscoreae Rhizoma. By means of HPLC analysis has demonstrated that allantoin act as the absorbed constituents in *Psammosilene tunicoides* medicinal materials, and as the index [53]. Chang Jinhua et al. [54] determined the concentration of diosgenin in rat plasma with UPLC-MS/MS and found diosgenin blood concentration is relatively reduced after oral administration and diosgenin is widely distributed and metabolized slowly after entering the blood. It is worthwhile to explore the metabolism of α -tocopherol and gallic acid, two main active components in Euryales Semen, in the body. α -tocopherol undergoes ω -oxidation, β -oxidation, and oxidation with free radicals in the body, and metabolites are excreted into urine mainly through conjugating with sulphate [55]. When being orally administered, gallic acid has relatively low absolute bioavailability [56], and its distribution is rapid and extensive, mainly in the small intestine, kidney, and liver tissues [57]. Ginkgolide A/B and bilobalide are primary components in Ginkgo Semen. YARO PETER [58] detected the contents of ginkgolide and bilobalide, two constituents of Ginkgo biloba extract, in rat plasma, kidney, liver, heart, lung,

brain and urine by UPLC/MS-MS and found that ginkgolide A/B and bilobalide have better oral bioavailability, were widely distributed and slowly eliminated. Berberine, phellodendrine, and magnoflorine are the components of Plantaginis Semen worthy of study. Kang Lixin et al. [59] applied UPLC-Orbitrap Fusion Lumos Tribrid-MS to test the prototype and metabolites of Phellodendri amurensis cortex aqueous extract in rat serum, urine and feces, and identified a total of 15 prototype components (higenamine, N-methyl tetrahydropapaverine, demethyleneberberine, berberrubine, jatrorrhizine, coptisine, dehydrocorydaline, obacuoic acid, etc.) and 55 metabolites. Xu Zhou [60] detected the concentrations of Plantaginis Semen extract in rat portal vein blood, liver and peripheral blood utilizing LC-MS/MS. The results revealed that phellodendrine had a strong liver first pass effect, and a small amount of phellodendrine enters the systemic circulation as a prototype; magnoflorine is not metabolized in the liver, some of which are excreted through bile, and about half of which enter the peripheral blood circulation system as a prototype; berberine has a strong hepatic first pass effect, which can be metabolized and eliminated by the gastrointestinal tract and first pass effect. Besides, geniposidic acid and verbascoside are components of Phellodendri Chinensis Cortex. Tan Qin [61] studied the absorption fluid of intestinal sac in everted intestinal sac model, and tested the absorbed components after rats were treated with Phellodendri Chinensis Cortex extract. The results unveiled that the intestinal sac absorbed 6 kinds of components, including geniposidic acid, acteoside and isoacteoside. Massive studies have analyzed the pharmacokinetics of geniposidic acid in vivo by UPLC/MS-MS, and the results showed that geniposidic acid has better bioavailability, is distributed more in the stomach and less in the liver, and has a fastest distribution rate in the spleen and kidneys [62], which is also rapidly metabolized and eliminated [63]. Verbascoside is

rapidly absorbed, and quickly and widely distributed after oral administration, with small amounts excreted from urine, bile, and feces, and its absolute bioavailability is as low as 1% [64].

3.2 Predictive analysis on the Q-marker of Yihuang Decoction based on component specificity

3.2.1 Dioscoreae Rhizoma

Dioscoreae Rhizoma contains abundant functional components such as polysaccharide, saponin, allantoin, polyphenol, fatty acid and protein. Among these, allantoin and diosgenin are important active substances of Dioscoreae Rhizoma. However, the content of these substances can vary significantly in medicinal materials sourced from different regions and can be influenced by factors such as harvesting time and processing methods [65-68]. Therefore, allantoin and diosgenin can serve as reliable Q-markers for assessing the quality of Dioscoreae Rhizoma medicinal materials.

3.2.2 Euryales Semen

Euryales Semen contains nutrients such as starch, protein, amino acids, etc., and medicinal ingredients including polysaccharides, polyphenols, lignans, tocopherols, cerebrosides, etc. Euryales Semen from different places of origin greatly vary in quality. Among its numerous medicinal components, tocopherols and polyphenols are important bioactive substances in Euryales Semen that contribute to various pharmacological effects. It has been documented that the content of α -tocopherol is greatly different in Euryales Semen from different regions, but is the highest in tocopherols from all Euryales Semen [69]. Polyphenols have complex components with various activities, and the combination of different polyphenols directly affects their efficacy. Among polyphenols, gallic acid has a relatively higher content [70]. Accordingly, α -tocopherol and gallic acid [8,71]

can serve as specific components in Euryales Semen.

3.2.3 Ginkgo Semen

Ginkgo Semen contains various active constituents including bilobols, terpene lactones, flavonoids, polysaccharides, and alkaloids. Terpene lactone is one of the main medicinal components in Ginkgo Semen, comprising diterpenoids (including ginkgolide A, B, C, etc.) and sesquiterpene lactones (including bilobalide). Ginkgo terpene lactones are specific components in ginkgo with profound pharmacological activities [72]. Bilobalide and ginkgolide have different contents in Ginkgo Semen from various places of origin or batches [17], making them reliable specific components for evaluating the quality of Ginkgo Semen.

3.2.4 Plantaginis Semen

Plantaginis Semen is composed of alkaloids, flavonoids, phenolic acids, triterpenoids, phenylpropanoids, amides, etc. Among these, alkaloids occupy the most content, primarily including berberine, phellodendrine, palmatine, magnoflorine, and jatrorrhizine. Berberine and phellodendrine have been identified as Q-markers of Plantaginis Semen in Pharmacopoeia of the People's Republic of China (2020 edition). Berberine occupies a large content in Plantaginis Semen, and phellodendrine is a specific component of Plantaginis Semen. Notably, the places of origin, harvesting time and stir-frying with salt-water process make significantly influence the levels of these two components [73-75]. The content of magnoflorine varies depending on the source of Plantaginis Semen [22]. Accordingly, berberine, phellodendrine, and magnoflorine can be used as the exclusive characteristic markers of Plantaginis Semen.

3.2.5 Phellodendri Chinensis Cortex

Phellodendri Chinensis Cortex contains a variety of chemical components, such as iridoids, phenylethanoid glycosides, polysaccharides, flavonoids and alkaloids. Iridoids (including

geniposidic acid) and phenylethanoid glycosides (including verbascoside) are the main active constituents of Phellodendri Chinensis Cortex, with several pharmacologic action [24]. Geniposidic acid and verbascoside have been listed as Q-markers of Phellodendri Chinensis Cortex in Pharmacopoeia of the People's Republic of China (2020 edition) and are indicators during processing of Phellodendri Chinensis Cortex [76]. Collectively, geniposidic acid and verbascoside can act as specific components of Phellodendri Chinensis Cortex.

3.3 Predictive analysis on the Q-marker of Yihuang Decoction with constituents related to medicinal effectiveness

3.3.1 Anti-inflammation and immune modulation

The immunity comprises cellular immunity, humoral immunity and non-specific immunity. LPS binding to TLR can trigger MyD88-dependent and MyD88-independent TLR signal cascade reaction, and activate NF- κ B, resulting in expressions of inflammatory factors, including TNF- α and IL-1 β . Dioscoreae Rhizoma and Euryales Semen can elevate thymus index, improve T cell proliferation, increase serum IL-2 and IL-4 levels, and decrease IFN- γ level, so as to ameliorate the immunity [77]. Moreover, diosgenin in Dioscoreae Rhizoma has been identified to downregulate expressions of inflammatory factors (IL-1 β , IL-6 and TNF- α), protein levels of COX-2 and iNOS, and augment levels of genes related to antioxidant (haem oxygenase-1, superoxide dismutase 3, etc.) [78]. Bilobalide can increase expressions of antioxidant genes (Nrf and Keap), dwindle levels of inflammatory factors (TNF- α , IL-1 β and VEGF) and NF- κ B, restore Th1/Th2, and thus promote the immunity [79,80]. Reportedly, Ginkgolide attenuates LPS-induced NF- κ B (p65) expression and inflammatory cell accumulation (neutrophils, lymphocytes and macrophages), and downregulates TNF- α and IL-1 β levels [81]. It has been documented

that total polysaccharide from *Phellodendri Amurensis* Cortex evidently suppress proliferation of T lymphocytes and spleen cells, and exert immunosuppressive effects through cellular immunosuppression [82]. Phellodendrine plays an anti-inflammatory role through blocking the activation of TLR-4/MyD88/NF- κ B (p65) pathway and inhibiting expressions of iNOS, COX-2 and TNF- α [83]. Additionally, verbascoside has been corroborated to promote the proliferation of mouse dendritic cells, stimulate the proliferation of naïve T cells, and inhibit transforming growth factor- β (TGF- β)-activated kinase 1 (TAK1)/JNK/AP-1 pathway and its downstream COX-2 and iNOS gene expressions, so as to generate anti-inflammatory and immunomodulatory effects [84,85]. Polysaccharides of *Phellodendri Chinensis* Cortex repress the overproduction of TNF- α , IL-2, IL-6, IL-10 and IL-1 β , elevate glutathione peroxidase and improve total antioxidant capacity in mouse liver [86]. Also, polysaccharides of *Phellodendri Chinensis* Cortex can enhance the phagocytic activity of peritoneal macrophages in immunosuppressed mice, potentiate the transformation function of splenic lymphocytes, increase hemolysin secretion, and promote the secretion of IL-2 by splenic lymphocytes, thereby strengthening the immunity [87]. Based on the above information, it can be concluded that Yihuang Decoction can exert anti-inflammatory and immunomodulatory effects via mediating inflammatory factors and immune cells.

3.3.2 Anti-bacteria

Several studies have indicated that TCM exerts antibacterial effects by disrupting the integrity of bacterial cell walls and membranes, and dampening the synthesis of proteins and nucleic acids. Polysaccharides in TCM have good antibacterial effects. For example, *Dioscoreae Rhizoma* polysaccharides [88], *Euryales Semen* polysaccharides [89] and

Ginkgo Semen polysaccharides [90] can suppress the growth of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*, among which *Ginkgo Semen* polysaccharides can play a bacteriostatic role through inhibiting the formation of *Staphylococcus aureus* biofilm. Moreover, gallic acid curbs the growth of *Staphylococcus aureus* via disrupting bacterial membrane structure and suppressing bacterial protein synthesis [91]. Berberine, the main active component of *Plantaginis Semen*, regulates colitis-triggered intestinal flora imbalance and restores the content of tryptophan, a metabolite of intestinal bacteria [92]. Berberine hydrochloride concentration-dependently produces bacteriostatic effects by limiting the number of *Staphylococcus aureus* and suppressing the formation of biofilm [93].

3.3.3 Anti-virus

HPV encodes two key oncoproteins E6 and E7. The dimer formed by E6 and E6AP can specifically bind to the tumor suppressor protein p53, and promotes the degradation of p53, thereby blocking cell apoptosis. E7 binds to the tumor suppressor protein pRB to dissociate E2F and pRB complexes and to make the cell cycle out of control. E6/E7 expression is regulated by URR which contains transcription factors that can bind to viral regulatory protein E2 and host-derived transcription factors (AP-1, STAT3). Further, berberine can inhibit AP-1 and the expressions of viral oncoproteins E6 and E7 [94]. *Berberis aquifolium* ethanolic plant extract alters STAT3 and AP-1 expressions to control E6 and E7 expressions, and its chemical components berberine, parmarine and magnoflorine can effectively suppress E6 expression [95]. *Plantaginis Semen* has been evidenced to inhibit cellular viral replication and upregulate the expressions of antiviral genes [96]. Verbascoside may exert antiviral effects through dwindling HBx protein level, reducing antigen secretion, and blocking the transcription of the viral genome [97].

3.4 Predictive analysis on the Q-marker of Yihuang Decoction based on compound compatibility

The therapeutic effect of drugs in traditional Chinese medicine (TCM) is influenced by the compatibility of compound prescriptions. Drug compatibility based on drug property can enhance curative effect and alleviate partial effect. In Yihuang Decoction, *Dioscoreae Rhizoma* and *Euryales Semen* are principle drugs with mild property, which are neither dry nor hard to digest, and can tonify Qi deficiency, strengthen astringent, invigorate spleen for diuresis, and arrest spontaneous emission and leukorrhagia. *Ginkgo Semen*, as the ministerial drug, has a sweet, bitter and astringent flavor and mild nature, which can reinforce Qi deficiency and reduce leucorrhea. *Plantaginis Semen* has a bitter flavor and cold nature, and *Phellodendri Chinensis Cortex* has a sweet flavor and cold nature, which together function as adjuvants for clearing dampness-heat in lower energizer. Fu Yuanyuan et al. [98] investigated the main chemical components of salt-fried *Phellodendri Chinensis Cortex* and Yihuang Decoction and found that the mass fraction of 4-O-feruloylquinic acid calculated based on salt-fried *Phellodendri Chinensis Cortex* in Yihuang Decoction is increased, while those of phellodendrine chloride, 5-O-feruloylquinic acid and berberine hydrochloride are decreased, indicating that the components of Yihuang Decoction may interact during the decoction process. Medicinal materials in Yihuang Decoction are matched with each other, and jointly clear heat, induce diuresis and tonify Chong and Ren channels, so that all diseases can be cured.

3.5 Predictive analysis on the Q-marker of Yihuang Decoction based on content detection

Q-marker of Yihuang Decoction is set based on its specificity and activity as well as components that can be easily detected. As per the Pharmacopoeia of the People's Republic of China (2020 edition), the

indicators for content detection of *Plantaginis Semen* and *Phellodendri Chinensis Cortex* are berberine, phellodendrine, geniposidic acid and verbascoside. The indicators of *Dioscoreae Rhizoma*, *Euryales Semen* and *Ginkgo Semen* are not listed in the Pharmacopoeia of the People's Republic of China (2020 edition). Through detection using HPLC, Li Hualu et al. [8] found that the contents of allantoin, gallic acid, geniposidic acid and berberine in 15 batches of Yihuang Decoction ranged from 0.0801% to 1.087%, 0.047% to 0.057%, 0.037% to 0.081% and 0.0250% to 0.387%, respectively. Luo Jing et al. [99] determined the content of diosgenin in 14 different places of origin with HPLC, and confirmed that its content from different places of origin is between 3.8694 to 21.0747 mg/g, among which the highest content is found in *Paris polyphylla* var. *yunnanensis* from Shege Village, Wusha Town, Xingyi City, Southwest Guizhou Autonomous Prefecture, Guizhou Province, as well as from Agricultural Science Extension Research Institute in Dali Prefecture (Yunnan Province) 2. Zhang Yu et al. [100] tested the content of tocopherol in vegetable oils utilizing HPLC, after which 4 tocopherols are successfully separated, and the detection limit of α -tocopherol is 0.16. Zhang Qunqun et al. [18] assayed 4 terpene lactones (bilobalide, ginkgolide A/B/C) in 5 *Ginkgo Semen*s from different places of origin through HPLC-ELSD technology. The results revealed that the contents of the 4 terpene lactones in *Ginkgo Semen*s from different places of origin are 75.55~219.73, 54.79~135.88, 200.37~853.39, and 119.31~498.06 μ g/g, respectively, and the total content of the 4 terpene lactones is the highest in *Ginkgo Semen* from Taixing City, Jiangsu Province. Pan Chao et al. [101] successfully determined the contents of phellodendrine, magnoflorine, jatrorrhizine, palmatine chloride and berberine hydrochloride in Ermiao Pill through HPLC, demonstrating that the method is simple, accurate and exclusive, and can be used as the

content detection of the indicators in *Plantaginis Semen*. Chen Qizhao [102] adopted HPLC to test the content changes of 6 components in *Phellodendri Chinensis Cortex* processed with/without salt, including geniposidic acid, plantagoside, quercetin, kaempferol, verbascoside and isoverbascoside. According to the data, there are certain differences in the contents of the above 6 components in *Phellodendri Chinensis Cortex* processed without salt from different batches or places of origin, but in *Phellodendri Chinensis Cortex* processed with salt, no

significant difference is observed in plantagoside content, the content of verbascoside is obviously reduced, and the contents of geniposidic acid, isoverbascoside and flavonoids are markedly increased. In view of the above information, allantoin, diosgenin, gallic acid, α -tocopherol, bilobalide, ginkgolide A, ginkgolide B, berberine, phellodendrine, magnoflorine, geniposidic acid and verbascoside can serve as the Q-markers of Yihuang Decoction (Table 1 and Figure 3).

Table 1 Information of Q-marker of Yihuang Decoction.

No.	Compound	Molecular formula	CAS No.	Molecular weight	Habitat
1	Allantoin	C ₄ H ₆ N ₄ O ₃	97-59-6	158.12	Dioscoreae Rhizoma
2	Diosgenin	C ₂₇ H ₄₂ O ₃	512-04-9	414.62	Dioscoreae Rhizoma
3	Gallic acid	C ₇ H ₆ O ₅	149-91-7	170.12	Euryales Semen
4	α -tocopherol	C ₂₉ H ₅₀ O ₂	10191-41-0	430.71	Euryales Semen
5	Bilobalide	C ₁₅ H ₁₈ O ₈	33570-04-6	326.30	Ginkgo Semen
6	Ginkgolide A	C ₂₀ H ₂₄ O ₉	15291-75-5	408.40	Ginkgo Semen
7	Ginkgolide B	C ₂₀ H ₂₄ O ₁₀	15291-77-7	424.40	Ginkgo Semen
8	Berberine	C ₂₀ H ₁₈ NO ₄ ⁺	2086-83-1	336.37	Plantaginis Semen
9	Phellodendrine	C ₂₀ H ₂₀ NO ₄ ⁺	6873-13-8	342.41	Plantaginis Semen
10	Magnoflorine	C ₂₀ H ₂₄ NO ₄ ⁺	2141-09-5	342.41	Plantaginis Semen
11	Geniposidic acid	C ₁₆ H ₂₂ O ₁₀	27741-01-1	374.34	Phellodendri Chinensis Cortex
12	Verbascoside	C ₂₉ H ₃₆ O ₁₅	61276-17-3	624.59	Phellodendri Chinensis Cortex

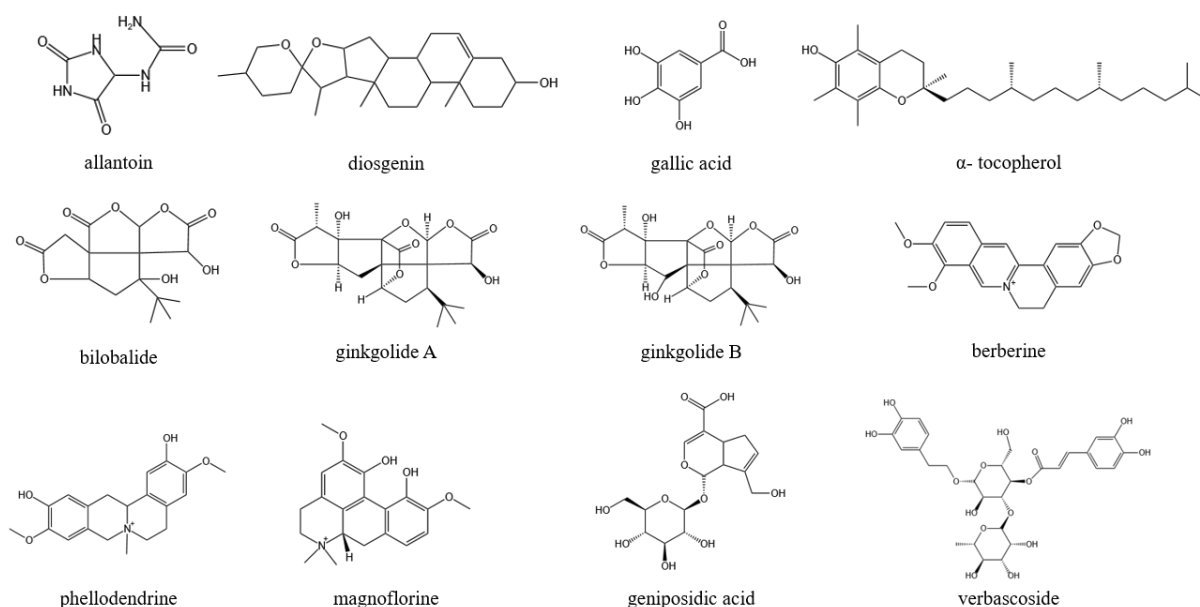


Figure 3 Chemical structures of twelve compounds in Yihuang Decoction.

4 Conclusion

Classical prescriptions are the essence of Chinese medicine and lay a crucial role in the discovery of novel drugs. In recent years, China has laid greater emphasis on the research and development of classical prescriptions. The quality of Chinese medicine is of utmost importance for ensuring clinical efficacy and drug safety, and it serves as the lifeline for the development of the Chinese medicine industry. The research on the quality of Chinese medicine has always been the difficulty and hot spot, which, together with standard setting, is the national strategic issue related to Chinese medicine science and industrial development. However, owing to the multi-families genera, complex compositions, unclear pharmacodynamic substances and undefined mechanism of action of Chinese medicine, coupled with the long industrial chain, the quality research and the quality standard setting for Chinese medicine face a great challenge.

Yihuang Decoction, included in the first batch of the 100 ancient classical prescriptions released in 2018, has been clinically used for the treatment of vaginitis, pelvic inflammation and other gynecological diseases for hundreds of years. It exhibits a wide range of pharmacological effects, such as anti-inflammation, anti-bacteria, immunoregulation, and antioxidation. This report summarizes the main chemical components, pharmacological effects and clinical application of Yihuang Decoction, and analyzes Yihuang Decoction from the perspectives of quality transmissibility and traceability, component specificity, component effectiveness, compound compatibility, and component measurability based on Chinese medicine Q-marker theory. The results revealed that allantoin, diosgenin, gallic acid, a-tocopherol, bilobalide, ginkgolide A, ginkgolide B, berberine, phellodendrine, magnoflorine, geniposidic acid and verbascoside can serve as Q-markers of Yihuang

Decoction, which lays a basis for the research and development of Yihuang Decoction and provides a reference for its quality evaluation system.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: Menggai Zhang and Xue Liu; Data curation: Hehe Shi and Yinyue Xu; Formal analysis: Longbiao Luo and Lijin Yu; Methodology: Yitao Wang and Sicen Wang; Writing – original draft: Wang-hui Jing and Menggai Zhang; Writing – review and editing: Xue Liu and Hehe Shi; All authors have read and agreed to the published version of manuscript.

Ethics Approval and Consent to Participate

This study was approved by Medical Ethics Committee, and patients were informed and agreed.

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Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Supplementary Materials

Not applicable.

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