

Review

Wuzi Yanzong prescription (五子衍宗丸) from Traditional Chinese Medicine for male infertility: a narrative review

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Abstract

Wuzi Yanzong prescription (WYP), as a classical prescription for male infertility with kidney essence deficiency, is composed of Gouqizi (*Fructus Lycii*), Tusizi (*Semen Cuscutae*), Wuweizi (*Fructus Schisandrae Chinensis*), Fupenzi (*Fructus Rubi Chingii*) and Cheqianzi (*Semen Plantaginis*). It has been used for hundreds of years in the treatment of male infertility, known as "the first prescription of ancient and modern seeds", with convincing clinical evidence. At present, more than 100 chemical compounds have been isolated from WYP, including polysaccharide, fatty acids, flavonoids,

phenylpropanoids, organic acids, alkaloids, terpenoids, etc. Pharmacological and clinical studies show that WYP has an obvious effect on reproductive system diseases, especially male infertility, which has a very wide application prospect. It also has effects on the nervous system, inhibiting liver injury, lowering blood sugar and blood lipid, anti-aging, improving immunity, resisting hypoxia and fatigue effects. This study reviewed the chemical constituents, quality control, pharmacology, and clinical application of WYP. There is no doubt about the clinical value of WYP, but its quality control system is not perfect, pharmacological mechanism is not fully explained, and clinical applications need to be reevaluated. Therefore, the follow-up researches should proceed from the theory of Traditional Chinese Medicine (TCM) and clinical applications, further explain the theoretical connotation, reveal the mechanism of action, and provide the basis for the secondary development of classic famous prescriptions. In addition, WYP is mostly used in combination with western medicines besides being used alone. Whether it can improve the efficacy and reduce side effects will also be a meaningful research direction in the future.

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Keywords: quality control; pharmacology; clinical applications; chemical constituents; Wuzi Yanzong prescription; review

1. INTRODUCTION

Infertility and subfertility affect a significant proportion of humanity. According to World Health Organization (WHO) and European Association of Urology (EAU), about 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility. One in every four couples in developing countries had been found to be affected by infertility¹ Infertility will become the third major disease affecting human health after cancer and cardiovascular disease. The incidence rate of infertility caused by male factors is 30% to 50%. Male fertility can be impaired as a result of semen abnormality, spermatogenesis dysfunction, vas deferens obstruction and other systemic factors (such as high altitude, high temperature, super intensity work and radiation work,

etc.).² The most common reason is oligoasthenospermia, with an incidence rate of about 75% and a trend of increasing year by year. At present, the treatment of male infertility by Western Medicine mainly includes primary diseases, semen drugs, assisted reproductive technology, etc., but the therapeutic effect is limited and can't solve the problem of male infertility fundamentally. However, the theory of TCM believes that male infertility with abnormal semen and few weak sperm is often caused by insufficient kidney essence, and should take the method of nourishing kidney and improving essence. With the main effect of tonifying kidney and essence, WYP is favored by ancient and modern doctors. It has unique advantages in the treatment of infertility, and has become a new research direction of infertility and adjuvant treatment.

WYP also known as Wuzi pill (五子丸) and Wuzi Bushen pill (五子补肾丸), being firstly detailed recorded in *Shesheng Zhongmiao Fang* (摄生众妙方) of the Ming Dynasty, as a classical prescription for male infertility with kidney essence deficiency, is composed of Gouqizi (*Fructus Lycii*), Tusizi (*Semen Cuscutae*), Wuweizi (*Fructus Schisandrae Chinensis*), Fupenzi (*Fructus Rubi Chingii*), Cheqianzi (*Fructus Rubi Chingii*). It is recognized as "the first prescription of ancient and modern seeds". "Wu" (五) means composed of five drugs, "Zi" (子) means that all drugs are seeds, "Yan" (衍) means breed offspring. The whole prescription and its medicines are shown in Figure 1. Among them, Gouqizi (*Fructus Lycii*) and Tusizi (*Semen Cuscutae*) are Monarch drugs, which can nourish the Yin of liver and kidney and make its essence and blood sufficient. Wuweizi (*Fructus Schisandrae Chinensis*) and Fupenzi (*Fructus Rubi Chingii*) are Minister drugs,

which not only supplement the kidney essence, but also have the effect of consolidating essence. Cheqianzi (*Semen Plantaginis*) as Adjuvant drug, its property is cold. It can release the fire of the bladder and prevent the essence of the kidney from leaking out. The compatibility of five seeds can not only tonify kidney Yin, but also tonify kidney Yang, so as to play the role of tonifying kidney and benefiting essence.^{3,4}

Now, WYP is often used to treat oligozoospermia and asthenospermia of male infertility. In addition, the combination of Chinese and Western medicine can be used to treat oligozoospermia or azoospermia caused by primary spermatogenesis dysfunction. Based on the theory of TCM, it is found that WYP also can prevent and treat Alzheimer disease, aging, neuroendocrine network disorders related diseases, neural tube malformations and other diseases. In recent years, with the rapid development of experimental technology, some achievements have been made in the study of the effective components, basic research, clinical research and mechanism of WYP. However, these studies are still in the exploratory stage, lacking breakthrough and leaving many unclear issues to further explore.

In this review, we not only analyze the chemical composition, quality control, pharmacology and clinical application of WYP, but also discuss the limitations of the current researches, in order to promote the basic research and modern development of it and serve the clinic better.

2. CHEMICAL CONSTITUENTS

2.1. Chemical constituents of single drug in Wuzi Yanzong prescription

WYP is composed of Gouqizi (*Fructus Lycii*), dried

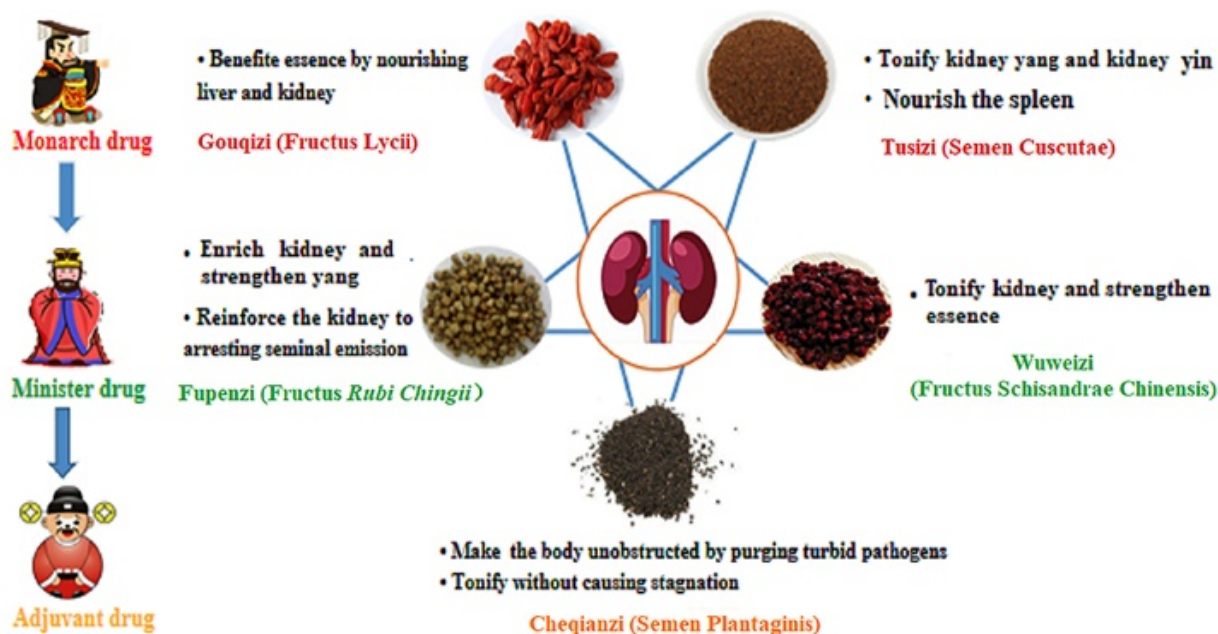


Figure 1 Composition and interpretation of Wuzi Yanzong prescription

fructus of *Lycium barbarum* L. Tusizi (*Semen Cuscutae*), dried semen of *Cuscuta australis* R.Br. or *Cuscuta chinensis* Lam. Wuweizi (*Fructus Schisandrae Chinensis*), dried fructus of *Schisandra chinensis* (Turcz.) Baill. Fupenzi (*Fructus Rubi Chingii*), dried fructus of *Rubus chingii* Hu. Cheqianzi (*Semen Plantaginis*), dried semen of *Plantago asiatica* L. or *Plantago depressa* Willd.

At present, there are few studies on chemical constituents of WYP, mainly focusing on single drug (Table 1). The main active ingredient of Tusizi (*Semen Cuscutae*) are flavonoids, which can improve the endocrine of germ cells and inhibit the apoptosis of spermatogenic cells. *Lycium barbarum* polysaccharide is the main effective component of Gouqizi (*Fructus Lycii*), which can promote the proliferation of spermatogonial stem cells *in vitro* and protect the development of germ cells. The main component of Fupenzi (*Fructus Rubi Chingii*) are terpenoids, which has obvious antioxidant and anti-inflammatory effects. Lignans and triterpenoids are the main active components in Wuweizi (*Fructus Schisandrae Chinensis*), which have antitumor and antioxidant activities. The main components of Cheqianzi (*Semen Plantaginis*) are flavonoids and phenylethanol glycosides, which have antioxidant activity and protect the male reproductive system.⁵

2.2. Chemical constituents of Wuzi Yanzong prescription

The five drugs of WYP are seeds or fruits of plants, which are rich in fatty acids. Tan *et al*²⁰ used GC-MS to determine 19 kinds of fatty acids in WYP, including 9 saturated fatty acids and 10 unsaturated fatty acids. With the development of modern analytical techniques, UPLC-ESI-LTQ-Orbitrap-MS was used to make the constituents of WYP more clear and comprehensible. A total of 106 compounds of WYP were identified or tentatively identified, including 35 flavonoids, 34 phenylpropanoids, 17 organic acids, 8 alkaloids, 11 terpenoids and 1 miscellaneous ingredient. Among them, 14 ingredients from Gouqizi (*Fructus Lycii*), 10 ingredients from Tusizi (*Semen Cuscutae*) (fried), 33 ingredients from Fupenzi (*Fructus Rubi Chingii*), 37 ingredients from Wuweizi (*Fructus Schisandrae Chinensis*) (steamed), and 20 ingredients from Cheqianzi (*Semen Plantaginis*) (fried with salt). Abromine, nicotinic acid, thiamine, riboflavin, taurine, quinic acid, atropine, ferulic acid, chlorogenic acid, scopoletin, rutin, esculin, apigenin, hesperidin, quercetin, kaempferol, luteolin, isorhamnetin and schisandrin were unambiguously identified with available standards.²¹ The structures of main components of WYP were shown in Figure 2.

In addition, Yan *et al*²² analyzed WYP by network pharmacology, the effective components of WYP in the treatment of infertility, the effective components of WYP are mainly steroids, phenylpropanoids, flavonoids, alcohols and alkaloids. Among them, the content of steroids 42%-48%, phenylpropanoids 11%-15%,

flavonoids 12%-14%, alcohols 9%-12%, and alkaloids 15%, which provides a theoretical basis for the researches and clinical application of its active ingredients.

3. QUALITY CONTROL

Chinese patent medicines with WYP as prescription include pill, tablet, capsule, soft capsule, oral liquid and granule. In order to ensure the safety, effectiveness and stability of WYP, it is particularly important to establish scientific and comprehensive quality control methods. With the rapid development of modern technology, accurate qualitative and quantitative analysis of multiple chemical components in Chinese polyherbal formula has been achieved, which provides a powerful methods for its quality control. This manuscript systematically summarizes the research progress of chemical analysis methods of WYP.

3.1. Thin layer chromatography (TLC) qualitative identification

TLC is a low-cost method for rapid qualitative analysis and identification by detecting the main or characteristic components of herbs. There are many TLC studies on WYP, such as Chinese Pharmacopoeia (2020 Edition), *Xin Yao Zhuan Zheng Biao Zhun*, *Zhong Yao Cheng Fang Zhi Ji*, and so on (Table 2). However, there are few chemical components involved, most of them are tested separately for individual components of a certain medicine.

3.2. HPLC quantitative analysis

High performance liquid chromatography (HPLC) is the most commonly methods for quantitative and qualitative analysis of chemical components in herbs. Previous literature reports mainly determined the content of single components such as betaine, hyperoside, kaempferin, schisandrin, schisandrin A, and schisandrin B, etc. With the introduction of advanced technologies and methods, the quality evaluation of WYP has been significantly improved (Table 3). However, more quantitative control methods for the effective ingredients need to be established to reflect the overall characteristics and internal quality of WYP.

3.3. Fingerprint or specific chromatogram analysis

In order to control the quality of WYP more effectively and control its internal quality from the perspective of multi drug and multi-component systematization, many researches have established the fingerprint of WYP (Table 4), identified the characteristic components, and matched with the fingerprint of single herbal medicine to obtain exclusive and overall comprehensive characteristic information. It can reflect the internal quality of WYP and the overall difference of preparation technology, provide a scientific, simple and feasible evaluation method for the qualitative and quantitative identification of WYP. However, further studies such as quantitative and structural determination are needed to control the quality of compounds more comprehensively.

Table 1 Chemical constituents of single drug in Wuzi Yanzong prescription

Chemical component	Source	Type	Reference
Flavonoids	Tusizi (<i>Semen Cuscutae</i>)	41	6
	Gouqizi (<i>Fructus Lycii</i>)	-	7
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	14	8
	Fupenzi (<i>Fructus Rubi Chingii</i>)	16	9
	Cheqianzi (<i>Semen Plantaginis</i>)	11, including flavonoids, flavonols and their glycosides	10-11
Polysaccharides	Tusizi (<i>Semen Cuscutae</i>)	Including neutral heteropolysaccharide, H3 acid polysaccharide	6
	Gouqizi (<i>Fructus Lycii</i>)	It's made up of six monosaccharides	12
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	The polysaccharide content is 85.56%, which is composed of rhamnose, galactose, arabinose and glucose	13
	Cheqianzi (<i>Semen Plantaginis</i>)	It contains 10%-30% arabinose, xylose, mannose and galactose (5.6:9.4:1.3:1)	10-11
Phenylethanoid glycosides	Cheqianzi (<i>Semen Plantaginis</i>)	20, it is composed of caffeic acid, phenyl glycoside and glycogen	10-11
Iridoids	Cheqianzi (<i>Semen Plantaginis</i>)	22	10-11
Steroids	Tusizi (<i>Semen Cuscutae</i>)	6, β -sitosterol, carotenoid, 7- α -hydroxy sitosterol, rape oleosterol, stigmastol, Δ 5-oatmasterol	6
	Fupenzi (<i>Fructus Rubi Chingii</i>)	6	9,14
Terpenoids	Cheqianzi (<i>Semen Plantaginis</i>)	-	10
	Tusizi (<i>Semen Cuscutae</i>)	9	6
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	9	8
	Fupenzi (<i>Fructus Rubi Chingii</i>)	19	14
	Cheqianzi (<i>Semen Plantaginis</i>)	-	10
Alkaloids	Tusizi (<i>Semen Cuscutae</i>)	4	6
	Gouqizi (<i>Fructus Lycii</i>)	Betaine	15
	Fupenzi (<i>Fructus Rubi Chingii</i>)	7	14
	Cheqianzi (<i>Semen Plantaginis</i>)	-	10,12
Amino acid	Tusizi (<i>Semen Cuscutae</i>)	15	6
	Gouqizi (<i>Fructus Lycii</i>)	18	12
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	16	16
	Cheqianzi (<i>Semen Plantaginis</i>)	-	11
Acids	Tusizi (<i>Semen Cuscutae</i>)	18	6
	Gouqizi (<i>Fructus Lycii</i>)	18%-22%	17
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	14	8
	Fupenzi (<i>Fructus Rubi Chingii</i>)	8	14
	Cheqianzi (<i>Semen Plantaginis</i>)	5	18
Trace elements	Tusizi (<i>Semen Cuscutae</i>)	Ca, Mg, Fe, Mn, Zn, Pb, Sn, etc.	6
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	K, Ca, Zn, Fe, etc.	8
	Cheqianzi (<i>Semen Plantaginis</i>)	Fe, Mg, Al, Zn, etc.	11
Lignans	Tusizi (<i>Semen Cuscutae</i>)	29	6
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	31 (8%)	8
Volatile components	Cuscutae Semen	11	6
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	5%-6%	16
Coumarins	Fupenzi (<i>Fructus Rubi Chingii</i>)	4, including esculetin, esculin, imperatorin, hexacosylp-coumarate	14
Others	Cuscutae Semen	Thymine, arbutin, etc.	19
	Gouqizi (<i>Fructus Lycii</i>)	Vitamins, 4-desmethylsterols, 4-methylsterols, 4'-dimethylsterols, taurine, etc.	12
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	neoisostegane, plaunol, gedunin, merulinic acid A, etc.	8
	Fupenzi (<i>Fructus Rubi Chingii</i>)	butyl dosocanoate, pentacosanol, liballino, 1H-2-indenone, etc.	9
	Cheqianzi (<i>Semen Plantaginis</i>)	Protein, Plantaginic Acid, Adenine, β -sitosterol, etc.	18

3.4. Other methods

Wei *et al*²⁰ used GC-MS qualitatively and quantitatively to determine the contents of 19 fatty acids in WYP. Based on previous studies, it is speculated that fatty acid may be the basis for the partial pharmacological effects. Multi index comprehensive quality control will become the trend of quality evaluation. Since Quantitative analysis of multicomponents by single-

marker (QAMS) was put forward, it has been recognized by many scholars. He *et al*²⁴ simultaneously determined Schisandrin, Hyperoside, quercitrin, kaempferol-3-o-rutinoside, schisandrin A and schisandrin B in WYP. This method can significantly reduce the cost of detection, improve the efficiency of analysis and test. Dong *et al*²⁷ determined the contents of hyperoside, isoquercitrin, verbascoside, astragaline,

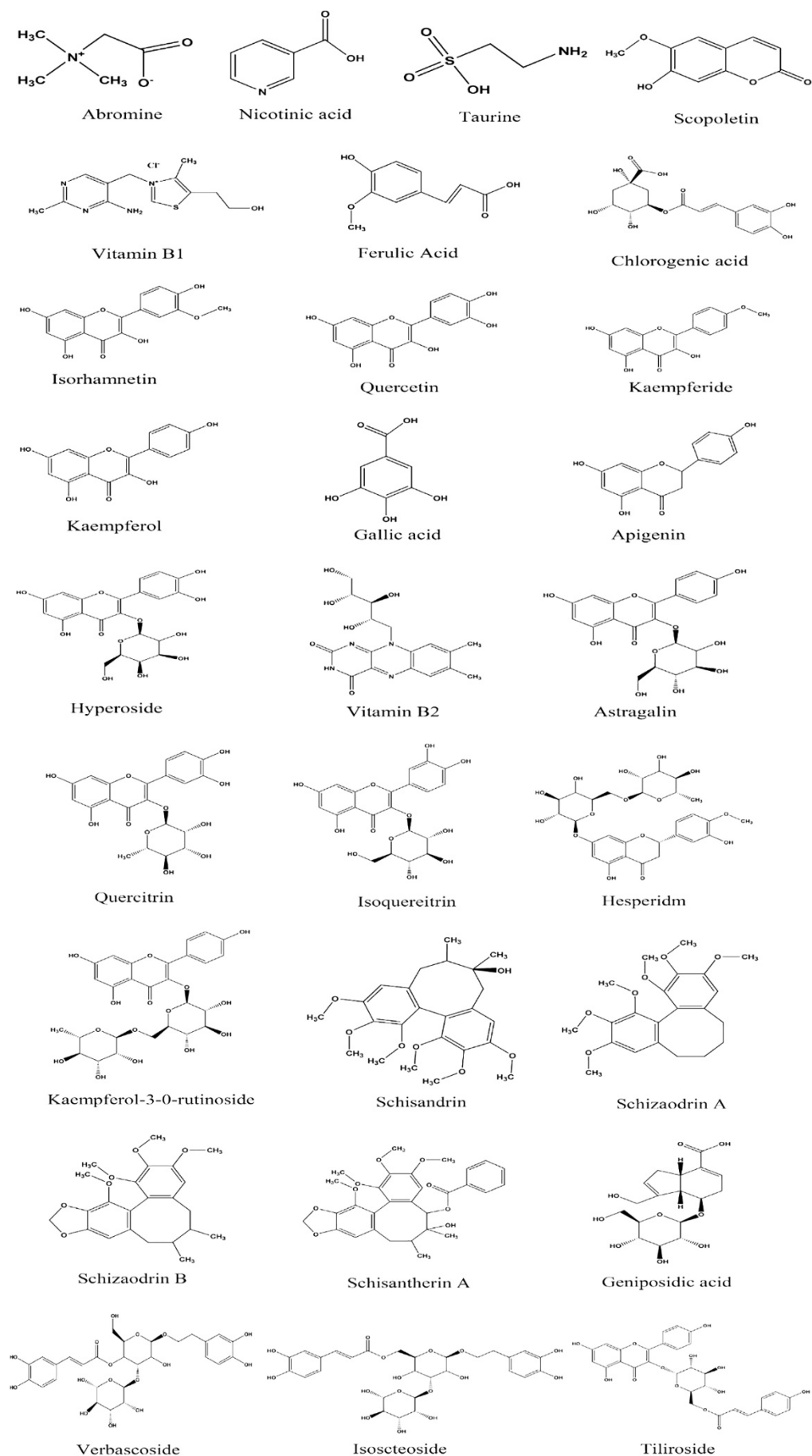


Figure 2 Structures of main components of Wuzi Yanzong prescription

Table 2 TLC identification of Wuzi Yanzong prescription

Dosage form	Control ingredients and medicinal materials	Developing solvent	Reference
Wuzi Yanzong Wan	Scopoletin	Petroleum ether (30-60)-ethyl formate-formic acid (20:20:0.1)	Chinese Pharmacopoeia (2020)
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>), Schisandrin	Methylbenzene-ethyl acetate (6:4)	
	Gouqizi (<i>Fructus Lycii</i>)	Ethyl acetate-trichloromethane-formic acid (3:2:1)	Xin Yao Zhuan Zheng Biao Zhun
Wuzi Yanzong Pian	Tusizi (<i>Semen Cuscutae</i>)	Petroleum ether (30-60)-butyl acetate-butanone-methyl alcohol-water (15:25:3:7:1)	Chinese Pharmacopoeia (2020)
	Schisandrin A	Methylbenzene-ethyl acetate (9:1)	
	Gallic acid	Methylbenzene-ethyl acetate-formic acid (6:3:1)	
	Tusizi (<i>Semen Cuscutae</i>)	Methylbenzene-ethyl acetate-formic acid (5:5:3)	
Wuzi Yanzong Ruanjiaonang	Scopoletin	Petroleum ether (30-60)-ethyl formate-formic acid (20:20:0.1)	Xin Yao Zhuan Zheng Biao Zhun
	Wuweizi (<i>Fructus Schisandrae Chinensis</i>), Gomisin A	Methylbenzene-ethyl acetate (6:4)	
	Gouqizi (<i>Fructus Lycii</i>)	Petroleum ether (60-90)-ethyl acetate-formic acid (13:5:1)	
	Tusizi (<i>Semen Cuscutae</i>)	Methylbenzene-ethyl acetate-formic acid (5:5:3)	
Wuzi Yanzong Jiaonang	Schisandrin A, Schisandrin	Petroleum ether (30-60)-ethyl acetate-formic acid (15:5:1)	Xin Yao Zhuan Zheng Biao Zhun
	Gouqizi (<i>Fructus Lycii</i>)	Acetate-trichloromethane-formic acid (2:3:0.1)	
	Quercetin	Acetate-trichloromethane-formic acid (26:13:5)	
	Gouqizi (<i>Fructus Lycii</i>)	Trichloromethane-ethyl acetate-benzene-formic acid (5:6:3:1)	
Wuzi Yanzong Koufuye	Wuweizi (<i>Fructus Schisandrae Chinensis</i>)	Petroleum ether (30-60)-ethyl acetate-formic acid (15:5:1)	Zhong Yao Cheng Fang Zhi Ji
	Gouqizi (<i>Fructus Lycii</i>)	Trichloromethane-ethyl acetate-benzene-formic acid (5:6:3:1)	

Notes: TLC: thin layer chromatography.

Table 3 HPLC methods developed for quantitative determination of Wuzi Yanzong prescription

Dosage form	Determination of components	Detection method (wavelength)	Reference
Wuzi Yanzong Wan	Hyperoside, Gomisin A	HPLC (360, 250 nm)	Chinese Pharmacopoeia (2020)
	Hyperoside, Kaempferide, Schisandrin, Schisandrin A, Schisandrin B	HPLC/PDA (360, 250 nm)	
	Hyperoside, Quercitrin, kaempferol-3-rutinoside, Schisandrin, Schisandrin A, Schisandrin B	HPLC/PDA (254 nm)	24
	Betaine	HPLC (192 nm)	25
	Scopoletin	HPLC-FLD (343 nm, 458 nm)	26
	Wuzi Yanzong Pian	Hyperoside, Gomisin A	HPLC (360, 250 nm)
Wuzi Yanzong Jiaonang	Schisandrin A	HPLC (230 nm)	Xin Yao Zhuan Zheng Biao Zhun
	Schisandrin	HPLC (254 nm)	Xin Yao Zhuan Zheng Biao Zhun
Wuzi Yanzong Keli	Rutin	HPLC (254 nm)	Xin Yao Zhuan Zheng Biao Zhun

Notes: HPLC: high performance liquid chromatography; PDA: photo-diode array.

kaempferol and schisandrin A by QAMS, and established HPLC fingerprint, which provides an accurate and scientific evaluation mode for the quality control of WYP.

4. PHARMACOLOGY

4.1. Effects on reproductive system

4.1.1 Effects on the male reproductive system

The strength of male fertility is intently associated to spermatogenic function and sperm quality. WYP can promote spermatogenesis, improve sperm quality and protect the integrity of sperm structure and function by

repairing the damage of spermatogenic cells and Sertoli cells, resisting excessive oxidative stress, inhibiting the decrease of Mitochondrial Membrane Potential (MMP) and alleviating the damage of mitochondrial microstructure to improve the reproductive function. The effects on the male reproductive system of WYP have been summarized in Table 5.

4.1.2 Effects on the female reproductive system

It is found that WYP also has a corresponding regulatory effect on the female reproductive system. It can up regulate the expression of S100A11 gene decreased by GnRHa long case COH, improve endometrial receptivity, and improve mouse pregnancy rate and embryo implantation rate.⁴⁰ Studies have shown that total flavonoids

Table 4 HPLC methods developed for fingerprint or specific chromatogram analysis of Wuzi Yanzong prescription

Dosage form	Detection Method (Wavelength)	Total chromatographic peak number	Chromatographic peak identification	Reference
Wuzi Yanzong Wan	HPLC (250 nm)	5	Hyperoside, Verbascoside, Kaempferide, Schisandrin	Chinese Pharmacopoeia (2020)
	HPLC (254 nm)	18	Hyperoside, Isoquercitrin, Kaempferol, Verbascoside, Astragaln, Gomisn A	27
	HPLC-DAD (254 nm)	24	Gallic acid, geniposidic acid, Chlorogenic acid, Hyperoside, Isoquercitrin, Verbascoside, Kaempferol-3-rutinoside, Isoverbascoside	28
	HPLC/PDA (360, 250 nm)	11	Chlorogenic acid, Hyperoside, Quercetin, Kaempferide, Verbascoside, Astragaln, Schisandrin, Gomisn A, Schisandrin A, Schisandrin B	23
	HPLC/PDA (254 nm)	10	Chlorogenic acid, Hyperoside, Verbascoside, Kaempferol, Schisandrin, Schisandrin A, Schisandrin B, Astragaln, Quercetin	29

Notes: HPLC: high performance liquid chromatography; HPLC-DAD: high-performance liquid chromatography-diode array detection; HPLC/PDA: high-performance liquid chromatography- photo-diode array.

Table 5 Effects on the male reproductive system of Wuzi Yanzong prescription

Pharmacological activity	Testing subject	Dosage	Effect	Reference
Effects on spermatogenesis	Oxidative damage of testis in mice	2, 4 g·kg ⁻¹ ·d ⁻¹ , 35 d	Significantly antagonize the testicular oxidative injury and spermatogenic cell apoptosis induced by cyclophosphamide	30
	Mice with oligospermia	1.56 g·kg ⁻¹ ·d ⁻¹ , 14 d	Regulate the meiosis of spermatogenic cells, homologous recombination of testis, renin-angiotensin recombination of testis, renin-angiotensin system, cholesterol metabolism pathway and others, so as to repair the blocking of testicular spermatogenesis, by affecting the gene expression of testis tissue in mice with oligospermia	31
	AT1KO mouse	5.8 g/kg, 2 times a day	Has a positive regulatory effect on the sex hormone levels of male AT1 ^{+/-} mice, increasing the levels of GnRH and FSH, and the degree of improvement is positively correlated with the intervention time, improving the reproductive function of male mice	32
Effects on sperm quality	Testicular tissue of rats with kidney essence deficiency	0.01 mL/g, 30 d	Can reduce the apoptosis of spermatogenic cells in rat testis by inhibiting Bax and promoting the expression of Bcl-2 protein	33
	Rat with oligospermia	1.96 g·kg ⁻¹ ·d ⁻¹ , 60 d	Can correct HPG secretion disorder and improve sperm quality	34
Effects on Sertoli cells	Mouse Testis TM4 Sertoli Cells	12.0 g/kg, 2 times a day, 7 d	Serum can improve the secretory function of TM4 Sertoli cells by regulating the levels of ROS and autophagy	35
	Human testicular support cell line TM4 cells	0.2, 1.0, 5.0 mg/mL	Can improve oxidative stress injury of testicular support cells and inhibit cell apoptosis	36
	Heat stress model of rat Sertoli cells	20 g/L	Can reduce the generation of reactive oxygen species and the expression of heat shock protein 70 to protect heat stress	37
Effects on sperm mitochondria	Rats with Oligoasthenozoospermia	1, 2, 4 g·kg ⁻¹ ·d ⁻¹ , 28 d	Can inhibit the opening of mitochondrial permeability transition pore (MPTP), improve the quality of spermatozoa, and reduce the apoptosis rate of spermatogenic cells (including spermatozoa)	38
	Rats with Oligoasthenozoospermia	4.0 g·kg ⁻¹ ·d ⁻¹ , 28 d	Can down-regulate the expression of Bax, VDACL1 and CypD proteins in rat testicular tissue, inhibit the over-opening of mPTP, and prevent the germ cell apoptosis caused by the activation of Caspase protein family	39

Notes: GnRH: gonadotropin-releasing hormone; HPG: human pituitary gonadotropin; TM4: testicular Sertolicells of normal mice; ROS: reactive oxygen species; MPTP: mitochondrial permeability transition pore; CypD: cyclophilin D; VDACL1: voltage-dependent anion-selective channel protein 1.

of *Cuscutae Semen* (TFSC) can significantly restore the ovarian function of rats with premature ovarian failure, increase the ovarian weight and number of follicles, improve the estrogen level, and have obvious curative effect on premature ovarian failure.⁴¹ TFSC can also increase the contents of serum E2 and FSH in letrozole induced polycystic ovary syndrome (PCOS) model rats.⁴²

4.2. Effect on nervous system

The theory of TCM believes that an important way to treat encephalopathy is to invigorate the kidney, replenish the essence and fill the marrow. WYP also plays a more important role in the field of nerve, and might be a useful agent for prevention and treatment of neuroinflammatory disease. WYP might act to suppress neuroinflammatory response in lipopolysaccharide stimulated rat astrocytes *via* NF- κ B and JNK/p38 MAPK signaling cascades.⁴³ It could also improve neurological function, regulate Rho/ROCK signaling pathway, reduce nerve tissue damage, inhibit the expression of inflammatory protein, promote the expression of anti-inflammatory macrophage cells and anti-inflammatory factor, and reduce the expression of pro-inflammatory factors in experimental autoimmune encephalomyelitis mice.^{44,45} WYP can reduce inflammatory factors interleukin (IL)-6, IL-1 β , TNF- α , and affect the expression of IL-4, improve the aggravation of inflammatory response in the brain, repair the damaged myelin sheath, promote myelin regeneration, and then improve the demyelination of cuprizone induced model.⁴⁶ WYP can improve the cognitive and learning status of patients with Parkinson's disease,⁴⁷ mild cognitive impairment and Alzheimer's disease by inhibiting the apoptosis of neural tube cells,⁴⁸ promoting the proliferation and differentiation of neural stem cells.⁴⁹ Besides, WYP has the effect of preventing neural tube defects (NTDs), and better than folic acid (FA). Its mechanism may be to up-regulate the expression levels of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and inhibit oxidative stress response.⁵⁰

4.3. Effect on urinary system

Urinary system is composed of kidney, ureter, bladder and urethra. The main function of it is to generate and excrete urine and excrete metabolic end products, regulate water and salt metabolism and maintain acid-base balance of body fluids.⁵¹ Diseases of urinary system can not only be caused by diseases of other systems, but also affect other systems and even the whole body. However, its specific diseases such as glomerulonephritis, urolithiasis and renal failure are mainly related to the kidney. Pharmacological studies have shown that WYP can improve the abnormal urination,⁵² nephromegaly and abnormal indexes of kidney and prostate in rats, and has a significant regulatory effect on urea nitrogen (BUN) and creatinine

(CRE).⁵³ However, *Fupenzi* (*Fructus Rubi Chingii*) has significant urinary contraction effect, and the effect of salt products is better. It can significantly improve aldosterone (ALD), urine volume and renal index in adenine induced polyuria model rats with kidney *Yang* deficiency.⁵⁴ Some scholars also found that kaempferol can inhibit apoptosis, inhibit inflammatory response and protect renal function.^{55,56} Isorhamnetin inhibits NF- κ B over activation, reduce the level of inflammatory factors to reduce proteinuria.⁵⁷

4.4. Other pharmacological effects

Modern researches also found that WYP also has the functions of inhibiting liver damage, lowering blood sugar and blood lipids, anti-aging, improving immunity, resisting hypoxia and fatigue, etc. WYP can regulate the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum, SOD and malondialdehyde (MDA) in liver tissue, and had a good protective effects on liver injury caused by cyclophosphamide (CTX).^{58,59} WYP can reduce the secretion of inflammatory factors, inhibit Rho signal pathway, improve the neurological function score of experimental autoimmune encephalomyelitis (EAE) mice, delay the onset time of EAE, and regulate the body immunity.⁴⁷ It can also regulate serum immunoglobulin G, IL-6, IL-2 and promote the balance of T lymphocyte network, improve the immune function of kidney essence deficiency rats.⁶⁰ WYP can significantly increase the activity of SOD and GSH-Px, reduce the content of MDA, alleviate the oxidative damage caused by D-galactose, inhibit the abnormal activity of polyol pathway in lens tissue of diabetic cataract mice, improve the opacity of lens in diabetic cataract mice.⁶¹ Through swimming test and survival time test under anoxic condition in mice, WYP can significantly prolong the swimming time, enhance the anoxic tolerance time and enhance the immune function of mice, etc.⁶² In addition, WYP can regulate tumor energy metabolism through SIRT1 signal pathway, so as to play a role in the treatment of tumor related fatigue (CRF).⁶³

5. CLINICAL APPLICATION

5.1. Reproductive system

5.1.1 Treatment of male reproductive system diseases

Clinical studies have found that WYP can reduce the levels of DNA fragmentation index (DFI) and ROS in semen, improve SOD activity and improve sperm DNA damage.⁶⁴ By observing the patient's TCM symptoms, sperm concentration, forward movement sperm rate, and normal sperm morphology rate, and comparing the therapeutic effects of taking WYP, vitamin C and vitamin E on male infertility patients, the result showed that WYP can significantly improve male infertility. The quality of semen in patients with symptom, and the improvement of semen parameters is significantly better

than the effect of vitamin C combined with vitamin E treatment.⁶⁵ WYP combined with Jinshuibao capsule can significantly improve semen volume, sperm density, grade a sperm motility, (a + b) sperm motility and sperm motility. It also can improve the level of serum hormone and the success rate of spouse pregnancy.⁶⁶ Yang *et al*⁶⁷ compared the efficacy and safety of WYP and Qilin pill (麒麟丸) in the treatment of idiopathic oligoasthenospermia according to the method of randomized double-blind parallel control study, and found that both WYP and Qilin pill can significantly improve sperm quality, regulate sex hormone level, present the efficacy law of time effect improvement, without obvious adverse reactions, and medication safety. Li *et al*⁶⁸ found that in infertile patients with oligospermia and asthenospermia, the sperm motility was significantly increased and the levels of caspase 3 and caspase 8 in semen and sperm were decreased after continuous use of WYP for 2 months. Tan *et al*⁶⁹ used WYP to treat poor semen liquefaction, the result showed that the contents of prostate-specific antigen and acid phosphatase in the seminal plasma of the patients were significantly increased, the semen liquefaction time was shortened, and the semen quality was improved.

5.1.2 Treatment of female reproductive system diseases

Clinical studies have shown that WYP not only has significant curative effect on male reproductive system diseases, but also has a certain conditioning effect on female reproductive system. Zhang *et al*⁷⁰ treated 86 cases of adolescent dysfunctional uterine bleeding with Jiawei Wuzi Yanzong pill (加味五子衍宗丸). Among them, 64 cases were cured, 19 cases were improved and 3 cases were not cured. The total effective rate was 96.51%. Sun *et al*⁷¹ used WYP to treat infertility patients with ovulation disorder. WYP can effectively improve the symptoms of kidney deficiency and essence deficiency, improve the level of sex hormone secretion and luteal function, and improve the endometrial receptivity and pregnancy rate of patients with infertility caused by kidney deficiency and essence deficiency symptom pattern and luteal insufficiency (LPD). It has high safety and is worthy of clinical promotion.⁷² Modified Wuziyanzong pill can regulate HPOA axis, improve luteal function, improve hormone level, reduce the score of kidney deficiency and essence deficiency symptom pattern, and improve endometrial receptivity, so as to improve pregnancy rate. Its clinical effect is better than that of Western Medicine.⁷³ WYP combined with clomiphene citrate in the treatment of infertility can improve the ovulation of patients and alleviate clinical symptoms. It has high safety and is worthy of clinical application.⁷⁴ WYP combined with clomiphene citrate capsule can improve the ovulation rate, pregnancy rate, endometrial thickness, basal body temperature (BBT) distribution and cervical mucus score (CMS) of patients with ovulatory infertility, and the scoring effect is better than that of TCM alone or western medicine alone.⁷⁵

5.2. Nervous system

Li *et al*⁷⁶ found that Jiawei Wuzi Yanzong pill can improve the ability of image free recall, pointing memory and associative learning of patients with mild cognitive impairment (MCI) to varying degrees, and significantly reduce the hippocampal index and temporal angle width, indicating that it can improve the memory ability of patients and delay hippocampal atrophy. Zhang *et al*¹⁷ found that after 12 weeks of treatment with Wuzi Yanzong decoction combined with donepezil, the total effective rate, MCI and mini mental state examination scores were significantly higher than those with donepezil alone.

5.3. Urinary system

In recent years, studies have found that WYP also has a certain effect on urinary system diseases. Female urinary incontinence and infantile enuresis belong to the category of “urinary incontinence” and “enuresis” in TCM. The pathogenesis is mostly kidney deficiency, resulting in kidney *Qi* deficiency, ineffective intake and storage, and the bladder loses its restraint ability. The main treatment methods are warming and tonifying kidney *Yang*, fixing and shrinking urine, strengthening spleen and tonifying lung. Pan *et al*⁷⁷ used Jiawei Wuzi Yanzong pills to treat female urinary incontinence. After a course of medication, the patient had normal urination and no urinary incontinence. Compound Wuzi oral liquid can significantly reduce the clinical symptoms of frequent micturition and urgency in women with urethral symptom pattern, and improve the level of estrogen.⁷⁸ Huangkuai capsule (黄葵胶囊) combined with WYP is effective in the treatment of immunoglobulin A nephropathy proteinuria damp heat symptom pattern.⁷⁹ In addition, WYP has good curative effect on enuresis, frequent urination and nephrotic symptom pattern in children.⁸⁰

5.4. Others system

WYP is still used in the treatment of stable stage of chronic obstructive pulmonary disease (COPD) with deficiency of lung and kidney *Qi* (*Yang*),⁸¹ and has certain curative effect on recurrent oral ulcer, alopecia areata, primary thrombocytopenic Pura and other diseases.⁸² Based on gonadotropin-releasing hormone (GnRH) antagonist program intervention, Jiawei Wuzi Yanzong pill was given to the patients with poor kidney deficiency symptom pattern with low ovarian response by cycle and stage. It can regulate the level of endocrine hormones, promote follicular development, improve ovarian reserve function, increase the number of ovums, improve the quality of ovums, improve the pregnancy outcome, and increase the chances of successful assisted pregnancy with autonomous rail rapid transit, but it is worthy of further study.⁸³ WYP combined with clomiphene citrate capsule can improve the ovulation rate, pregnancy rate, endometrial thickness, BBT distribution and CMS in patients with ovulatory infertility. It is better than using Chinese medicine or

Western Medicine alone.⁷⁵ In addition, the combination of WYP and compound glycyrrhizic acid S injection can significantly improve the liver function and clinical symptoms of lung cancer, gastric cancer and breast cancer chemotherapy induced liver injury, and is better than the injection alone.⁸⁴

6. DISCUSSION

WYP as a classic famous prescription for nourishing kidney and producing essence. It has been used in the symptom patterns of *Yang* phlegm infertility, spermatorrhea and premature ejaculation caused by kidney essence deficiency for thousands of years and is still widely used today. Its composition is scientific and the curative effect is definite. It has a significant effect on reproductive system diseases, especially male infertility, as well as neurological diseases and endocrine system diseases. This article reviews its chemical composition, quality control, pharmacology and clinical application. We found that WYP had the value of further development and utilization, but the production practice and clinical application showed that there were still some problems in the development and utilization of WYP.

First of all, WYP was first recorded in the 1985 edition of the Pharmacopoeia of People's Republic of China. As a traditional pill, its preparation process is simple. At present, a series of preparations have been developed on the basis of WYP. However, we found that WYP produced by different companies has great differences in properties, specifications, usage, dosage and content of active ingredients, which inevitably lead to the phenomenon of "different effects of the same drug". In order to improve the quality of WYP, it is recommended to fully consider the nature of raw materials, processes and the principle of facilities in the production to promote the technological innovation of the preparation of WYP. At the same time, it is necessary to introduce relevant indicators such as biological potency, effective component index, biomarkers and other related indicators into quality control and quality evaluation of WYP, construct the quality evaluation method of WYP based on efficacy, further improve the existing quality evaluation system, ensure the quality of WYP, and make it better serve the clinical.

Pharmacodynamic researches of WYP are still in the basic stage, and the researches on its target and related molecular mechanism are mostly single perspective or level. Hyperoside, Verbascoside, Gomisin A, Schisandrin A, Schisandrin B and Kaempferol have good protective effects on central nervous system diseases such as Alzheimer's disease, Parkinson's disease, depression, cerebral ischemia and glioma, especially on neurodegenerative diseases.⁸⁵ Quercetin has a certain protective function on the reproductive system and can protect the structure and function of testicular tissue and maintain normal.⁸⁶ Kaempferol has the functions of anti-oxidation, anti-inflammatory, anti-cancer, prevention

and treatment of diabetes, atherosclerosis and inhibition of protein kinase.⁸⁷ The mechanism mainly includes regulating the activity of proinflammatory enzymes and controlling the expression of inflammation related genes.⁸⁸ Stigmasterol and β -Sitosterol have many functions such as reducing blood lipid, anti-inflammatory, antioxidant, regulating growth and hormone like functions.⁸⁹ After oxidation, they will be transformed into estrogen like substances in the body, which will improve the level of sex hormones in the plasma, which is beneficial to the development of the body's reproductive system, enhance the body's reproductive ability, and reduce the level of reproductive steroid hormones in the plasma.⁹⁰ In addition, based on network pharmacology, Yan *et al*²² concluded that the pharmacodynamic compounds of WYP in the treatment of infertility, impotence and premature ejaculation are steroids, phenylpropanoids, flavonoids, alcohols and alkaloids, which provides a theoretical basis for its active ingredient research and clinical application. Modern scientific research requires us not only to use a variety of modern technology to explore the changes of effective substances of it, but also to study its compatibility significance from the theory of TCM, understand the special relationship between individual herbs and the whole prescription in terms of nature, taste, meridian tropism and efficacy, carry out the researches on material basis and mechanism of action, provide scientific proof for the main efficacy of WYP, and make it glow with new brilliance.

With the deepening of modern research, the clinical application of WYP has become more and more extensive. It has a significant effect on a variety of diseases such as reproductive system, urinary system and nervous system, especially reproductive system diseases. WYP is mainly used to treat male infertility caused by oligospermia and asthenospermia, it is not suitable for all male infertility. It should be reasonably on the basis of symptom pattern identification and treatment. Most literatures mainly focus on clinical efficacy, lack of in-depth study on the mechanism of male infertility, and most of them use modified WYP or combination drugs. The specific medicinal ingredients and pharmacological mechanisms of WYP have not been studied. Meanwhile, Tusizi (*Semen Cuscutae*), Wuweizi (*Fructus Schisandrae Chinensis*), and Cheqianzi (*Semen Plantaginis*) have a wide variety of four herbs, and they are widely distributed, and there is no uniform medication standard. The specific modified drugs and dose changes are also uncertain, which brings many unknown factors to clinical research. Therefore, it is still an arduous task to establish a standardized mechanism for the treatment of male infertility. In addition, WYP is also used in the treatment of female urinary incontinence, alopecia areata, purpura and other diseases, but they are mostly case reports, lack of large sample of clinical randomized trial data, its effectiveness needs to be further verified.

7. CONCLUSIONS

WYP as a classic prescription for nourishing kidney and producing essence. Its composition is scientific and the curative effect is definite. On the one hand, with the expansion of clinical application, it has the value of further development and utilization. On the other hand, it also exposed some problems that need further research. It is very important to study the chemical components for the identification of material basis of efficacy and the exploration of its mechanism. There are few studies on the multiplicity of new preparations, new processes, effective components, effective parts, compatibility and combination, therapeutic approaches and targets of WYP, the researches in related fields should be further strengthened. In addition, WYP is mostly used in combination with western medicines besides being used alone. Whether it can improve the efficacy and reduce side effects will also be a meaningful research direction in the future.

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