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The mechanism of Xuefu Zhuyu decoction in treating erectile dysfunction based on Arrowsmith tool

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Abstract

Arrowsmith, a literature retrieval tool, was used to explore the theoretical pharmacological mechanism of action of Xuefu Zhuyu decoction and its single herb against erectile dysfunction. Cytoscape 3.6.1 software was used to analyze the correlation between the components of Xuefu Zhuyu decoction and the identified mechanism of action. Different herbs in Xuefu Zhuyu decoction may play a therapeutic role by regulating iNOS, COX-2, mTOR, Wnt, AMPK, Nrf2, TLR4, CYP2D6, BDNF, MAPK, Akt, STAT3, PI3K, ERK1, P38MAPK, JNK, VEGF, and other targets. The treatment of erectile dysfunction with Xuefu Zhuyu decoction embodied the multicomponent, multitarget, and multipathway. The Arrowsmith tool retrieval and analysis provided a scientific basis for further elucidation of the mechanism of Xuefu Zhuyu decoction against erectile dysfunction.

Key words: Arrowsmith; erectile dysfunction; mechanism; Xuefu Zhuyu decoction

***Abbreviations*:** ED, rectile dysfunction; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; ET-1, endothelin-1; ApoE, apolipoprotein E; AGD, Amygdalin; Nrf2, NF-E2-related factors; TLR4, Toll-like receptor; CYP2D6, cytochrome P4502D6; JNK, C-Jun amino-terminal kinase; Akt, protein kinase B; AMPK, AMP-activated protein kinase; BDNF, brain-derived neurotrophic factor; MAPK, mitogen-activated protein kinase; ERK, extracellular regulating kinase; PI3K, phosphatidylinositol-4,5-bisphosphate3-kinase; mTOR, mammalian target of rapamycin; STAT3, signal transducer and activator of transcription 3; SSd, Saikosaponin D; eNOS, endothelial nitric oxide synthase; FA, ferulic acid; VEGF, vascular endothelial growth factor.

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Background

Erectile dysfunction (ED) is a common male disease characterized by difficulties in achieving and maintaining erection, which affects patient's sexual life [1]. The etiology and pathogenesis of ED are complex. Currently, it is believed the main causes are related to vascular and metabolic dysfunction [2]. Organic ED requires drug treatment and phosphodiesterase 5 inhibitors, which are first-line drugs in western medicine. Although phosphodiesterase 5 inhibitors have apparent effects and rapid onset, they are highly-priced and impose a substantial financial burden on patients or health systems. Side effects, such as muscle pain and facial flushing, are also common. If patients have systemic symptoms due to these drugs, the achievable results may not be significant. Traditional Chinese medicine presents several treatments for ED, which became a research hotspot in recent years, with clear advantages in the overall ED therapy.

ED belongs to "impotence" category in the traditional Chinese medicine theory. Xuefu Zhuyu decoction, from the book *Yilin Gaicuo* (*Correction on Errors in Medical Classics*; 1830 C.E.), which proposed new anatomical maps, physiological perspectives and mechanisms of action for some conditions, correcting previous mistakes. Xuefu Zhuyu decoction includes Chuanxiong (*Rhizoma Chuanxiong*), Chishao (*Radix Paeoniae Rubar*), Taoren (*Semen Persicae*), Honghua (*Flos Carthami*), Niuxi (*Radix Achyranthis Bidentatae*), Dihuang (*Radix Rehmanniae*), Chaihu (*Radix Bupleuri*), Zhike (*Fructus Aurantii*), Jiegeng (*Radix Platycodonis*) and Danggui (*Radix Angelicae Sinensis*). It promotes blood circulation, eliminates blood stasis, and regulates Qi. Taoren (*Semen Persicae*) and Honghua (*Flos Carthami*) in the formula could remove blood stasis, and the two drugs supplement each other for a total of sovereign (Jun) drugs. Chishao *(Radix Paeoniae Rubra)*, Chuanxiong (*Rhizoma Chuanxiong*) and Niuxi (*Radix Achyranthis Bidentatae*) are known for promoting blood circulation, counteracting blood stasis, and relieving pain. Dihuang (*Radix Rehmanniae*) and Danggui (*Radix Angelicae Sinensis*) could nourish blood Yin, clear heat, and activate blood circulation. Chaihu (*Radix Bupleuri*), Zhike (*Fructus Aurantii*), and Jiegeng (*Radix Platycodonis*) is believed to sooth the liver and regulate Qi. *Radix Platycodonis* carries medicine upwards, and *Radix Achyranthis Bidentatae* guides blood down, *Radix Glycyrrhiza* coordinates all drugs. This prescription is widely used in the treatment of andrologic diseases. Its complex components have the characteristics of traditional Chinese medicine—multicomponent, multitarget, and multipathway—in treating illness. At present, the mechanism of action of Xuefu Zhuyu decoction for the treatment of ED has not been fully elucidated.

Arrowsmith knowledge retrieval has been widely used in biomedical research in recent years [3]. Based on a large number of literature datasets, the identification of associations provided clues and ideas for clinical or experimental research. Therefore, based on a PubMed database literature, we used the Arrowsmith tool to find standard correlation terms between subject headings on known literature, which could be extended to WOS, Embase, CNKI, and other databases [4]. We used the correlation analysis of the literature to explore the mechanism of action of Xuefu Zhuyu decoction and its single herb against ED.

Literature retrieval using Arrowsmith

Arrowsmith (http://arrowsmith.psych.uic.edu/) online retrieval interface was used on September 10th, 2020. The retrieval words in the subject of A collection were "Xue Fu Zhu Yu Tang" or single flavor medicine, such as peach walnut ("*Semen Persicae*" or "taoren"), and the retrieval words in the subject of C collection were "Erectile Dysfunction." After connecting A and C sets with the Arrowsmith tool to form a B set, the association word was set. To eliminate the undesired field-related words, the semantic filter was used to select Chemicals & Drugs, Disorders, Genes & Protein Names, Gene & Protein Sequences, and the remaining related terms were formed into a new set B. Then irrelevant terms were manually excluded. The relevant statistics are shown in Table 1.

Comprehensive analysis of literature retrieval results

By determining the word bank correlation of a single drug, the top 20 related words were selected for analysis. At least two or more associated words were chosen for Xuefu Zhuyu decoction or its single herb. Cytoscape3.6.1 software was used to visualize the essential correlation.

Analysis of Single Herb and ED

Analysis of *Semen Persicae* and ED. *Semen Persicae*, or peach kernel, promotes blood circulation, removes blood stasis, moistens bowel laxity, and relieves cough and asthma. It is a common herb for the clinical treatment of blood stasis block. The related words (gene or protein) of ED in peach kernel treatment include nitric oxide synthase, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), endothelin-1 (ET-1), and apolipoprotein E (ApoE), as shown in Table 2. The main components of peach kernel are lipid-soluble substances, proteins, sterols, glycosides, flavonoids, and phenolic acids [5].

Vascular endothelial dysfunction and atherosclerosis

are important in the pathogenesis of ED [6]. Some studies have found that the cavernous artery, the cavernous smooth muscle, and endothelium presents structural changes in diabetic patients [7]. ED is considered an indicator of systemic endothelial dysfunction and a sentinel of asymptomatic systemic cardiovascular disease [8]. Fermino Sanches Lizarte Neto et al. [9] studied the iNOS gene and protein expression in the cavernous smooth muscle of alcoholic, diabetic, and alcoholic diabetic rats. NO and iNOS are involved in inflammatory regulation *in vivo*. NO generation by iNOS plays an essential role in inflammatory responses and tissue repair, inducing both inflammatory and cytotoxic effects [10−12]. Amygdalin (AGD) is one of the main active components of peach kernel glycosides and has antiinflammatory activity. AGD inhibits the expression of COX-2 and iNOS and reduces the inflammatory response [13]. AGD also improves the inflammatory response by inhibiting the production of inflammatory factors, including iNOS and COX-2 [14]. ET-1, a potent vasoconstrictor peptide, is involved in the physiopathology of ED and is key component in the pathogenesis of ED mediated by hypercholesterolemia and diabetes [15−17]. A study also showed that the level of ET-1 in peripheral blood and cavernous blood in the ED case group was higher than in healthy controls [18]. Zhang et al. [19] treated chronic pancreatitis with AGD *in vivo*, and found that AGD may improve pancreatic microcirculation by downregulating ET-1. Peach kernel compounds could also inhibit significantly the expression of ET-1 in rabbit serum and improve hemorheology [20]. The same compounds were able to induce development of mature plaques in ApoE gene-deficient mice, stabilizing the plaques to some extent. This mechanism may be related to the regulation lipid metabolism and inhibition of the inflammatory response [21].

Table 1 Using the Arrowsmith tool to retrieve the information table

Job Id	Subject Search Terms	A Set	C Set	B Set	b Set	closely related word set
25715	*Semen Persicae* or taoren (TR)	76	25000	502	185	20
25744	*Flos Carthami* or honghua (HH)	127	25000	756	237	32
25774	*Fructus Aurantii* or zhike (ZK)	199	25000	925	287	35
25854	*Radix Rehmanniae* or dihuang (DH)	239	25000	1235	427	42
25356	*Radix Bupleuri* or chaihu (CH)	280	25000	1232	421	37
25903	*Radix Glycyrrhiza* or gancao (GC)	25	25000	174	53	9
25939	*Radix Angelicae Sinensis* or danggui (DG)	501	25000	1786	622	61
260701	*Radix Paltycodonis* or jiegeng (JG)	25000	25000	14384	4324	66
26292	*Radix Achyranthis Bidentatae* or niuxi (NX)	48	25000	345	89	6
26121	*Rhizoma Chuanxiong* or chuanxiong (CX)	328	25000	1401	460	56
26359	*Radix Paeoniae Rubra* or chishao (CS)	1	25000	5	1	0
26496	Xue Fu Zhu Yu Tang (XFZYT)	3	25000	27	9	1

Table 2 The top 20 related words of *Semen Persicae* and erectile dysfunction

number	relevance	related words	number	relevance	related words
1	0.99	nitric oxide donor	11	0.96	hypercholesterolemic rabbit
2	0.99	sirtuin	12	0.95	nitric oxide prostaglandin
3	0.99	nitric oxide synthase	13	0.94	endothelin-1
4	0.99	oxide synthase	14	0.94	ApoE
5	0.99	ginseng	15	0.93	neurotrophic
6	0.98	iNOS	16	0.93	antimutagenic
7	0.98	inducible nitric oxide	17	0.88	chronic liver disease
8	0.98	COX-2	18	0.86	coronary artery disease
9	0.97	hypercholesterolemic	19	0.84	calcitonin gene related
10	0.96	health related	20	0.84	gene related peptide

Analysis of *Flos Carthami* and ED. *Flos Carthami* has the effect of promoting, or safflower, can promote blood circulation, disperse circulation, disperse blood stasis and relieve pain. Clinically, the medicinal herb has been used to improve micro-vascular diseases, such as coronary heart disease and angina pectoris. The related words (gene or protein) of ED in safflower therapy included NF-E2-related factors (Nrf2), Toll-like receptor (TLR4), iNOS, COX-2, and cytochrome P4502D6 (CYP2D6), as shown in Table 3. The main components of safflower are flavonoids, alkaloids, polyacetylene, arginine, and lignans [22].

Previous studies have shown that TLR4 was associated with vascular homeostasis and vascular dysfunction in chronic diseases [23]. It was also found that TLR4 mediates vascular diseases, such as diabetes [24], hypertension [25], and vascular ED [26]. COX-2 regulates prostaglandins, which play an essential role in inflammation and are controlled by TLR4 signals [27, 28]. TLR4 and COX-2 expressions were significantly increased in ED model rats [29]. Kenia Pedrosa Nunes et al. [30] found that long-term inhibition of TLR4 could slightly improve diabetic-induced ED in rats. Studies have shown that the aqueous extract of safflower has potent antioxidant activity [31]. Dan Han et al. [32] observed that hydroxyl safflower yellow pigment A alleviated the inflammatory damage in hyperlipidemia rat models by inhibiting *TLR4* signal transduction. The spongy tissue of ED patients showed reduced Nrf2 and increased superoxide content [33]. Nrf2 is a central regulator that coordinates cells' response to oxidative stress and protects the endothelium from injury by upregulating some effector enzymes [34]. A study also showed that the deletion of the *Nrf2* gene in rats could lead to endothelial dysfunction, oxidative stress, and microvascular changes [35]. Javier Angulo et al. [33] found that *Nrf2* activation could improve endothelial dysfunction and enhance ED conventional drug therapy in human target tissues. It is known that safflower can significantly enhance Nrf2 [36]. Zhang et al. [37] used safflower extract combined with aceglutamide injection in a brain injury rat model. The results showed that aceglutamide injection enhanced the Nrf2 system, inhibited the activation of apoptosis signal-regulated kinase 1, prevented the subsequent activation of p38 and C-Jun amino-terminal kinase (JNK) signaling cascade, and reduced oxidative damage and cell apoptosis. Liu Feng et al. [38] proved that safflower extract could inhibit the subtype CYP2D6 in rats, suggesting that adverse effects may be avoided when combined with other drugs affecting the CYP2D6 metabolism.

Analysis of *Fructus Aurantii* and ED. *Fructus Aurantii* regulates Qi and eliminates distension. The associated proteins (genes or proteins) in ED treatment of *Fructus Aurantii*, include protein kinase B (Akt), AMP-activated protein kinase (AMPK), brain-derived neurotrophic factor (BDNF), and mitogen-activated protein kinase (MAPK), as shown in Table 4. The main components of *Fructus Aurantii* are volatile oils, flavonoids, coumarins and alkaloids [39].

The PI3K-Akt-MTOR pathway regulates various cellular processes, including cell apoptosis, proliferation, and metabolism, and has been shown to be vital in the generation of vascular endothelial cells [40]. Naringin, a flavonoid present in *Fructus Aurantii*, inhibits autophagy by activating the PI3K-Akt-MTOR pathway to improving endothelial cell dysfunction, a possible protection and treatment for diabetic vascular diseases [41]. The PI3K/Akt signaling pathway also regulates fat formation [42−43]. AMPK is a critical point in metabolic and catabolic pathways and a classic target for diabetes treatment. P-syringolin is a primary alkaloid in *Fructus Aurantii* [44]. It has been found that through the activation of the Akt pathway,

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P-syringolin acted as antilipid with protective effect against damage in the vascular system [45]. Acid heterosaccharide of *Fructus Aurantii*i was found to activate Akt to play the same role [46]. Limonene, one natural product of the volatile essential oil in *Fructus Aurantii*i, was able to regulate fat formation and blood glucose through the Akt signaling pathway and increase AMPK content [47, 48]. In conclusion, the practical components of *Fructus Aurantii* can be important in the treatment of metabolic diseases by targeting Akt and AMPK. Nf-κB comprises a variety of cytokines including the tumor necrosis factor-alpha, IL-1β, and IL-6, involved in the regulation of the inflammatory process factor [49]. MAPK is also involved in the inflammatory process. Evidence suggests that the MAPK family (including p38MAPK, ERK and JNK) plays an essential role in signal transduction pathways and mediates a variety of physiological and pathological changes in cells. Therefore, the inhibition of NF-κB and MAPKs may have potential therapeutic effects in inflammatory diseases [50]. Limonene was shown to reduce the production of proinflammatory cytokines by inhibiting the activation of NF-κB and MAPK [51], and *Fructus Aurantii* inhibited MAPK and NF-κB signaling pathways and activated the AMPK signaling pathway, causing a significant decrease in the inflammatory response, oxidative stress, and apoptosis [52]. BDNF, a member of the neurotrophic factor family, activates the JAK kinase/signal transduction and transcriptional activator pathway in Schwann cells, which mediates the effect of nerve regeneration after penis-spongiform nerve injury [53−55]. Lujie Song et al. [56] proved that BDNF could enhance the recovery of erectile function and inhibit penile cavernous fibrosis after cavernous nerve injury. Additionally, naringin was able to upregulate BDNF expression [57].

Table 3 The top 20 related words of *Flos Carthami* and erectile dysfunction

number	relevance	related words	number	relevance	related words
1	0.99	liquid chromatography tandem	11	0.98	iNOS
2	0.99	endothelial nitric oxide	12	0.97	COX-2
3	0.99	Sirtuin	13	0.96	angiogenic
4	0.99	Nrf2	14	0.96	hypercholesterolemic
5	0.98	nitric oxide synthase	15	0.93	endothelial inflammation
6	0.98	oxide synthase	16	0.95	autophagy
7	0.98	ginseng	17	0.95	hypercholesterolemic rabbit
8	0.98	TLR4	18	0.95	nitric oxide prostaglandin
9	0.98	metoprolol	19	0.94	hyperlipidemia
10	0.98	lipid lowering	20	0.93	cyp2d6

Table 4 The first 20 related words of *Fructus Aurantii* and erectile dysfunction

number	relevance	related words	number	relevance	related words
1	0.99	liquid chromatography tandem	11	0.97	autophagy
2	0.99	Akt	12	0.97	MAPK
3	0.99	chronic pelvic pain	13	0.97	x receptor
4	0.99	major depressive disorder	14	0.97	unpredictable mild stress
5	0.99	pelvic pain	15	0.96	depressive disorder
6	0.99	sirtuin	16	0.94	anti-apoptotic
7	0.99	AMPK	17	0.94	mild stress
8	0.99	herb drug interaction	18	0.93	antidepressant
9	0.98	BDNF	19	0.93	ms
10	0.97	hypertensive rat	20	0.92	coronary artery disease

Analysis of *Radix Rehmanniae* and ED. *Radix Rehmanniae* nourishes Yin, clears heat, cools blood, and enriches the blood. The related words (gene or protein) for the treatment of ED included Akt, mammalian target of rapamycin (mTOR), Wnt signaling pathway, phosphatidylinositol-4, 5-bisphosphate3-kinase (PI3K), TLR4, MAPK, ERK, CYP2D6, and COX-2, as shown in Table 5. The main components of *Radix Rehmanniae*, include iridoid, glycosides, glycosides, and amino acids [58].

Catalpa alcohol, an iridoid glycoside extracted from *Radix Rehmanniae*, has been shown to improve endothelial dysfunction by inhibiting the NF-κB/iNOS pathway and activating the PI3K/Akt/eNOS pathway [59]. In addition, catalpa inhibited the expression of TLR4, iNOS, and COX-2, and the production and presentation of proinflammatory cytokines [60, 61]. Cavernous fibrosis also contribute for the deterioration of the erectile function. Altered or dysregulated Wnt activity in adults is associated with pathological angiogenesis, atherogenesis, and tissue fibrosis [62−64]. S.H. Shin et al. [65] demonstrated the differential expression of Wnt family members in the penis of diabetic mice and speculated that modulation of the Wnt pathway might represent a novel therapeutic target for the treatment of diabetic ED. Total glucoside from leucoside was able to inhibit renal fibrosis by regulating the Wnt/-catenin signaling pathway [66]. PI3K/AKT/mTOR pathway was also involved in inflammatory, oxidative stress, cellular fibrosis, and other pathological processes [67−69]. Catalpa significantly activated the PI3K/Akt/mTOR signaling pathway and inhibited oxidative damage and cell apoptosis [70]. Rehmannia showed a significant dose-dependent inhibition on the PROBE substrate in a CYP2D6 model [71].

Table 5 The top 20 related words of *Radix Rehmanniae* and erectile dysfunction

number	relevance	related words	number	relevance	related words
1	0.99	Akt	11	0.96	streptozotocin
2	0.99	mTOR	12	0.96	kinase erk
3	0.99	Wnt	13	0.95	lc
4	0.98	induced diabetic rat	14	0.95	CYP2D6
5	0.98	chronic mild stress	15	0.95	induced diabetic
6	0.98	PI3K	16	0.95	ginseng
7	0.98	anxiety depression	17	0.94	oxide nanoparticle
8	0.97	beta catenin signaling	18	0.92	beta catenin
9	0.97	TLR4	19	0.92	COX-2
10	0.96	MAPK	20	0.92	deficit hyperactivity disorder

Table 6 The top 20 related words of *Radix Bupleuri* and erectile dysfunction

number	relevance	related words	number	relevance	related words
1	0.99	mTOR	11	0.96	ginsenoside
2	0.99	chronic pelvic pain	12	0.96	STAT3
3	0.99	pelvic pain	13	0.96	herbal product
4	0.98	sirtuin	14	0.96	lc
5	0.98	Nrf2	15	0.96	BDNF
6	0.98	ginseng	16	0.95	antipsychotic
7	0.97	TLR4	17	0.93	MAPK
8	0.97	iNOS	18	0.93	hedgehog
9	0.97	inducible nitric oxide	19	0.93	schisandra
10	0.96	autophagy	20	0.91	caspase-3

Analysis of *Radix Bupleuri* and ED. *Radix Bupleuri*, or Buchaihu, contributes for dispelling appearance, soothing liver, and relieving depression, by raising Yang to lift depression, and reducing fever and malaria. mTOR, Nrf2, TLR4, iNOS, signal transducer and activator of transcription 3 (STAT3), BDNF, MAPK, and Caspase-3 were related words (gene or protein) of ED treated by Buchaihu, as shown in Table 6. The active ingredients of *Radix Bupleuri* include saikosaponin A, saikosaponin D (SSd), quercetin, and kaempferol. [72].

Saikosaponin A inhibits the level of inflammatory factors, the expression of COX-2 and iNOS in inflammatory endothelial cell models, reduces TLR4 dimerization and lipid raft translocation, presents anti-inflammatory and antioxidant effects [73], and upregulates the BDNF expression [74]. SSd and quercetin upregulate Nrf2 expression, reducing inflammation [75, 76], and the *Radix Bupleuri* extract can improve the antioxidant capacity by activating the Nrf2 signaling pathway [77]. Bupleuri polysaccharides can inhibit the TLR4 signaling pathway, present anti-inflammatory activity, and contribute to the immune regulation [78]. STAT3 is a transcription factor. Phosphorylation of STAT3, a transcription factor, regulates the transcription of a series of targeted genes and plays an essential role in the PDGFR/STAT3 signaling pathway, involved in ED's pathogenesis and often associated with atherosclerosis [79]. In addition, STAT3 is known to be involved in COX-2 protein expression regulation [80]. SSd can significantly inhibit STAT3 phosphorylation, thereby inhibiting COX-2 expression [81]. Quercetin shows antifibrotic activity by inhibiting fibrogenic factors and the AKT/mTOR signaling pathway [82]. The caspase family is essential for cell apoptosis, particularly for the effector enzyme Caspase-3, located in the apoptotic pathway [83]. SSd can trigger apoptosis by activating Caspase-3 to delay fibrosis [84].

Analysis of *Radix Glycyrrhiza* and ED. *Radix Glycyrrhiza*, or licorice, is able to tonify the spleen, invigorate Qi, clear heat, detoxify, expectorate phlegm, relieve cough, relieve pain, and harmonize all kinds of drugs. The role of licorice on the treatment of ED is still unknown. The Arrowsmith tool is based on the available literature and finds relevant target information through literature correlation. Therefore, no related proteins or genes were found in the intersection target of the licorice root and ED. The main components or licorice are flavones and saponins [85].

Analysis of *Radix Angelicae Sinensis* and ED. *Radix Angelicae Sinensis* is believed to enrich blood, activate blood circulation, regulating menstruation, relieve pain, moisten bowel, and constipation. The related words (gene or protein) of ED treated by angelica, include Akt, eNOS, PI3K, ERK1, Nrf2, p38MAPK, MAPK, and JNK, as shown in Table 7. The main active components of *Radix Angelicae Sinensis* are the volatile essential oil, organic acids, polysaccharides, and flavonoids [86].

Nitric oxide (NO) is a critical factor in regulating endothelial function. Phosphorylation of Akt promotes endothelial nitric oxide synthase (eNOS) in endothelial cells to protect the endothelium. Angelica polysaccharides can regulate NO levels and protect vascular endothelium through VEGF/Akt signaling [87], and reduce Nrf2 protein expression [88]. Ferulic acid (FA), one of angelica's organic acids, has anti-inflammatory and antioxidant activity. The combination of FA and astragaloside IV was able to promote NO and eNOS release and to have a significant protective effect on vascular endothelial dysfunction in diabetic rats [89]. FA's antioxidant role is thought to be due to activation of the Nrf2 pathway mediated by ERK1/2 [90], promoting the activation of PI3K/AKT signaling pathway [91], and inhibiting phosphorylation of MAPKs, including P38 and JNK [92].

Table 7 The top 20 related words of *Radix Angelicae Sinensis* related to rectile dysfunction

number	relevance	related words	number	relevance	related words
1	0.99	Akt	11	0.98	ginseng
2	0.99	pi3k	12	0.98	signal regulated kinase
3	0.99	ERK1	13	0.97	MAPK
4	0.99	endothelial nitric oxide	14	0.97	inducible nitric oxide
5	0.98	Sirtuin	15	0.97	kinase erk
6	0.98	Nrf2	16	0.97	essential oil
7	0.98	p38 MAPK	17	0.97	oxide synthase activity
8	0.98	hypoxia inducible	18	0.96	lc
9	0.98	nitric oxide synthase	19	0.96	regulated kinase
10	0.98	oxide synthase	20	0.96	JNK

Analysis of *Rhizoma Chuanxiong* and ED. *Rhizoma Chuanxiong* promotes blood circulation, dispels wind, and relieves pain. The related words (gene or protein) of ED treated by *Rhizoma Chuanxiong* were vascular endothelial growth factor (VEGF), Akt, and Nrf2, as shown in Table 8. The active ingredients of *Rhizoma Chuanxiong* are volatile essential oils, alkaloids, and polysaccharides [93].

In diabetic or hypercholesterolemic ED animal models, VEGF can effectively target penile angiogenesis [94]. Ligustrazine is a lacustrine alkaloid that may inhibit angiogenesis, by inhibiting VEGFR2 expression [95] and activate PI3K/Akt [96]. Studies have shown that [97] Chuanxiong and its active components can affect PI3K-Akt, a signaling pathway closely related to vascular endothelial injury with blood stasis syndrome. VEGF expression significantly promotes the tubulogenesis and proliferation of endothelial progenitor cells [98]. Jingchang Du [99] found that the volatile oil of *Rhizoma Chuanxiong* was able to upregulate the expression of VEGF. Ligustilide, the main component of the volatile oil of Ligusticum Chuanxiong, inhibits the expression of Akt/NF-κB signaling [100]. Ligusticonolactone can activate the Nrf2 pathway by upregulating the phosphorylation of ERK1/2 [101].

Analysis of *Radix Platycodonis* and ED. *Radix Platycodonis* can promote the lung, benefits the pharynx, eliminates phlegm, and dischares pus. *Radix Platycodonis* treatment ED associated words (genes or proteins) were Wnt, Akt, mTOR, STAT3, Erk1, VEGF, p38 MAPK, PI3K, JNK, MAPK, CYP3A, JAK-STAT, Lname, Nrf2, BDNF and iNOS, as shown in Table 9. The primary chemical constituents of *Radix Platycodonis* are triterpenoid saponins, flavonoids, phenolic acids, and polyalkenes [102].

Platycodin, the main active component of *Radix Platycodonis*, can downregulate VEGF expression [103] and regulate the proliferation and metastasis of vascular endothelial cells. It also activates AMPK/PI3K/Akt signaling pathway and inhibits the NF-κB signaling pathway toward its antiapoptosis, antiinflammatory, and antioxidant activities [104, 105]. Platycodin D inhibits vascular endothelial cells' proliferation due to the phosphorylation of ERK1/2, P38 MAPK, and JNK [106]. Platycodin D can also regulate the Wnt pathway, protecting blood vessels [107], and activate ERK to trigger autophagy [108]. Consequently, NF- B and JAK2/STAT3 pathways are inhibited [109] as well as the expression of iNOS and iNOS mRNA [110]. Furthermore, Nrf2 expression can be activated [111] and BDNF upregulated [112]. Additionally, CYP450 is involved in the biosynthesis of *Radix Platycodonis* and presents an antioxidant role [113].

Analysis of *Radix Achyranthis Bidentatae* and ED. *Radix Achyranthis Bidentatae* is believed to promote blood stasis and passing through meridians, strengthening muscles and bones of liver and kidney, diuresis and circulation, and drawing blood down. The related word (gene or protein) of *Radix Achyranthis Bidentatae* and treatment ED was Wnt (Table 10). Its main active components of this root are polysaccharides, saponins, and phytosterols [114]. Kalyan Ghatak et al. found that Wnt signaling antagonists could restore erectile function in experimental mice by inducing nerve regeneration and angiogenesis [115]. Zhang et al. found that the active ingredients of *Radix Achyranthis Bidentatae* have a significant inhibitory effect on endothelial cell proliferation [116]. It is speculated that the *Radix Achyranthis Bidentatae* may play a role through the Wnt pathway by significantly inhibiting vascular endothelium proliferation.

***Radix Paeoniae Rubar* and ED analysis**. *Radix Paeoniae Rubar* has the following effects: clearing heat and cooling blood, dispersing blood stasis, and relieving pain. No clear target was found with this root, and its active ingredients are mainly monoterpenes and their glycosides [117]. Paeoniflorin can inhibit the expression and secretion of inflammation-related proteins and chemokines and improve inflammatory endothelial cells [118]. Studies have also shown that [119] paeoniflorin has a strong effect on improving hemorheology abnormalities and protecting vascular endothelial function.

Table 8 The top 20 related words of *Rhizoma Chuanxiong* and erectile dysfunction

number	relevance	related words	number	relevance	related words
1	0.99	vegf	11	0.97	Nrf2
2	0.98	Akt	12	0.97	streptozotocin induced
3	0.98	endothelium dependent	13	0.97	microalbuminuria
4	0.98	microrna	14	0.97	endothelial dysfunction
5	0.98	liquid chromatography tandem	15	0.96	estrogen progesterone receptor
6	0.98	streptozotocin induced diabetic	16	0.96	nitric oxide synthase
7	0.98	endothelial nitric oxide	17	0.96	oxide synthase
8	0.98	major depressive disorder	18	0.96	ginseng
9	0.98	chronic mild stress	19	0.96	hypoxia inducible
10	0.97	Sirtuin	20	0.95	streptozotocin

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Table 9 The top 20 related words of *Radix Platycodonis* and erectile dysfunction

number	relevance	related words	number	relevance	related words
1	0.86	Wnt	11	0.86	mapk
2	0.87	Akt	12	0.86	cyp3a
3	0.87	mtor	13	0.86	jak stat
4	0.87	stat3	14	0.85	l name
5	0.87	erk1	15	0.85	sirtuin
6	0.87	vegf	16	0.85	ampk
7	0.87	p38 mapk	17	0.84	inos
8	0.87	smad	18	0.84	bdnf
9	0.86	pi3k	19	0.84	Nrf2
10	0.86	jnk	20	0.84	genome wide

Table 10 The top 20 related words of *Radix Achyranthis Bidentatae* and erectile dysfunction

number	relevance	related words	number	relevance	related words
1	0.99	prostatitis chronic pelvic	11	0.57	tcm
2	0.95	chronic prostatitis chronic	12	0.46	improving blood circulation
3	0.95	chronic pelvic pain	13	0.43	gout
4	0.94	pelvic pain	14	0.30	saponin
5	0.94	Wnt	15	0.24	osteoporosis
6	0.94	chronic prostatitis	16	0.17	pill
7	0.93	pelvic pain syndrome	17	0.17	endurance
8	0.87	prostatitis	18	0.16	femur
9	0.85	beta catenin signaling	19	0.14	ms
10	0.63	triterpenoid saponin	20	0.14	osteonecrosis

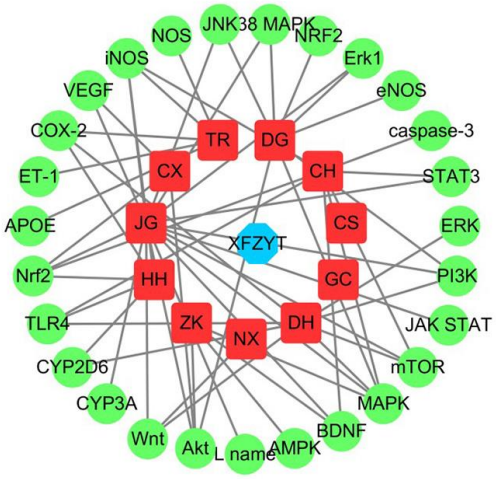

Figure 1 Analysis of common targets of Xuefu Zhuyu decoction and its single drug in the treatment of erectile dysfunction

Analysis of Xuefu Zhuyu decoction and ED

Xuefu Zhuyu decoction is a representative prescription for promoting blood circulation and avoiding blood stasis. This decoction is believed to have good efficacy in treating of ED, but there are no studies on its action *in vivo*. The main gene or protein associated with the treatment of ED is VEGF. VEGF can enhance vascular permeability, promote neovascularization, stimulate the proliferation and metastasis of vascular endothelial cells. Xuefu Zhuyu decoction was able to regulate the proliferation and apoptosis of smooth muscle cells [120].

Analysis of common targets

As shown in Figure 1, the typical marks of Xuefu Zhuyu decoction and its single drugs are iNOS, COX-2, mTOR, Wnt, AMPK, Nrf2, TLR4, CYP2D6, BDNF, MAPK, Akt, STAT3, PI3K, ERK1, P38MAPK, JNK, and VEGF. These targets are involved in pathways and contribute to the inhibition of the inflammatory response, reduction of apoptosis, and protection of vascular endothelial cells.

Discussion

ED is a chronic physical and psychological disease with a severe impact on its patients. The etiology and pathogenesis of ED are complex. Currently, it is believed the main causes are related to vascular and metabolic dysfunction [2]. In recent years, we have gained a deeper understanding of ED's pathophysiology. Many emerging therapies, such as angiogenesis therapy, aim to treat vascular and spongiform injuries, to regenerate new penis tissue [121]. As a classic prescription, more clinical trials have proved that Xuefu Zhuyu decoction has a good effect in treating ED.

In this study, the Arrowsmith tool was used to correlate Xuefu Zhuyu decoction and its single herbs with ED literature. It was found that Xuefu Zhuyu decoction and its single herbs had an indirect therapeutic effect on ED based on multiple components, multiple pathways and various targets. Besides, among the 17 common targets, mTOR, Wnt, iNOS, COX-2, Nrf2, TLR4, BDNF, MAPK, Akt, PI3K, and other targets had a high degree of common correlation, providing an entry point for subsequent analysis of the therapeutic ED mechanism of Xuefu Zhuyu decoction and its single herbs. This study on different and common targets also provides new data toward the compatibility and synergistic effect of traditional Chinese medicine.

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