Isatis Indigotica: A Review of Phytochemistry, Pharmacological Activities and Clinical Applications

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Abstract

Objectives *Isatis indigotica* Fort. (*I. indigotica*) is an herbaceous plant belonging to *Cruciferae* family. Its leaf (IIL) and root (IIR) are commonly used in traditional Chinese medicines (TCMs) with good clinical efficacies such as clearing away heat and detoxification, cooling blood and reducing swelling. This review aimed to provide a systematic summary on the phytochemistry, pharmacology and clinical applications of *I. indigotica*.

Key Finding This plant contains alkaloids, organic acids, flavonoids, lignans, nucleosides, amino acids, and steroids. Previous pharmacological researches indicated that *I. indigotica* possesses promising antivirus, antibacterial, immunoregulatory, anti-inflammation, and cholagogic effects. Importantly, it can inhibit various viruses, such as influenza, hepatitis B, mumps, herpes simplex, cytomegalovirus, and coxsachievirus. Clinically, it is frequently used to treat various viral diseases like viral influenza, parotitis and viral hepatitis. Consequently, *I. indigotica* may be beneficial for the prevention and treatment of COVID-19.

Summary This paper reviewed the chemical constituents, pharmacological effects and clinical applications of *I. indigotica* which may guide further research and application of this plant.

Keywords: Traditional Chinese Medicine, *Isatis indigotica*, Phytochemistry, Pharmacology, Clinical application

Introduction

Isatis indigotica Fort., a biennial herb of *Isatis* genus in *Cruciferae*, is mainly distributed in Gansu, Shaanxi, Hebei, Shandong, Jiangsu, Zhejiang, Anhui, and Guizhou provinces of China.[1] Owing to the efficacies of heat-clearing and detoxifying, cooling blood and eliminating ecchymoses, antibiosis and anti-inflammation,[2] its root (IIR, Chinese name *Ban-lan-gen*) and leaf (IIL, Chinese name *Da-qing-ye*) have been widely used in combination with other Chinese medicines to treat and prevent a variety of diseases such as influenza, parotitis, epidemic encephalitis B, epidemic myelitis, epidemic cerebrospinal meningitis, acute infectious hepatitis and sore throat.[3,4] In recent years, studies have shown that the indigotin and indirubin, present in *I. indigotica*, display many important pharmacological activities such as liver protection and anti-microbial, and indirubin also has anti-tumor effects.[5] Furthermore, the leaves have the highest content of indigotin and indirubin followed by stems and roots.[6,7]Besides alkaloids, there are many other active constituents such as organic acids, flavonoids, lignans, nucleosides, steroids, and amino acids, among which, flavonoids and nucleosides are two main components also present in the leaf.[6] In addition, amino acids, and organic acids, sinigrin and sulfur ingredients are also presented in the roots and display antiviral properties.[8]

Chemical constituents

Leaf

The fresh leaves contain isatan B, 3-indlymethyglucosinolate, glucobrassicin, neoglucobrassicin, 1-sulpho-3-indolymethy glucosinolate.[9] While the dried leaves contain alkaloids, including indigotin, indirubin,[10] 2,4(1H,3H) - quinazolinedion, 5-hydroxy-2-indolinone, 10H-indolo[3,2-b]quinolone,[11] 4(3H)-quinazolinone,

deoxyvascinone, tryptanthrin,[12] Isatisine A.[13] Indigotin and indirubin are fat soluble compounds displaying poor solubility and are only soluble in chloroform, acetone and other organic solvents. They have a life span of only 24 hours in the dark after which they begin to decompose.[14]

Some of the other components in the leaves are: **(1) Organic acids**: [15,16] 3,5-dimethoxy-4-hydroxy benzoin acid, syringic acid, nicotic acid, succinic acid, salicylic acid, anthranilic acid. **(2) Flavonoids**:[17] isovitexin, 6-β-D-glucopyranosyldiosmetin. **(3) Lignans**:[18] (-)-lariciresinol, (+)-isolariciresinol. **(4) Nucleosides**:[19] uridine, adenosine, xanthine, hypoxanthine. **(5) Steroids**:[20] β-rosasterol, β-sitosterol, γ-sitosterol. **(6) Amino acid**:[21] L-pyroglutamic acid. **(7) Minerals**:[22] Iron, titanium, manganese, zinc, copper, cobalt, nickel, selenium, chromium, arsenic, etc. There are also volatile oil components present in folium isatidis.

Roots

The roots include the following chemical constituents **(1) Alkaloids**: indigotin, isatin, indirubin,[10] indoxyl-β-glucoside, 2,5-dihydroxy-indole, 2,3-dihydro-4-hydroxy-2-oxo-indole-3-acetonitrile, indole-3-acetonitrile-6-*O*-β-*D*-glucopyranoside,[23] hydroxyindirubin, isaindigodione, (*E*)-3-(3',5'-dimethoxy-4'-hydroxybenzylidene)-2-indolinone, 3-formyl-indole, deosyvasicinone, isaindigotone, tryptanthrin,[24] 3-(2'-carboxyphenyl)-4(3H)-quinazolinone, 4(3H)-quinazolinone, 3-(2'-hydroxyphenyl)-4(3H)-quinazolinone, isaindigotidione, Isatan A,[25] 3-[2'-(5'-hydroxymethyl)furyl]-1(2H)-isoquinolinone-7-*O*-β-*D*-glucoside, 2,3-dihydro-1*H*-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10*H*,11aH)-dione.[26] **(2) Flavonoids**:[27-29] neohesperidin, liquiritigenin, isoliquiritigenin, isovitexin, linarin,

eupatorin. **(3) Lignans** [18]: (-)-lariciresinol, lariciresinol-4-*O*-β-*D*-glucopyranoside, lariciresinol-4,4'-di-*O*-β-*D*-glucopyranoside, 4-(1,2,3-trihydroxypropyl)-2, 6 - dimethoxyphenyl-1- *O*- β- *D*- glucopyranoside, syringin, (+)-isolariciresinol. **(4) Organic acids**:[30] 3-pyridinecarboxylic acid, maleic acid, 2-hydroxy-1,4-benzenedicarboxylic acid, benzoic acid, salicylic acid, syringic acid, palmitic acid, succinic acid, 2-amino benzoic acid, 5-hydroxymethyl furoic acid. **(5) Anthraquinones**:[31] emodin, emodin-8-*O*-β-*D*-glucoside. **(6) Steroids**:[32] β-sitosterol, daucosterol, γ-sitosterol. **(7) Sinigrins**:[33] 3-indolylmethyl gluosinolate, neoglucobrassicin, 1-sulpho-3-indolylmethylgluosinolate. **(8) Sulfur compounds**:[34] epigoitrin, 1-thiocyano-2-hydroxy-3-butene. **(9) Amino acids**:[35] praline, arginine, tyrosine, valine, glutamic acid, γ-aminobutyric acid, leucine, tryptophan, aspartic acid, L-threonine, β-hydroxyalanine, glycine, isoleucine, phenylalanine, histidine, lysine. **(10) Nucleotides**:[36] uridine, hypoxanthine, uracil, adenosine, guanine. **(11) Others**:[37-40] ammonium formate, sucrose, 5-hydroxymethyl-furaldehyde, *n*-butyl-*O*-β-*D*- fructopyranose, mannitol, pyrophaeophorbideα, polygalitol. The main chemical constituents and chemical structures of *I. indigotica* are presented in Table 1 and Figure 1-6, respectively [9-40].

Pharmacological activities

Antiviral activity

Epigoitrin, an alkaloid from *I. indigotica*, can reduce the susceptibility to H1N1 virus and the production of pro-inflammatory cytokines to alleviate pneumonia in restraint-stressed mice.[41] Plant-derived compounds such as indigotin, sinigrin, aloe emodin and hesperetin display anti-SARS coronavirus effects, effectively blocking the cleavage processing of the 3C-like protease.[40,41] The injection of IIL extracts can inhibit the infection and proliferation of influenza A, encephalitis B, mumps viruses,

etc.[42] The result from the hemagglutination titer test showed a direct inhibitory effect of IIL against influenza A virus.[43] However, there are few studies on its antiviral mechanism of action. 4(3H)-quinazolinone, a compound isolated from the leaves, has the capacity to inhibit influenza and coxsackie virus.[44] In the early stage of viral myocarditis (VMC), the leaves may improve and protect the myocardial cells by inhibiting the synthesis of virus, enhancing the phagocytosis of leukocytes and reducing the permeability of capillaries.[45] The root aqueous extract can inhibit human H7N9 avian influenza virus *in vitro* possibly by blocking the absorption of H7N9 avian influenza virus to host cells by inhibiting the hemaglutinin of H7N9 avian influenza virus, so as to prevent the virus invading the host cells.[46] It has a good curative effect on virus-caused pharyngitis, acute upper respiratory tract infection and pneumonia, especially catarrhal inflammation such as cough, nasal obstruction, runny nose and sneeze.[47] Polysaccharides from *I. indigotica* can inhibit hepatitis B virus (HBV) *in vitro*, reduce extracellular and intracellular DNA level of HBsAg, HBeAg and HBV in HepG2.2.15 cells in a time and dose-dependent manner.[48-49] Peptides reduces the mortality of mice infected with influenza virus and inhibits the proliferation of virus.[50] Aqueous extract of leaves can anti–virus such as HSV-II, Dengue virus II and Cytomegalovirus.[51,53] Aqueous extract of roots can anti HSV-I, inhibits virus replication and proliferation in cells. [52]

Antibacterial activity

The aqueous, ethanol and n-butanol extracts of the leaves have antibacterial effects on *Staphylococcus aureus* and *Escherichia coli*.[53,54] The leaf decoction showed antibacterial effect *in vitro* on *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus* A and *Streptococcus* B by use of disk diffusion test.[55] Tryptanthrin, a component isolated from the leaves, has strong inhibitory effects on *Trichophyton*

mentagrophytes, *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Microsporum canis*, which can cause tinea pedis.[56,57] The roots have a broad-spectrum antibacterial effect, in which tryptanthrin is the main antibacterial active ingredient. The root aqueous extract can inhibit *Escherichia coli*, *Staphylococcus epidermidis*, *Pneumococcus*, *Himophilus influenzae*, and *Streptococcus*.[58] The total organic acids from roots also show strong antibacterial activity on *Escherichiacoli* by cylinder-plate test.[59,60] Salicylic acid can inhibit excessive release of TNF-α and NO in serum of mice [61], and the roots decoction can decrease the levels of TNF - α and IL-6 in peritoneal macrophages of mice.[62]

Anti-endotoxin

Bacterial endotoxin is the lipopolysaccharide component existing in the extracellular of gram-negative bacteria, which can stimulate the body's defense system to release inflammatory factors, such as tumor necrosis factor and nitric oxide, causing fever, disseminated intravascular coagulation, multiple organ failure, and even death.[63,64] The leaf extract can directly neutralize and degrade endotoxin to reduce the thermophilic and lethality of endotoxin in actinomycin D sensitized mice with endotoxin lethal attack.[65] The chloroform extract of the leaves has the anti-endotoxin effect on *Escherichia coli* $O_{111}B_4$ with dilution *in vitro* to 64 times still destroying the endotoxin, and the endotoxin dripped into the vein of rabbits is also destroyed, suggesting that the leaves contain anti-endotoxin active substances.[66,67] IIR can significantly reduce the level of serum lipid peroxide and improve the activity of superoxide dismutase, suggesting its functions of anti-lipid peroxidation, scavenging free radicals and antagonizing endotoxin.[68] The result of bacterial endotoxin destruction test showed that the different pH value significantly affected the action intensity of the root aqueous extract against bacterial endotoxin, the reason

being that the active ingredients contained in the roots against bacterial endotoxin are extracted more easily in an acid environment.[69]

Immunopotentiation

The leaf decoction can promote IL-2 secretion of spleen lymphocytes induced by concanavalin A in normal mice to enhance immunity, but has no effect on TNF-α secretion of peritoneal macrophages and the activity of leukocytes, pathological damage and dysfunction.[70,71] Polysaccharide of the roots has immunopotentiation effects, which can promote specific immune, non-specific immune, humoral immune or cellular immune affects.[72] Intraperitoneal injection of polysaccharide 50mg/kg significantly enhanced the immune function of normal mice with increasing the spleen weight and total number of leukocytes and lymphocytes.[73,74] However, it also markedly reduced spleen index and total number of leukocytes and lymphocytes in the immunosuppressed mice induced by hydrocortisone, and inhibited the delayed anaphylaxis in immunosuppressed mice induced by dinitrochlorobenzene and cyclophosphamide.[75] Further study showed that lectin from the roots could bind to glycoprotein on the cell surface to promote the development of thymus and the proliferation of thymocytes, indirectly maintaining the microenvironment of thymus, promoting the secretion of thymosin and cytokines by T-lymphocytes and thymic epithelial cells, and improving the immunity of the body.[76]

Anti-inflammation

The leaf decoction has a significant inhibitory effect on methanal induced arthritis in mice and suppresses the local inflammatory reaction and capillary permeability of rabbit skin caused by xylene.[77,78] Total alkaloids and amino acids from the leaves also alleviate mouse ear edema, suggesting the anti-inflammatory effects.[79] 70% ethanol extract of the roots can inhibit ear swelling of mice caused by

xylene and foot swelling of rats caused by egg white to a certain extent.[80]

Anti-tumor

Indirubin, an alkaloid from *I. indigotica*, possesses an anti-tumor activity, which strongly inhibits transplanted tumor growth of animals and alleviates chronic myeloid leukemia.[81,82] Owing to poor water-soluble and liposoluble properties, the indirubin's derivatives named derivative III were designed and synthesized to increase solubility with a inhibitory rate of 58% against leukemia cells.[83] Indirubin is likely to participate in regulating the metabolism of lung cancer cells by inducing the activity of cytochrome P4501A1 and 1B1mRNA enzyme in MCF-7 lung cancer cells.[84,85] Curdione isolated from the roots can inhibit the proliferation of hepatocarcinoma BEL-7402 cells and ovarian cancer A2780 cells, induce differentiation, reduce the telomerase activity and boost the conversion of tumor cells into normal cells.[86] Indirubin displays significant cytotoxicity in HL-60 cells, eliciting cell pyknosis, condensation and even lyses.[87]

Others

IIL also has a cholagogic effect, which can promote bile excretion and relieve pain.[88,89] It can depress adenosine diphosphate-elicited platelet aggregation in rabbits due to the efficacy of promoting blood circulation and removing stasis.[90] Indigotin has significant protective effect against liver injury caused by carbon tetrachloride[91,92] and the leaves can detoxify the effects of lead poisoning mice.[93] All the pharmacological effects of this plant are summarized in Table 2.

Toxicity

I. indigotica is generally considered nontoxic, however, the adverse reactions of its leaves occur from time to time as reported in the literature.[94,95] The extracts of

roots of *I. indigotica*, also called *Banlangen*, can induce the micronucleus rate of polychromatic erythrocytes in mouse bone marrow and increase the sperm deformity rate of mice, suggesting a certain genotoxicity in mammalian somatic cells and germ cells.[96,97]

Clinical application

Hepatitis

The leaves of *I. indigotica* show significantly improvement effects on acute common infectious hepatitis. 32 cases of icterohepatitis were treated with the leaves of *I. indigotica* in combination with roots of *Salviae miltiorrhizae*, roots of *Curcumae longae*, roots of *Dryopteridis crassirhizomatis* and fruits of *Ziziphus jujuba*, and the effective rate was 94%.[98,99] *Yigan-Jiedu* decoction composed of the leaves and roots of *I. indigotica*, roots of *Salviae miltiorrhiza*, roots of *Astragalus membranaceus*, and whole herb of *Lysimachia christinae* apparently improved the symptoms and signs of 86 cases with chronic hepatitis B when compared with the control group.[100] Another injection named *Shu-gan-ning*, composed of roots of *I. indigotica*, *Ganoderma lucidum*, fruits of *Kochia scoparia*, fruits of *Gardenia jasminoides*, and roots of *Scutellaria baicalensis*, quickly alleviated jaundice symptoms of 45 cases with acute icteric hepatitis, and the clinical effective rate was 91%.[101,102] *Qinggan-Lidan* decoction, consisted by the roots of *I. indigotica*, whole herb of *Artemisia carvifolia*, fruits of *Gardenia jasminoides*, barks of *Phellodendri chinensis,* whole herb of *Bupleurum chinense*, *Poria cocos*, roots of *atractylodis macrocephalae*, and semens of *Coix lacryma-jobi*, treated 100 cases with acute icteric hepatitis and the effective rate was 100%. The compound decoction is simple, easy to use, economical and cheap, and has few reported side effects.[103]

Parotitis

Total 92 cases of children mumps were treated with the formula containing the leaves combined with ganciclovir. The time of fever abatement, parotid swelling abatement and parotid pain abatement was significantly shortened in the treatment group when compared with the control group, and their effective rates were 97.83% and 80.43%, respectively.[104,105] The formula comprised of the roots of *I. indigotica*, borneolum syntheticum and cactus cured all 45 cases of epidemic parotitis, with 15 cases cured in two days, accounting for 33%, 21 cases in three days accounting for 47%, 9 cases in four days accounting for 20%.[106] The external application of jinhuang ointment combined with the oral administration of the root granules has an effective rate of 100% when treating 60 cases of children mumps and no adverse reactions and complications were reported in any of the patients.[107]

Upper respiratory tract infection

Total 56 cases of upper respiratory tract infection were treated with the root granules, and the effective rate was 98.21%, which is higher than that of 80.36% observed in the control group treated with ribavirin only.[108,109] A similar result for the root granules was observed in another 60 cases of upper respiratory tract infection, with the effective rate of 100% versus 87% in the control group treated with ribavirin only.[110] Oseltamivir phosphate combined with the root granules showed significant clinical efficacy in the treatment of influenza A (H1N1) when compared the control group of patients received oseltamivir phosphate alone, and the total effective rate was 97.14%.[111]

Others

The decoction comprised of the leaves and roots of *I. indigotica*, herba lysimachiae and radix et rhizoma rhei displayed significant improvement effects in the treatment of pointed condyloma 28 cases, among whom, 14 cases were cured, 12

improved and 2 ineffective, having an effective rate of 92.8% when oral decoction was combined with fumigation and washing.[112] 35 cases of palmoplantar pustulosis were treated topically with the formula consisting of the leaves, herba violae, flos lonicerae, radix sophorae flavescentis, fructus kochiae, fructus cnidii, semen plantaginis, rhizoma atractylodis, and alum, and the total effective rate was 68.57%.[113] 136 cases of epidemic kerato-conjunctivitis were treated with the root granules in combination with herba houttuyniae injection, 110 cases recovered, and the cure time was 2-15 days, averaging 5.6 days.[114] The compound granule could treat viralmyocarditis, which consists of the leaves and roots of *I. indigotica*, fructus forsythiae, and rhizoma bistortae, and the effective rate were 85.5%, among whom, 23 cases were excellent, 77 fine, 17 ineffective for ventricular premature beats symptom.[115]

Conclusions and perspectives

Natural agents which are commonly derived from plants or herbs could not only give us essential foods for living, including sugars, lipids, proteins and vitamins, but also supply us some precious medicinal secondary metabolites for preventing various diseases, such as berberine, artemisinin, emodin, and taxol.[116-118] As a natural plant, *I. indigotica* contains alkaloids, organic acids, flavonoids, lignans, nucleosides, amino acids, and steroids. Previous pharmacological researches indicated that *I. indigotica* possesses promising antivirus, antibacterial, immunoregulatory, anti-inflammation, and cholagogic effects. Importantly, it can inhibit various viruses, such as influenza, hepatitis B, mumps, herpes simplex, cytomegalovirus, and coxsachievirus. Clinically, it is frequently used to treat various viral diseases like viral influenza, parotitis and viral hepatitis. Consequently, *I. indigotica* may be beneficial for the prevention and treatment of COVID-19. *I. indigotica* has the function of immune regulation, which

reinforces its anti-virus effects in turn. Therefore, *I. indigotica* may be effective for the prevention and treatment of COVID-19, however, this need to be investigated further. Although numerous chemical constituents have been isolated and identified from *I. indigotica*, the active components, mechanisms of action and their target remain unknown. As the clinic application of Chinese medicines is characterized by compatibility, the therapeutic mechanism of *I. indigotica* combined with other medicines should be investigated further. However it is rather difficult to clarify the mechanism at the molecular level based on the compatibility of the crude extracts or components. The compound-based Chinese medicine formula (CCMF) may be promising for clarification of the mechanism and target due to its clear composition of compounds derived from Chinese medicines. The action targets of compounds can be investigated through such techniques as CETSA, DARTS, and MST. When the mechanism of compatibility for CCMF is defined, the scientific connotation for the TCM compatibility theory will probably be clarified.

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Conflicts of interest

The authors confirm that this article content has no conflicts of interest.

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Table 1. Chemical constituents isolated from *Isatis indigotica*

Classification	No.	Chemical constituents	Part of plant	Ref.
	1	Indigotin	whole herb	[11]
	2	Indirubin	whole herb	[11]
	3	Isaindigotone	Whole herb	[11]
	4	Tryptanthrin	Whole herb	[11]
	5	2,5-dihydroxy-indole	Root	[10]
	6	2,3-dihydro-4-hydroxy-2-oxo-indole-3-acetonitrile	Root	[10]
	7	Indole-3-acetonitrile-6-*O*-B-*D*-glucopyranoside	Root	[10]
Alkaloids	**8**	Hydroxyindirubin	Root	[10]
	9	Isatin	Root	[10]
	10	2,4(1*H*,3*H*)-quinazolinedion	Aerial part	[11]
	11	5-hydroxy-2-indolinone	Aerial part	[11]
	12	10H-indole[3,2-b]quinoline	Aerial part	[11]
	13	Isatan A	Root	[10]
	14	3-formyl-indole	Root	[10]
	15	Deoxyvascinone	Root	[10]

	16	4(3*H*)-quinazolinone	Aerial part	[11]
	17	3-(2'-hydroxyphenyl)-4(3H)-quinazolinone	Root	[10]
	18	3-[2'-(5'-hydroxymethyl)furyl]-1(2H)-isoquinolinone-7-*O*-β-*D*-glucoside	Root	[10]
	19	3-dihydro-1*H*-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10H,11aH)-dione	Root	[10]
	20	(*E*)-3-(3',5'-dimethoxy-4'-hydroxybenzylidene)-2-indolinone	Root	[10]
	21	Nicotic acid	Aerial part	[15]
	22	Anthranilic acid	Aerial part	[15]
	23	3-pyridinecarboxylic acid	Root	[30]
	24	Maleic acid	Root	[30]
	25	2-hydroxy-1,4-benzenedicarboxylic acid	Root	[30]
	26	Benzoic acid	Root	[30]
Organic acids	**27**	Palmitic acid	Root	[30]
	28	Salicylic acid	Whole herb	[15]
	29	Syringic acid	Whole herb	[15]
	30	Succinic acid	Whole herb	[15]
	31	2-amino benzoic acid	Root	[30]
	32	5-hydroxymethyl furoic acid	Root	[30]
Flavonoids	**33**	Isovitexin	Whole herb	[17]

	34	Neohesperidin	Root	[28]
	34	Liquiritigenin	Root	[28]
	36	Isoliquiritigenin	Root	[29]
	37	Linarin	Root	[29]
	38	Eupatorin	Root	[29]
	39	(-)-lariciresinol	Aerial part	[18]
	40	(+)-isolariciresinol	Whole herb	[18]
Lignans	**41**	lariciresinol-4-*O*-β-*D*-glucopyranoside	Root	[18]
	42	4-(1,2,3-trihydroxypropyl)-2,6-dimethoxyphenyl-1-*O*-β-*D*-glucopyranoside	Root	[18]
	43	Uridine	Whole herb	[36]
	44	Adenosine	Whole herb	[36]
	45	Hypoxanthine	Whole herb	[36]
Nucleosides	**46**	Xanthine	Aerial part	[36]
	47	Uracil	Root	[36]
	48	Guanine	Root	[36]
	49	Rosasterol	Aerial part	[20]
Steroids	**50**	β-sitosterol**[**	Whole herb	[20]
	51	Daucosterol	Root	[32]

	52	L-pyroglutamic acid	Aerial part	[21]
	53	Arginine	Root	[21]
	54	Tyrosine	Root	[21]
	55	Valine	Root	[21]
	56	Glutamic acid	Root	[21]
	57	γ-aminobutyric acid	Root	[21]
Amino acids	**58**	Tryptophan	Root	[35]
	59	Aspartic acid	Root	[35]
	60	L-threonine	Root	[35]
	61	Isoleucine	Root	[35]
	62	Histidine	Root	[35]
	63	Lysine	Root	[35]
	64	Emodin	Root	[31]
	65	Emodin-8-*O*-β-*D*-glucoside	Root	[31]
	66	Epigoitrin	Root	[34]
Others	**67**	Sucrose	Root	[37]
	68	5-hydroxymethyl-furaldehyde	Root	[37]
	69	*n*-butyl-*O*-β-*D*- fructopyranose	Root	[37]

70	Mannitol	Root	[37]
71	1-thiocyano-2-hydroxy-3-butenen	Root	[38]
72	Sinigrin	Root	[38]
73	Syringin	Root	[38]
74	4-(4’-hydroxy-3’,5’-dimethoxyphenyl)-3-buten-2-one	Root	[38]
75	Indoxyl-O-glucoside	Root	[38]
76	(*E*)-2-[(3’-indole)cyanomethylene]-3-indolinone	Root	[38]
77	1-methoxy-3-acetonitrile indole	Root	[39]
78	3-acetate indole	Root	[39]
79	3- indole aldehyde	Root	[39]
80	1-methoxy-3-indolealdehyde	Root	[39]
81	Qingdainone	Aerial part	[40]
82	Linolenic	Root	[40]
83	Erueic acid	Root	[40]

Table 2. Pharmacological activities of *Isatis indigotica*

Pharmacological effect	Tested substance	Model	Tested living system/organ/cell	Result	Dose	Ref.
Anti-virus	Epigoitrin	H1N1	KM mice	Reduces the production of pro-inflammatory cytokines to alleviate pneumonia.	88mg/kg (ig)	[41]
	Indigotin	SARS-coronavirus	SARS-CoV 3C-like protease	Blocks the cleavage processing of the 3C-like protease	1, 10, 100μg/ml	[41]
	Alkaloid	Influenza A virus	ICR mice	Prolongs the survival time of infected mice.	0.65g/kg (ig)	[42]
	Indirubin	Influenza virus	NCI-H292 cells	Inhibits transcription and production of RANTES.	0.01, 0.1, 1, 10μM/ml	[43]
	4(3H)-quinazolinone	Escherichiacoli	Rabbit	Reduces high body temperature in rabbits caused by endotoxin.	5ml/kg (ip)	[44]
	Alkaloid	Newcastle disease virus	Chicken embryo fibroblasts	Blocks the absorption of virus, protects cells and reduces virus infection.	7.8–31.3μg/ml	[45]
	Root aqueous extract	H7N9 avian influenza virus	Chicken embryos	Inhibit human H7N9 avian influenza virus in vitro by blocking the absorption of H7N9 avian influenza virus to host cells.	IC-50=5000μg/mL	[46]
	Unamed Compounds from leaves	Respiratory syncytial virus	Hep-2 cells	Inhibits the proliferation of respiratory syncytial virus after invading Hep-2 cells.	10–120μg/ml	[47]
	Polysaccharide	HSV-II	BALB/C mice	Reduces the incidence rate, mortality and prolongs the average survival time in mice.	0.5 and 1.0mg/kg (ip)	[48]
		HBV	HepG2/2-15 cells	Reduces extracellular and intracellular levels of HBsAg, HBeAg and HBV DNA in cells.	50, 100 and 200 μg/ml	[49]
	Peptides	H1N1	KM mice	Reduces the mortality of mice infected with influenza virus and inhibits the proliferation of virus.	50, 100 and 200mg/kg(ig)	[50]
	Leaf aqueous extract	HSV-II	Vero cells	Inhibits the replication and Inhibits proliferation of HSV-II in cells.	0.25–16mg/ml	[51]

	Root aqueous extract	HSV-I	Hep-2 cells	Inhibits biosynthesis of HSV-I in vitro.	2–128mg/ml	[52]
	Leaf aqueous extract	Dengue virus II	C6/36 cells	Inhibits virus replication and proliferation in cells	0.5–4.0mg/ml	[53]
	Leaf ethanol extract	Cytomegalovirus	Guinea pig embryo lung cells	Antiguinea pig cytomegalovirus activity.	$3g\cdot ml^{-7}$–$3g\cdot ml^{-1}$	[54]
	Leaf aqueous extract	Shigella Castellani Streptococcus pneumoniae Staphylococcus aureus	Tube method	Obvious inhibitory effect	25–400mg/kg	[55,56]
Antibacterial	Organic acid Alkaloid Nucleoside Anthraquinone	Escherichia coli	Oxford Cup	Components have strong antibacterial activity.	2.0g/mL	[57,58]
	Salicylic acid	Lipopolysaccharide	Balb/c mice	Inhibits excessive release of TNF-α and NO in serum of mice.	20mL/kg (ip)	[59]
	Root decoction	Lipopolysaccharide	Peritoneal macrophage	Decreases the levels of TNF - α and IL-6 in peritoneal macrophages of mice.	1g/mg	[60]
Immunomodulatory	Polysaccharide	Lymphocyte	KM mice	Enhances peripheral blood lymphocytes in mice.	2mg/mL	[61]
			Balb/c mice	Promotes the humoral immune response of the body and produces immune effect.	4mg/mL	[62]

	Fructopyrano-(1→4) -glucopyranose	Macrophage phagocytosis	KM mice	Enhance the phagocytic function of peritoneal macrophages in mice.	100, 200 mg/kg (ig)	[63]
	Root ethanol extract	Lipopolysaccharide	RAW264.7 cells	Inhibits the release of PGE 2 and TNF-α.	0.1,0.5,1.0,2.5mg/mL	[64]
Antitumor	Polysaccharide	S-180 cells	ICR mice	Enhances the immune function of tumor bearing mice and prolongs the survival time of tumor bearing mice	50,100mg/kg (ig)	[65]
	Indirubin-3'-oxime	MV4-11 cells	BALB/c nude mice	Increases the anti-proliferative efficacy of MV4-11 cells	20mg/kg(ig)	[66]
	Indirubin	leukemia	HL-60 cells	Elicits pyknosis, condensation and lyses in cells.	25, 50, 100, 200, 400μg/mL	[67]
	Leaf ethanol extract	Medicated serum	K562 cells	The drug containing serum inhibits the proliferation of cells.	1g/mL	[68]

Figure captions:

Figure 1. The chemical structures of alkaloids isolated from *Isatis indigotica*

Figure 2. The chemical structures of organic acids isolated from *Isatis indigotica*

Figure 3. The chemical structures of flavonoids and lignans isolated from *Isatis indigotica*

Figure 4. The chemical structures of nucleosides and steroids isolated from *Isatis indigotica*

Figure 5. The chemical structures of amino acids isolated from *Isatis indigotica*

Figure 6. The chemical structures of other compounds isolated from *Isatis indigotica*