Chen, Q, Lan, H-Y, Peng, W, Rahman, K, Liu, Q-C, Luan, X and Zhang, H

Isatis indigotica: a review of phytochemistry, pharmacological activities and clinical applications.

http://researchonline.ljmu.ac.uk/id/eprint/14818/

Article

Citation (please note it is advisable to refer to the publisher’s version if you intend to cite from this work)

Chen, Q, Lan, H-Y, Peng, W, Rahman, K, Liu, Q-C, Luan, X and Zhang, H (2021) Isatis indigotica: a review of phytochemistry, pharmacological activities and clinical applications. Journal of Pharmacy and Pharmacology. ISSN 0022-3573

LJMU has developed LJMU Research Online for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

http://researchonline.ljmu.ac.uk/
*Isatis Indigotica: A Review of Phytochemistry, Pharmacological Activities and Clinical Applications*

Qiong Chen¹,²#, Hai-Yue Lan¹#, Wei Peng², Khalid Rahman³, Qing-Chun Liu⁴*, Xin Luan¹, Hong Zhang¹,²*

¹Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

²School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, 611137, China

³School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, Liverpool L3 3AF, England, UK

⁴Department of Medicine, Chengdu Brilliant Pharmaceutical Co. Ltd., Chengdu 610041, China

# These authors contributed equally to this work.

*Corresponding authors:*

Dr. Hong Zhang, No. 1200, Cailun Road, Pudong New Area, Shanghai 201203, China.
Email: issuepreparation@163.com (H.Z.)

Dr. Xin Luan, No. 1200, Cailun Road, Pudong New Area, Shanghai 201203, China.
Email: luanxin@shutcm.edu.cn (X.L.)

Dr. Qingchun Liu, College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, 611137, China. Email: abc_23@126.com (Q.C.L)
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 coxsachieivirus. 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 indigo tin 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, Isatisine A. 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.

Some of the other components in the leaves are: (1) **Organic acids**: 3,5-dimethoxy-4-hydroxy benzoin acid, syringic acid, nicotic acid, succinic acid, salicylic acid, anthranilic acid. (2) **Flavonoids**: isovitexin, 6-β-D-glucopyranosyldiosmetin. (3) **Lignans**: (-)-lariciresinol, (+)-isolariciresinol. (4) **Nucleosides**: uridine, adenosine, xanthine, hypoxanthine. (5) **Steroids**: β-rosasterol, β-sitosterol, γ-sitosterol. (6) **Amino acid**: L-pyroglutamic acid. (7) **Minerals**: 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, 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, hydroxyindirubin, isaindigodione, (E)-3-(3’,5’-dimethoxy-4’-hydroxybenzylidene)-2-indolinone, 3-formyl-indole, deosyvascinone, isaindigotone, tryptanthrin, deoxyvascinone, isaindigotone, tryptanthrin, Isatan A, 3-[2′-(5′-hydroxymethyl)furyl]-1(2H)-isoquinolinol-7-O-β-D-glucoside, 2,3-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine-5,11(10H,11aH)-dione. (2) **Flavonoids**: 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-thiocyno-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, pyropheophorbidea, 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,
The result from the hemagglutination titer test showed a direct inhibitory effect of IIL against influenza A virus. 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. 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. 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. 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. 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. Peptides reduces the mortality of mice infected with influenza virus and inhibits the proliferation of virus. Aqueous extract of leaves can anti–virus such as HSV-II, Dengue virus II and Cytomegalovirus. Aqueous extract of roots can anti HSV-I, inhibits virus replication and proliferation in cells.

Antibacterial activity

The aqueous, ethanol and n-butanol extracts of the leaves have antibacterial effects on Staphylococcus aureus and Escherichia coli. The leaf decoction showed antibacterial effect in vitro on Staphylococcus aureus, Staphylococcus albus, Streptococcus A and Streptococcus B by use of disk diffusion test. 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.\cite{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.\cite{58} The total organic acids from roots also show strong antibacterial activity on Escherichiacoli by cylinder-plate test.\cite{59,60} Salicylic acid can inhibit excessive release of TNF-α and NO in serum of mice \cite{61}, and the roots decoction can decrease the levels of TNF - α and IL-6 in peritoneal macrophages of mice.\cite{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.\cite{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.\cite{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.\cite{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.\cite{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.\cite{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-\(\alpha\) secretion of peritoneal macrophages and the activity of leukocytes, pathological damage and dysfunction.\cite{70,71} Polysaccharide of the roots has immunopotentiation effects, which can promote specific immune, non-specific immune, humoral immune or cellular immune affects.\cite{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.\cite{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.\cite{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.\cite{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.\cite{77,78} Total alkaloids and amino acids from the leaves also alleviate mouse ear edema, suggesting the anti-inflammatory effects.\cite{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.\cite{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.\cite{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.\cite{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.\cite{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.\cite{86} Indirubin displays significant cytotoxicity in HL-60 cells, eliciting cell pyknosis, condensation and even lyses.\cite{87}

**Others**

IIL also has a cholagogic effect, which can promote bile excretion and relieve pain.\cite{88,89} It can depress adenosine diphosphate-elicited platelet aggregation in rabbits due to the efficacy of promoting blood circulation and removing stasis.\cite{90} Indigotin has significant protective effect against liver injury caused by carbon tetrachloride\cite{91,92} and the leaves can detoxify the effects of lead poisoning mice.\cite{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.\cite{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 miltiorrhiza*, roots of *Curcumaes 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 *attractylodis 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.\cite{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%.\cite{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.\cite{114} The compound granule could treat viral myocarditis, 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.\cite{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.\cite{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.

**Acknowledgments**

This work was supported by funds from the National Natural Science Foundation of China (No. 81773941), National Key Subject of Drug Innovation (2019ZX09201005-007), National key R & D program for key research project of modernization of traditional Chinese medicine (2019YFC1711602), and Xinglin Scholar Discipline Promotion Talent Program of Chengdu University of Traditional Chinese Medicine (no. BSH2018006).

**Conflicts of interest**

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

1. Liang ZB, et al. Study on the effective components of *Isatis indigotica* root, stem and leaf. *Guangzhou Chem* 2016; 44: 156-157.
2. Pei Y. Pharmaceutical research on the medicinal parts of *Isatis indigotica* and indigo *indigotica*. Heilongjiang Univ Trad Chin Med, 2007.
3. Qu RJ, et al. Selection of reference genes for the quantitative real-time PCR normalization of gene expression in *Isatis indigotica* fortune. *BMC Mol Biol* 2019; 20: 9.
4. Yan ZH. Clinical practical Chinese medicine 1984.
5. Li HY, et al. Extraction and content comparison of indigo and indirubin in *Isatis indigotica* root, stem and leaf. *Guangdong Chem* 2016; 43: 27-28.
6. Yu YP, et al. Quality consistency evaluation of Isatidis Folium combined with equal weight quantified ratio fingerprint method and determination of antioxidant activity. *J Chromatogr B* 2018; 1095: 149-156.
7. Liao BL, et al. Four Natural Compounds Separated from Folium Isatidis: Crystal Structures and Antibacterial Activity. *Chem Biodiver* 2018; 15.
8. Li X. Study on chemical composition and quality control of isatis root. Shanxi Medical University 2010.
9. Zheng HZ, et al. Modern research and application of Traditional Chinese Medicine. Xueyuan Press 1993.
10. Li L, et al. Chemical constituents of *Isatis indigotica*. *Chin Herb Med* 1996; 389-391.
11. Deng XY, et al. Chemical constituents of Folium Isatidis. *J Shenyang Pharm Univ* 2009; 26: 274-278.
12. Liu JF, et al. Chemical constituents of Folium Isatidis. *Chin J Trad Chin Med* 2006; 1961-1965.
13. Liu JF, et al. Isatisine A, a novel alkaloid with an unprecedented skeleton from leaves of *Isatis indigotica*. *Organic Lett* 2007; 9: 4127-4129.
14. Li W, et al. Chemical constituents of Folium Isatidis. *Chin J Shenyang Pharm Univ* 2005; 15-16.
15. Liu R, et al. Identification of five chemical components in the aqueous extract of Folium Isatidis by HPLC-MS2. *Trad Chin Med* 2005; 33-35.
16. Ruan JL, Zou JH, Cai YL. Chemical constituents of Folium Isatidis. *Chin J Trad Chin Med* 2005; 49-50.
17. Gao GH. Study on the separation, identification and determination of flavonoids in Folium Isatidis. Shenyang Pharmaceutical University 2008.
18. Chen XH. Study on the extraction and refining technology of effective components from *Isatis indigotica*. Chengdu University of Technology 2005.
19. Wu X, et al. Chemical Constituents of *Isatis indigotica*. *Planta Medica* 1997;63:55-57.
20. Li WJ. Study on the extraction and refining process of the effective components of *Isatis indigotica* leaves. *Biotechnol World* 2016; 222.
21. Gong MG. Studies on the dynamics of the synthesis and accumulation of the active components of *Isatis indigotica* and the differences of their contents. Northwest Agricultural and Forestry University of science and technology 2005.
22. Lv WY, LV P. Determination of eight inorganic elements in the Folium Isatidis of Flos Lonicerae and Forsythia suspense. *Microelement Health Res* 2003; 26-27.
23. Liu YH, et al. Chemical constituents of isatis root (I). *Chin Herb Med* 2001; 4-7.
24. Ding SP, et al. Study on chemical constituents of isatis root (II). *Med J* 2001; 475-476.
25. Fang JG, et al. Chemical constituents of isatis root (I). Chin Herb Med 2004; 9-10.
26. Liu HL, et al. Chemical constituents of Radix isatidis. J Shenyang Pharm Univ 2002; 93-95.
27. Liang YN, et al. Optimization of ultrasonic extraction process and antioxidant activity of total flavonoids from Radix Isatidis by box Behnken response surface methodology. Journal of Changchun Univ Trad Chin Med 2019; 35: 119-124.
28. Zhang HJ, et al. Molecular docking study on anti influenza virus of flavonoids in isatis root. Chem Time 2018; 32: 19-21.
29. Zhao WT. Optimization of ultrasonic assisted extraction technology and antioxidant activity of Flavonoids from Isatis indigotica root by response surface methodology. Chin Modern Appl Pharm 2016; 33: 313-317.
30. Liu YH, et al. Study on chemical constituents of isatis root (III). Chin Herb Med 2002: 3-5.
31. Liu YH, et al. Study on chemical constituents of isatis root (IV). Med J 2003: 591-594.
32. Chen Y, et al. Chemical constituents of Radix isatidis. Chin J Trad Chin Med 2018; 43: 2091-2096.
33. Liu LF, Li FZ. Chemical composition, pharmacology and quality control management of Radix Isatidis. Chin Health Indu 2018; 15: 36-37.
34. Sun Q, et al. Chemical constituents of isatis root Radix isatidis. Chin J Exp Pharmacol 2012; 18: 74-75.
35. Wang XL, et al. Studies on the chemical constituents of Radix isatidis. 9th Natural Organic Chemistry Academic Conference of China Chemical Society: Haikou, Hainan, China, 2012.
36. Liu YH, et al. Study on chemical constituents of Radix isatidis (V). Zhongnan Pharm 2003: 302-305.
37. Yan J. Study on chemical constituents and activity evaluation of Radix isatidis and flaxseed. Jilin University 2011.
38. Wang T, et al. Antiviral activity of a polysaccharide from Radix Isatidis (Isatis indigotica Fortune) against hepatitis B virus (HBV) in vitro via activation of JAK/STAT signal pathway. J Ethnopharmacol 2020; 112782.
39. Kurihara H, et al. Isatis indigotica Epigoitrin, an Alkaloid From Reduces H1N1 Infection in Stress-Induced Susceptible Model and. Front Pharmacol 2019; 10: 78.
40. Tsai FJ, et al. Anti-SARS coronavirus 3C-like protease effects of Isatis indigotica root and plant-derived phenolic compounds. Antiv Res 2005; 68: 36-42.
41. Peng W, et al. Polygonum cuspidatum Sieb. et Zucc.: a review of its botany, phytochemistry, pharmacology, and potential applications. J Ethnopharmacol 2013, 148: 729-745.
42. Zhou LN, et al. Study on the in vitro antibacterial effect and anti endotoxin effect of Compound Folium Isatidis injection. J Shenyang Pharm Univ 2006: 247-50.
43. Liu S, et al. Anti influenza A virus effects of different germplasm of isatis root and Folium Isatidis. J Second Milit Med Univ 2000; 204-206.
44. Xu T. Anti-viral Effects of 4(3H)quinazolinone from Folium Isatidis against Influenza Virus A and PRRS Virus in vitro. Gansu Agricultural University 2008.
45. Li XQ. Mechanism of Astragalus membranaceus and Folium Isatidis in the treatment of viral myocarditis in mice. The Fourth Military Medical University 2002.
46. Li ZT, et al. Study on the efficacy of aqueous extract of radix isatidis in inhibiting human H7N9 avian influenza virus in vitro. J Moder Chin Wester Med 2016; 25: 3877-3879.
47. Yan XS, et al. Clinical observation on 179 cases of influenza A H1N1 treated with non Tamiflu.
drugs. Med Innov Chin 2010: 130-131.

48. Zheng JL, et al. Study on Bacteriostasis of extracts from Folium Isatidis and Radix Isatidis. Chin J Microbiol 2003: 21-22.

49. Zhang LT, et al. Antibacterial effect of Folium Isatidis in vitro. Lishizhen Med Mater Med Res 2002: 283-284.

50. Hou JY, Fang TH. Pharmacology of Traditional Chinese Medicine [M]. Beijing: China Press of Traditional Chinese Medicine 2007: 54-5.

51. Ma JM. Analysis of modern pharmacology and clinical application of radix isatidis. Chin Health Standard Management 2014; 5: 65-66.

52. Hu XY, et al. Study on the spectrum effect relationship of the antibacterial active parts of radix isatidis. Chin Herba Med 2013; 44: 1615-1620.

53. Yao XY. Study on the regulatory mechanism of CD11b activation on the pathogenesis of endotoxic shock and related immune cell activation. Jinan University 2019.

54. Lejars M, Hajnsdorf E. The world of asRNAs in Gram-negative and Gram-positive bacteria. BBA - Gene Regulatory Mechanisms 2020; 1863.

55. Fang JG, et al. Screening of anti endotoxin active parts of folium isatidis. Chinese Herbal Medicine 2004: 64-66.

56. Li Y. Study on the basis of antiviral active substances of Folium Isatidis. Chengdu University of Traditional Chinese Medicine 2006.

57. Tang J, et al. Tang Effect of Radix Isatidis on serum LPO and SOD levels in rabbits with endotoxic DIC. Herald Med 2004: 4-5.

58. Hu WB, et al. The destructive effect of Radix Isatidis extracted by different methods on bacterial endotoxin. Res Prac Chin Med 2003: 60-61.

59. Zhao H, et al. In vitro study on the regulation of IL-2 and TNF-α secretion by mouse immune cells by folium isatidis decoction. Shaanxi J Trad Chin Med 2003: 757-9.

60. Jing XP, et al. Immunomodulatory mechanism of Astragalus Polysaccharide and Isatis polysaccharide based on antibody chip technology. Chin J Trad Chin Med Pharm 2013; 28: 3420-3423.

61. Geng CJ, et al. Immunomodulatory effect of Radix Isatidis polysaccharide on immunosuppressive mice. Agri Prod Process 2012: 36-39.

62. Wang HY, et al. Toxicity test of Isatis polysaccharide and its effect on immune system of mice. J Mianyang Norm Univ 2012; 31: 75-80.

63. Zhang J, et al. Two way immunoregulation of Isatis polysaccharide on cyclophosphamide model rats. Drug Eva Res 2016; 39: 531-538.

64. Wang L, et al. Experimental study on Antipyretic, anti-inflammatory, analgesic and bacteriostatic effects of new compound folium isatidis tablets. Chin J Trad Med Sci Technol 2007; 14: 412-413.

65. Shi GJ, Zhang J. Experimental study on the pharmacological effect of ethanol precipitate of Folium Isatidis. J Henan Univ Chin Med 2006: 15-6.

66. Wei CL, Yan XL. Anti inflammatory effect of isatis root. J Henan Univ Chin Med 2000: 53-54.

67. Yu MF. Synthesis and antitumor activity of indirubin analogues. Shang Hai JiaoTong University 2009.

68. Wu KM, et al. Synthesis of indirubin, indigo and isoindigo derivatives. Acta Pharm Sin 1985: 821-826.

69. Wang Y, et al. Research progress on antitumor and neuroprotective effects of indirubin and its
analogues. Chin Med Herb 2014; 45: 2404-2411.
70. Liang YH, et al. Anticancer activity of radix isatidis diketone B in vitro. Chin Med Herb 2000: 53-55.
71. Hsuan SL, et al. The cytotoxicity to leukemia cells and antiviral effects of Isatis indigotica extracts on pseudorabies virus. J Ethnopharmacol 2009; 123: 61-67.
72. Tian DH. Pratical Dictionary of Traditional Chinese Medicine. Beijing: People's Medical Publishing House 2002.
73. Yu S, et al. Study on the active components of promoting blood circulation in Radix Isatidis. Bull Chin Mater Med 1988: 31-32.
74. Hou JY, Fang TH. Pharmacology of traditional Chinese medicine. Chin Press Trad Chin Med 2007: 54-55.
75. Zhao L, et al. Antagonistic effect of Mungbean and Folium Isatidis on lead toxicity. Chin J Public Heal 2004: 74.
76. Pang ZL, et al. Effect of Radix Isatidis on genotoxicity of experimental mice. Acad J Guangzhou Med Coll 2000: 41-44.
77. Jin MZ, et al. Effect of Radix Isatidis on immune function and influenza virus FM1. Lishizhen Med Mater Med Res 2007: 394-396.
78. Hou XB. Study on the discovery and mechanism of potential pharmacodynamic components of Radix Isatidis. Nanjing University of Chinese Medicine 2017.
79. Liu QW. Quality standard research of isatis indigotica. Gansu Agricultural University 2018.
80. He LW, et al. Extraction and purification of total alkaloids from Radix Isatidis and their antiviral pharmacological effects. Chin Patent Drug 2014; 36: 2611-2614.
81. Xu YF, et al. Effect of acid of radix isatidis alkaloid on adsorption and release of Newcastle disease virus. J Nanjing Agri Univ 2010; 33: 90-94.
82. Zuo Y, et al. Experimental study on the anti herpes simplex virus type II effect of Isatis polysaccharide. West Chin J Pharm Sci 2013; 28: 267-269.
83. Mak NK, et al. Inhibition of RANTES expression by indirubin in influenza virus-infected human bronchial epithelial cells. Biochem Pharmacol 2004; 67: 167-174.
84. Liu XJ, Lin SJ. Study on Anti-virus Effect of Peptides from Isatis indigotica on the Mice Infected by Influenza Virus. Chin Pharm 2014; 25: 590-592.
85. Yu SQ, et al. In vitro experimental study on the anti herpes simplex virus type II effect of extracts from Folium Isatidis. Herald Med 2008: 394-396.
86. Fang JG, et al. Effect of Radix Isatidis on herpes simplex virus type I in vitro. Chin Trad Herb Drugs 2005: 242-244.
87. Li XQ, et al. A comparative study of Astragalus and Folium Isatidis in the treatment of viral myocarditis in mice. Chin J Contemp Pediatr 2003: 439-442.
88. Liu Z, et al. Experimental study on the effect of effective monomer of Folium Isatidis on respiratory syncytial virus. Lishizhen Med Mater Med Res 2009; 20: 1977-1979.
89. Hong WY, et al. In vitro experimental study on the anti dengue virus type II effect of extracts from Folium Isatidis. Chin J Moder Drug Appl 2010; 4: 161-162.
90. Liu HZ, et al. Preliminary study on the effect of Folium Isatidis on cytomegalovirus induced cytopathy. Chin J Birth Heal Heredity 2006: 58-60.
91. Chang XB. Study on the chemical basis of endotoxin and pharmacodynamics of Radix Isatidis and resistance. Jilin Med J 2013; 34: 5539.
92. Chu YF, et al. Effects of Chinese herbal medicinal ingredient on cells mediated immunity in mice. *J Nanjing Agri Univ* 2004: 97-100.

93. Xu YQ. Chemical separation of Isatis polysaccharide and its immunoenhancement activity. *Biotechnol World* 2015: 140.

94. Liu MH, et al. Effects of Fructopyranosyl(1→4)-Glucopyranose Extracted from Radix Isatidis on Tumor Growth and Immune Function in Tumor-Bearing Mice. *Chin Pharm J* 2012; 47: 1542-1546.

95. Ma YM, et al. Comparative study on anti-inflammatory and analgesic activities of different extracts of radix isatidis. *Chin Trad Herb Drugs* 2014; 45: 2517-2521.

96. Li JP, et al. Experimental study on antitumor effect and immune function regulation of Isatis polysaccharide in vivo. *Nat Prod Res Develop* 2017; 29: 2010-6.

97. Jeong P, et al. Discovery of orally active indirubin-3’-oxime derivatives as potent type 1 FLT3 inhibitors for acute myeloid leukemia. *Eur J Med Chem* 2020; 195: 112205.

98. Jian XS, et al. *In vitro* antitumor activity of ethanol extract of Folium Isatidis containing serum. *J Chin Med Mater* 2013; 36: 633-635.

99. Huo XY. 32 cases of icteric hepatitis treated with folium isatidis mixture. *Shaansi J Trad Chin Med* 1985: 222.

100. Yan Q. Clinical observation on 86 cases of chronic hepatitis B treated with Yiganjiedu Decoction and entecavir. *Clin J Chin Med* 2017; 9: 42-43.

101. Tai J, et al. Clinical analysis of 45 cases of acute icteric hepatitis treated with Shuganning injection. *J Clin Int Med* 2007: 103.

102. Zhang JL, et al. 6th National Congress of difficult and severe liver diseases: Lanzhou, Gansu, China, 2011.

103. Liu Y, Lu P. Clinical study of compound folium isatidis mixture combined with ganciclovir in the treatment of children mumps. *Mod Pharm Clin* 2019; 34: 1414-1417.

104. Han H. Application of JinHuang ointment combine with radix isatidis granules in children mumps *Chin Forei Med Treatment* 2012; 31: 117.

105. Hu XY, et al. Study on the spectral activity relationship of the antibacterial active parts of Isatidis Radix. *Chin Trad Herbal Drugs* 2013; 44: 1615-1620.

106. Li J, et al. Anti-endotoxic effects of 4(3H)-quinazolinone from RadixIsatidis. *West Chin J Pharm Sci* 2008: 7-9.

107. Li J, et al. Anti-endotoxic effects of salicylic acid from Radix Isatidis. *Chin J Hosp Pharm* 2007: 1349-1352.

108. Huang SX. Clinical observation on the treatment of upper respiratory tract infection with Radix Isatidis Granules. *J North Pharm* 2018; 15: 149.

109. Zhan Y. Radix Isatidis, Borneolum Syntheticum and Cactus in the treatment of Epidemic Parotitis. *Moder J Integr Trad Chin West Med* 2002; 2033.

110. Liu CJ. Clinical observation on the treatment of upper respiratory tract infection with Radix Isatidis Granules. *Asia-Pacific Trad Med* 2012; 8: 82-83.

111. Huang YQ. Observation on the efficacy of oseltamivir phosphate combined with Radix Isatidis Granules in the treatment of influenza A (H1N1) and Discussion on nursing care. *Strait Pharm J* 2019; 31: 233-234.

112. Luo CY, Hu NY. "Root and leaf Decoction" in the treatment of 28 cases of male condyloma acuminatum. *J Integr Chin West Med* 1990: 537.
113. Zhang DL, et al. Clinical efficacy and mechanism of folium isatidis for pasfulosis palmaris et plantaris: a pilot study. J Dermatol Venereol 2019; 41: 476-478.
114. Dai LB, et al. Herba houttuyniae injection and radix isatidis granule in the treatment of epidemic kerato-conjunctivitis. Chin Naturopath 2001: 47-48.
115. Ma WY, et al. An observation on the therapeutic efficacy of compound radix isatidis on viral myocarditis. Prac J Cardiac Cere Pneumal Vascular Dis 2003: 135-138.
116. Zhang Q, et al. A network pharmacology approach to investigate the anticancer mechanism and potential active ingredients of Rheum palmatum L. against lung cancer via induction of apoptosis. Front Pharmacol. 2020; 11:528308.
117. Long Y, et al. Nose to brain drug delivery - a promising strategy for active components from herbal medicine for treating cerebral ischemia reperfusion. Pharmacol Res. 2020; 159,104795.
118. Newman D J, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. J Nat Prod. 2016; 79(3):629-661.
Table 1. Chemical constituents isolated from *Isatis indigotica*

| Classification | No. | Chemical constituents                                      | Part of plant | Ref. |
|----------------|-----|------------------------------------------------------------|---------------|------|
| Alkaloids      | 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] |
|                | 8   | Hydroxyindirubin                                           | Root          | [10] |
|                | 9   | Isatin                                                     | Root          | [10] |
|                | 10  | 2,4(1H,3H)-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] |
| No. | Compound Name                                      | Plant Part | Reference |
|-----|---------------------------------------------------|------------|-----------|
| 16  | 4(3H)-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-1H-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  | Nicotinic 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]      |
| 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]      |
| 33  | Isovitexin                                        | Whole herb | [17]      |

**Organic acids**

**Flavonoids**
| # | Compound                  | Source       | Reference |
|---|---------------------------|--------------|-----------|
| 34 | Neohesperidin             | Root         | [28]      |
| 34 | Liquiritigenin            | Root         | [28]      |
| 36 | Isoliquiritigenin         | Root         | [29]      |
| 37 | Linarin                   | Root         | [29]      |
| 38 | Eupatorin                 | Root         | [29]      |
| 39 | (-)-lаричирезинол          | Aerial part  | [18]      |
| 40 | (+)-lаричирезинол         | Whole herb   | [18]      |
| 41 | lаричирезинол-4-O-β-D-глюкопираносид | Root | [18] |
| 42 | 4-(1,2,3-тригидроксиэтил)-2,6-диметоксибензил-1-O-β-D-глюкопираносид | Root | [18] |
| 43 | Uridine                   | Whole herb   | [36]      |
| 44 | Adenosine                 | Whole herb   | [36]      |
| 45 | Hypoxanthine              | Whole herb   | [36]      |
| 46 | Xanthine                  | Aerial part  | [36]      |
| 47 | Uracil                    | Root         | [36]      |
| 48 | Guanine                   | Root         | [36]      |
| 49 | Rosasterol                | Aerial part  | [20]      |
| 50 | β-ситостерол              | 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 |
| 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 |
| 67 | Sucrose                        | Root     | 37 |
| 68 | 5-hydroxymethyl-furaldehyde    | Root     | 37 |
| 69 | n-butyl-O-β-D- fructopyranose   | Root     | 37 |

### Amino acids

### Others
| # | Name                                                                 | Location         | Reference |
|---|----------------------------------------------------------------------|------------------|-----------|
| 70 | Mannitol                                                            | Root             | [37]      |
| 71 | 1-thiocyno-2-hydroxy-3-butene                                       | 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]      |
| 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     | Respiratory syncytial virus  | Hep-2 cells                    | Inhibits the proliferation of respiratory syncytial virus after invading Hep-2 cells. | 10–120μg/ml  | [47]   |
|                        | from leaves          |                              |                                |                                                                         |            |        |
|                        | 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 200μg/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]   |
| Plant Part          | Virus/Pathogen                          | Cell Type/Activity                                      | Effect/Measurement                           | Concentration/Citation |
|---------------------|-----------------------------------------|----------------------------------------------------------|-----------------------------------------------|------------------------|
| 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·ml⁻¹– 3g·ml⁻³ [54] |

**Shigella Castellani**

| Plant Part          | Pathogen                                | Method          | Effect/Measurement                           | Concentration/Citation |
|---------------------|-----------------------------------------|-----------------|-----------------------------------------------|------------------------|
| Leaf aqueous extract| Streptococcus pneumoniae                | Tube method     | Obvious inhibitory effect                     | 25–400mg/kg [55,56]    |
|                     | Staphylococcus aureus                   |                 |                                               |                        |

**Antibacterial**

| Plant Part          | Pathogen                                | Method          | Effect/Measurement                           | Concentration/Citation |
|---------------------|-----------------------------------------|-----------------|-----------------------------------------------|------------------------|
| Organic acid        | Escherichia coli                        | Oxford Cup      | Components have strong antibacterial activity.| 2.0g/mL [57,58]        |

**Nucleoside**

| Plant Part          | Pathogen                                | Method          | Effect/Measurement                           | Concentration/Citation |
|---------------------|-----------------------------------------|-----------------|-----------------------------------------------|------------------------|
| Anthraquinone       | Lipopolysaccharide                      | Balb/c mice     | Inhibits excessive release of TNF-α and NO in serum of mice. | 20mL/kg (ip) [59]     |
|                     | Root decoction                          | Peritoneal macrophage | Decreases the levels of TNF - α and IL-6 in peritoneal macrophages of mice. | 1g/mg [60] |

**Immunomodulatory**

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

**Antitumor**
**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*