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ORIGINAL ARTICLE

# Hypoglycemic effect and active ingredients screening of *Isodon Japonicus* based on network pharmacology and experimental validation

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**KEYWORDS**

*Isodon Japonicus*;
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**Abstract** *Isodon Japonicus* (IJ) has historically been widely used as herbal functional food. To date, there has been no publication regarding the hypoglycemic mechanism of IJ. This study was to study the hypoglycemic ingredients and mechanisms of IJ. The network pharmacology method was used to explore the ingredient-target-pathway relationship. There were 11 hypoglycemic ingredients, 12 hypoglycemic targets, and 130 signaling pathways for IJ on diabetes. To confirm the hypoglycemic impacts, cell tests and animal experiments were jointly carried out. With the human umbilical vein endothelial cells (HUVECs) treated by water extract (0.06–2.00 g/L) and alcohol extract (0.06–2.00 g/L) of IJ, the cell viability in the water extract group (0.06–2.00 g/L) and the alcohol extract group (0.25–2.00 g/L) showed significant viability compared to that of the model group ($P < 0.05$), respectively. Animal experiments showed that both the water extract and the alcohol extract of IJ could lower diabetic rats' blood glucose levels compared to the model group ($P < 0.05$). This study proposed an effective method to explore the potential hypoglycemic ingredients, targets, signaling pathways, and pharmacological effects for IJ on diabetes. This strategy may be useful for other herbs to explore the active ingredients and pharmacological mechanism.



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## 1. Introduction

*Isodon Japonicus* (IJ), known as 'Xiang-Cha-Cai' in Chinese, is the dry aerial part of *Isodon japonicus* (Burm.f.) H.Hara (Isodon genus, Lamiaceae family) (Sun et al., 2019). It is widely distributed in China and other Asian countries (Han et al., 2004). In China, IJ has historically been widely used as a cold dish (young parts) and herbal functional food with a variety of pharmacological effects, including treating gastritis and hepatitis, clearing heat and detoxifying, and invigorating the spleen and activating blood (Kim et al., 2011, Hwang et al., 2012, Hai et al., 2015). In Japan, it is traditionally known as a stomachic treatment for anorexia and dyspepsia (Hai et al., 2015, Matsumoto et al., 2017).

The chemical substances contained in IJ are primarily diterpenoids. In addition, it also contains triterpenoids, flavonoids, lignans, steroids, etc. With the advantage of the main origin area and continuous study of our institute, more than 50 ingredients had been isolated from IJ by our institute, including 2 new compounds (15α, 20β-Dihydroxy-6β-m ethoxy-6, 7-seco-6, 20-epoxy-1, 7-olide-ent- kaur-16-ene and 6β-Acetoxy-1α, 7β, 11β, 15β-tetrahydroxy-7α, 20-epoxy-ent-kaur-16-ene) and 8 compounds (Rabdosichuanin C, Rabdosinate, Longirabdolide C, Eriocaside A, Parvifoline G, Taihangexcisoidesin C, Dayecrystal A and hyptadienic acid) which were found in *Isodon Japonicus* for the first time (Yan et al., 2009, Yan et al., 2010, Yan et al., 2010, Xie et al., 2011, Hou et al., 2012, Liang et al., 2012, Liu et al., 2013, Hai et al., 2015). Recent pharmacological studies on IJ showed that it has anti-tumor, anti-inflammation, anti-bacteriostasis, anti-allergy, and anti-oxidation properties (Kim et al., 2004, Hai et al., 2015, Matsumoto and Watanabe 2020, Xing et al., 2020).

After cardiovascular disease and cancer, diabetes has become the third leading non-communicable disease in recent years. The incidence of diabetes was at an alarming rate, and it was becoming a higher and higher concerning issue of global health. High blood glucose levels were considered as a core feature and a high-risk factor of diabetes (Niu et al., 2017, Wang et al., 2019). Diabetes, with long-term hyperglycemia, could lead to a series of problems, including stroke, coronary heart disease, and even organ failure (Schmidt 2018). At present, many studies have highlighted traditional herbs in the treatment of diabetes (Gao et al., 2019, Sun et al., 2019, Jintao et al., 2020). Sun Jiayi *et al* found rosmarinic acid and its substructures (caffeic acid and 3,4-dihydroxyphenyllactic acid) of IJ showed significant inhibitory activity to amyloid β and human islet amyloid polypeptide, and good antioxidant activity on the therapy of diabetes (Sun et al., 2019). In our previous study, *Isodon rubescens*, a plant of the same genus with IJ, had revealed good hypoglycemic effect on diabetes (Jintao et al., 2020). As IJ contains many active ingredients, screening hypoglycaemic ingredients and revealing hypoglycaemic mechanisms is particularly difficult. As systems biology, computational biology, multi-direction pharmacology, computational biology, network analysis, and other technologies are all integrated in network pharmacology, network pharmacology could explore the relationship between traditional herbs and disease, and screen targets and signaling pathways from a holistic perspective to improve drug discovery efficiency (Zhang et al., 2019, Huang et al., 2020, Yuan et al., 2022). So, network pharmacology would be a useful tool for studying traditional herbs with multiple components, multiple targets and multiple mechanisms.

To better understand and evaluate the pharmacological effect and active ingredients of IJ on diabetes, the network pharmacology method was used to explore the ingredient-target-pathway relationship of IJ Integrated with experimental validation, and the hypoglycemic effect of alcohol extract and water extract from IJ was studied by cell tests and animal experiments.

## 2. Material and methods

### 2.1. Reagents

Streptozotocin (No. WXBC5204V) was got from Sigma-Aldrich Company (Saint Louis, Missouri, USA). Metformin (No. H31022081) was purchased from Shangyao Xinyi Pharmaceutical Company (Shanghai, China). DMEM low glucose culture medium (No. 31600) was the product of Beijing Solarbio Science & Technology Company (Beijing, China). Ethanol (No. 20170220, purity greater than 99.5%) was supplied by Tian-Jin-Heng-Xing Chemical Reagent Manufacturing Co. Ltd. (Tianjing, China). The glucometer was the product of Changsha Sannuo Biosensor Technology Company (Changsha, China). Cell Counting Kit-8 (No. C0038) was from Shanghai Beyotime Biotechnology Company (Shanghai, China). Water was purified twice. All other reagents were of analytical grade.

### 2.2. Isodon Japonicus

*Isodon Japonicus* (IJ), known as 'Xiang-Cha-Cai' in Chinese, was the dry aerial parts of *Isodon japonicus* (Burm.f.) H.Hara in the genus *Isodon* (family Lamiaceae). The plant name has been confirmed in the Index Kewensis (electronic Plant Information Centre ePIC, Royal Botanic Gardens, Kew, UK: https://www.kew.org/epic) and The Plant List (https://www.theplantlist.org). IJ samples (No. 201807011) were collected from Wanxianshan Mountain, Xinxiang, China. The collection process of IJ samples were followed the Convention on Biological Diversity and the Nagoya Protocol. The IJ samples were identified by Li Chunyan in Aug. 2018. Voucher specimens (No. 201807XY01IJ) were deposited at the School of Pharmacy, Xinxiang Medical University, Xinxiang, China.

Water extract of IJ: 10 g of IJ was decocted in 100 mL of water for 2 h, and then the extract solution was filtrated. With the same procedure, the second decoction was with 80 mL of water for 1.5 h, and the third time with 60 mL of water for 1 h. The filtrate from the above three times was concentrated to 20 mL. So, each 1 mL of extract solution is equivalent to 0.5 g of IJ raw herbs, and the dosage in the following study was calculated on this base.

Alcohol extract of IJ: with the same procedure of water extract, 10 g of IJ was first extracted with 100 mL of 95% ethanol for 2 h by reflux, then second time with 80 mL of 95% ethanol for 1.5 h. The combined filtrate was condensed without ethanol by rotary evaporation. The concentrated solution is fixed to 20 mL (1 mL of extract solution is equivalent to 0.5 g of raw IJ, and the dosage in the following study was calculated on this base).

### 2.3. Network pharmacology

According to the literature and our previous research, the ingredients of IJ were gathered (Han et al., 2004, Yan et al., 2009, Yan et al., 2010, Yan et al., 2010, Xie et al., 2011, Hou et al., 2012, Liang et al., 2012, Liu et al., 2013, Tanaka et al., 2014, Hai et al., 2015, Liu et al., 2017, Xing et al., 2020, Liu et al., 2021, Liu et al., 2022). Each chemical structure of IJ was drawn with Personalize ChemDraw software (Version 20.0), and converted to inchi and smiles formats with Open Babel GUI software (Version 2.4.1). Then, the inchi and smile files were imported into the Batman-TCM database (https://bionet.ncpsb.org/batman-tcm/index.php/Home/Index/index), TCMSP database (https://www.tcmsp-e.com/) and NPASS database (https://bidd2.nus.edu.sg/NPASS/) to obtain the corresponding targets for each ingredient. Targets

of diabetes were obtained from the DrugBank database (Version 5.0, https://www.drugbank.ca/). To select the potential hypoglycemic targets and ingredients of IJ, the intersection analysis was performed between the targets of ingredients and the targets of diabetes. The Z-score was used to show the matching rate between the ingredient and the target.

The hypoglycemic targets were imported to the String database (Version11.0, https://www.string-db.org/) for Gene Ontology (GO) study and KEGG pathway analysis. The biological processes, molecular functions and cellular components were studied with GO enrichment analysis, respectively. Then, a network of the ingredient-target-pathway relationship was constructed with Cytoscape software (Version 3.5).

### 2.4. Cell test

The human umbilical vein endothelial cells (HUVECs, No. JH-H1285) were provided by the American Type Culture Collection (ATCC). HUVECs were cultured in humidified air at 37 ℃ with 5% $CO_2$, and incubated in DMEM low glucose culture medium supplemented with free serum medium and 5.5 mmol/L glucose solution for 24 h. Then, with the cells at the same passage stage, HUVECs were divided into 5 groups: (I) normal group: it was treated with the normal culture medium; (II) model group: it was cultured in the culture medium containing 33 mmol/L of glucose solutions; (III) positive group: after incubating in the culture medium containing 33 mM of glucose solutions for 48 h, it was treated with 1.0 μmol/L of metformin; (IV) alcohol extract group: with the same procedure with the positive group, it was treated with different concentrations (2.0 g/L, 1.0 g/L, 0.50 g/L, 0.25 g/L, 0.13 g/L and 0.06 g/L) of alcohol extract (instead of metformin), respectively; (V) water extract group: as same with the alcohol extract group, it was treated with the water extract at 2.0 g/L,1.0 g/L, 0.50 g/L, 0.25 g/L, 0.13 g/L, and 0.06 g/L, respectively. After 24 h of treatment, cell viability was analyzed with the Cell Counting Kit-8 method in three independent experiments (Li et al., 2017). In the normal group, cell viability was set to 100% and cell activity was calculated as a percentage of cell viability of the normal group.

### 2.5. Animal experiments

Adult male Sprague-Dawley rats (8 weeks), were obtained from the Laboratory Animal Center of Xinxiang Medical University (Henan, China). All rats were maintained in standard conditions with the temperature at 20 ± 2 ℃ and 12 h of light–dark cycles with free access to water and diet. After one week of adaptive feeding, all rats were randomly divided into 6 groups: the normal group, the model group, the positive group, the alcohol extract group and the water extract group. Except for the rats in the normal group, all other rats were fed with the high sugar and fat diet (adding 10% sucrose and 10% lard to the normal diet). Then, after 12 h of fasting with free access to water, diabetic rats were induced with an intraperitoneal injection of freshly prepared streptozotocin (50 mg/kg). The streptozotocin was dissolved at a concentration of 0.5 % (m/v) in 0.1 mol/L citrate buffer (pH 4.5). The rats of normal group were injected with the same dose of sodium citrate-sodium citrate buffer as those in model group. After three days, the fasting-blood glucose (FBG) was measured with the tail vein puncture method. With an FBG value above 11.1 mmol/L, the rat was considered as diabetic rat for the following study (Kamble et al., 2016). The rats of the positive group were administrated with metformin (0.18 g/kg) by gavage; the alcohol and water extract groups were given by gavage the corresponding alcohol extracts or water extracts (2.7 g/kg), respectively. Other rats in the normal group and the model group were treated with the same dose of normal saline. All the above groups were continuously administered for four weeks. At last, the rats were anesthetized with isoflurane and sacrificed by pressing down on the neck and pulling the tail to the rear. The pancreas was collected for Hematoxylin and Eosin Staining.

The experiment was approved by the Animal Ethics Committee of Xinxiang Medical University (XYLL-2020055). All the procedures were performed in accordance with the Animal Ethics protocol, the internationally accepted principles of EU Directive 2010/63/EU, and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

### 2.6. Statistical analysis

All data were listed as mean ± standard deviations (SD). The statistical difference was analyzed by one-way ANOVAs (2-tailed) via SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). The significance level of $P < 0.05$ means there was significant difference between two groups, and $P < 0.01$ represents a very significant difference.

## 3. Results and discussion

### 3.1. Hypoglycemic ingredients and targets

A total of 233 ingredients (as listed in **Fig. A**) of IJ were collected from the related literature and our previous study (Han et al., 2004, Yan et al., 2009, Yan et al., 2010, Yan et al., 2010, Xie et al., 2011, Hou et al., 2012, Liang et al., 2012, Liu et al., 2013, Tanaka et al., 2014, Hai et al., 2015, Liu et al., 2017, Xing et al., 2020, Liu et al., 2021, Liu et al., 2022). For these ingredients, 206 un-repeated targets with 583 frequencies were obtained from the Batman-tcm database, TCMSP database and NPASS database. After intersection analysis of the targets, 12 hypoglycemic targets were got for IJ on diabetes. Then, 11 ingredients (Table 1 and Fig. 1) were related to the above12 hypoglycemic targets (Table 2), so these 11 ingredients were considered as the potential hypoglycemic ingredients of IJ on diabetes. The Z-score value of more than 22 would reflect a good match between compounds and targets. The higher the Z-score, the better the match between the ingredient and the target has.

As shown in Table 1 **and** Table 2, the targets, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), progesterone receptor (PGR), integrin alpha-L (ITGAL) and integrin beta-2 (ITGB2), played an essential role in the hypoglycemic effect of IJ on diabetes. A study by Takei S. *et al* showed that when β cells lacked HMGCR, the number of β cells would decrease, and insulin secretion would be damaged, so HMGCR would play a key role in the insulin secretion and the development of β cells (Takei et al., 2020). Picard F. *et al* found knockdown of PGR could improve glucose toler-

**Table 1** Chemical information and targets of hypoglycemic ingredients of *Isodon Japonicus*.

| No. | Ingredients | Molecular formula | CAS No. | log P | Target gene (Z'-score) |
|---|---|---|---|---|---|
| 1 | alboatisin A | $C_{20}H_{30}O_4$ | 1393090–51-1 | 2.0684 | HMGCR (22.37), DRD2 (22.37), DRD1 (22.37), AVPR2 (22.37), ADRA2A (22.37), ADRA1B (22.37) |
| 2 | rabdoternin B | $C_{20}H_{28}O_7$ | 128887–81-0 | −0.3060 | ITGB2 (80.88), ITGAL (80.88), HMGCR (80.88) |
| 3 | enmein | $C_{20}H_{26}O_6$ | 3776–39-4 | 1.1954 | GAA (22.37), CA2 (22.37) |
| 4 | hebeiabinin B | $C_{20}H_{34}O_5$ | 934832–65-2 | 1.2243 | PGR (122.78) |
| 5 | isolushinin G | $C_{22}H_{32}O_7$ | 1233704–14-7 | 0.5808 | PGR (48.00) |
| 6 | eriocasin C | $C_{22}H_{32}O_6$ | 1254953–48-4 | 2.0642 | PGR (48.00) |
| 7 | nervonin I | $C_{24}H_{36}O_8$ | 1011715–08-4 | 0.9418 | HMGCR (23.00) |
| 8 | nervonin D | $C_{28}H_{40}O_{10}$ | 1011715–03-9 | 2.0834 | HMGCR (23.00) |
| 9 | tormentic acid | $C_{30}H_{48}O_5$ | 119725–19-8 | 5.1752 | PGR (48.00) |
| 10 | β-sitosterol | $C_{29}H_{50}O$ | 83–46-5 | 8.4149 | DPP4 (22.37) |
| 11 | forrestin C | $C_{26}H_{38}O_9$ | / | / | HMGCR (23.00) |

*PS:* ADRA2A: alpha-2A adrenergic receptor; ADRA1B: alpha-1B adrenergic receptor; AVPR2: vasopressin V2 receptor; CA2: carbonic anhydrase 2; DPP4: dipeptidyl peptidase 4; DRD1: d(1A) dopamine receptor; DRD2: d(2) dopamine receptor; GAA: lysosomal alpha-glucosidase; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; ITGAL: integrin alpha-L; ITGB2: integrin beta-2; PGR: progesterone receptor.

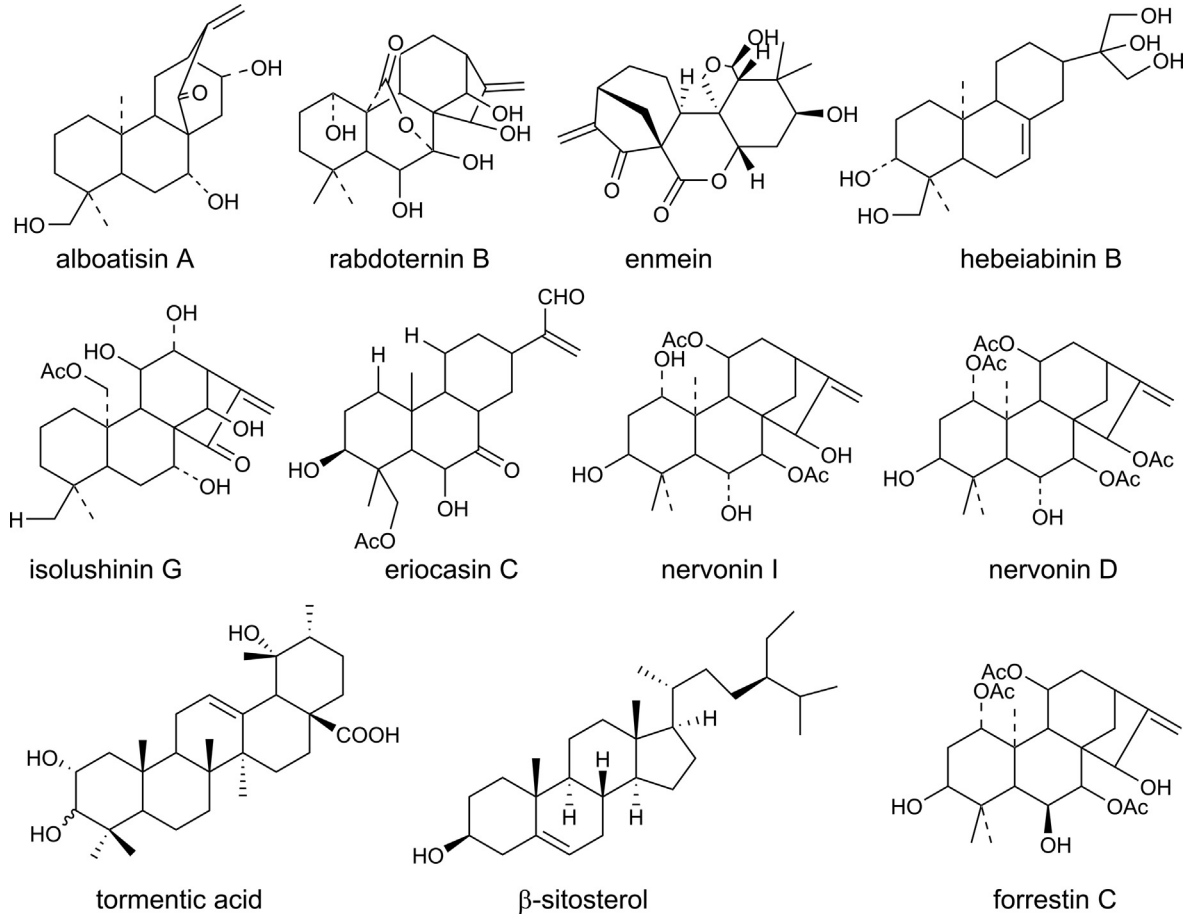


**Fig. 1** Chemical structural of the hypoglycemic ingredients.

**Table 2** Information of hypoglycemic targets of *Isodon Japonicus*.

| Target | Target gene | Uniprot ID | Ingredients (Z'-score) |
|---|---|---|---|
| 3-hydroxy-3-methylglutaryl-coenzyme A reductase | HMGCR | P04035 | rabdoternin B (80.88); nervonin D (23.00); nervonin I (23.00); forrestin C (23.00); alboatisin A (22.37) |
| progesterone receptor | PGR | P06401 | hebeiabinin B (122.78); tormentic acid (48.00); eriocasin C (48.00); isolushinin G (48.00) |
| integrin alpha-L | ITGAL | P20701 | rabdoternin B (80.88) |
| integrin beta-2 | ITGB2 | P05107 | rabdoternin B (80.88) |
| alpha-1B adrenergic receptor | ADRA1B | P35368 | alboatisin A (22.37) |
| alpha-2A adrenergic receptor | ADRA2A | P08913 | alboatisin A (22.37) |
| vasopressin V2 receptor | AVPR2 | P30518 | alboatisin A (22.37) |
| carbonic anhydrase 2 | CA2 | P00918 | enmein (22.37) |
| dipeptidyl peptidase 4 | DPP4 | P27487 | β-sitosterol (22.37) |
| d (1A) dopamine receptor | DRD1 | P21728 | alboatisin A (22.37) |
| d (2) dopamine receptor | DRD2 | P14416 | alboatisin A (22.37) |
| lysosomal alpha-glucosidase | GAA | P10253 | enmein (22.37) |

ance through the proliferation of β cells and secreting higher levels of insulin (Picard et al., 2002). The research of Huang M. *et al* revealed that the main pathological features of autoimmune diabetes were the recruitment of T cells and its destruction to islet cells, and ITGAL could inhibit the adhesion of T cells to pancreatic islet endothelium (Huang et al., 2005). The study of Barlow S. C. *et al* displayed that ITGB2 was an important mediator of leukocyte-mediated tissue damage in the development of autoimmune diabetes (Barlow et al., 2004).

### *3.2. Gene ontology and pathways analysis*

Gene Ontology (GO) study and KEGG pathway analysis of the hypoglycemic targets of IJ were analyzed in the String database. The GO study showed that there were 643 biological processes with 884 frequencies (**Table A.1**), 128 molecular functions with 157 frequencies (**Table A.2**), and 127 cellular components with 194 frequencies (**Table A.3**). As indicated in Fig. 2**, 17** biological processes occurred at the frequency of more than 4 times, and 5 molecular functions had more than 3 times with 8 cellular components at a frequency of more than 4 times.

The KEGG Pathways study showed that 130 signaling pathways at 264 frequencies (**Table A.4**) were related with the hypoglycemic targets of IJ. Fig. 3 showed 16 signaling pathways at a frequency of more than 4 times. To better understand and evaluate the pharmacological mechanism of IJ on diabetes, as illustrated in Fig. 4, a network of 11 hypoglycemic ingredients, 12 hypoglycemic targets, and 43 signaling pathways with more than 3 frequencies were established. This network revealed that the ingredients of IJ could regulate multiple targets, while these targets also could control multiple signaling pathways.

As shown in Fig. 3 **and** Fig. 4, the signaling pathways, Rap1 signaling pathway, cAMP signaling pathway and Oxytocin signaling pathway, might played an important role in the hypoglycemic effect of IJ on diabetes. Rap1 is a monomeric small GTPase belonging to the Ras family. Ablation of the Rap1 gene would significantly decrease the blood glucose and serum insulin levels, and improve the glucose and insulin resistance (Kaneko et al., 2021). So, the Rap1 signaling pathway could be a very effective mechanism for therapeutic intervention on diabetes. As an important cell signaling molecule, CAMP could adjust the insulin and glucagon secretion of pancreatic β cells and α cells, so the cAMP signaling pathway could regulate blood glucose levels by normalizing the insulin and glucagon secretion (Tengholm and Gylfe 2017). In Oxytocin Signaling Pathway, Oxytocin could significantly decrease the overall blood glucose level through stimulating insulin secretion and increasing plasma insulin concentration (Mohan et al., 2018).

### *3.3. Cell viability analysis*

Cell viability of human umbilical vein endothelial cells (HUVECs) was showed in Fig. 5. Compared to the cell viability of the normal group which was set to 100% of viability, the viability of the model group significantly decreased to 75.96 % ± 3.07% ($P < 0.01$), so it indicates the cell viability treated by the high concentration of glucose solutions (33 mmol/L) would be greatly reduced. Compared with the cell viability of the model group, the alcohol extract at the concentration from 0.25 g/L – 2.00 g/L and the water extract at 0.06 g/L – 2.00 g/L could significantly increase the viability ($P < 0.01$), respectively. Especially, the 0.13 g/L and 0.25 g/L of water extracts would make the viability to be higher than that of the positive group ($P < 0.01$). However, the viability of alcohol extract at 0.06 g/L – 2.00 g/L was lower than that of positive group, while the viability of water extract at 0.06 g/L – 0.50 g/L were higher than that of the positive group. In summary, the results of the cell test showed that both the water extract and the alcohol extract of IJ had a protective effect on the cell viability under high glucose treatment, and the water extract, especially at the concentrations of 0.13 g/L and 0.25 g/L, showed higher efficiency than the alcohol extract.

### *3.4. Hypoglycemic effect on diabetic rats*

As listed in Table 3, the fasting-blood glucose (FBG) of each group after modeling, except for that of the normal group,

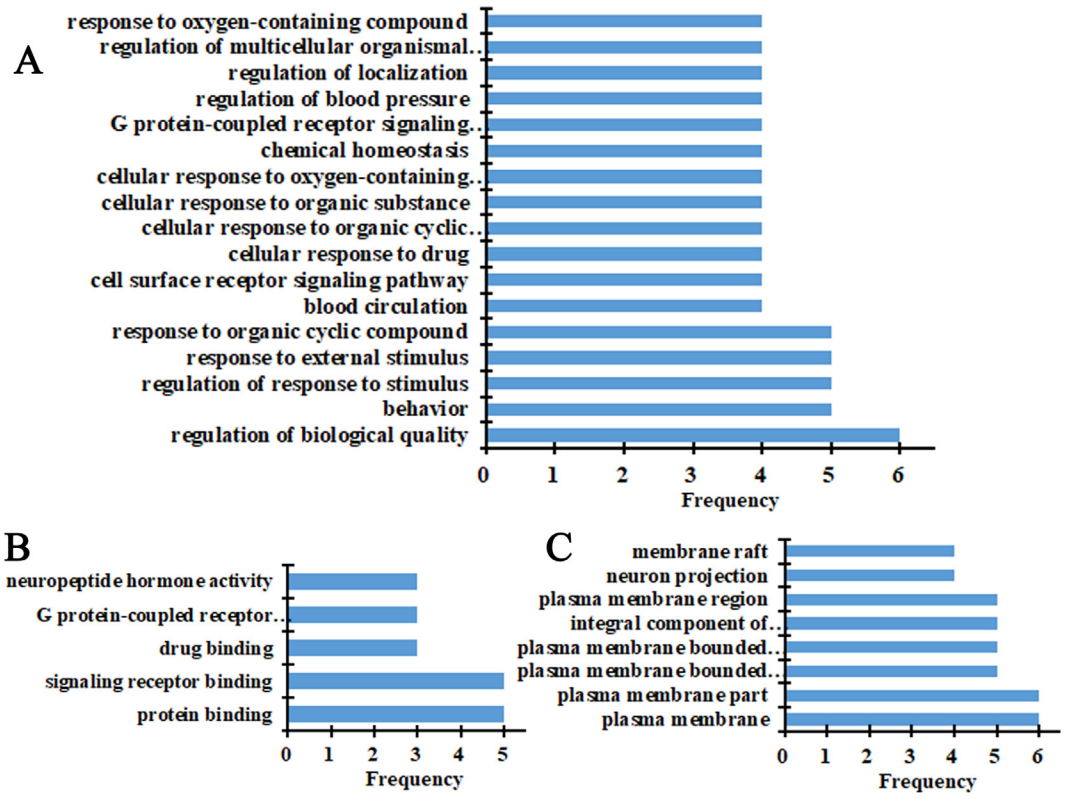


**Fig. 2** Biological processes (A), cellular components (B), and molecular functions (C) of gene ontology analysis for hypoglycemic targets.

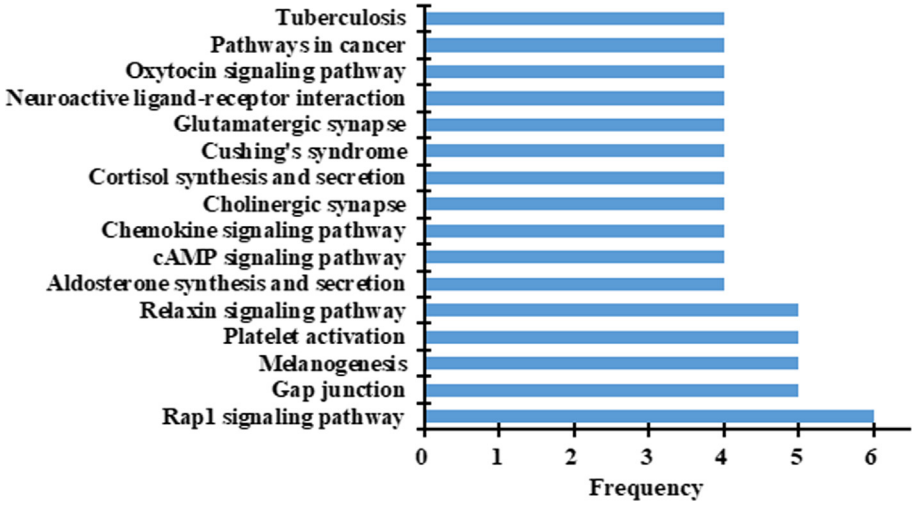


**Fig. 3** KEGG signaling pathways for hypoglycemic targets of *Isodon Japonicus*.

was more than 16.6 mmol/L, and it increased significantly ($P < 0.05$). As shown in Fig. 6**A**, the pancreatic tissues of the normal rat were observed to be clear and intact, and the islet cells were arranged neatly in good shape. After modeling (Fig. 6**B**), vacuole-like changes appeared in disorganized form, and the number of pancreatic cells in the diabetic rats decreased with sparse distribution. So, the results of Table 3 and Fig. 6 indicated that the experimental diabetic rat model was successfully induced. As shown in Table 3, after administration with IJ for 4 weeks, compared with the model group, the FBG of the water extract group and the alcohol extract group decreased significantly ($P < 0.05$), respectively. Moreover, for both the water extract group and the alcohol extract group, there was no significant difference with the positive group. So, both the water extract and the alcohol extract of IJ had a good hypoglycemic effect for diabetic rats.

Both the cell test and the animal experiment showed that the ingredients in the water extract and the alcohol extract of IJ had a good hypoglycemic effect on diabetes. Usually, the log P of a chemical ingredient could reflect its solubility in

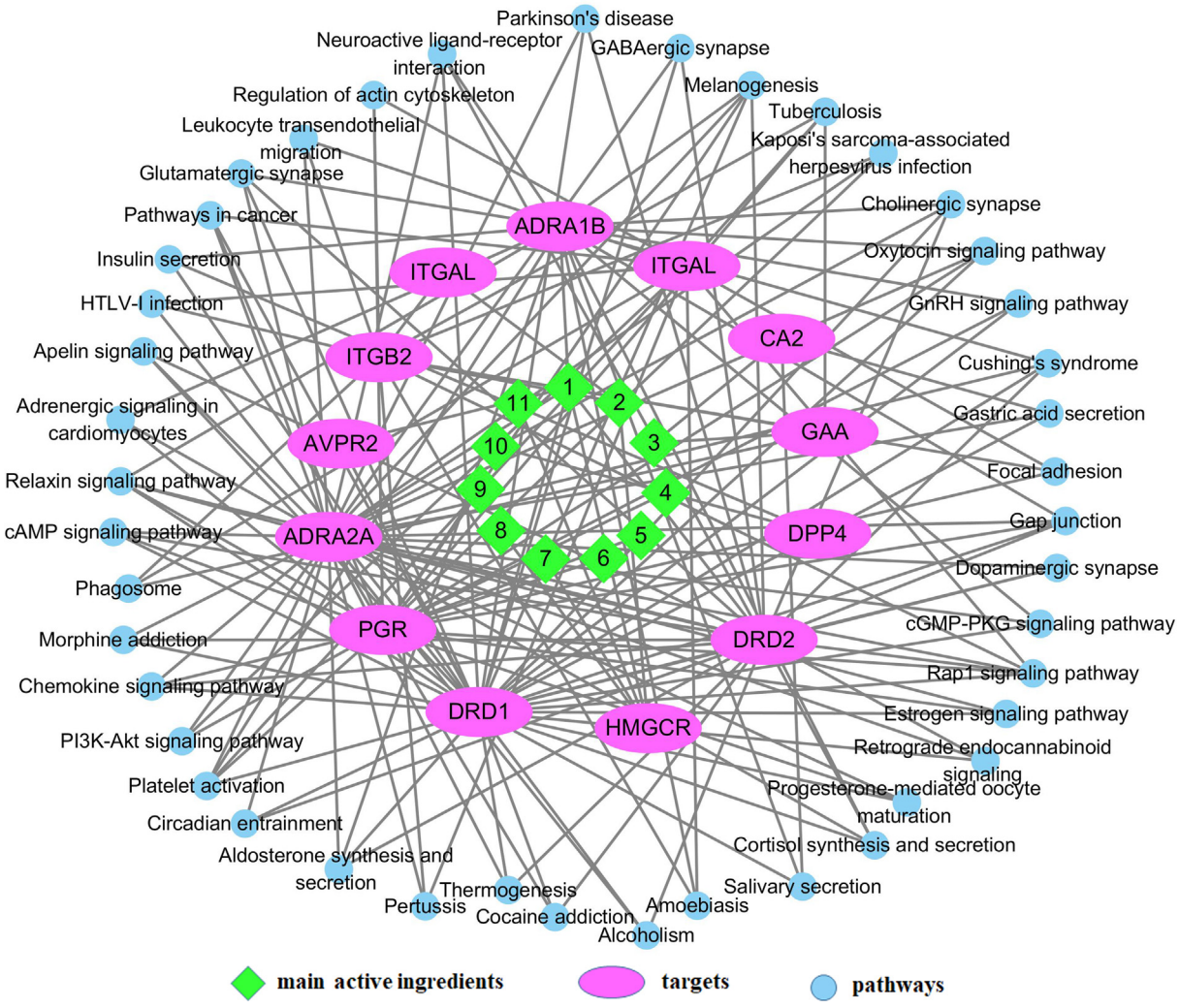


**Fig. 4** The ingredient-target-pathway network of *Isodon Japonicus* on diabetes (ingredients No. 1–11 listed in Table 1).

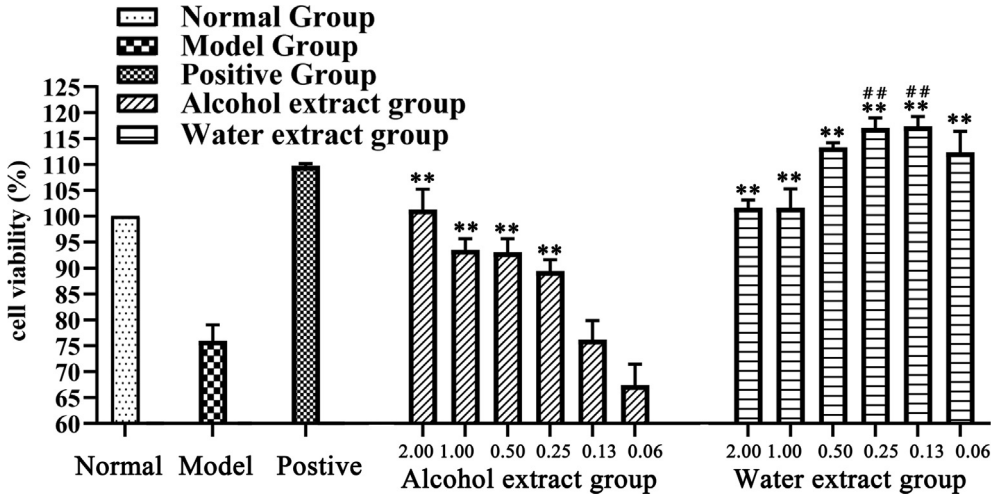


**Fig. 5** Cell viability treated with the extracts of *Isodon Japonicus* (n = 3, data = mean ± SD). **: $P < 0.01$ in comparison with model group; ##: $P < 0.01$ in comparison with positive group.

**Table 3** Blood glucose level of each group (mean ± SD).

| Group | N | Blood glucose (mmol/L) | | |
|---|---|---|---|---|
| | | Before modeling | After modeling | 4 weeks of administration |
| Normal group | 6 | 5.1 ± 1.2 | 4.6 ± 1.0 | 5.5 ± 1.2[&] |
| Model group | 6 | 4.8 ± 17 | 20.9 ± 5.9*[#] | 21.4 ± 4.9 |
| Positive group | 6 | 4.5 ± 1.6 | 19.8 ± 5.1*[#] | 6.9 ± 3.0[&] |
| Alcohol extract group | 6 | 5.9 ± 1.1 | 20.1 ± 6.3*[#] | 6.5 ± 3.4[&] |
| Water extract group | 6 | 5.6 ± 0.9 | 18.5 ± 5.7*[#] | 5.9 ± 1.8[&] |

*PS*: *: $P < 0.05$ in comparison with normal group after modeling. [#]: $P < 0.05$ in comparison before and after modeling for each group, respectively. [&]: $P < 0.05$ in comparison with model group after 4 weeks of administration.

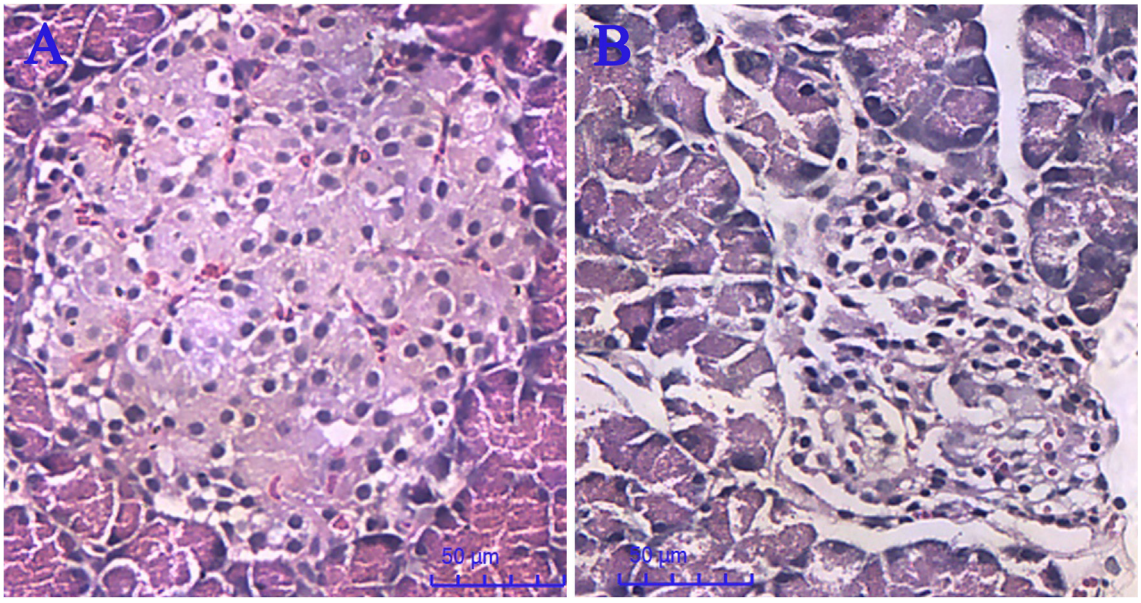


**Fig. 6** Pancreatic morphology of normal rats (A) and diabetic rats (B).

water and ethanol, and its membrane permeability *in vivo*. More than 3 of log P value means the ingredient had good performance in ethanol, while < 0 of log P value indicates good water solubility. The log P value at the range of 0–3 is much better, which means the ingredient could be dissolved in both water and ethanol, and had good performance in cell membrane permeability. Integrated with the results of the cell test, the animal experiment, and the network pharmacology (Table 1), Rabdoternin B of IJ should theoretically have good water solubility, and tormentic acid and β-sitosterol may be mainly existed in alcohol extract of IJ. Other 7 hypoglycemic ingredients, such as alboatisin A, enmein, hebeiabinin B, and so on, were with 0–3 of log P, so this might explain the results that both water extract and the alcohol extract of IJ had a good hypoglycemic effect. However, further study *in vivo* and *in vitro* experiments should be carried out to confirm the above results.

## 4. Conclusion

Historically, IJ has been widely utilized as healthy food and traditional herb. To better understand and evaluate its pharmacological effect and active ingredients on diabetes, the ingredient-target-pathway relationship and hypoglycemic effect of IJ were investigated by network pharmacology, cell tests and animal experiments. Based on network pharmacology method, 11 hypoglycemic ingredients, 12 hypoglycemic targets and 130 signaling pathways were got. The cell test and animal experiment verified the hypoglycemic effect of IJ. The cell viability in water extract group (0.06–2.00 g/L) and alcohol extract group (0.25–2.00 g/L) showed significant viability compared to that of the model group ($P < 0.05$), respectively. Animal experiments showed that both water extract and alcohol extract of IJ could lower diabetic rats' blood glucose levels when compared to the model group ($P < 0.05$). Based on network pharmacology technology combined with cell and animal experiments, 11 ingredients may be the main hypoglycemic ingredients, and HMGCR, PGR, ITGAL and ITGB2 may be the potential therapeutic targets. The hypoglycemic mechanisms may be through Rap1 signaling pathway, cAMP signaling pathway, Oxytocin signaling pathway. This study provided some references for our following study. However, to provide support for our findings, further study *in vivo* and *in vitro* experiments needed be carried out to confirm the hypoglycemic ingredients, targets and signaling pathways of IJ on diabetes. On the other hand, this strategy may be an effective method for other herbs to explore the active ingredients and pharmacological mechanisms.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Authorship contribution statement**

Xue Jintao, Zhang Nan and Yang Pengfei designed this study. Xue Jintao, Lu Yusi, Xiao Xian, Li Chunyan, Wang Canyu, Zhu Huiqing, Liu Xiaolong, Song Liaofan, Zhang Nan, and Yang Pengfei participated in the collecting the samples, acquisition of data, and analysis and interpretation of the data. Xiao Xian and Xue Jintao aided in review, editing and revising the manuscript. All the authors had approved the final version of the manuscript to be submitted.

**Appendix A. Supplementary data**

**Fig. A**: The active ingredients of *Isodon Japonicus*. **Table A.1**: The biological processes of the hypoglycemic targets for *Isodon Japonicus*. **Table A.2**: The molecular functions of the hypoglycemic targets for *Isodon Japonicus*. **Table A.3**: The cellular components of the hypoglycemic targets for *Isodon Japonicus*. **Table A.4**: the KEGG results of the hypoglycemic targets for *Isodon Japonicus*.

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