

# Research on Saponin Active Ingredient of Stir-Fried Dolichos Lablab L. Kernel for Treatment of Type-2 Diabetes Based on UHPLC-Q-Exactive Orbitrap MS and Network Pharmacology

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## Research

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# Research on Saponin Active Ingredient of Stir-fried *Dolichos lablab* L. Kernel for Treatment of Type-2 Diabetes based on UHPLC-Q-Exactive Orbitrap MS and Network Pharmacology

Jun Han, Liangzi Fang, Qinfang Zheng

**Abstract: Background:** Although the clinical effect of stir-fried *Dolichos lablab* L. kernel has been approved in modern traditional Chinese medicine, existing associated studies mainly focus on its clinical studies and chemical ingredients. However, there are few studies on pharmacodynamics material basis and molecular mechanism of stir-fried *Dolichos lablab* L. kernel in treatment of type-2 diabetes(T2DM), thus restricting the further development and utilization of stir-fried *Dolichos lablab* L. kernel.**Methods:** A qualitative analysis on saponin chemical ingredients of stir-fried *Dolichos lablab* L. kernel was performed using UHPLC-Q-Exactive Orbitrap MS. A total of 10 saponin ingredients were selected. Moreover, target screening, biological process and metabolism pathway analysis were accomplished by network pharmacology. Four key proteins (EGFR, IGF1, MAPK1 and PIK3R1) of type-2 diabetes were selected for molecular docking verification with saponin ingredients. Specifically, molecular dynamics simulation of ingredients which have strong bindings with proteins was conducted. **Results:** In this study, 16 saponin ingredients were identified from stir-fried *Dolichos lablab* L. kernel. There were 91 intersection targets and the KEGG pathway enrichment involved 20 relevant pathways. According to the molecular docking verification, saponin ingredients of stir-fried *Dolichos lablab* L. kernel can form stable binding with key protein targets. The molecular dynamics simulation further verifies stability and reasonability of the docking results. **Conclusions:** This study provides references to identification of efficient ingredients of stir-fried *Dolichos lablab* L. kernel, screening of quality markers and explanation of relevant action mechanism by combining UHPLC-Q-Exactive Orbitrap MS and network pharmacology.

**Keywords:** UHPLC-Q-Exactive Orbitrap MS, network pharmacology, stir-fried *Dolichos lablab* L. kernel, type-2 diabetes, molecular docking, molecular dynamics simulation, ligand channels

## Background

Diabetes is directly related with pancreas which is an important component of the digestive system. Pancreas generates enzyme and hormones to help digestion of foods. Diabetes belongs to the “consumptive thirst” category in traditional Chinese medicine and it is closely related with spleen and stomach. In five elements, spleen and stomach of people are corresponding to the earth. They cover several digestive organs including pancreas of human body. According to the etiology and pathogenesis that diabetes is caused by Yin deficiency and manifested by dry heats, nitrifying spleen and Yin is the key of Yin nutrition. *Radix Trichosanthis*, *Radix Ophiopogonis* and *Dolichos lablab* L. which are characteristic of sweet and cool or sweet and cold are suggested as the basic medicines for symptoms of spleen and Yin deficiency. Among them, *Dolichos lablab* L. belongs to the spleen channel and it can

invigorate spleen to eliminate dampness. Stir frying is equivalent to increase the strengthening effect of spleen. In traditional Chinese medicine, *Dolichos lablab L.* is a kind of white seeds of *leguminosae* plants, *Dolichos L.* Some study demonstrated that triterpenoid saponin has been separated from other similar plants of *Dolichos L.* Triterpenes and glucosides of sterols can inhibit absorption of alcohol and sugar<sup>[1]</sup>. Although the clinical effect of stir-fried *Dolichos lablab L.* kernel has been approved in modern traditional Chinese medicine, existing associated studies mainly focus on its clinical studies and chemical ingredients. However, there are few studies on pharmacodynamics material basis and molecular mechanism of stir-fried *Dolichos lablab L.* kernel in treatment of type-2 diabetes(T2DM), thus restricting the further development and utilization of stir-fried *Dolichos lablab L.* kernel.

Hence, saponin chemical ingredients of stir-fried *Dolichos lablab L.* kernel were identified in the present study by using UHPLC-Q-Exactive Orbitrap MS technology. On this basis, active saponin ingredients related with T2DM were screened by network pharmacology. Moreover, action mechanism of screened active ingredients was interpreted. Results could provide references to screen quality markers. Molecular docking and molecular dynamics simulation were carried out based on structures of key proteins and saponin ingredients. Binding free energy was calculated and main thrust force for binding between proteins and saponin ingredients was analyzed. Besides, amino acids which play the key role in the binding between proteins and saponin ingredients were disclosed and the relevant binding mechanism was discussed. Research conclusions can provide theoretical references for development of more efficient saponin for treatment of T2DM.

## **Experiment and Methods**

### **Instruments and materials**

Instruments and materials used in the experiment include the series connection of four-stage rod-electrostatic field orbital hydrazine high resolution mass spectrometer and the Ultimate 3000 ultra-high performance liquid chromatography system (Seymour Fisher Technologies Inc, USA), BT25S electronic scales (Sartorius Company), KQ-300DB numerical control ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd) and methyl alcohol (Sinopharm Group Chemical Reagent Co. LTD, batch No.: 20190910).

### **Preparation of solution samples**

Stir-fried *Dolichos lablab L.* kernel powder (0.5g) was taken and 10% methyl alcohol (10ml) was added in for ultrasonic processing by 30min. Later, the mixture was filtered and the solution samples were gained.

### **Chromatography and mass spectrometry conditions**

ZORBAX SB-C18 chromatographic columns (2.1×100mm,1.8μm) were used, with a column temperature of 35°C. The flowing phase used methyl alcohol (A)-0.1%formic acid (B), which was

processed by gradient elution (0-5min, 2%A, 5-20min, 25%-100%A; 20-25min, 100%-100%A; 25-27min, 100%-2%A; 27-30min, 2%-2%A). The flow rate and feed rate were set as 0.3ml/min and 3 $\mu$ L, respectively. Ultraviolet full wavelength was applied. Mass spectrometry conditions were introduced as follows: temperature of capillary tube=320°C, voltage of electronic spraying=3.0kv, flow rate of sheath gas (N<sub>2</sub>)= 30arb, flow rate of auxiliary gas (N<sub>2</sub>)= 10arbm and collection range= m/z 133-2000.

### **Structural identification**

According to the principle that the error between testing value and theoretical value is smaller than 5ppm and nitrogen laws, the expected compounds were gained through high-resolution extraction ion chromatography by combining the Compound Discover version 3.0. MS2 data of the expected compounds was collected through the PRM mode. Finally, saponin candidates were determined according to fragment ions, retention time and references.

### **Screening of compounds and disease targets**

3D structural files of compounds were established using chemdraw and the corresponding SMILES numbers were gained. Targets of compounds were gained through PharmMapper(<http://www.lilab-ecust.cn/pharmmapper/>) and SwissTargetPrediction(<http://www.swisstargetprediction.ch/>)、TargetNet(<http://targetnet.scbdd.com/calcnnet/index/>). The 3D structural files were submitted to PharmMapper to get targets and each compound returned 300 results. According to instructions of websites, the higher z'-score reflects the higher possibility of becoming the target of compound and negative z'-score represents insignificant results. Therefore, results with positive z'-score were selected. The SMILES numbers were submitted to SwissTargetPrediction and each compound returned 100 results. Since published literatures of SwissTargetPrediction have explained that the top 15% results represent 75% possibility, the top 15 returning results of each compound in SwissTargetPrediction were selected. Later, the SMILES numbers were submitted to TargetNet and results with prob higher than 0 were chosen. Results of the above three websites were summarized and used as the compound target library.

Relevant targets of type-2 diabetes were searched from CTD(<http://ctdbase.org/>), TTD(<http://db.idrblab.net/ttd/>) and GeneCards(<https://www.genecards.org/>), PharmGKB(<https://www.pharmgkb.org/>) with keywords of CTD: type 2 diabetes mellitus; TTD: type 2 diabetes; GeneCards: type 2 diabetes mellitus; PharmGKB: Diabetes Mellitus, Type 2. To increase reliability of data and eliminate data with low scores, GeneCards selected targets with Relevance score higher than or equal to 30. CTD chose targets with M or T (marker/mechanism|therapeutic).

Intersection between compound targets and disease targets was selected, which covered 91 genes. The Venny website is <http://bioinformatics.psb.ugent.be/webtools/Venn/>.

### **Protein interaction network**

The 91 treatment genes were input into the STRING database (<https://string-db.org/>) and species was limited within human. To increase reliability of results, the lowest interaction score was limited at high confidence (0.700) and results were input into cytoscape3.8.0 for visualization and analysis. The network was analyzed by cytoscape plug-in and individual node parameters in the network were calculated. In this study, 22 key genes were screened by using means of Degree, BetweennessCentrality and ClosenessCentrality as the thresholds. These 22 genes might be key targets for traditional Chinese medicine treatment of type-2 diabetes.

### **Active ingredients-target network**

Compounds corresponding to 91 therapeutic targets were identified one by one. Besides, an active ingredients-therapeutic target network was built up and input into cytoscape3.8.0 for visualization and analysis. The network was analyzed by cytoscape plug-in and individual node parameters in the network were calculated. GO and KEGG enrichment analyses of 91 therapeutic genes were finished using clusterprofiler package of Rstudio. Moreover, results were visualized by ggplot2 and put in a descending order according to pvalue. GO shows top 10 items of BP, CC and MP, respectively. KEGG shows top 20 significant pathways.

### **Molecular docking**

Molecular docking experiment between active ingredients and key targets was carried out based on AutoDock Vina\_1.1.2 to verify the interaction activity between ingredients and key targets. AutoDock Vina applied the semi-flexible molecular docking. In other words, pharmacologic molecules are flexible and rigidity of proteins is kept constant in the docking process, and docking results are evaluated by a semi-experience free energy function<sup>[2]</sup>.

### **Molecular dynamics simulation and ligand channels**

The orbit of molecular dynamics simulation was finished by gromacs 2019-3. Graphs of root-mean-square deviation (RMSD), rotating rate, mean-square deviation (MSD), total energy, root-mean-square fluctuation (RMSF) of ligands and proteins were drawn. Clustering analysis was calculated. All potential channels for binding between ligands and proteins were explored using caver 2.0. Parameters were set as follows: minimum Probe radius =0.9, shell radius =3, and maximum shell depth in the surface region =4.

## **Results and analysis**

### **Structural identification based on UHPLC-Q-Exactive Orbitrap MS**

Ingredients of stir-fried *Dolichos lablab L.* kernel were identified based on the UHPLC-Q-Exactive Orbitrap MS technology through abovementioned sample processing and analysis conditions, and then analyzed. Firstly, samples were injected into UHPLC-Q-Exactive Orbitrap MS and high-

resolution mass data were acquired through MS full scan. Secondly, molecular formula of saponin was predicted by setting parameters of metabolic working flow for Compound Discover, and then confirmed by MS full scan and high-resolution extraction ion chromatography (HREIC). In this way, an ion list was generated. Finally, fragment ions were gained using UHPLC-Q-Exactive Orbitrap MS through parallel reaction monitoring mode (the above constructed ion list). Finally, saponin candidates were determined according to fragment ions, retention time and references. In this study, 16 saponin ingredients were identified from stir-fried *Dolichos lablab* L. kernel under the negative ion mode. Results are shown in Table 1.

Table 1 Ingredient identification results of stir-fried *Dolichos lablab* L. kernel

No.	RT/min	Extracted ions	Testing value	Theoretical value	Error (ppm)	Molecular formula	Primary secondary fragment ions (MS/MS)	Identification result	References
1	8.85	[M-H] <sup>-</sup>	1409.6586	1409.6594	-0.6	C <sub>66</sub> H <sub>106</sub> O <sub>32</sub>	1329.1364,1248.6079,1007.5526,727.3286	Lablaboside E	3
2	8.94	[M-H] <sup>-</sup>	1393.6636	1393.6645	-0.6	C <sub>66</sub> H <sub>106</sub> O <sub>31</sub>	1263.6006,1007.5516,719.3311,654.2996	Lablaboside F	3
3	9.50	[M-H] <sup>-</sup>	1117.5431	1117.5436	-0.4	C <sub>54</sub> H <sub>86</sub> O <sub>24</sub>	955.4912,909.4147,768.3499,630.2892,581.2709	Lablaboside B	4
4	9.63	[M-H] <sup>-</sup>	1101.548	1101.5487	0.6	C <sub>54</sub> H <sub>86</sub> O <sub>23</sub>	809.4331,638.2866	Lablaboside A	4
5	9.77	[M-H] <sup>-</sup>	1261.5848	1261.5859	-0.9	C <sub>60</sub> H <sub>94</sub> O <sub>28</sub>	1117.5416,971.4852,823.4119,630.2890	Lablaboside D	3
6	9.96	[M-H] <sup>-</sup>	957.5054	957.5065	-1.1	C <sub>48</sub> H <sub>78</sub> O <sub>19</sub>	807.4170,630.2891,557.2599	Oleanane, β-D-glucopyranosiduronic acid deriv.	5
7	10.07	[M-H] <sup>-</sup>	955.4901	955.4908	-0.7	C <sub>48</sub> H <sub>76</sub> O <sub>19</sub>	955.4902,807.4169,630.2890,557.2598	Chikusetsusaponin V	5
8	10.25	[M-H] <sup>-</sup>	809.4325	809.4329	-0.5	C <sub>42</sub> H <sub>66</sub> O <sub>15</sub>	783.4016,630.2888,557.2599	Bonusenricoside B	7
9	10.35	[M-H] <sup>-</sup>	939.4958	939.4959	-0.1	C <sub>48</sub> H <sub>76</sub> O <sub>18</sub>	823.4124, 783.4023,697.3077,630.2898,503.3377	3-O-[R-L-Rhamnopyranosyl(1-2)a-D-glucopyranosyl(1-2)-a-D-glucuronopyranosyl]olean-12-en-22-oxo-3a,-24-diol	8
10	10.53	[M-H] <sup>-</sup>	793.4378	793.4380	-0.3	C <sub>42</sub> H <sub>66</sub> O <sub>14</sub>	741.7372, 630.2897,459.0940	Chikusetsusaponin IVa	5
11	10.88	[M-H] <sup>-</sup>	1081.5219	1081.5225	-0.6	C <sub>54</sub> H <sub>82</sub> O <sub>22</sub>	955.4909,865.4230,793.4379,621.3490	Lablab saponin I	3
12	11.23	[M-H] <sup>-</sup>	971.4853	971.4857	-0.4	C <sub>48</sub> H <sub>76</sub> O <sub>20</sub>	935.4647,793.4379,647.3796	Caryocaroside II-11	9
13	11.41	[M-H] <sup>-</sup>	941.5110	941.5115	-0.5	C <sub>48</sub> H <sub>78</sub> O <sub>18</sub>	896.207	Soyasaponin I	3
14	11.67	[M-H] <sup>-</sup>	795.4536	795.4536	0.0	C <sub>42</sub> H <sub>68</sub> O <sub>14</sub>	580.3254,387.2862	3-O-Cellobiosyl-hederagenin	10
15	11.88	[M-H] <sup>-</sup>	925.5164	925.5166	-0.2	C <sub>48</sub> H <sub>78</sub> O <sub>17</sub>	795.4528,645.3643,580.3259,455.0136	Chikusetsusaponin Ib	6
16	12.28	[M-H] <sup>-</sup>	779.4583	779.4587	-0.5	C <sub>42</sub> H <sub>68</sub> O <sub>13</sub>	661.3594,593.1298,549.1036,459.2959	Erythrosaponin A	11

By combining with previous researches, hypoglycemic active compounds like Chikusetsusaponin Iva, Chikusetsusaponin V, Chikusetsusaponin Ib, Soyasaponin I, 3-O-Cellobiosyl-hederagenin, Oleanane,  $\beta$ -D-glucopyranosiduronic acid derive, lablaboside B, Lablaboside D, Lablaboside E and Lablaboside F were chosen as the representative saponin ingredients.

### Venn analysis of active ingredient targets and type-2 diabetes target set

In this study, 292 targets of saponin ingredients and 792 targets of T2DM were screened from PharmMapper, SwissTargetPrediction, TargetNet, CTD and PharmGKB databases. The 91 intersection targets for T2DM treatment based on saponin were gained through Venn analysis of saponin ingredient targets and relevant targets of T2DM.

### Construction and analysis of PPI network

Based on PPI relationship, the interaction relations among therapeutic targets of T2DM based on stir-fried *Dolichos lablab L.* kernel were acquired from the STRING database in order to understand its action mechanism better. A total of 22 key genes were screened by using means of Degree, BetweennessCentrality and ClosenessCentrality as thresholds (Table 2). These targets are hub nodes of the network and they are core targets for treatment of T2DM.

Table 2 22 key genes

Gene	Degree	BetweennessCentrality	ClosenessCentrality
STAT3	33	0.080071042	0.572463768
MAPK1	32	0.081962504	0.580882353
SRC	29	0.056634532	0.530201342
IGF1	27	0.050415988	0.533783784
MAPK8	27	0.065736027	0.560283688
JUN	25	0.046517234	0.54109589
EGFR	25	0.050964745	0.54109589
PIK3R1	25	0.050340626	0.506410256
ALB	23	0.135327763	0.548611111
CASP3	20	0.045545357	0.523178808
HGF	20	0.033453303	0.506410256
AR	20	0.037843527	0.509677419
ESR1	20	0.02296979	0.512987013
MMP9	18	0.024822659	0.509677419
NOS3	17	0.046045739	0.516339869
SIRT1	17	0.036262484	0.503184713
CAT	12	0.065146404	0.475903614
HMOX1	12	0.037773667	0.478787879
F2	12	0.04549904	0.456647399
NR3C1	11	0.037173508	0.454022989
PPARG	10	0.041550519	0.448863636

Four key proteins of T2DM (EGFR, IGF1, MAPK1 and PIK3R1) were chosen for molecular docking verification.

**GO bioprocess analysis and KEGG metabolic pathway analysis**

GO enrichment analyzes functional distribution of key targets and key targets are in a descending sequence according to pvalue. GO shows top 10 items of BP, CC and MF, respectively (Fig.1). KEGG shows the top 20 significant pathways. Additionally, the bubble graph is drawn (Fig.2). PI3K-AKT signaling pathway (Fig.3) and EGFR tyrosine kinase inhibitor resistance (Fig.4) process.

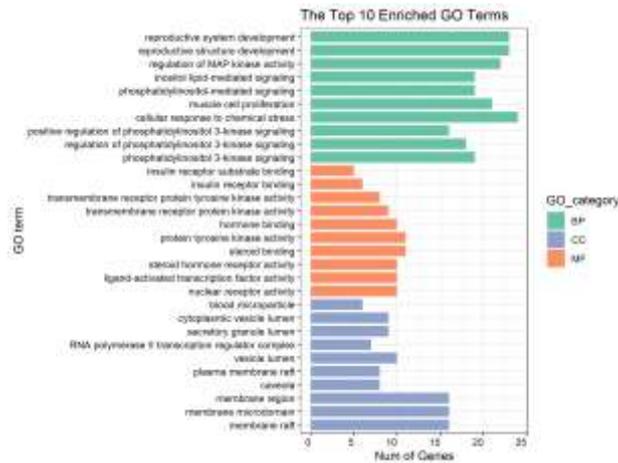


Fig.1 GO enrichment analysis of main active ingredients of stir-fried *Dolichos lablab L.* kernel

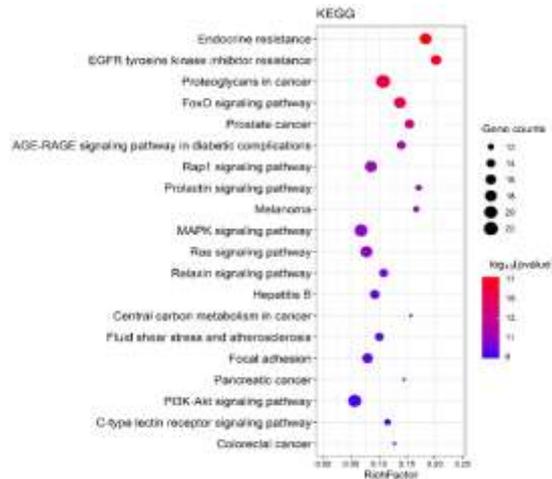


Fig.2 KEGG bubble graph of action targets of stir-fried *Dolichos lablab L.* kernel on T2DM

Extracellular cytokines like HGF and IL-2 of PI3K-AKT signaling pathway can activate the downstream AKT signal pathway through PI3KR1 and PIK3CG after combined with receptors (e.g. EGFR and CSFR) on membrane. The activated AKT2 can realize phosphorylation of NOS1, RAF1, BAD, CHUK and MDM2, thus promoting releasing of NO, activating MAPK signal pathway and facilitating generation of BCL2L1 and BCL2. These can influence apoptosis and NF-κB cell pathways, regulate effects of TP53 on p53 signal pathway, and control cell proliferation, angiogenesis, DNA

repair and cell survival.

The PIK3R1/AKT signal shaft, JAK/STAT3, SRC/STAT3 signal shaft and MAPK signal shaft are activated by combinations of EFG, TGFA, HGF, IGF1, VEGFA and FGF with receptors on membrane like EGFR, ERBB2, ERBB3, MET, IGF1R, KDR and FGFR2. AKT2 can promote phosphorylation of MTOR, BAD, GSK3B and FOXO3. Phosphorylation of MAPK1 can inhibit expression of BAX by promoting expression of BCL2L1 and BCL, thus inhibiting apoptosis. The phosphorylated FOXO3, STAT3 and MAPK1 can develop the transcriptional control effect after entering into nucleus. All of these downstream molecules regulate differentiation, growth, proliferation, survival and activity of cells as well as angiogenesis.

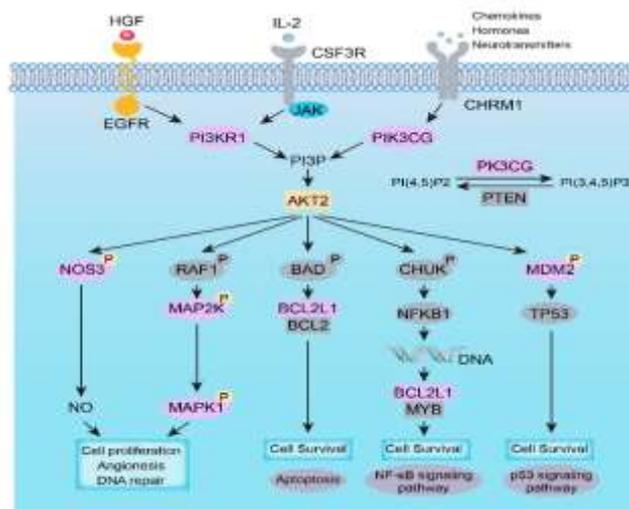


Fig.3 PI3K-AKT signaling pathway

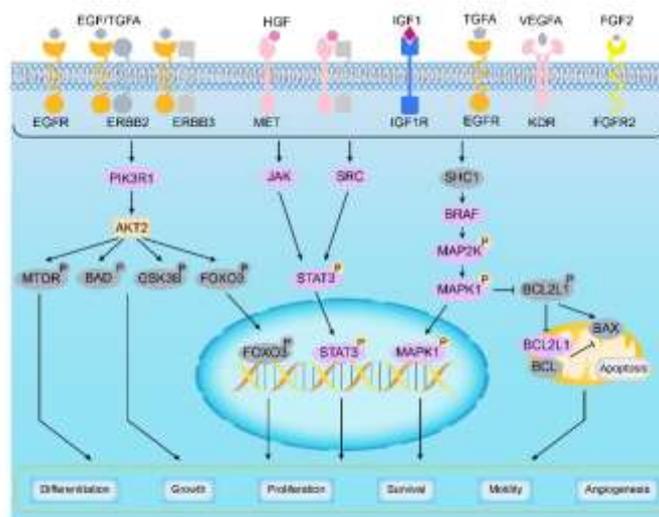


Fig.4 EGFR tyrosine kinase inhibitor resistance

### Building of an active ingredients-targets-pathway network

The Saponin active ingredients-targets-signal pathway network was built using Cytoscape3.8.0

(Fig.5). It can be seen from Fig.5 that there are 101 nodes (10 compounds and 91 targets) and 593 sides. The V-shaped nodes represent compounds, while the round nodes represent genes. Colors of nodes from light to dark represent the increasing values of node degree. The therapeutic mechanism of stir-fried *Dolichos lablab L.* kernelsaponin ingredients to T2DM is related with inflammatory response, autoimmune damage and several other morbidity theories. This study interpreted the therapeutic mechanism of stir-fried *Dolichos lablab L.* kernelsaponin ingredients to T2DM from different perspectives.

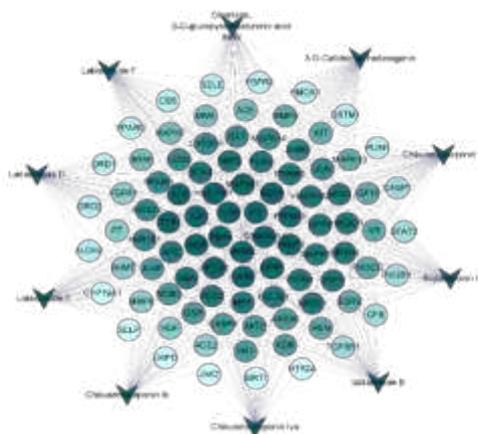


Fig.5 Saponin active ingredients-targets-signal pathway network

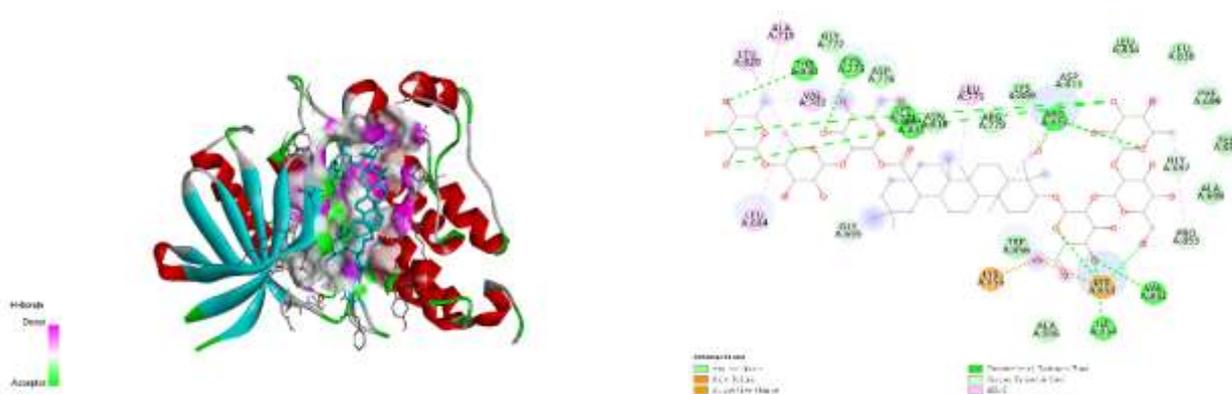
### Molecular docking

Molecular docking experiment between 10 saponin active ingredients and the screened key protein targets related with T2DM was carried out using AutoDock Vina\_1.1.2. AutoDock Vina evaluates the bonding strength between micromolecules and proteins mainly through affinity, that is, the fitted  $\Delta G$  value after calculation. If  $\Delta G$  value is lower than 0, the ligands can bind with receptors spontaneously. Moreover, the lower  $\Delta G$  value brings the higher affinity and the active ingredients are easier to bind with receptors<sup>[12]</sup>. Results are listed in Table 3. Graphs are drawn for each protein and the best docked compound in Table 3 (Fig.6). Clearly, lablaboside\_E enters into the active pockets of EGFR completely and forms one or two hydrogen bonds with active site residues Thr830, Cys773, Lys721, Asp831, Arg817, Val852, Ile854 and Lys851, thus forming 12 hydrogen bonds. Additionally, lablaboside\_E not only forms salt bridges with residues Lys855 and Lys851, but also forms Van Der Waals' force with residues Asp776, Asn818, Gly772, Arg779, Lys889, Leu834, Leu838, Phe699, Ser696, Ala698, Trp856 and Ala896. It also forms alkyl interactions with residues Ala719, Leu820, Val702, Leu775 and Leu694. The docking results reflect that lablaboside\_F is bonded on the surface of IGF1 and forms three hydrogen bonds with active sites. These hydrogen bonds involve residues Asp12, Glu9 and Cys6, respectively. Lablaboside\_F forms alkyl interactions with residues Ala13, Leu5, Leu57, Leu54 and Val11, and forms Van Der Waals' force with residues Phe16, Gln15, Glu3 and Phe23.

According to docking results, lablaboside\_F enters into the active pocket of MAPK1 and forms 14 hydrogen bonds with active site residues, mainly involving Gln105, Lys54, Asp167, Asn154, Tyr36, Ala35, Val188, Thr190, Trp192 and Arg191. Residues Val39, Ile31, Tyr113 and Tyr30 participate in the formation of interactions with alkyl groups or pi-alkyl groups, while residues Ile84, Leu156, Thr110, Ala52, Cys166, Lys114, Gly230, Ala189, Asp149, Leu170, Gly34, Ser153, Glu33 and Asp111 form Van Der Waals' force with lablaboside\_F. It can be seen from Fig.6 that chikusetsusaponin\_Iva enters into active site of PIK3R1 completely. It forms not only four hydrogen bonds with residues Arg1818, Gln1815, Gln1630 and Glu1849, but also Van Der Waals' force with residues Arg1852, Arg1281, Ser1275, Leu1279, His1759, Asn1756, Phe1794, Ile163, Met1811, His1670, Tyr1836, Pro1835, Gly1837 and Leu1755. Chikusetsusaponin\_Iva also forms alkyl interactions with residues Leu1834 and Val1851.

Table 3 Molecular docking affinity between saponin and key proteins

COMPOUND	EGFR	IGF1	MAPK1	PIK3R1
Lablaboside_F	-10	-7.6	-10.2	-9.5
Lablaboside_E	-10.7	-7.3	-10.2	-8.8
3-O-Cellobiosyl-hederagenin	-9.3	-6.8	-9.8	-9.5
Chikusetsusaponin_Ib	-9.7	-6.4	-9.7	-8.7
Oleanane	-8.8	-6.7	-9.7	-8.6
Soyasaponin_I	-10.1	-7.2	-9.5	-9.9
Chikusetsusaponin_V	-9.7	-6.9	-9.5	-8.7
Chikusetsusaponin_Iva	-10	-7	-9.4	-10.3
Lablaboside_D	-10.2	-5.7	-9.3	-7.8
lablaboside_B	-9.1	-6.3	-8.9	-7.2



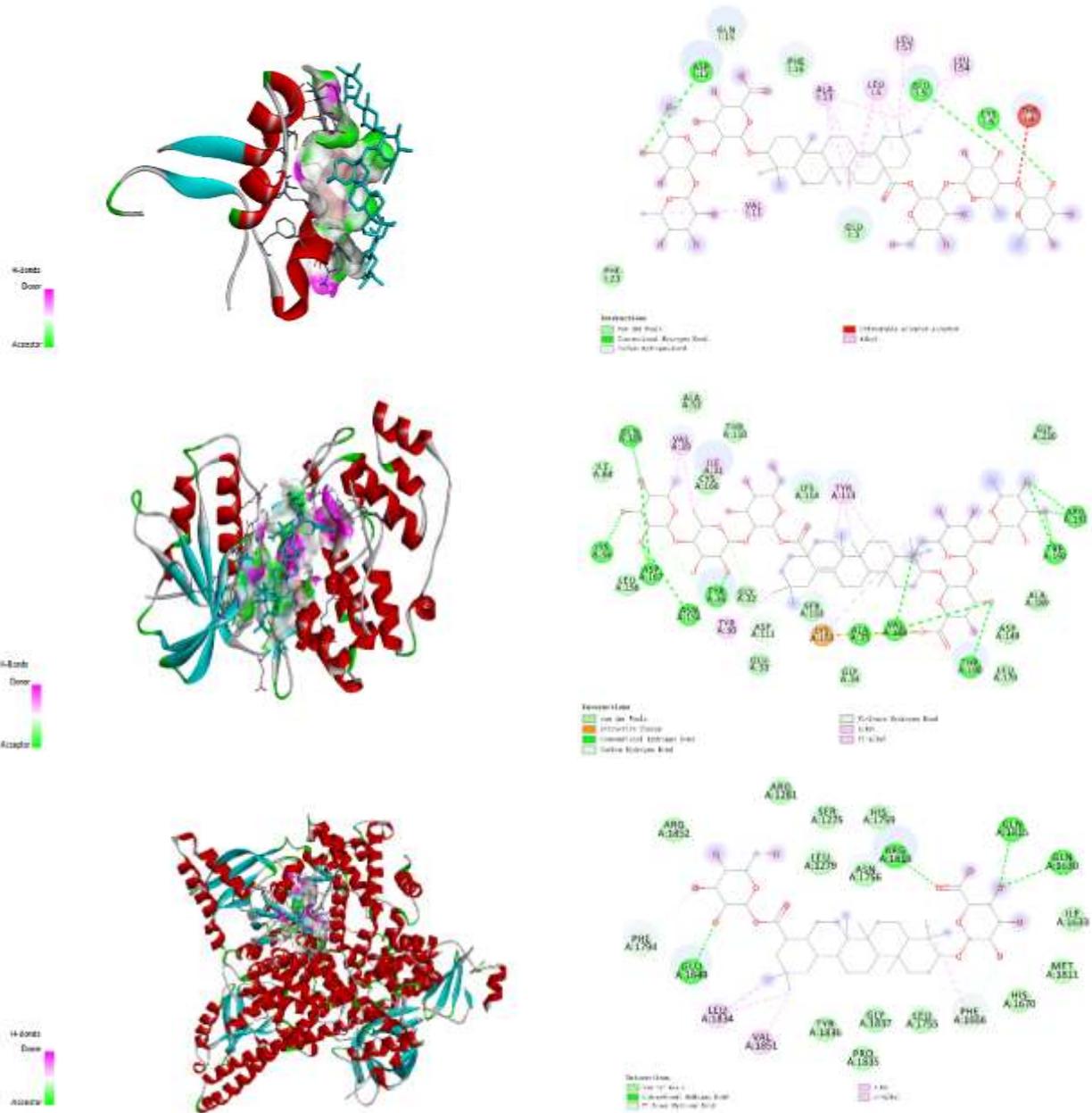


Fig.6 Docking mode analysis between the lowest-affinity ingredients and targets

### Molecular dynamics simulation and ligand channels

According to molecular docking results, molecular dynamics simulation on Chikusetsusaponin Iva which has relatively binding with the key protein PIK3R1 was carried out, which further verified stability and reasonability of docking results. The trajectory of protein rotating value is shown in Fig.7. This trajectory is used to study the mean distance of each protein atom to the center, thus illustrating closeness of each corresponding proteins. The trajectory of protein MSD is used to calculate the error between each ligand and protein.

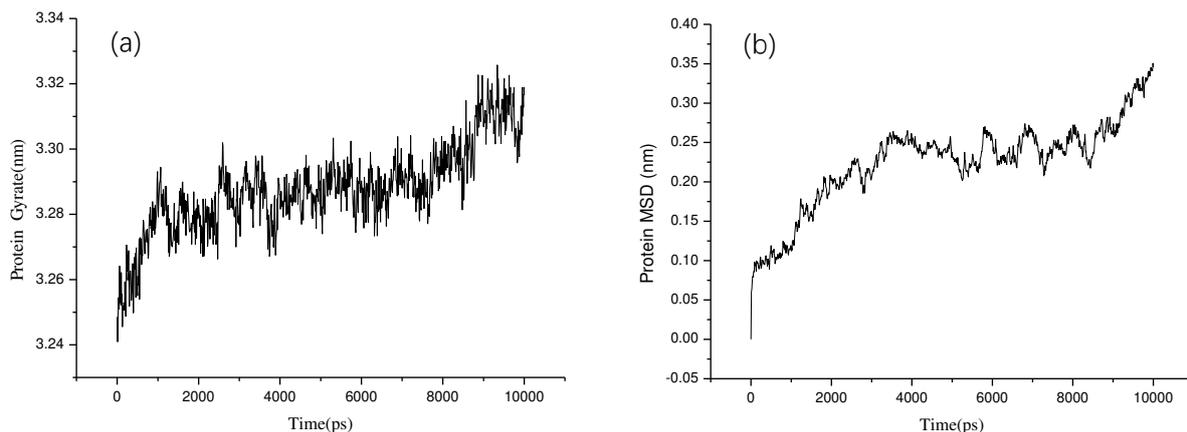


Fig.7 (a) Protein rotating value (b) MSD of proteins

The total energy trajectory graph can be used to evaluate stability of ligand-protein compound. The mean potential energy, kinetic energy and total energy which are needed for ligand-protein binding were calculated as -2923388.5KJ/mol, 552577.6KJ/mol and -2370811KJ/mol, respectively. Based on these parameters, binding stability of ligand-protein compounds was compared.

Representative structure of the ligand-protein compounds was discussed through clustering analysis. There are 7 classes of typical structures of Chikusetsusaponin Iva-protein compounds (Fig.8).

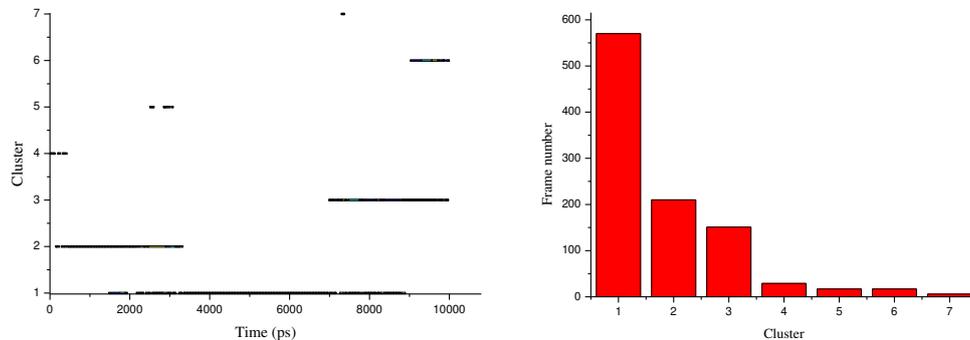


Fig.8 Clustering analysis

Three-dimensional simulation of ligand channels was constructed to estimate all possible channels for combination of ligands and proteins. Chikusetsusaponin Iva has 15 possible pathways (Fig.9).

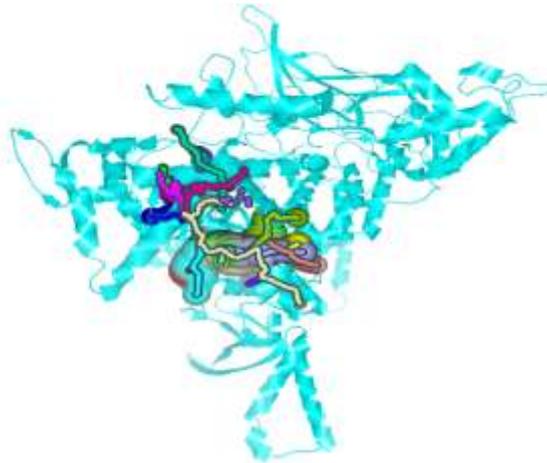


Fig.9 Three-dimensional simulation of ligand channels

## Discussions

Four key proteins, including EGFR, IGF1, MAPK1 and PIK3R1, were chosen. Chu et al. [13] discovered that systematic insulin-like growth factor-1(IGF1-1) therapy can reverse the hyperpathia phenomena of mice with diabetic peripheral neuropathy and improve their mobility. Mauvaris Jarvis<sup>[14]</sup> pointed that PI3 kinase plays the crucial role in metabolism of insulin. Moreover, the levels of p85 $\alpha$  and AS53 could be decreased by hybrid damages to PIK3R1 genes, thus enabling to increase insulin sensitivity and lower fasting and postprandial blood glucose levels. As result, morbidity of diabetes which is related with genetic insulin resistance is decreased significantly. MAPK3 and MAPK1 are two crucial MAPKs in MAPK/ERK cascading. MAPK/ERK pathway plays an important role in diabetes and its complications. It is reported that blocking EGFR can inhibit infiltration and oxidative stress of kidney immune cells and strengthen autophagy activity of pancreas, finally improving diabetic nephropathy<sup>[15]</sup>.

Compounds (Lablaboside E, Lablaboside F, Chikusetsu saponin Iva and Chikusetsu saponin Iva) which have strong bindings with 4 key proteins can protect hyperglycemia-induced INS-1 cells from functional damages. The possible molecular mechanisms might be attributed to the anti-apoptosis effect by activating the Akt/mTOR signal pathway. Mixtures of glucosides were gained through separation of seeds of *Dolichos lablab L.*. These mixtures possess outstanding activity of immunologic adjuvants. Six oleanane-type bisaponin compounds which are separated and identified from these glucoside mixtures with activity of immunologic adjuvants are named Lablaboside A, B, C, D, E and F. Lablaboside A, B, C, D, E and F not only have immunological enhancement, but also can lower blood glucose<sup>[1]</sup>. The blood glucose lowering mechanism might be related with improving immunological functions of patients with diabetes, adjusting combination of insulin and receptors, and increasing sensitivity of body to insulin.

According to metabolic pathway analysis, EGFR signal pathway is the pathway with a relatively

high enrichment factor. Pathways which have relatively more enrichment targets include PI3K-Akt signal pathway, MAPK signal pathway, Ras signal pathway, Rap1 signal pathway, and pathways of proteoglycans in cancer. Some studies demonstrate that therapeutic pathways of T2DM based on saponin of stir-fried *Dolichos lablab L.* kernel include cancer pathways like proteoglycans in cancer. According to a large-scaled epidemiological investigation, morbidity of cancers at some specific positions in patients with T2DM is significantly higher compared to that of other patients<sup>[16]</sup>. In addition, cancer mortality of patients with T2DM is relatively higher. The mechanism can be interpreted by that the effect of hyperinsulinemia on microenvironment of cancer cells and signal transduction in cells of diabetic patients is in favor of tumor growth<sup>[17]</sup>. Rap which belongs to Ras family has two subtypes: Rap1 and Rap2. Rap controls many important signal pathways in cells. On one hand, these pathways are closely related with important biological functions, such as formation of cell polarity, cell proliferation, differentiation and canceration, cell adhesion and movement. On the other hand, they further affect some important physiological functions of tissues and organs, such as establishing of nerve polarity, synaptic growth, synaptic plasticity, neuronal migration, and so on.

GO analysis finds that main enrichment entries of molecular functions include steroid hormone receptor activity, steroid binding, protein tyrosine kinase activity, insulin receptor binding and hormone binding. Main enrichment entries in the biological process (BP) include cellular response to chemical stress, regulation of MAP kinase activity and reproductive system development. Main enrichment entries of cellular component (CC) mainly distribute in membrane region, vesicle lumen and membrane microdomain. According to GO analysis, therapeutic mechanism of T2DM based on saponin of stir-fried *Dolichos lablab L.* kernel might lie in the binding between insulin and specificity insulin receptor on plasma membrane. Defects of receptors can influence the downstream signal network. Insulin receptor level covers the affinity between insulin and insulin receptor as well as the insulin receptor tyrosine kinase (IRTK).

It can be seen from molecular docking experiment that all compounds can enter into the active sites of targets and form 3-14 hydrogen bonds with active site residues. Moreover, Van der Waals' force exists universally between compounds and active sites. According to affinity, all compounds form strong binding with targets, indicating that compounds can treat T2DM by adjusting activity of these targets.

## **Conclusion**

Based on acquisition of accurate chemical ingredients, active ingredients of stir-fried *Dolichos lablab L.* kernel for treatment of T2DM and relevant therapeutic mechanism are discussed in this study by combining UHPLC-Q-Exactive Orbitrap MS analysis and network pharmacology. Research conclusions lay a solid foundation for identification of effective ingredients, screening of mass markers

and interpretation of action mechanism of stir-fried *Dolichos lablab* L. kernel.

#### **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

#### **Abbreviations**

T2DM: type-2 diabetes

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##### **Contributions**

J H responsible in the study design and experiments and wrote the manuscript. LZF and QFZ carried out the experiments and MS analysis. All authors read and approved the final manuscript.

##### **Corresponding authors**

Correspondence to Jun Han

##### **Ethics declarations**

##### **Ethics approval and consent to participate**

Not applicable.

##### **Consent for publication**

Not applicable.

##### **Competing interests**

The authors declare that they have no competing interests.

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ginseng in treatment of type-2 diabetes [J]. Journal of Guangdong Pharmaceutical University, 2020, 36(01): 50-59.

# Figures



**Figure 1**

GO enrichment analysis of main active ingredients of stir-fried *Dolichos lablab* L. kernel

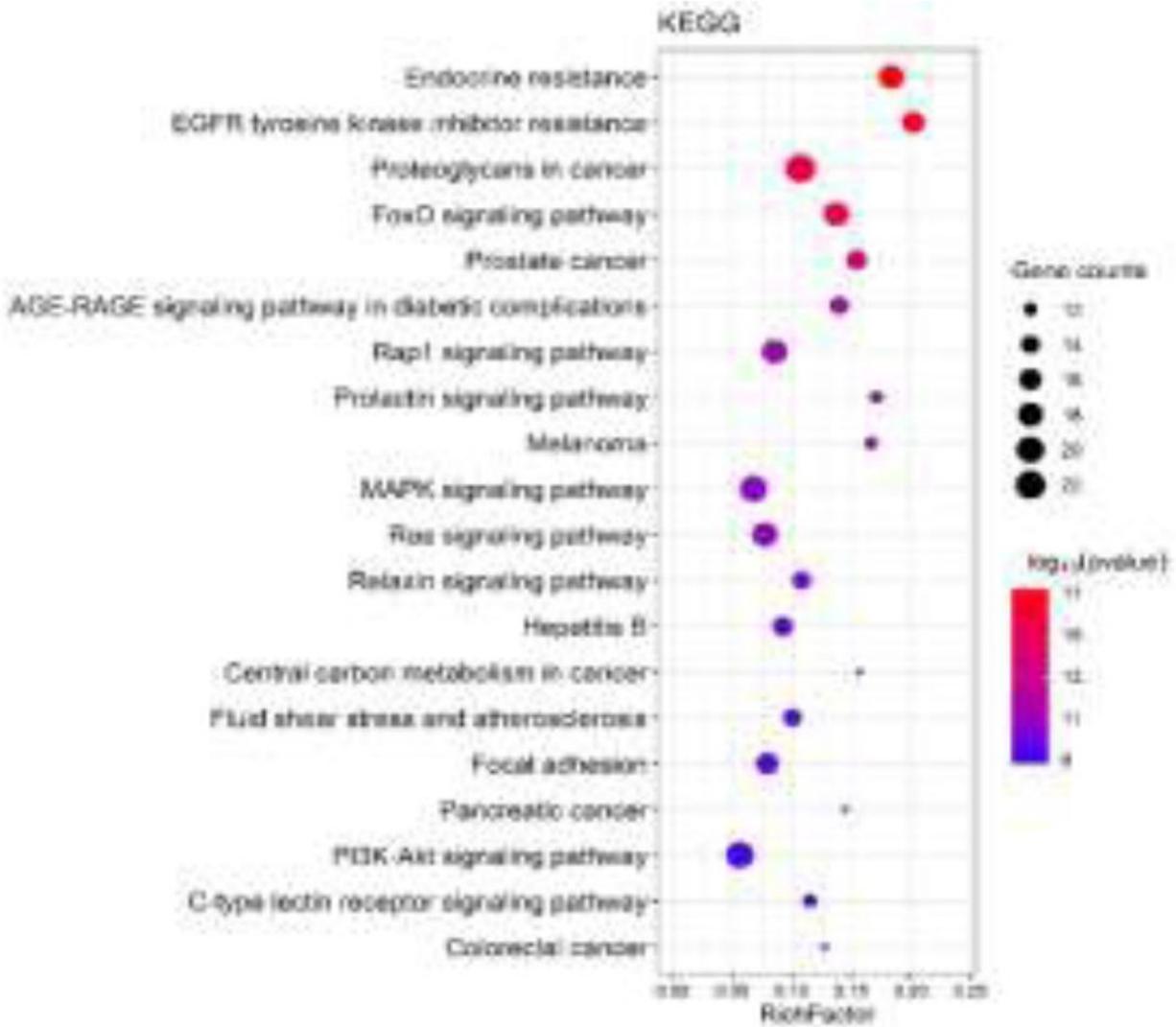


Figure 2

KEGG bubble graph of action targets of stir-fried *Dolichos lablab* L. kernel on T2DM

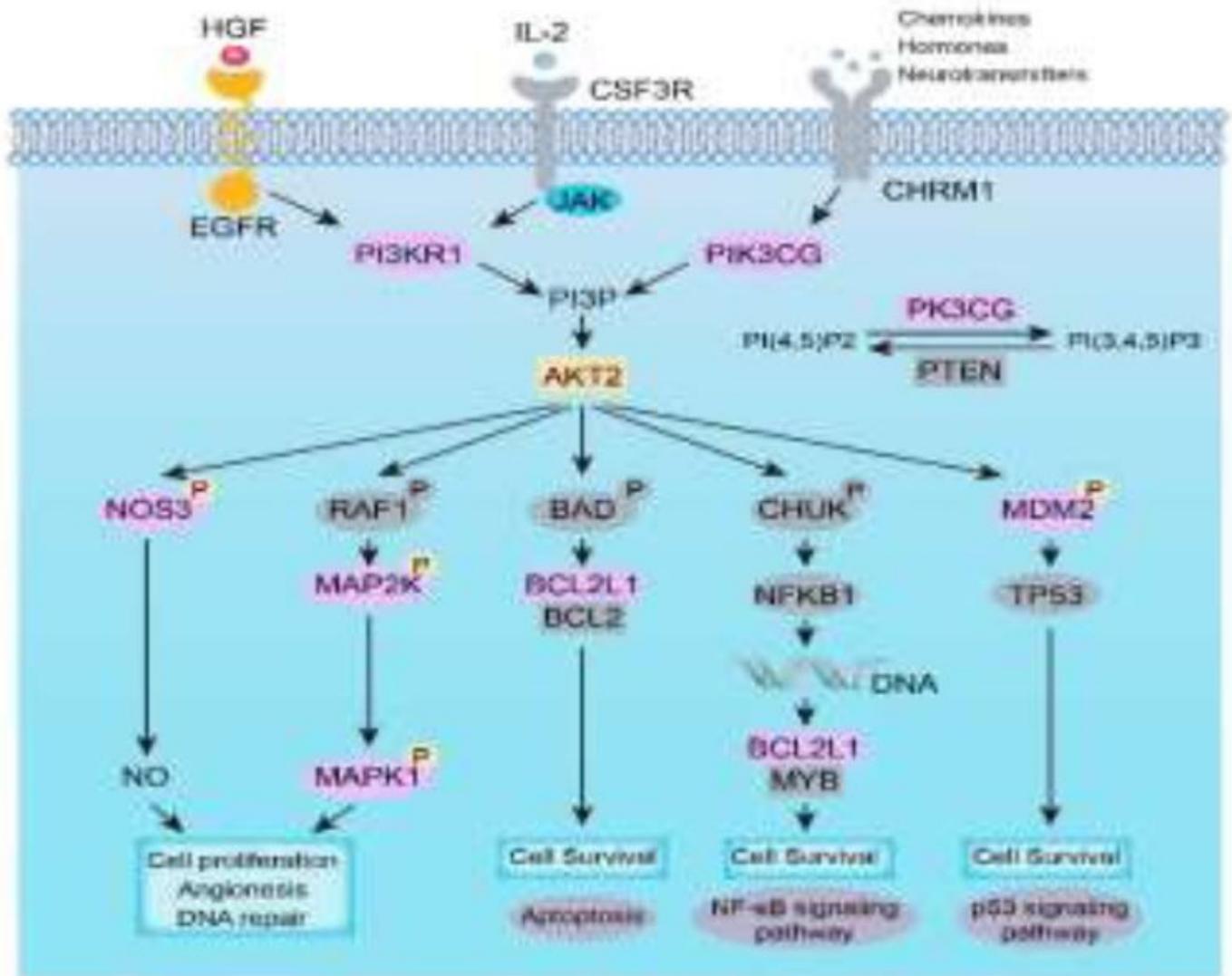


Figure 3

PI3K-AKT signaling pathway

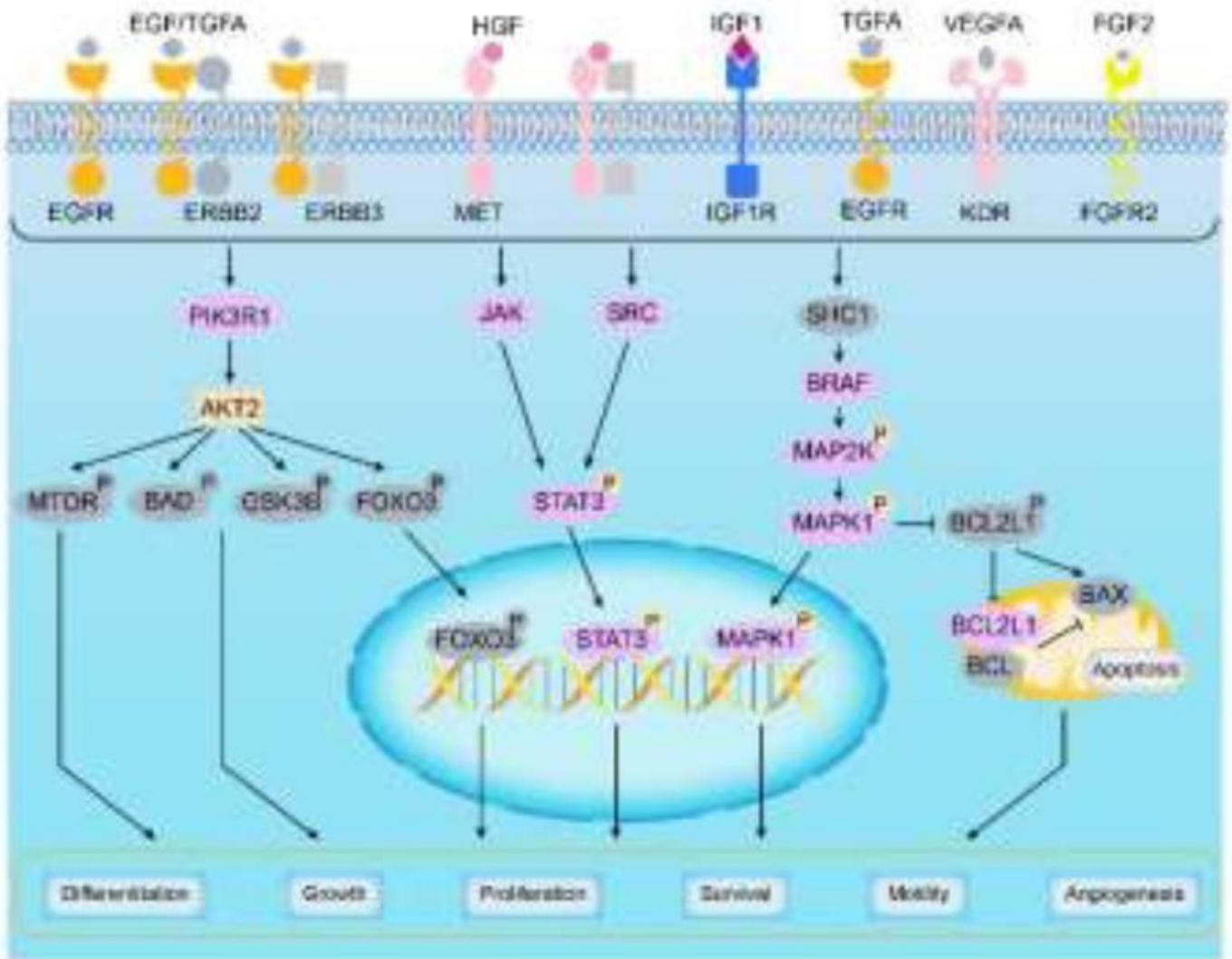


Figure 4

EGFR tyrosine kinase inhibitor resistance

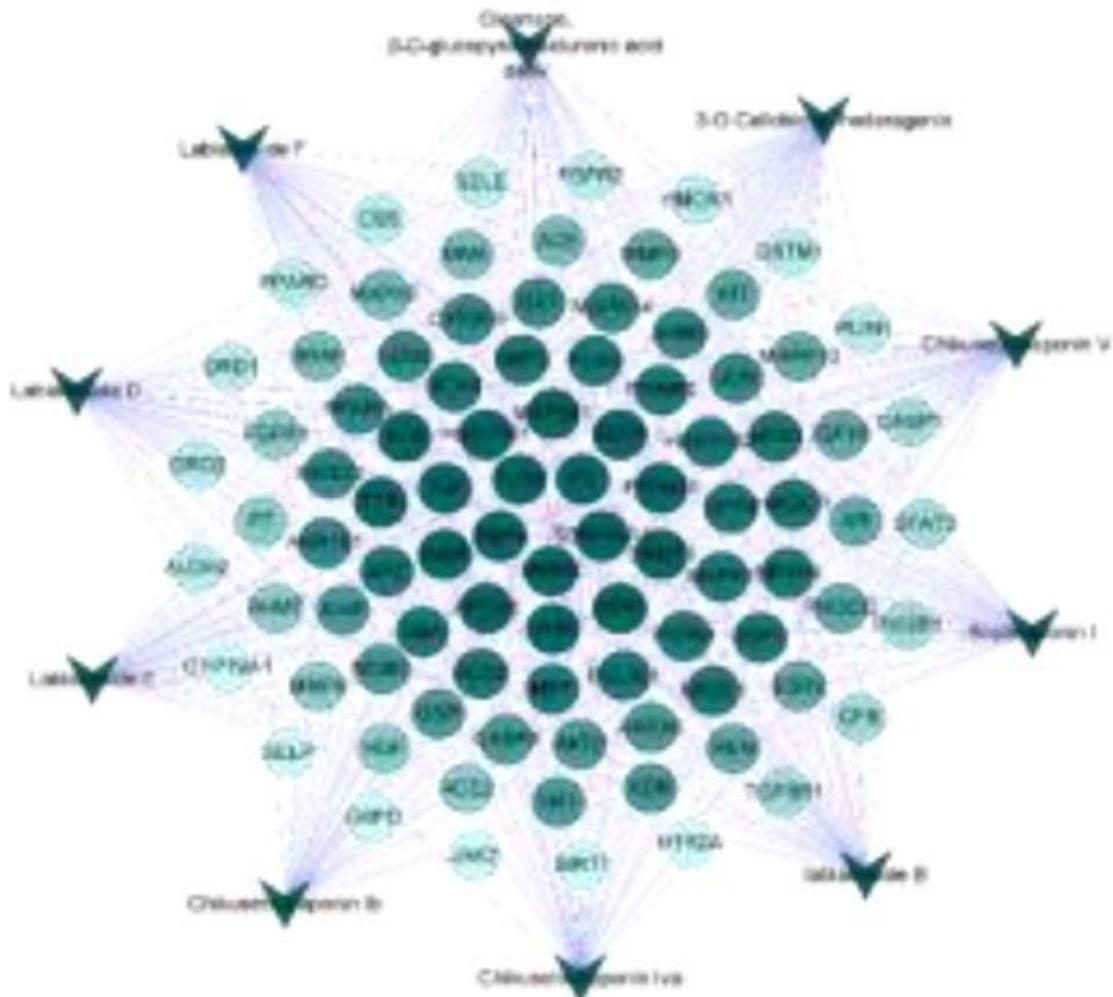
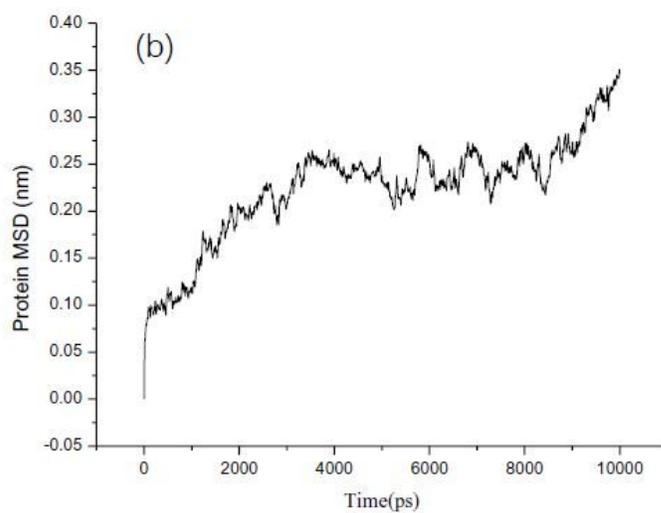
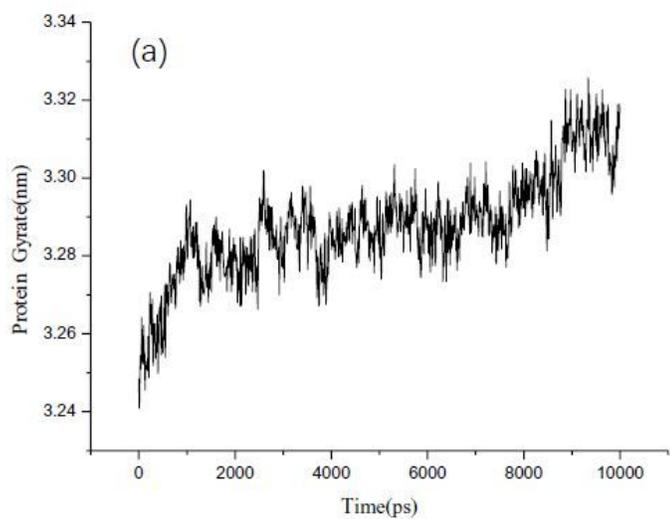


Figure 5

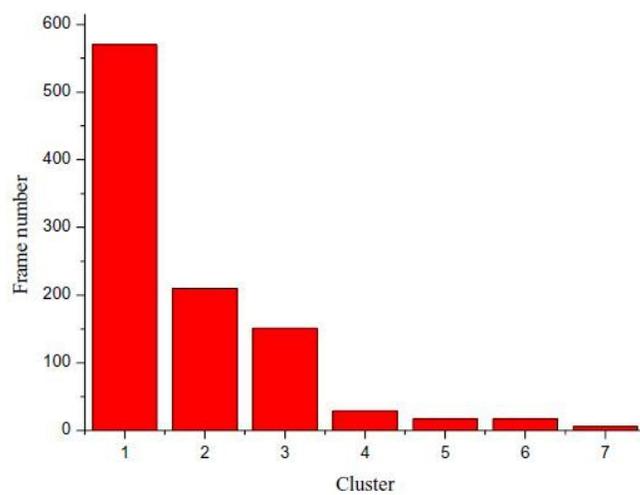
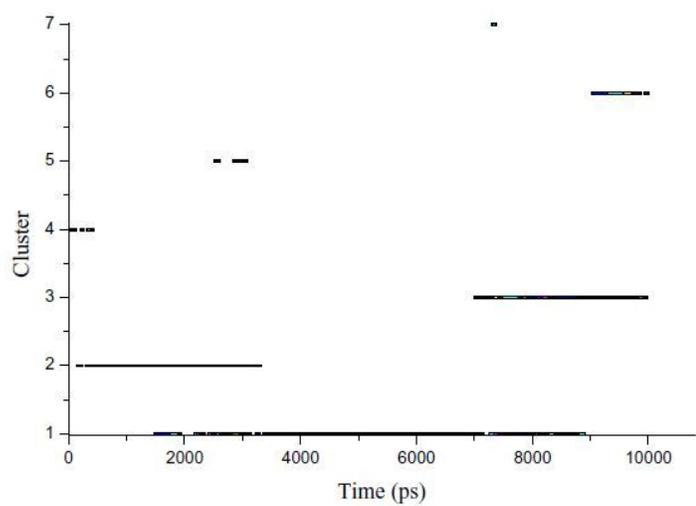
Saponin active ingredients-targets-signal pathway network





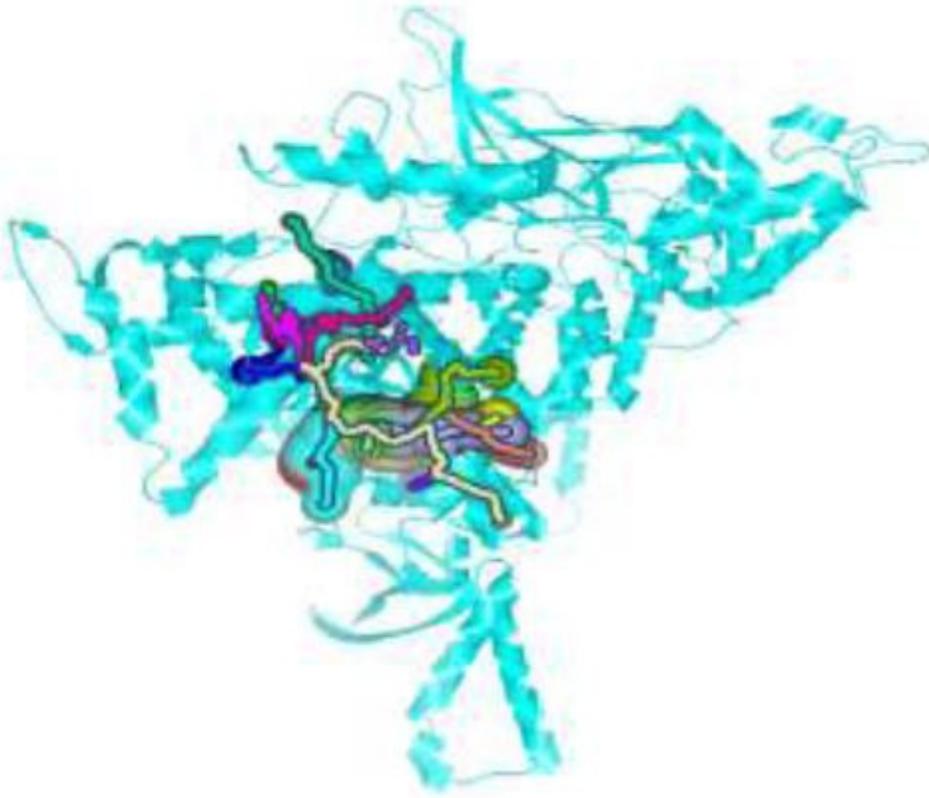
**Figure 7**

(a) Protein rotating value (b) MSD of proteins



**Figure 8**

Clustering analysis



**Figure 9**

Three-dimensional simulation of ligand channels