

# Phytonutrient and anti-diabetic functional properties of flavonoid-rich ethanol extract from *Angelica Keiskei* leaves

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**Abstract** An extraction method was optimized to get a flavonoid-rich ethanol extract from *Angelica keiskei* leaves (FREE-AK). Trace elements and total flavonoid content of FREE-AK were identified, and the hypoglycemic and hypolipidemic effects of FREE-AK were studied in streptozotocin-induced diabetic mice. For FREE-AK extraction the optimal conditions were 65% ethanol, 45 °C and 15 min, resulting in a total flavonoid content up to 10.18% and K, Mg, Na, and Ca, content of about 36.59, 1.52, 14.51 and 7.486 mg/g, respectively. FREE-AK uptake could cause a marked decrease of fasting blood glucose and a significant improvement on glucose tolerance in diabetic mice. In addition, FREE-AK treatment with a dose of 800 mg/kg b.w. resulted in a reduction in the total triglyceride level (TC) in serum. Results demonstrated the effectiveness of FREE-AK for hypoglycemia and hypolipidemia in streptozotocin-induced mice and FREE-AK may be a potential dietary treatment for type 2 diabetes.

**Keywords** *Angelica keiskei* leaves · Flavonoid · Type 2 diabetes · Blood glucose · Glucose tolerance

## Introduction

*Angelica keiskei*, a perennial plant which belongs to the *Umbelliferae* family, is a dark green leafy vegetable that has been widely cultivated in Asian countries, mainly China and Japan. It is used as an important natural medicinal herb, studies have demonstrated that *Angelica keiskei* possesses several bioactivities, suppression of gastric acid secretion, anti-tumourigenic properties, anti-thrombosis properties, anti-hypertension properties, suppression of histamine secretion, and vasodilation (Li et al. 2009). Because of these perceived medicinal properties, fresh leaves of *Angelica keiskei* are typically either stir-fried and eaten as salad or consumed as a raw vegetable. In addition, the roots and stems of these plants are used to generate health-care products, including tea bags and capsules (Chen 2004).

The benefits of *Angelica keiskei* to human health are due to nutrients contained, such as trace elements (Chen 2004), lutein, trans- $\beta$ -carotene, and total phenols (Li et al. 2009). Literatures have demonstrated that the roots of *Angelica keiskei* are rich in chalcones, including xanthoangelols and 4-hydroxyderricin. These special chalcones have been found to provide beneficial effects including vasodilatation, anti-tumor and anti-metastatic activities (Kimura and Baba 2003; Sugii et al. 2005). While the majority of studies have focused on the root of *Angelica keiskei* due to its high levels of chalcones, there also exists evidence that extract of *Angelica keiskei* leaves also exerts numerous bioactivities. However, few studies have been carried out to evaluate the functional effects of *Angelica keiskei* leaves.

Diabetes mellitus (DM) is a metabolic disorder that is characterized by a lack of insulin secretion or weakened sensitivity to insulin, resulting in chronic hyperglycemia (Zheng et al. 2011). Current treatments for DM are largely

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based on oral hypoglycemic agents, including biguanides and insulin. However, these drugs possess toxic side effects following prolonged use (Birsoy et al. 2014).

Numerous medicinal plants have been reported to be effective for the treatment of DM. These plants contain specific chemical constituents, such as flavonoids, which have been shown to lower both blood glucose and lipids (Ogawa et al. 2007; Zheng et al. 2011). Compounds containing flavonoids that were isolated from *Angelica keiskei* have been reported to function as an  $\alpha$ -glucosidase inhibitor and gene regulator (Ohnogi et al. 2012). This type of inhibitor has been shown to prevent dysfunction of  $\beta$ -cell insulin secretion in diabetic patients and could also potentially act to suppress the progression of diabetes (Luo et al. 2012; Sugii et al. 2005), while the gene being regulated to more expression were acyl-CoA oxidase 1, medium-chain acyl-CoA dehydrogenase (MCAD), ATP-binding membrane cassette transporter A1 (ABCA1) and apolipoprotein A1 which are related to high-density lipoprotein.

Type 2 diabetes have developed into a worldwide concern health problem. The goal of type 2 diabetes treatment is to control the blood glucose and avoid diabetes-related complications, such as weight gain, worsened insulin resistance, high cardiovascular (Bodegard et al. 2013; Ross et al. 2011). Metformin is a classical oral anti-diabetes drug that has been used for more than half a century for management of type 2 diabetes. It is a good sodium-glucose co-transporter 2 inhibitors which could reduce blood glucose and body weight significantly and has a positive impact on blood pressure (Yang and Chan Chan 2014). Also streptozotocin (STZ) is the most usual substances used to induce type 2 diabetes in the rat (Szkudelski 2001). The aim of the current study is to prepare and study phytonutrients of FREE-AK to further investigate its anti-diabetic activity in STZ-induced diabetic mice.

## Materials and methods

### Materials

*Angelica keiskei* was cultivated under the woods and harvested in Qibao Agricultural Experiment Field of the College of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China. Preparation of *Angelica keiskei* leaves was carried out by washing residue with water and drying naturally. Fresh *Angelica keiskei* leaves were provided by Experimental Center belonged to the College of Agriculture and Biology College of Agriculture and Biology. Streptozotocin (STZ) and metformin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Male ICR mice were obtained from The Experimental

Animal Breeding Centre (Shanghai, China). All other chemicals used in these studies were of analytical grade.

### Preparation of flavonoid rich ethanol extract from *Angelica keiskei* leaves

Fresh *Angelica keiskei* leaves were cut into small pieces, freeze-dried at  $-70\text{ }^{\circ}\text{C}$  for 20 h, and converted into a powder in order to ensure that all material could pass through mesh with a pore size no greater than 0.5 mm. A total of 500 g of sample powder was placed into a beaker and was macerated using ethanol–water mixtures as extraction solvents for the reason that ethanol is less toxic and can be easily recovered by reduced pressure distillation, factors related to flavonoids extraction-ethanol concentration (v/v), extraction time and temperature were investigated, appropriate experimental ranges of the factors were established by single factor experiment to optimize the *Angelica keiskei* leave extraction process. Samples were first extracted using different ethanol concentrations (40–90%), different time points (20–100 min) and different temperatures (30–50  $^{\circ}\text{C}$ ). Temperature was controlled using a thermostat-controlled water bath. After the extraction, sample was cooled with water and centrifuged at 8000 rpm at 4  $^{\circ}\text{C}$  for 20 min. The supernatant was collected and dried in a rotatory evaporator then lyophilized and stored at 4  $^{\circ}\text{C}$ , protected from light. Based on the information provided by single factor experiments, the  $L_9$  ( $3^4$ ) orthogonal table was chosen in order to optimize the extraction conditions in this study (Table 1).

### Flavonoids

The determination of flavonoids was carried out according to a previously described procedure with minor modifications (Zhishen et al. 1999). Briefly, 1 mL of 1 g/kg ethanol extract solution was placed in a 10 mL volumetric flask for 6 min, then 0.4 mL  $\text{NaNO}_2$  solution (5%, w/v) and 0.4 mL of  $\text{Al}(\text{NO}_3)_3$  solution (10%, w/v) were added and kept for 6 min. Then 4 mL of NaOH solution (5%, w/v) was added and the final volume was brought to 10 mL with distilled water. The solution was mixed for 15 min and the absorbance was measured against a blank at 510 nm using a

**Table 1** Three-factor-three-level orthogonal design for optimizing experiment factors

Level	A Ethanol concentration (%)	B Time (min)	C Temperature ( $^{\circ}\text{C}$ )
1	60	15	40
2	65	25	45
3	70	35	50

spectrophotometer (Unico 2100, Shanghai, China). Rutin was used as a standard.

### Nutritional constituents

The nutritional constituents of ethanol extract powder of *Angelica keiskei*, including protein, polysaccharide, and mineral elements were analyzed in this study.

The total sugar content in freeze-dried ethanol extract powder was measured with the phenol–sulfuric acid assay using ultraviolet spectrophotometry. Glucose was used as a standard in this assay, according to the procedure described by Pierre et al. (2012).

Protein content was determined using Lowry protein assay, and bovine serum albumin was used as a standard (Wang et al. 2014).

Mineral elements were measured using induced coupled plasma-atomic emission spectroscopy (ICP-AES, USA), as described by Woo et al. (2002).

### Animal experimentation

The Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985) were strictly followed throughout animal studies (Zhu et al. 2008). The protocol was carried out as follows:

Male ICR mice weighed between 17 and 20 g were maintained in polypropylene cages (seven mice per cage) at an ambient temperature of  $23 \pm 2$  °C with  $55 \pm 10\%$  relative humidity with 12 h light/12 h dark cycle in the animal house. Mice were treated according to the ethical guidelines of the Animal Center, Shanghai Jiao Tong University.

35 mice were on a conventional diet (5 g/day for feed and 6 mL/day for water) for a week and then divided into two species based on body weight, non-diabetic control mice and STZ-induced diabetic mice. Seven mice were prepared for non-diabetic control mice and twenty-eight for STZ treatment. STZ treatment was prepared by intraperitoneal injection of a freshly prepared solution of STZ dissolved in citrate buffer (pH 4.5). A dose of 40 mg/kg b.w. was given once a day, for five consecutive days. Five days following STZ administration, whole blood samples were obtained from the tail vein of the mice and glucose levels were measured using a One Touch glucometer (Johnson & Johnson Medical. Ltd. USA). Mice with blood glucose levels greater than 16.7 mmol/L were considered to be diabetic and used for the study.

The mice were divided into the following five groups with seven mice per group.

Group I (NC): Normal control mice were administrated 10 mL/kg of 0.9% saline solution containing 1% Tween 80.

Group II (DM-C): Diabetic control mice were administrated 10 mL/kg of 0.9% saline solution containing 1% Tween 80.

Group III (DM-M): Diabetic mice were administrated 200 mg/kg b.w. metformin dissolved in 0.9% saline solution containing 1% Tween 80.

Group IV (DM-LFREE-AK): Diabetic mice were administrated 400 mg/kg b.w. FREE-AK powder dissolved in 0.9% saline solution containing 1% Tween 80.

Group V (DM-HFREE-AK): Diabetic mice were administrated 800 mg/kg b.w. FREE-AK powder dissolved in 0.9% saline solution containing 1% Tween 80.

All groups were administered an oral treatment once a day for 28 days. On the 28th day of the experiment, the animals were deprived of food overnight prior to being sacrificed. Blood samples obtained from tails were separated by centrifugation for 5 min and stored at  $-80$  °C.

### Biochemical assays

Throughout the course of the 4 weeks treatment period, the fasting blood glucose (BG-F) was measured weekly from tail vein blood samples using the One Touch glucometer.

Oral glucose tolerance test (OGTT) was carried out after the 28 days treatment. Briefly, animals were fasted for 12 h and then orally fed with 2 g/kg b.w. of glucose following a 60 min drug administration. BG-F was measured at 0, 30, 60, 90 and 120 min with a One Touch glucometer.

Following 28 days of treatment, blood samples were obtained from the eyeballs of mice. Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were measured using an automatic blood chemical analyzer (Beckman Coulter DXC800, CA 92821, American).

### Statistical analysis

Results are presented as mean  $\pm$  SE. Test were performed in triplicate for each group and sample. The significance of difference versus the control group was determined by Student's *t* test (SPSS Program; Version 13.0). Difference among groups were analyzed by one-way ANOVA. The level of difference was set at  $p < 0.05$  or  $p < 0.01$ .

## Results and discussion

### Optimization of extraction of total flavonoids from *Angelica keiskei* leaves

*Angelica keiskei* has been reported to contain bioactive chalcone and flavanone, which have been characterized as

**Table 2** Results of orthogonal experiment for extraction flavonoid from *Angelica Keiskei* leaves

No.	A	B	C	TFA (wt%)
1	1	1	1	7.18
2	1	2	2	7.69
3	1	3	3	8.85
4	2	1	2	10.34
5	2	2	3	7.62
6	2	3	1	8.55
7	3	1	3	6.30
8	3	2	1	6.53
9	3	3	2	5.74
K1	7.907	7.940	7.420	
K2	8.837	7.280	7.923	
K3	6.190	7.713	7.590	
R	2.647	0.660	0.503	

special anti-diabetic flavonoid substances (Salahuddin and Jalalpure 2010). Thus, flavonoids which are reported to have the ability of anti-diabetic were studied in ethanol extract of *Angelica keiskei*. Results demonstrated significant difference in total flavonoid (TFA) content depending on the ethanol extraction parameters. The highest TFA yield was achieved when the extraction was carried out using 60% (v/v) ethanol concentration, extraction time of 20 min, and an extraction temperature of 45 °C.

In order to optimize the extraction process to generate the highest TFA yield from *Angelica keiskei* leaves, we used the extract conditions described above which we showed to yield the highest content of TFA. These different factors were chosen as the central condition of the L<sub>9</sub> (3<sup>4</sup>) orthogonal experiment, with results shown in Table 2. According to the value of extreme difference (R value), which reflects the difference between the maximal mean value and the minimum mean value for TFA content recovered with each factor, we found that the ethanol concentration produced the most significant effect on TFA yield (2.65), followed by extraction time (0.66), and then extraction temperature (0.50). Moreover, the K value for these three factors had the highest value at level 2, level 1 and level 2, respectively. This indicated that the optimal parameters for extraction were an ethanol concentration of

65%, an extraction time of 15 min, and an extraction temperature of 45 °C. Results from experiments repeated in triplicate that were carried out using the above predicted optimal conditions demonstrate that the mean TFA yield in the ethanol extract was increased to 10.18 ± 0.2%. This yield was higher than that of flavonoids identified in methanol extract from *Angelica keiskei* leaves, which was reported to be 7.58 ± 0.35 mg/g (D. W. Kim et al. 2014).

**Phytonutrients of powdered FREE-AK**

The main phytonutrient contents of powdered FREE-AK, with the exception of TFA, were analyzed (Table 3). Protein and total sugar content were 23.50 and 17.80%, respectively. Both protein and sugar content were determined to be significantly higher than that of TFA, demonstrating that the powdered FREE-AK was consisted of numerous nutrients. In addition, powdered FREE-AK was found to contained high levels of essential mineral elements, including K (36.59 mg/g), Mg (1.52 mg/g), Na (14.51 mg/g), and Ca (7.486 mg/g). These levels were significantly higher than the concentrations of corresponding elements found in fresh leaves and stems of *Angelica keiskei*, as reported by Chen (2004) and as shown in Table 3. Moreover, the levels found in general plants were 0.5–1.0 mg/g of Ca, which was significantly lower than that found in powdered FREE-AK from *Angelica keiskei* leaves, as shown in Table 3 (Fang and Li 2014). The high levels of the above-mentioned mineral elements play numerous critical roles in maintaining human health. For example, potassium (K) is an essential element of life that plays a role in maintaining stable blood pressure, or even lowering both systolic and diastolic blood pressure (Houston and Harper 2008). Therefore, because powdered FREE-AK was found to contain numerous phytonutrients, with notably high levels of total flavonoids and potassium, powdered FREE-AK could have numerous applications as a type of functional anti-diabetic food.

**Effect of FREE-AK on BG-F of mice in the experimental period**

The effect of FREE-AK on the fasting blood glucose levels of diabetic mice was determined and the results are

**Table 3** Phytonutrients contained in FREE-AK

Phytonutrients	Content (%)	Phytonutrients	Content (%)
Total sugars	17.80 ± 0.50	K	3.659 ± 0.030
Total protein	23.50 ± 1.10	Mg	0.152 ± 0.002
Ca	0.7486 ± 0.0044	Mn	0.0015 ± 0.0002
Cu	0.0088 ± 0.0002	Na	1.4510 ± 0.0060
Fe	0.0038 ± 0.0000	P	0.1353 ± 0.0003

summarized in Table 4. The steady increase of blood glucose levels in Group II over the 4 weeks demonstrated that a diabetic animal model was successfully established.

The BG-F levels (8.56 mmol/L) of mice in the NC group remained stable throughout the course of the 4 weeks and were found to be significantly lower ( $p < 0.05$ ) than those of the STZ-induced mice in the other four experimental groups. Blood glucose levels of the treatment groups (III), DM-LFREE-AK(IV), DM-HFREE-AK(V) changed from 9.92 to 11.25, 12.66–13.96, and 11.04–12.63 mmol/L, respectively. Fasting blood glucose levels of the treatment groups, DM-LFREE-AK, DM-HFREE-AK, and the positive control group, DM-M, were found to be significantly decreased with time ( $p < 0.001$ ), but still much higher than the DM-C group. However, BG-F levels of the DM-HFREE-AK and DM groups were found to be lower than 16.7 mmol/L at week 4, which is considered to be a tipping point for a diabetic. The levels of BG-F were found to decrease in proportion to the amount of FREE-AK consumed.

Factors that contribute to abnormal blood glucose levels are primarily the body's resistance to insulin and a decreased ability of pancreatic  $\beta$  cells to produce insulin (Schrauwen 2007). Blood glucose levels are closely related to HbA1c, and detection of them can provide clear data for diabetes. Plant extracts were reported to act on blood levels through insulin release-stimulatory effects (Kumar and Dey 2003). This may be the mechanism by which FREE-AK extract possesses significant effects on hyperglycemia in diabetic mice induced by streptozotocin.

### Effect of FREE-AK on oral glucose tolerance

In OGTT, the BG-F levels of all groups of animals were estimated at time points from 0 to 120 min. The blood

glucose reached maximum levels in all five groups 30 min following oral glucose administration. During the 120 min, all the groups indicated significant difference ( $p < 0.001$ ) with DM-C group (II). The levels of BG-F in DM-C were higher than other group during the 120 min indicated that STZ-induced diabetic mice model was successfully established. The decreases of BG-F in group III, IV and V during 30–120 min indicated that metformin and FREE-AK with different doses had positive effects on controlling BG-F. These suppression effects on blood glucose levels was found to persist until the blood glucose level reached the initial level. As come to the treatment groups, though the BG-F was higher than NC group, it appeared that the levels of BG-F in FREE-AK treated rats reduced to near nearly two folds below as compared to BG-F in STZ-rats. Furthermore, the effect of HFREE-AK group on a reduction of BG levels in STZ-rats was nearly equal to BG in MF-treated rats (Table 5).

These findings also confirmed that action of antihyperglycemic begins during 30–120 min after treatment, and the results were consistent with what was observed with STZ-rats that were fed *Selaginella tamariscina* flavonoids (Zheng et al. 2011). Connecting peptide, which is necessary for the formation of insulin by helping to link chains and fold, was reported to increase following flavonoid consumption (Kim et al. 2017; Steiner et al. 1967). Thus, FREE-AK may affect the regulation of insulin levels in order to enhance the utilization of glucose and result in a significant decrease in blood glucose levels in glucose-loaded mice.

### Effects of FREE-AK on body weight

The changes in body weight as an effect of FREE-AK are depicted in Table 4. At the beginning of the experiment, there were no statistically significant differences in body

**Table 4** Effect of powdered FREE-AK on BG-F and bodyweight in diabetic mice

Group	I	II	III	IV	V
BG-F (mmol/L)					
Week 0	6.01 ± 0.59***	22.36 ± 2.21	21.80 ± 0.91	21.89 ± 1.39	21.67 ± 0.85
Week 1	6.28 ± 0.60***	23.37 ± 3.19	20.26 ± 0.89	20.17 ± 2.56	20.02 ± 5.33
Week 2	6.39 ± 0.64***	24.71 ± 5.54	19.47 ± 4.92*	19.84 ± 6.78	19.59 ± 4.19*
Week 3	6.45 ± 0.85***	25.01 ± 4.06	18.85 ± 3.35*	18.96 ± 6.36*	16.84 ± 4.84***
Week 4	6.61 ± 0.92***	26.26 ± 2.77	16.07 ± 1.52***	17.90 ± 5.29***	16.14 ± 4.39***
Body weight (g)					
Week 0	34.9 ± 2.3***	31.4 ± 1.7	28.9 ± 2.4	31.0 ± 3.5	31.6 ± 3.2
Week 1	38.2 ± 2.7**	32.0 ± 2.1	32.3 ± 3.1	34.3 ± 4.1	35.2 ± 4.2
Week 2	38.3 ± 2.4**	32.7 ± 2.1	32.7 ± 4.2	35.3 ± 2.9	36.0 ± 3.3
Week 3	39.0 ± 2.7**	32.7 ± 2.2	33.2 ± 3.0	35.5 ± 3.9	35.8 ± 4.1
Week 4	39.6 ± 3.1***	32.9 ± 2.3	34.2 ± 1.6	36.2 ± 4.0	36.4 ± 4.0

\* $p < 0.05$  as compared with DM-C group; \*\* $p < 0.01$  as compared with DM-C group; \*\*\* $p < 0.001$  as compared with DM-C group



**Table 5** Effect of FREE-AK on oral glucose tolerance of normal and diabetic mice

BG-F (mmol/L)	I	II	III	IV	V
0 min	6.24 ± 0.59***	18.40 ± 4.41	9.92 ± 3.52**	12.66 ± 2.08*	11.04 ± 1.72***
30 min	14.80 ± 3.00***	30.66 ± 3.21	17.98 ± 3.04***	21.43 ± 1.63***	19.44 ± 4.63**
60 min	10.49 ± 1.48***	29.16 ± 2.91	14.93 ± 3.21***	17.31 ± 3.34***	16.47 ± 2.47***
90 min	9.37 ± 1.32***	27.63 ± 4.01	13.47 ± 2.17***	15.43 ± 2.35***	14.83 ± 4.27***
120 min	8.56 ± 0.91***	25.24 ± 4.72	11.25 ± 1.48***	13.96 ± 2.25***	12.63 ± 2.45***

\* $p < 0.05$  as compared with DM-C group; \*\* $p < 0.01$  as compared with DM-C group; \*\*\* $p < 0.001$  as compared with DM-C group

**Table 6** Effect of FREE-AK extract on lipid levels in serum of normal and diabetic mice

Lipid levels (mmol/L)	I	II	III	IV	V
TG <sup>a</sup>	2.67 ± 0.48***	3.59 ± 0.41	2.64 ± 0.57***	3.09 ± 0.29	2.79 ± 0.58
TC <sup>a</sup>	1.86 ± 0.38***	3.33 ± 0.41	2.51 ± 0.58***	2.86 ± 0.47	2.29 ± 0.39***
HDL <sup>a</sup>	2.72 ± 0.38*	2.11 ± 0.48	2.47 ± 0.43	2.34 ± 0.43	2.52 ± 0.36
LDL <sup>a</sup>	0.22 ± 0.05	0.57 ± 0.09	0.30 ± 0.08	0.40 ± 0.09	0.31 ± 0.05

\* $p < 0.05$  as compared with DM-C group; \*\* $p < 0.01$  as compared with DM-C group; \*\*\* $p < 0.001$  as compared with DM-C group

<sup>a</sup>TG is short for serum triglycerides, TC is short for total cholesterol, HDL is short for high-density lipoprotein, LDL is short for low-density lipoprotein

weight among the experiment groups (II, III, IV and V). However, the body weights were found to be significantly higher in the NC group compared to the DM-C group ( $p < 0.05$ ) from week 1 to week 4.

The loss in body weight observed in the STZ-induced diabetic group (after a period of 28 days) may be due to a loss of both muscle and tissue proteins based on the induction of diabetes with STZ (Swanston-Flatt et al. 1990). The increase in body weight was observed both in normal treated and diabetic treated groups.

#### Effect of FREE-AK on lipid levels in serum in normal and diabetic mice

Table 6 shows the serum levels of TC, TG, LDL, and HDL cholesterol measured in normal and experimental animals in each group. The TG, TC, and HDL levels of the DM-C group were found to be significant lower than the NC group. When the mice were given oral administrations for 28 days, the serum TG and TC were found to be significantly lower ( $p < 0.001$ ) in the NC group compared to the DM-C group, HDL was slight higher than DM-C group ( $p < 0.05$ ) and there was no significant difference of LDL between NC group and DM-C group. The results suggested that LDL independent of diabetic induced by STZ. There was no significantly difference of HDL and LDL among treatment groups. It was interesting to find that FREE-AK treatment with a dose of 800 mg/kg b.w. had an equal positive effect on TC with metformin ( $p < 0.001$ ). Both

HFREE-AK group and LFREE-AK group showed no significantly difference in TG compared to DM-C group.

Diabetes mellitus is typically correlated with remarkably high levels of serum lipids, with such an increase posing as a risk factor for coronary heart disease (Kim et al. 2014). Due to insulin deficiency or insulin resistance, a variety of changes in metabolic and regulatory mechanisms result in the observed accumulation of lipids. STZ-induced diabetes was also found to give rise to hyperlipidemia, which agrees with previous observations (Fatima et al. 2010; Sireesha et al. 2011). In the current study, the FREE-AK was found to reduce TC, TG, and LDL levels and increase HDL levels in diabetic control mice compared to normal control mice (Table 6). This could be attributed to the insulin tropic effect or the insulin secretagogue activities FREE-AK caused by FREE-AK.

#### Conclusion

The ethanol concentration played the most important role in optimization of extracting flavonoid from *Angelica keiskei* leaves. FREE-AK contained different types of phytonutrients and mineral element, such as potassium (K), which plays a key role in maintaining blood pressure. FREE-AK was found to be flavonoid-rich and possessed the equal ability to low blood glucose with metformin. FREE-AK with a dose of 800 mg/mg/kg b.w. could exhibit the same effect on TC with metformin. However, whether

flavonoids and other phytonutrients produced synergistic effects remains unclear, thus the identification of flavonoids in FREE-AK and the mechanism of FREE-AK function in vivo needs to be further studied.

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#### Compliance with ethical standards

**Animal rights statements** The in vivo experiment was still a animal experiment which is carrying out according guidelines of Shanghai Jiao Tong University.

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