Effects of Traditional Chinese Medicinal Plants on Anti-insulin Resistance Bioactivity of DXMS-Induced Insulin Resistant HepG2 Cells

Jun-Zeng Ma · Li-Xin Yang · Xiao-Ling Shen · Ji-Huan Qin · Li-Lan Deng · Selena Ahmed · Hong-Xi Xu · Da-Yuan Xue · Jiang-Xia Ye · Gang Xu

Received: 21 May 2014 / Accepted: 16 June 2014 / Published online: 10 July 2014 © The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract Medicinal plants have a long history of use in China to treat diabetic symptoms. Ancient Chinese medical manuscripts and ethnobotanical surveys document plant remedies that continue to be actively used in China for the treatment of diabetic symptoms. Based on a systematic ancient Chinese medical manuscripts review in combination with ethnobotanical survey, 16 medicinal plants for the traditional treatment of diabetic symptoms were identified for the evaluation of anti-insulin resistance bioactivity. The biological activity of 16 medicinal plants was tested on dexamethasone (DXMS)-induced insulin resistant HepG2 cells. The result shows that 11 of the 16 medicinal plants enhanced glucose uptake of DXMS-induced insulin resistant HepG2 cells, thereby demonstrating their ability to increase insulin sensitivity, other five medicinal plants including *Astragalus membranaceus* were found ineffective. The study shows that ancient Chinese medical manuscripts and ethnobotanical surveys on plants for the prevention and treatment of diabetic symptoms provide a promising knowledge base for drug discovery to mitigate the global diabetes epidemic.

Keywords Traditional medicinal plants · Diabetes · Anti-insulin resistance bioactivity · DXMS-induced insulin resistant HepG2 cells

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| DM           | Diabetes mellitus |
| IR           | Insulin resistant |
| IS           | Insulin sensitive |
| DXMS         | Dexamethasone |
| RGC          | Relative glucose consumption |
| CV           | Cell viability |

Jun-Zeng Ma and Li-Xin Yang have contributed equally in this work.

J.-Z. Ma · L.-X. Yang · G. Xu
State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People’s Republic of China
e-mail: xugang008@mail.kib.ac.cn

L.-X. Yang · S. Ahmed · D.-Y. Xue
College of Life and Environmental Science, Minzu University of China, 27 ZhongGuanCun South Avenue, Beijing 100086, China
e-mail: xuedayuan@hotmail.com

X.-L. Shen · J.-H. Qin
Laboratory of Chinese Herbal Drug Discovery, Tropical Medicine Institute, Guangzhou University of Chinese Medicine, Guangzhou 510405, People’s Republic of China
e-mail: xlshen66@126.com

L.-L. Deng · J.-X. Ye
Southwest Forestry University, Kunming 650224, People’s Republic of China

S. Ahmed
Sustainable Food and Bioenergy Systems Program, Department of Health and Human Development, Montana State University, Bozeman, MT 59717, USA

H.-X. Xu
Shanghai University of Traditional Chinese Medicine, Shanghai 201203, People’s Republic of China
1 Introduction

Diabetes mellitus (DM) is an increasingly prevalent group of metabolic diseases affecting hundreds of millions of people worldwide and costing billions of dollars in healthcare. This heterogeneous group of disorders is characterized by hyperglycemia [1] and results from absolute insulin deficiency, insulin resistance, and/or abnormal insulin secretion [2]. DM may lead to a series of complications including blindness, renal failure, nerve damage, stroke, and limb amputation [3]. In 2012, there were approximately 4.8 million deaths resulting from DM with 471 billion USD spent on healthcare costs globally. Approximately 371 million people today have DM [4].

Currently available drugs for the treatment of DM include sulphonylureas, biguanides, α-glucosidase inhibitors, and thiazolidinediones [5]. However, these treatments are noted to have varying degrees of adverse side effects such as gastrointestinal disturbances and hypoglycemia [6]. Given the side effects from current treatments, it is necessary to search safer agents for the treatment of DM, including remedies that may be more efficacious. Traditional medicinal plants have an extensive history for the treatment of diseases [7] and offer promising leads for the treatment of DM. Increased attention has been given to these resources for their complementary therapeutic effects to supplement western medicine [8]. Ethnopharmacological studies have noted that many traditional remedies have relatively fewer side-effects, better patient tolerance in diverse cultural contexts, less toxicity, and lower costs compared to modern drugs for DM treatment [9].

Traditional Chinese Medicine (TCM) is a major global healthcare system that relies on medicinal plants and plays a crucial role in healthcare for hundreds of millions of people in China including for the treatment of DM. In TCM, DM is categorized as “xiaoke” (polydipsia) with complex symptoms of excessive eating, drinking, polyuria, emaciation and urine-sweeting [10].

“Xiaoke” is recorded in many ancient Chinese medical manuscripts including “Shen Nong Ben Cao Jing”, “Ming Yi Bie Lu”, “Bei Ji Qian Jin Yao Fang”, “Qian Jin Yi Fang”, “Dian Nan Ben Cao”, and “Ben Cao Gang Mu”. Numerous medicinal plants have been reported in TCM for the treatment of “xiaoke” [9, 11]. For example, a powdered mixture of Coptis chinensis Franch. (Ranunculaceae), Astragalus membranaceus var. mongolicus (Bunge) P.K. Hsiao. (Leguminosae) and Lonicera japonica Thunb. (Caprifoliaceae) was found to improve insulin resistance for Type 2 DM [11]. Several medicinal plants from traditional Chinese pharmacopeia have been found to exert beneficial action on diabetes and related complications via multi-mechanisms [9].

The rising interest and use of traditional plant remedies has been met with concerns over the lack of quality control and scientific evidence for the efficacy and safety of herbal medicine [12]. Scientific research has responded to this concern with increased attempts to search for common ground between traditional healing systems and western medicine including clinical and animal studies to validate the efficacy and safety of traditional herbal medicine [13, 14]. However, few studies have been devoted to investigating the anti-diabetic activity of traditional medicinal plants by integrating ethnobotanical, phytochemical, and pharmacological approaches. The objective of this study is to evaluate the anti-insulin resistance bioactivity of 16 selected traditional Chinese medicinal plants in DXMS-induced IR (insulin resistant) HepG2 cells. HepG2 cells are a pure cell line of human liver carcinoma derived from the liver tissue with a well-differentiated hepatocellular carcinoma [15]. The model of DXMS-induced insulin resistance in HepG2 cells is recognized as important for interpreting insulin resistance mechanisms and drug screening [16].

2 Results and Discussion

2.1 Inventory of Target Anti-diabetic Plants

Table 1 shows traditional medicinal plants used in the treatment of diabetic symptoms. Plants of Group I were found to be mentioned with relatively high frequency in the literature review for their anti-diabetic activities including: A. membranaceus, C. chinensis, Morus alba, Pueraria lobata, Trichosanthes kirilowii, Alisma orientale, Schisandra ningpoensis, Cuscuta chinensis, Schisandra chinensis. Of these, seven plants were mentioned with high frequency for the treatment of “xiaoke” and diabetes reported in articles published between 1980 and 2003 including: A. membranaceus, C. chinensis, P. lobata, T. kirilowii, A. orientale, S. ningpoensis and S. chinensis [17–20]. Plants of Group II were collected through ethnobotanical survey in Lijiang, Dali, and Dongchuan etc. during 2011–2013. Plants were identified through semi-structured interviews with traditional herb doctors. Medicinal plants usage frequency of Han, Naxi, Bai and Lisu socio-linguistic groups for the treatment of “xiaoke” and diabetic symptoms was studied.

Table 2 shows review of previous studies of traditional Chinese medicinal plants for treating diabetic symptoms including: A. membranaceus, C. chinensis, M. alba, P. lobata, T. kirilowii, Agrimonia pilosa, C. chinensis, S.
| No. | Latin name                          | Family       | Local name | Used part | Function                                                                 | Frequency | Source | Yield (%) MEs | Voucher |
|-----|-------------------------------------|--------------|------------|-----------|--------------------------------------------------------------------------|-----------|--------|---------------|---------|
| A1  | Astragalus membranaceus var. mongholicus (Bunge) P.K. Hsiao. | Leguminosae  | Huang Qi   | Root      | Tonifying Qi and lifting yang, inducing diuresis for removing edema      | 50        | 7      | 22.8          | KUNX01  |
| A2  | Coptis chinensis Franch.            | Ranunculaceae| Huang Lian | Root      | “Xiaoke” and excessive urine, urine like oil                            | 69        | 2, 3, 4, 5, 6, 7 | 19.0    | KUNX02  |
| A3  | Morus alba Linn.                   | Moraceae     | Sang Bai Pi| Root skin | Inducing urination, to treat “xiaoke” and excessive urine               | 3         | 4, 6     | 11.6          | KUNX03  |
| A4  | Pueraria lobata (Willd.) Ohwi.      | Leguminosae  | Ge Gen     | Root      | To treat “xiaoke”, heat, stomach weaken, dysphoria                     | 57        | 1, 2, 3, 4, 5, 7 | 6.7     | KUNX04  |
| A5  | Trichosanthes kirilowii Maxim.     | Cucurbitaceae| Tian Hua Fen| Root      | To treat “xiaoke”, dysphoria, and heat                                 | 66        | 1, 2, 3, 4, 6, 7 | 3.9     | KUNX05  |
| A6  | Alisma orientale (Samuel.) Juz.     | Alismataceae | Ze Xie     | Root      | To treat “xiaoke”                                                       | 58        | 2, 3, 4, 7 | 12.6          | KUNX07  |
| A7  | Scrophularia ningpoensis Hems.      | Scrophulariaceae | Xuan Shen | Root      | To treat polydipsia and pyreticosis, removing heat to cool blood       | 50        | 7      | 22.4          | KUNX08  |
| A8  | Cuscuta chinensis Lam.              | Convolvulaceae| Tu Si Zi   | Seeds     | To treat “xiaoke” and dribbling urination                               | 3         | 3, 4, 6 | 11.5          | KUNX09  |
| A9  | Schisandra chinensis (Turcz.) Baii. | Magnoliaceae | Wu Wei Zi  | Fruits    | To treat edema from nephritis, using diuretic of hydragogue to alleviate water retention | 53 | 3, 4, 7 | 43.9 | KUNX10 |
| A10 | Viburnum odoratissimum Ker-Gawl.    | Caprifoliaceae| Jia Mi     | Branch leaves | Removing heat to cool blood, inducing diuresis to alleviate edema, diffusing wind-heat, clearing heat-fire, tonifying spleen and dampness | 12 | Dali | 13.2 | KUNX11 |
| A11 | Polygonatum verticillatum (L.) All. | Liliaceae    | Lun Ye Huang Jing | Bulb | Tonifying spleen and dampness, “xiaoke”, tonifying Qi | 8 | Lijiang | 9.7 | KUNX12 |
| A12 | Hypericum henryi Levl. et fan.      | Clusiaceae   | Xi Nan Jin Si Mei | Aerial plant | Clearing away heat and toxic materials, diuresis, promoting blood circulation to restore menstrual flow | 3 | Redland | 10.5 | KUNX14 |
| A13 | Lobaria yunnanensis Yoshim.         | Lobariaceae  | Qing Wa Pi | Whole plant | Inducing diuresis to alleviate edema                                   | 7         | Dali     | 7.3           | KUNX17  |
| A14 | Agrimonia pilosa Ldb.              | Rosaceae     | Xian He Cao | Whole plant | Promoting blood circulation to restore menstrual flow                   | 9         | Lijiang  | 9.1           | KUNX18  |
| A15 | Fragaria nilgerrensis Schlecht. ex Gay var. mairei (Levl.) Hand.-Mazz. | Rosaceae | Ye Cao Mei | Whole plant | Clearing heat                                                          | 6         | Lijiang  | 16.6          | KUNX19  |
| A16 | Fagopyrum dibotrys (D. Don) Hara     | Polygonaceae | Jin Qiao Mai | Root | Tonifying spleen and dampness                                           | 10        | Lijiang  | 20.2          | KUNX20  |

---

*a* Frequency means recorded frequency in ancient Chinese medical manuscripts or experiences from herbalists for diabetes treatment

*b* 1 “Shen Nong Ben Cao Jing”, 2 “Ming Yi Bie Lu”, 3 “Bei Ji Qian Jin Yao Fang”, 4 “Qian Jin Yi Fang”, 5 “Dian Nan Ben Cao”, and 6 “Ben Cao Gang Mu”, 7 Ref. [21]
According to GCA IR values of medicinal plants. HepG2 cells treated with 1 μmol/L of DXMS (IR control) exhibited comparable cell viabilities (CV IR > 95 %) to DXMS free insulin sensitive HepG2 cells (IS control) but consumed much less glucose (RGC IR = 82.1 %, RGC: Relative Glucose Consumption), implying that insulin resistance in HepG2 cells was successfully induced by DXMS. The IR state of cells was further supported by a GCA IR value of 0.86.

Among the 16 analyzed medicinal plants, A. orientale was found to be both highly effective as well as no toxicity. Five species including C. chinensis, M. alba, F. dibotrys, H. henryi and F. nilgerrensis were shown to be highly effective but toxic. One species, V. odoratissimum, was found to be highly effective but only at toxic concentrations. S. chinensis was found to be moderately effective. Three species, P. lobata, T. kirilowii, L. yunnanensis, were shown to be moderately effective with low toxicity. The remaining five of the 16 test extracts from A. membranaceus, S. ningpoensis, C. chinensis, P. verticillatum, Agrimonia pilosa were found to be ineffective as anti-insulin resistance agents in DXMS-induced IR HepG2 cells.

2.3 Anti-insulin Resistance Bioactivity of Traditional Medicinal Plants

This study supports that traditional Chinese medicinal plants have the potential for development as anti-diabetic drugs. All plants identified through the literature review of traditional Chinese medical texts and through ethnobotanical surveys exhibited efficacy as anti-insulin resistance agents. Eleven of the 16 tested traditional medicinal plants were able to enhance glucose uptake in DXMS-induced IR HepG2 cells, thereby demonstrating their in vitro anti-insulin resistance bioactivity. One of these species, A. orientale, was found to have both high efficacy and non-toxicity. A. orientale is an aquatic herbaceous plant that is cultivated widely in Sichuan, Jiangxi and Fujian Provinces of China and is used as a key ingredient in some TCM prescriptions such as hachimijioigan (Ba Wei Di Huang Wan). Previous studies have shown that hachimijioigan increased insulin secretion and decreased postprandial glucose in type-2 diabetic Goto-Kakizaki rats. In addition, A. orientale has been shown to have extensive bioactivities including diuresis, modulating immune system, hypotensive and anti-atherosclerosis. Further research is called for towards the development of this plant as a widespread drug for the treatment of DM. In vivo studies are particularly called for as extracts that show efficacy in vitro will not necessarily lead to a corresponding response in vivo due to the dose used and associated metabolic responses.

Six of the nine medicinal plants identified through the literature review exhibited high or moderate anti-insulin resistance bioactivity in this study while five of the seven medicinal plants identified through the ethnobotanical surveys exhibited efficacy in the study screenings. Previous research has analyzed nine of the 16 medicinal plants examined here (Table 2) for their anti-diabetic activity using in vitro and/or in vivo drug screenings and have found these species to be efficacious for improving glucose metabolism through different mechanisms including stimulating insulin secretion of pancreas, increasing insulin sensitivity of IR cells, and delaying intestinal absorption of glucose.

The three plants that have previously been reported to have anti-diabetic activity and had no activity in the present study (A. membranaceus, C. chinensis and A. pilosa) along with the two plants identified in the ethnobotanical surveys that showed no bioactivity (S. ningpoensis and P. verticillatum) may be due to various factors regarding the specific bioactivity assessed here and the preparation protocols. Anti-insulin resistance bioactivity is one of several indicators of anti-diabetic activity and other mechanisms are involved in diabetes pathology. For example, β-hydroxy-2-oxopomolic acid in Agrimonia pilosa works through regulating lipogenesis but not through...
| No. | Latin name                          | Anti-diabetic constituents reported | Activity* | Mechanism                                                                 | References |
|-----|-------------------------------------|-------------------------------------|-----------|---------------------------------------------------------------------------|------------|
| A1  | *Astragalus membranaceus*           | Isoastragaloside I, Astragaloside II and IV | b         | Elevating adiponectin production                                          | [25, 26]   |
|     |                                     | Formononetin, calycosin             | c         | Activating PPARz and PPARγ                                                | [27]       |
|     |                                     | Astragalus polysaccharide           | b         | Regulating PKB/GLUT4 signaling in skeletal muscle, inhibiting PTP1B       | [28, 29]   |
| A2  | *Coptis chinensis*                  | Berberine                           | b         | Modulating the structure of gut microbiota                               | [30]       |
|     |                                     | Polysaccharide                      | a, b      | Stimulating AMPKα1, AMPKα2 and inhibiting hepatic gluconeogenesis         | [31, 32]   |
| A3  | *Morus alba*                        | Polysaccharides                     | a, d      | Inhibiting inflammatory response and attenuate oxidative stress in pancreas tissue | [34]       |
|     |                                     | Moracin M, Mullberroside A etc.     | a         |                                                                             | [35]       |
|     |                                     | Extract                             | a, b      |                                                                             | [36]       |
| A4  | *Pueraria lobata*                   | Puerarin                            | a, c, d   | Promoting expression of insulin etc., activating α1-adrenoceptors. Upregulating the expression of PPARγ | [37–40]   |
| A5  | *Trichosanthes kirilowii*           | Lectin                              | a         | Increasing glucose uptake of liver cells                                 | [41]       |
| A6  | *Alisma orientale*                  |                                     | a         | Inhibiting α-glucosidase activity                                         | [42, 43]   |
| A8  | *Cuscuta chinensis*                 | Polysaccharide                      | a, c      | Inhibiting α-amylase activity                                              | [44, 45]   |
| A9  | *Schisandra chinensis*              | Lignan-rich fraction                | a, b      | Activating PPAR-γ                                                        | [46]       |
| A14 | *Agrimonia pilosa*                  | 1β-Hydroxy-2-oxopomolic acid        | c         | Blocking PPARγ and C/EBPα expression                                      | [47]       |

* a, improving glucose metabolism; b, improving insulin sensitivity or enhancing insulin level; c, regulating lipid metabolism; d, improving the function of pancreas
| Sample                  | C (µg/mL) | RGC (%)       | CV (%)     | GCA   | Comments                               |
|-------------------------|-----------|---------------|------------|-------|----------------------------------------|
| Alisma orientale        | 100       | 105.0 ± 4.4*  | 103.2 ± 5.1| 1.02  | Highly effective and non-toxic         |
|                         | 50        | 97.2 ± 7.2    | 104.5 ± 2.9| 0.93  | Highly potential                        |
|                         | 25        | 84.2 ± 2.1    | 100.7 ± 0.1| 0.83  |                                        |
| Viburnum odoratissimum  | 100       | 88.6 ± 4.8    | 68.8 ± 8.9 | 1.29  | Highly effective at toxic concentrations|
|                         | 50        | 88.0 ± 5.6    | 85.2 ± 3.0 | 1.03  |                                        |
|                         | 25        | 89.6 ± 9.5    | 92.6 ± 1.8 | 0.97  |                                        |
| Coptis chinensis        | 100       | 75.9 ± 3.5    | 23.3 ± 1.9 | 3.26  | Highly effective but toxic             |
|                         | 50        | 94.4 ± 8.8    | 43.0 ± 5.4 | 2.19  |                                        |
|                         | 25        | 142.4 ± 0.1   | 65.6 ± 5.8 | 2.17  |                                        |
| Morus alba              | 100       | 82.6 ± 2.0    | 45.6 ± 2.1 | 1.81  | Highly effective but toxic             |
|                         | 50        | 90.1 ± 5.1    | 65.9 ± 8.0 | 1.37  |                                        |
|                         | 25        | 83.9 ± 1.7    | 80.2 ± 14.3| 1.05  |                                        |
| Hypericum henryi        | 100       | 82.9 ± 0.3    | 51.0 ± 4.9 | 1.62  | Highly effective but toxic             |
|                         | 50        | 110.8 ± 1.0** | 71.8 ± 6.6 | 1.54  |                                        |
|                         | 25        | 94.6 ± 1.6*   | 77.7 ± 1.3 | 1.22  |                                        |
| Fragaria nilgerrensis   | 100       | 100.0 ± 3.3** | 87.9 ± 4.4 | 1.14  | Highly effective but toxic             |
|                         | 50        | 96.8 ± 2.6**  | 88.2 ± 5.1 | 1.10  |                                        |
|                         | 25        | 95.7 ± 6.9*   | 91.5 ± 7.9 | 1.04  |                                        |
| Fagopyrum dibotrys      | 100       | 37.6 ± 0.6    | 4.7 ± 2.4  | 8.00  | Highly effective but toxic             |
|                         | 50        | 47.5 ± 2.1    | 20.5 ± 2.8 | 2.32  |                                        |
|                         | 25        | 71.1 ± 3.4    | 69.8 ± 6.3 | 1.02  |                                        |
| Schisandra chinensis    | 100       | 71.6 ± 2.6    | 79.6 ± 3.8 | 0.90  | Moderately effective                   |
|                         | 50        | 86.8 ± 5.0    | 96.3 ± 3.4 | 0.90  |                                        |
|                         | 25        | 96.6 ± 4.9    | 101.3 ± 2.7| 0.95  |                                        |
| Pueraria lobata         | 100       | 85.8 ± 8.4    | 87.7 ± 7.4 | 0.98  | Moderately effective with low toxicity|
|                         | 50        | 85.4 ± 6.6    | 90.9 ± 8.6 | 0.94  |                                        |
|                         | 25        | 83.5 ± 4.5    | 89.2 ± 6.3 | 0.94  |                                        |
| Trichosanthes kirilowii | 100       | 75.5 ± 5.9    | 85.8 ± 2.6 | 0.88  | Moderately effective with low toxicity|
|                         | 50        | 77.4 ± 6.4    | 83.6 ± 4.3 | 0.93  |                                        |
|                         | 25        | 81.6 ± 8.2    | 87.8 ± 4.8 | 0.93  |                                        |
| Lobaria yunnanensis     | 100       | 82.5 ± 5.3    | 85.6 ± 8.1 | 0.96  | Moderately effective with low toxicity|
|                         | 50        | 80.8 ± 6.4    | 90.0 ± 7.0 | 0.90  |                                        |
|                         | 25        | 68.2 ± 6.2    | 89.7 ± 6.8 | 0.76  |                                        |
| Astragalus membranaceus | 100       | 68.8 ± 1.6    | 89.0 ± 10.3| 0.77  | Ineffective                            |
|                         | 50        | 71.3 ± 1.1    | 88.6 ± 7.5 | 0.80  |                                        |
|                         | 25        | 68.8 ± 1.4    | 92.1 ± 6.2 | 0.75  |                                        |
| Scrophularia ningpoensis| 100       | 71.5 ± 6.7    | 85.8 ± 6.5 | 0.83  | Ineffective                            |
|                         | 50        | 65.7 ± 7.9    | 89.0 ± 5.7 | 0.74  |                                        |
|                         | 25        | 66.2 ± 6.2    | 90.2 ± 4.8 | 0.73  |                                        |
| Cuscuta chinensis       | 100       | 78.5 ± 3.9    | 91.3 ± 9.2 | 0.86  | Ineffective                            |
|                         | 50        | 80.3 ± 4.9    | 93.2 ± 9.8 | 0.86  |                                        |
|                         | 25        | 78.8 ± 4.4    | 101.0 ± 3.8| 0.78  |                                        |
| Polygonatum verticillatum| 100    | 70.6 ± 1.6    | 87.8 ± 7.8 | 0.80  | Ineffective                            |
|                         | 50        | 73.8 ± 0.2    | 86.5 ± 4.6 | 0.85  |                                        |
|                         | 25        | 75.3 ± 7.5    | 87.0 ± 3.0 | 0.86  |                                        |
| Agrimonia pilosa        | 100       | 56.7 ± 3.7    | 85.2 ± 3.9 | 0.66  | Ineffective                            |
|                         | 50        | 59.7 ± 1.5    | 90.7 ± 6.8 | 0.66  |                                        |
|                         | 25        | 69.1 ± 2.5    | 92.8 ± 5.8 | 0.74  |                                        |
directly affecting the insulin-signaling pathway of cells. Traditional medicinal plants are most often used in complex formulas with other species where each species has a distinct effect and their synergistic effect is responsible for the ultimate bioactivity. The methanol preparation used to evaluate medicinal plants in this study may not best extract active constituents. Methanol is relatively efficient in extracting hydrophobic compounds while water may be more efficient in extracting hydrophilic compounds and macromolecules such as polysaccharides. Previous studies have shown methanol extracts of plant material to exhibit different activities compared to water extracts [48]. Future ethnopharmacological studies on the medicinal plants examined here should incorporate traditional preparation protocols.

### Table 3 continued

| Sample       | C (µg/mL) | RGC (%) | CV (%) | GCA  | Comments |
|--------------|-----------|---------|--------|------|----------|
| IR control   | 82.1 ± 5.1 | 95.2 ± 3.9 | 0.86   |      |          |
| IS control   | 100       | 100     | 1.00   |      |          |

RGC and CV were expressed as Mean value ± standard deviation (n = 3)
GCA ≥ 1, highly effective; 0.86 < GCA < 1, moderately effective; GCA < 0.86, ineffective
Compared to IS control, data were significantly different at *P < 0.05
Compared to IR control, data were significantly different at *P < 0.05, **P < 0.01 and ***P < 0.001

### Fig. 1 Map of ethnobotanical survey and medicinal plants material collection sites in Yunnan, China

#### 2.4 Toxicity of Traditional Medicinal Plants

Ten of the 11 plants that were found to be efficacious in this study demonstrated varying degrees of toxicity to IR HepG2 cells at different concentrations. However, standard methanol laboratory extractions rather than traditional preparations were used for the evaluation of these species. For example, preparation using traditional protocols such as hot water infusions may be less effective in extracting particular hydrophobic compounds that contribute to toxicity. Traditional preparation protocols may also call for more dilute solutions compared to the methanol extractions used in this study. In addition, traditional medicinal remedies are often complex formulas involving multiple plants where one plant may offset the toxicity of another plant.
Further research is needed that incorporates traditional preparation of the target medicinal plants found as efficacious towards their potential development as anti-diabetic drugs. In addition, further studies should explore the toxicity of extracts prepared with protocols involving solvents of different polarity and varying concentrations. Further research should also examine toxicity levels in vivo as extracts with toxicity in vitro will not necessarily lead to a corresponding response in vivo due to the dose used and metabolic responses in vivo.

2.5 GCA as an Evaluation Indicator

Findings support that a more effective indicator than the current indicator involving RGC would incorporate cell viability. The glucose consumption assay is commonly used to evaluate the anti-insulin resistance bioactivity of a test sample at non-toxic concentrations. In such cases, increased RGC indicates increased anti-insulin resistance bioactivity of cells by the test sample. In this study, bioactivity of traditional medicinal plants was investigated using their methanol extracts. Crude extracts are complicated in constituents of various chemical structures and diverse bioactivities. Therefore, the active components in test extracts may increase glucose uptake of living cells and at the same time the toxic components in the extract reduce the number of living cells. In such cases, \( \text{RGC}_{\text{Extract}} \leq \text{RGC}_{\text{Control}} \) does not mean that anti-insulin resistance bioactivity was unchanged or reduced and thus the index RGC is not suitable to evaluate anti-insulin resistance bioactivity.

Findings support that an evaluation index of glucose consumption ability (GCA = RGC/CV) that takes cell viability (CV) into account along with RGC is a more rational indicator than simply RGC for assessing anti-insulin resistance bioactivity of medicinal plant extracts. Among the eight traditional medicinal plants that demonstrated activities in increasing anti-insulin resistance bioactivity or enhancing glucose uptake, six were identified as effective according to the GCA indicator including *C. chinensis*, *M. alba*, *P. lobata*, *T. kirilowii*, *A. orientale* and *S. chinensis*. In addition, *A. membranaceus* and *C. chinensis* were identified as ineffective, while these species have previously been reported to show anti-diabetic capacity.

3 Experimental Section

3.1 General

Dulbecco’s modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Shanghai, China). Glucose assay kits were purchased from Changchun Huili Biotech Co., Ltd (Changchun, China). Cell counting kit-8 (CCK-8) was provided by Dojindo Laboratorise (Shanghai). Dexamethasone (DXMS), insulin and DMSO were purchased from Sigma (Shanghai, China).

3.2 Identification of Target Anti-diabetic Plants

Plants for the evaluation of anti-insulin resistance bioactivity were identified using an ethnobotanical approach based on the literature as well as primary research by this study’s authors. In addition, plants were selected on the basis of the criteria that they have not previously been evaluated for their anti-insulin resistance bioactivity in DXMS-induced IR HepG2 cells.

The ethnobotanical survey involved plants mentioned in 2011–2013 during the author’s surveys in Lijiang, Dali, and Dongchuan of northern Yunnan Province of southwestern China (Fig. 1) with Han, Naxi, Bai, and Lisu socio-linguistic groups for the treatment of “xiaoke” and diabetic symptoms. Plants were identified through semi-structured interviews with traditional herb doctors that were over 50 years of age with an average age of 62. A total of ten key informants were interviewed including eight men and two women. A total of seven plants were identified through literature reviews and were delineated as Group I plants (No. A1–A9 in Table 1).

The ethnobotanical survey involved plants mentioned in 2011–2013 during the author’s surveys in Lijiang, Dali, and Dongchuan of northern Yunnan Province of southwestern China (Fig. 1) with Han, Naxi, Bai, and Lisu socio-linguistic groups for the treatment of “xiaoke” and diabetic symptoms. Plants were identified through semi-structured interviews with traditional herb doctors that were over 50 years of age with an average age of 62. A total of ten key informants were interviewed including eight men and two women. A total of seven plants were identified through ethnobotanical survey and were delineated as Group II plants (No. A10–A16 in Table 1).

A total of 16 species from 14 plant families were selected for bioactivity screening and were divided into two groups (Table 1). Group I were purchased from Jv Hua Cun in Kunming, Yunnan Province of southwest China. The second group of focal plants were collected from ethnobotanical surveys in northern Yunnan Province. Voucher specimens of the 16 study species were deposited at the State Key Laboratory of Phytochemistry and Plant Resources Sustainability at the Kunming Institute of Botany of the Chinese Academy of Sciences (Kunming, China).

3.3 Cell Line and Cell Culture

Human hepatoma cell line HepG2 was purchased from the Laboratory Animal Center of Sun Yat-Sen University.
Drug treatment proceeded by seeding $8 \times 10^3$ HepG2 cells in 100 µL growth medium in 96-well plates. Plates were incubated for 24 h to allow cells to adhere to the well bottom. The medium was replaced with 100 µL fresh medium containing 100, 50 or 25 µg/mL of the medicinal plant extract or a blank without any extract. Drug treatment lasted for 48 h. To induce insulin resistance of cells, 1 µmol/L of DXMS was added to the medium with the medicinal plant extract [49]. After 48 h of drug treatment, 5 µL of medium was taken from each well for measurement of glucose concentration by the glucose assay kits. Glucose consumed in drug-treated wells was calculated and expressed as Relative Glucose Consumption (RGC) over that in drug free wells (insulin sensitive control, IS control). In this assay, RGC in drug free wells was set as 100 %.

3.6 CV Assay

After removing 5 µL of medium from each well for the glucose consumption assay, 10 µL of CCK-8 was added per well in the 96-well plate and was further incubated at 37 °C for 1.5 h. Optical density (OD), which is positively correlated with the number of living cells, was read at 450 nm. Wells that were free of cells and that contained equivalent volumes of medium as the treated cells were set as blanks. Cell Viability (CV) in the IS control well was set as 100 %. CV in drug treated wells was calculated as CV (%) = 100 \times \frac{(OD_{\text{Drug}} - OD_{\text{Blank}})}{(OD_{\text{IS Control}} - OD_{\text{Blank}})} where CV < 90 % indicates toxicity to cells [50].

4 Conclusion

This study used interdisciplinary research methods that integrated ethnobotany, phytochemistry, and pharmacology towards exploring traditional medicinal plants for drug discovery. Findings validate traditional usage of medicinal plants through scientific explanation of their efficacy for the prevention and treatment of diabetic symptoms and provide a promising knowledge base for drug discovery to mitigate the global diabetes epidemic. Further studies should incorporate traditional as well as novel experimental preparation protocols of plant remedies for drug discovery to evaluate both efficacy and safety. In addition, future research should adopt an activity-guided fractionation approach to drug discovery by assessing different fractions and chemical constituents of the medicinal plants examined in this study along with in vivo screening. The evaluation model used here, which combines cell viability of DXMS-induced IR HepG2 cells and GCA, is a reasonable method for evaluating anti-insulin resistance bioactivity and should be utilized widely as a screening technique for drug discovery from plant resources. Glucose consuming ability of cell (GCA) is only one of useful evaluation indicators in screening of anti-insulin resistance bioactivity and different evaluation models need to be tested and verified. The integration of traditional healing systems with scientific research can contribute to the management of global health epidemics including DM while providing economic incentives to conserve plant resources of traditional systems and their associated cultural practices and knowledge base.

Acknowledgments The work was financially supported by China National Major Projects (2009ZX09103-436) and 973 Program (2011CB915503) of Science and Technology of P. R. China, the reservation-talent project of Yunnan Province (2009C103), the foundation of study abroad returnees from Ministry of Personnel for
financial support (Ms. Li-Xin Yang), and the foundations from CAS (Dr. Gang Xu).

Conflict of interest The authors declare no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. G.I. Bell, K.S. Polonsky, Nature 414, 788–791 (2001)
2. P. Zimmet, K.G. Alberti, J. Shaw, Nature 414, 782–787 (2001)
3. M. Brownlee, Nature 414, 813–820 (2001)
4. International Diabetes Federation, Diabetes Atlas, 5th edn. (International Diabetes Federation, Brussels, 2012)
5. A.J. Krentz, C.J. Bailey, Drugs 65, 385–411 (2005)
6. B.J. Goldstein, D. Müller-wieland, Type 2 Diabetes: Principles and Practice, 2nd edn. (Informa, New York, 2008)
7. J.B. Won, J.Y. Ma, C.T. Ma, Arch. Pharm. Res. 33, 619–625 (2010)
8. S.Z. Liu, Y.X. Deng, B. Chen, X.J. Zhang, Q.Z. Shi, X.M. Qiu, J. Ethnopharmacol. 145, 490–498 (2013)
9. K. He, X.G. Li, X. Chen, X.L. Ye, J. Huang, Y.N. Jin, P.P. Li, Y.F. Deng, Q. Jin, Q. Shi, H.J. Shu, J. Ethnopharmacol. 137, 1135–1142 (2011)
10. F.L. Guo, Shanghai J. Tradit. Chin. Med. 3, 57 (1991)
11. M.L. Chao, D.J. Zou, Y.F. Zhang, Y.H. Chen, M. Wang, H. Wu, N. Wang, W.Q. Wang, Endocrine 36, 268–274 (2009)
12. F. Firenzuoli, L. Gori, Evid-Based Compl. Altern. Ther. 4, 37–40 (2007)
13. Y.J. Kang, Exp. Biol. Med. 233, 1059–1065 (2008)
14. J. Van der Greef, H. Van Wietmarschen, J. Schroën, M. Wang, T. Hankemeier, G.W. Xu, Planta Med. 76, 2036–2047 (2010)
15. S. Costantini, G.D. Bernardo, M. Cammarota, G. Castello, G. Colonna, Gene 518, 335–345 (2013)
16. L.J. Dong, Y.Z. Jin, L. Shi, G.M. Song, J. Third Mil. Med. Univ. 34, 671–673 (2012)
17. R.Z. Yu, China J. Tradit. Chin. Med. Pharm. 9, 57 (1991)
18. H.Y. Zhang, Beijing J. Tradit. Chin. Med. 3, 49–51 (1998)
19. Y.Q. Zhang, G.Z. He, Q. Han, X.M. Kong, Y. Li, P.A. Zeng, Y. Zhang, Q.G. Zhang, X.S. Zhou, Chin. J. Basic. Med. Tradit. Chin. Med. 10, 45–47 (2004)
20. C.Y. Hu, S.H. Gao, Chin. J. Inf. TCM 12, 93–94 (2005)
21. W.Y. Kang, Y.L. Song, L. Zhang, Med. Chem. Res. 20, 809–816 (2011)
22. Ö.D. Can, Y. Öz Türk, N. Öztürk, G. Sagratini, M. Ricciutelli, S. Vittori, F. Maggi, Fitoterapia 82, 576–584 (2011)
23. J.X. Wang, Chin. Med. Mod. Dist. Edu. China 7, 93–94 (2009)
24. Y.H. Zhang, Z.X. Zheng, Y.H. Liu, J.Y. Teng, R.X. Zhang, X.H. Liu, C.Y. Xue, Chin. J. Clin. Rehabil. 10, 111–113 (2006)
25. A.M. Xu, H.B. Wang, L.C. Hoo, R.G. Sweeney, P.M. Vanhoutte, Y. Wang, D.H. Wu, W.J. Chu, G.W. Qin, K.S.L. Lam, Endocrinology 150, 625–633 (2009)
26. M.E. Xu, S.Z. Xiao, Y.H. Sun, Y. Ouyang, X.Z. Zheng, Acta Pharmacol. Sin. 27, 229–236 (2006)
27. P. Shen, M.H. Liu, T.Y. Ng, Y.H. Chan, E.L. Yong, J. Nutr. 136, 899–905 (2006)
28. Y. Wu, J.P. Ouyang, K. Wu, Y. Wang, Y.F. Zhou, C.Y. Wen, Acta Pharmacol. Sin. 26, 345–352 (2005)
29. M. Liu, K. Wu, X.Q. Mao, Y. Wu, J.P. Ouyang, J. Ethnopharmacol. 127, 32–37 (2010)
30. X. Zhang, Y.F. Zhao, M.H. Zhang, X.Y. Pang, J. Xu, C.Y. Kang, M. Li, C.H. Zhang, Z.G. Zhang, Y.F. Zhang, X.Y. Li, G. Ning, L.P. Zhao, PLoS One 7, 1–12 (2012)
31. X. Ma, T. Egawa, H. Kimura, K. Karaike, S. Masuda, N. Inawaka, T. Hayashi, Metabolism 59, 1619–1627 (2010)
32. X. Xia, J.H. Yan, Y.F. Shen, K.X. Tang, J. Yin, Y.H. Zhang, D.J. Yang, H. Liang, J.P. Ye, J.P. Weng, PLoS One 6, 1–10 (2011)
33. S. Jiang, P.G. Du, L.P. An, G.X. Yuan, Z.W. Sun, Int. J. Biol. Macromol. 55, 118–122 (2013)
34. C. Guo, R. Li, N. Zheng, L.Y. Xu, T. Liang, Q.L. He, Int. Immunopharmacol. 16, 93–99 (2013)
35. M. Zhang, M. Chen, H.Q. Zhang, S. Sun, B. Xia, F.H. Wu, Pharmacoepidemiol. 80, 475–477 (2009)
36. A.N.B. Singab, H.A. El-Beshbishy, M. Yonekawa, T. Nomura, T.J. Fukai, Ethnopharmacology 100, 333–338 (2005)
37. K. Wu, T. Liang, X.Q. Duan, L.Y. Xu, K.F. Zhang, R. Li, Food Chem. Toxicol. 60, 341–347 (2013)
38. W.C. Chen, S. Hayakawa, T. Yamamoto, H.C. Su, J.M. Liu, J.T. Cheng, Planta Med. 70, 113–116 (2004)
39. O.H. Lee, D.H. Seo, C.S. Park, Y.C. Kim, BioFactors 36, 459–467 (2010)
40. F.L. Xiong, X.H. Sun, L. Gan, X.L. Yang, H.B. Xu, Eur. J. Pharmacol. 529, 1–7 (2006)
41. Q. Li, X.L. Ye, H. Zeng, X. Chen, X.G. Li, J. Chin. Med. Mat. 35, 475–479 (2012)
42. X.B. Yang, Z.M. Huang, W.B. Cao, H.Y. Chen, W.H. Tian, J.H. Wang, Chin. J. Exp. Tradit. Med. Form. 8, 24–26 (2002)
43. C.H. Lau, C.M. Chan, Y.W. Chan, K.M. Lau, T.W. Lau, F.C. Lam, C.T. Che, P.C. Leung, K.P. Fung, Y.Y. Ho, C.B.S. Lau, Phytother. Res. 22, 1384–1388 (2008)
44. D.Z. Li, D.Y. Peng, X.X. Xu, R. Zhang, Chin. Arch. Tradit. Chin. Med. 26, 2717–2718 (2008)
45. X.X. Xu, D.Z. Li, D.Y. Peng, R. Zhang, Chin. J. Exp. Tradit. Med. Form. 17, 232–234 (2011)
46. D.Y. Kwon, D.S. Kim, H.J. Yang, S. Park, J. Ethnopharmacol. 135, 455–462 (2011)
47. E.K. Ahn, J.A. Lee, D.W. Seo, S.S. Hong, J.S. Oh, Biol. Pharm. Bull. 35, 643–649 (2012)
48. U. Unachukuwa, S. Ahmed, A. Kavaler, J. Lyles, E.J. Kennelly, Food Sci. 75(6), C541–C548 (2010)
49. H.Y. Chen, X.G. Li, X.L. Ye, Y.N. Jin, J. Huang, J.F. Wu, China J. Chin. Mat. Med. 37, 1771–1774 (2012)
50. Y. Jiang, E.Y. Ahn, S.H. Ryu, D.K. Kim, J.S. Park, H.J. Yoon, S. You, B.J. Lee, D.S. Lee, J.H. Jung, BMC Cancer 4, 70 (2004)