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ORIGINAL ARTICLE

# *In vitro* Screening and Evaluation of 37 Traditional Chinese Medicines for Their Potential to Activate Peroxisome Proliferator-activated Receptors-γ

**Die Gao, Yonglan Zhang, Fengqing Yang, Yexin Lin, Qihui Zhang, Zhining Xia**

Department of Pharmacy, College of Chemistry and Chemical Engineering, Chongqing University, Chongqing 400030, P.R. China

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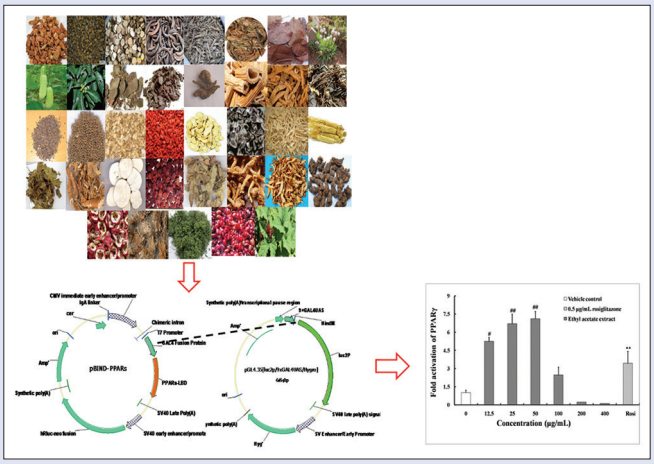
## ABSTRACT

**Background:** Peroxisome proliferator-activated receptors (PPAR)-γ is widely used as an attractive target for the treatment of type 2 diabetes mellitus. Thiazolidinediones, the agonists of PPARγ, has been popularly utilized as insulin sensitizers in the therapy of type 2 diabetes whereas numerous severe side-effects may also occur concomitantly. **Objective:** The PPARγ activation activity of different polar extracts, including petroleum ether, ethyl acetate, n-butanol, residual of ethanol, the precipitate part of water and the supernatant of water extracts, from 37 traditional Chinese medicines were systematically evaluated. **Materials and Methods:** HeLa cells were transiently co-transfected with the re-constructed plasmids of GAL4-PPARγ-ligand binding domain and pGL4.35. The activation of PPARγ by different polarity extracts were evaluated based on the PPARγ transactivation assay and rosiglitazone was used as positive control. **Results:** Seven medicines (root bark of *Lycium barbarum*, *Anoectochilus sroxburghii*, the rhizome of *Phragmites australis*, *Pterocephalus hookeri*, *Polygonatum sibiricum*, fruit of *Gleditsia sinensis*, and *Epimedium brevicornu*) were able to significantly activate PPARγ. **Conclusion:** As seven medicines were able to activate PPARγ, the anti-diabetic activity of them is likely to be mediated by this nuclear receptor.

**Key words:** Diabetes mellitus, different polarity extracts, peroxisome proliferator-activated receptor-γ, traditional anti-diabetic medicines

## SUMMARY

- Lots of the tested medicinal products had activation effects on activating PPARγ
- Ethyl acetate extracts of root bark of *L.barbarum*, rhizome of *P.saustralis* and fruit of *G.siasinensis* showed good PPARγ activation effect similar or higher than that of positive control, 0.5 μg/mL rosiglitazone
- Petroleum ether extracts of *A.roxburghii, P. hookeri, P. sibiricum, E.brevicornu* also can significantly activate PPARγ, the effects of them were higher than t0.5 μg/mL rosiglitazone
- *Schisandra chinensis* (Turcz.) Baill., the fruit *Cornus officinalis* Siebold and Zucc., *Alisma plantago-aquatica L.* and the root of *Trichosanthes Kirilowii* Maxim., traditional anti-diabetic mediciness in China, had no effects on the activation of PPARγ.




**Abbreviations used:** PPARγ: Peroxisome Proliferator-activated Receptors-γ, TCMs: Traditional Chinese medicines, TZDs: Thiazolidinediones, LBD: Ligand binding domain, DMSO: Dimethyl sulfoxide, FBS: Fetal bovine serum, DMEM: Dulbecco's modified Eagle's medium.

**Correspondence:**

Prof. Zhining Xia,
College of Chemistry and Chemical Engineering,
Chongqing University, Chongqing 400030, P.R. China.
E-mail: tcm_anal_cqu@163.com

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## INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by insulin resistance coupled with a lackage of enough insulin to control blood glucose.[1] It is well-known that chronic hyperglycemia results in many diabetic complications such as diabetic nephropathy, obesity, and atherosclerosis.[2]

Peroxisome proliferator-activated receptors (PPARs) have been implicated to participate in many critical physiological and pathological processes, especially in the treatment of diabetes mellitus, obesity, and atherosclerosis.[3,4] They play important roles in the expression of various genes which are crucial to lipid and glucose metabolism.[5] There are three PPAR subtypes, PPARα, PPARβ/δ, and PPARγ, which have respective expression patterns and regulate different biological processes based on the requirement of a specific tissue.[6] PPARγ is expressed in adipose cells as well as islet beta cells.[7] Activation of PPARγ can improve insulin sensitization and reduce the risk of the insulin resistance in adipose tissue, liver tissue, and *et al.*[8,9] The activation of it affects the



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glucose homeostasis and lipid metabolism as well as inflammation.[10] Increasing evidence indicated that the activation of PPARγ can promote the adipocyte differentiation, decrease the accumulation of glucose in adipose tissue.[11,12] Moreover, PPARγ plays an important role in the regulation of pancreatic functions and activation of PPARγ can decrease β-cell apoptosis.[13] It is also known that PPARγ affects glucose-stimulated insulin secretion.[14,15] Therefore, the screening of drugs which can activate PPARγ has great significance in the treatment of diabetes mellitus and related metabolic syndromes.

Thiazolidinediones (TZDs), the agonists of PPARγ, had been demonstrated to have a variety of clinical effects such as enhancing insulin sensitivity and improving glucose tolerance.[16,17] However, recently evidence demonstrates that TZDs had several side-effects such as cardiovascular risks, liver damage, weight gain, and peripheral edema.[18,19] On the other hand, traditional Chinese medicines (TCMs) with fewer side-effects have proven historically to be a potential source for drug discovery. Therefore, numbers of researches have been done to explore the activators of PPARγ from TCMs.[10,20]

In previous study, we developed a cell-based PPARs screening model. The model is a stable and effective assay tool to characterize the interactions between PPAR subtype and PPARs activating drugs. Only when a drug bound and activated GAL4-PPARγ-ligand binding domain (LBD) could the luciferase be expressed. The Z'-factor, which was a useful tool and a statistical characteristic of any given assay, was used to evaluate the reliability and the stability of our model. When the value of Z'-factor was between 0.5 and 1, suggesting that the model was suitable for drug screening. The value of Z'-factor of our model was 0.64.[21]

Herein, this study aimed to preliminary screen and to evaluate the activation of PPARγ by different polarity extracts from 37 TCMs on the basis of PPARγ transactivation assay, laying the basis of further identifying the effects of active extracts on PPARγ-mediated gene expression, and biological responses and isolating the active compounds from active extracts.

## MATERIALS AND METHODS

### Cells and reagents

HeLa cells were purchased from the Cell Center of the Chinese Academy of Medicinal Sciences (Shanghai, China). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were products of Gibco BRL (Gaithersburg, MD, USA). FUGene® HD Transfection Reagent and Bright-Glo™ Luciferase Assay were purchased from Promega (Madison, WI, USA). Dimethyl sulfoxide (DMSO), penicillin, streptomycin, and rosiglitazone were provided from Sigma-Aldrich Chemical Co, Ltd (St. Louis, MO, USA).

### Plasmid construction

pGL4.35 (luc2P/9XGAL4UAS/Hygro) Vector (Product No. E1370) and GAL4-GR Vector (Product No. E1581) were from Promega Corporation (Madison, WI, USA). The synthetic PPARγ-LBD gene sequences were inserted into GAL4-GR vector, and the sequencing of PPARγ-LBD genes was analyzed by restriction enzyme digestion (XhoI and XbaI) and gel electrophoresis. Then PPARγ-LBD replaced glucocorticoid receptor-LBD (GR-LBD) gene fusion in original GAL4-GR Vector and then formed the GAL4-PPARγ-LBD fusion protein. The pGL4.35 (luc2P/9XGAL4UAS/Hygro) vector containing GAL4 special response element of firefly luciferase was used as reporter gene.

### Collection of medicines material

The TCMs were obtained from the local drug stores, TCMs markets or production place [Table 1]. Further identification of the specimens was authenticated by Professor GuoyueZhong (Jiangxi University of TCM). All voucher specimens have been deposited at Chongqing Academy of Chinese Materia Medica, Chongqing, China.

### Preparation of different polarity extracts

The dried materials of 37 TCMs were ground into fine powder in a pulverizer, respectively. Reflux extraction was conducted in turn by petroleum ether, 70% ethanol, and water. Then, 70% ethanol extract was liquid-liquid extracted by ethyl acetate, while the concentrated segments of water were treated with absolute ethanol until the absolute ethanol reached to 60%. Afterward, the precipitate and the supernatant were collected, respectively. Finally, petroleum ether, ethyl acetate, the residual of ethanol, the precipitate, and the supernatant of water extracts were obtained. Each part was concentrated to dryness in a vacuum to afford samples for biological tests.

The positive drugs, petroleum ether, and ethyl acetate extracts were dissolved in DMSO. The residual of ethanol, the precipitate part of water, and the supernatant of water extracts were dissolved in water. All of them were stored at −20°C.

### Cell culture

HeLa cells were grown in DMEM, containing 10% FBS and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin). All cells were cultivated at 37°C in 5%$CO_2$ atmosphere.

### *IN VITRO* PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS γ TRANSACTIVATION BIOASSAY

HeLa cells were seeded into 6 well-plates at a density of $2 \times 10^5$ cells/well in DMEM containing 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin for 24 h. Then, 0.4 μg GAL4-PPARγ-LBD vector and 1.6 μg of pGL4.35 vector were transiently co-transfections into cells under the condition of FuGENE® HD Transfection reagent. After an overnight culture, the cells were replaced into 96 well-plates with new culture medium. 24 h later, negative control, 0.5 μg/mL rosiglitazone, or test samples were added, respectively. After 24 h, the luciferase activity was detected with Bright-Glo™.

### Statistical analysis

Each of these samples was measured in triplicate at various concentrations. Each concentration was tested for sextuplicate. All data are expressed as the mean ± standard deviation. The results were analyzed by one-way, and significant differences were determined by the origin 8.0. Statistical significance is displayed as $P < 0.05$.

## RESULTS

### The dose relationship between positive drug and the activation of peroxisome proliferator-activated receptors γ

To select an appropriate concentration of positive drug, the activation of PPARγ by rosiglitazone was tested (concentration from 0.5 μg/mL to 4.0 μg/mL) by PPARγ transactivation bioassay. The fold activation of rosiglitazone in dose-dependence manner is shows in Figure 1. The maximum fold on the activation of PPARγ was 1 μg/mL, and the range of fold activation was from 3.8-to 5.8-folds activation compared to vehicle control (DMSO). On the other hand, there was little change of the fold activation when the concentration of rosiglitazone exceeded 0.5 μg/mL (the range of fold activation was from 3.2 to 5.1 folds activation). Hence, we chose 0.5 μg/mL rosiglitazone as a positive control in later experiments.

**Table 1:** The plants species tested for potential peroxisome proliferator-activated receptor gamma activation properties in the screening model

| Number | Botanical name | Family | Part of plant | Voucher specimennumber | Local name |
|---|---|---|---|---|---|
| 1 | *Alisma plantago-aquatica* L. | Alismataceae | Root | GH828 | Ze Xie |
| 2 | *Ampelocissus artemisiifolia* Planch. | Vitaceae | Leaves | GH829 | Suan Lian Teng |
| 3 | *Anemarrhena asphodeloides* Bunge | Asparagaceae | Rhizome | GH830 | Zhi Mu |
| 4 | *Anoectochilus roxburghii* (Wall.) Lindl. | Orchidaceae | Leaves | GH831 | Jin Xian Lian |
| 5 | *Astragalus membranaceus* (Fisch.) Bunge | Leguminosae | Root | GH832 | Huang Qi |
| 6 | *Atractylodes lancea* (Thunb.) DC. | Compositae | Rhizome | GH833 | Cang Zhu |
| 7 | *Clematis chinensis* Osbeck | Ranunculaceae | Root stem | GH834 | Wei Lin Xian |
| 8 | *Cornus officinalis* Siebold & Zucc. | Cornaceae | Fruit | GH835 | Shan Zhu Yu |
| 9 | *Crataegus pinnatifida* Bunge | Rosaceae | Fruit | GH836 | Shan Zha |
| 10 | *Cuscuta chinensis* Lam. | Convolvulaceae | Seed | GH837 | Tu Si Zi |
| 11 | *Cyelcobalanpsis glauca* (Thunb. ex Murray) Oerst | Fagaceae | Leaves | GH838 | Tie Zhou |
| 12 | *Dendrobium moniliforme* (L.) Sw. | Orchidaceae | Root | GH839 | Shi Hu |
| 13 | *Epimedium brevicornu* Maxim. | Berberidaceae | Aerial parts | GH840 | Yin Yang Huo |
| 14 | *Gleditsia sinensis* Lam. | Leguminosae | Fruit | GH841 | Zhu Ya Zao |
| 15 | *Gynostemma pentaphyllum* (Thun b.) Makino | Cucurbitaceae | Whole plant | GH842 | Jiao Gu Lan |
| 16 | - | - | - | GH843 | Hui Dou Ba |
| 17 | *Ligustrum lucidum* W. T. Aiton | Oleaceae | Fruit | GH844 | Nv Zhen Zi |
| 18 | *L. barbarum* L. | Solanaceae | Root bark | GH845 | Di Gu Pi |
| 19 | *L. barbarum* L. | Solanaceae | Fruit | GH846 | Gou Qi |
| 20 | *Malus doumeri* (Bois) Chev. | Rosaceae | Leaves | GH847 | Lin Qin Ye |
| 21 | *Momordica charantia* L. | Cucurbitaceae | Fruit | GH848 | Ku Gua |
| 22 | *Moringa oleifera* Lam. | Moringaceae | Leaves | GH849 | La Mu Ye |
| 23 | *M. alba* L. | Moraceae | Leaves | GH850 | Sang Ye |
| 24 | *M. alba* L. | Moraceae | Root bark | GH851 | Sang Bai Pi |
| 25 | *Ophiopogon japonicus* (Thunb.) Ker Gawl. | Asparagaceae | Tuber | GH852 | Mai Dong |
| 26 | *Panax ginseng* C. A. Mey. | Araliaceae | Tuber | GH853 | Ren Sen |
| 27 | *Phragmites australis* (Cav.) Trin. ex Steud. | Gramineae | Rhizome | GH854 | Lu Gen |
| 28 | *Polygonatum odoratum* (Mill.) Druce | Asparagaceae | Rhizome | GH855 | Yu Zhu |
| 29 | *Polygonatum sibiricum* F. Delaroche | Liliaceae | Rhizome | GH856 | Huang Jin |
| 30 | *Pouzolzia zeylanica* (L.) Benn. | Schisandraceae | Fruit | GH857 | Wu Shui Ge |
| 31 | *Pterocephalus hookeri* (C. B. Clarke) Hoeck | Dipsacaceae | Leaves | GH858 | Yi Shou Cao |
| 32 | *Pueraria lobata* (Willd.) Ohwi | Leguminosae | Root | GH859 | Ge Gen |
| 33 | *R. glutinosa* (Gaertn.) DC. | Scrophulariaceae | Prepared tuber | GH860 | Shu Di Huang |
| 34 | *R. glutinosa* (Gaertn.) DC. | Scrophulariaceae | Tube | GH861 | Sheng Di Huang |
| 35 | *Salvia miltiorrhiza* Bunge | Labiatae | Root | GH862 | Dan Shen |
| 36 | *Schisandra chinensis* (Turcz.) Baill. | Schisandraceae | Fruit | GH863 | Wu Wei Zi |
| 37 | *Trichosanthes kirilowii* Maxim. | Cucurbitaceae | Root | GH864 | Tian Hua Fen |

*L. barbarum*: *Lycium barbarum*; *M. alba*: *Morus alba*; *R. glutinosa*: *Rehmannia glutinosa*

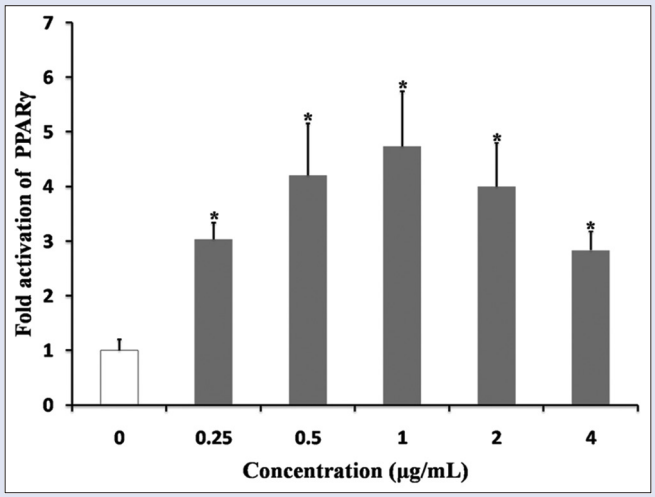


**Figure 1:** Fold activation of PPARγ by different dosages of rosiglitazone. The data are expressed as the means and standard deviations of three independent experiments with triplicate well. *$P$ < 0.05 vehicle control dimethyl sulfoxide (set to 1.0)

## Effects of different polarity extracts on the activation of peroxisome proliferator-activated receptors γ

The activation of PPARγ resulted from 185 different kinds of prepared extracts from selected TCMs were tested for six concentrations, respectively. The results [Table 2] indicated that nine extracts resulted in stronger activation on PPARγ than rosiglitazone based on the comparisons of the maximum folds on the activation of PPARγ.

Ethyl acetate extract of root bark of *Lycium barbarum* had the strongest effect on activating PPARγ in all extracts. The maximum fold was 7.11-fold (50 μg/mL), which equal to 207% of positive drug [Figure 2a]. Besides, ethyl acetate extracts of rhizome of *Phragmites australis* and fruit of *Gleditsia sinensis* also could significantly activate PPARγ. The maximum fold on the activation of PPARγ by rhizome of *P. saustralis* was 5.69 fold, which was 137% of 0.5 μg/mL rosiglitazone [Figure 2b]. The maximum fold activation of the fruit of *G. sinensis* was 5.02-fold, about 105% that of positive control [Figure 2c].

Petroleum ether extracts of *Anoectochilu sroxburghii*, *Pterocephalus hookeri*, *Polygonatum sibiricum*, *Epimedium brevicornu* also can significantly activate PPARγ. The maximum folds on the activation of PPARγ by them were higher than that of positive drug. The maximum

**Table 2:** Summary of the results obtained by applying the screening model to 185 different polarity extracts from 37 traditional Chinese medicines

| Number | Botanical name | Extracts[a] | Concentration at max fold activation (μg/mL) | Max fold activation mean±SD (positive drug fold activation) | Percentage versus positive drug |
|---|---|---|---|---|---|
| 1 | Root bark of *L. barbarum* | Ethyl acetate | 50 | 7.11±0.60 (3.43) | 207 |
| 2 | *Anoectochilus roxburghii* | Petroleum ether | 200 | 5.15±0.50 (3.22) | 160 |
| 3 | Rhizome of *P. australis* | Ethyl acetate | 200 | 5.69±0.27 (4.16) | 137 |
| 4 | *P. hookeri* | Petroleum ether | 400 | 4.06±0.49 (3.36) | 121 |
| 5 | *P. sibiricum* | Petroleum ether | 100 | 4.67±0.57 (3.99) | 117 |
| 6 | *P. hookeri* | The residual of ethanol | 400 | 3.65±0.58 (3.36) | 109 |
| 7 | *P. hookeri* | The supernatant of water | 25 | 3.61±0.62 (3.36) | 107 |
| 8 | Fruit of *G. sinensis* | Ethyl acetate | 50 | 5.02±0.63 (4.78) | 105 |
| 9 | *E. brevicornu* | Petroleum ether | 200 | 5.76±0.72 (5.54) | 104 |
| 10 | *Root bark of M. alba* | Petroleum ether | 50 | 3.17±0.22 (3.20) | 99 |
| 11 | *A. membranaceus* | Ethyl acetate | 100 | 3.31±0.62 (3.36) | 99 |
| 12 | *A. membranaceus* | Petroleum ether | 50 | 3.71±0.26 (3.73) | 99 |
| 13 | *Clematis chinensis* | Petroleum ether | 200 | 4.83±0.76 (4.94) | 98 |
| 14 | *Leaves of M. alba* | Petroleum ether | 12.5 | 4.37±0.56 (4.53) | 96 |
| 15 | *P. lobata* | Petroleum ether | 400 | 4.31±0.37 (4.57) | 94 |
| 16 | *P. sibiricum* | Ethyl acetate | 400 | 3.67±0.62 (3.99) | 92 |
| 17 | *P. lobata* | Ethyl acetate | 200 | 4.13±0.38 (4.57) | 91 |
| 18 | Leaves of *M. alba* | The residual of ethanol | 12.5 | 3.99±0.41 (4.53) | 88 |
| 19 | *A. artemisiifolia* | Petroleum ether | 200 | 4.40±0.47 (5.07) | 87 |
| 20 | *Cuscuta chinensis* | Petroleum ether | 200 | 4.29±0.73 (4.91) | 87 |
| 21 | *E. brevicornu* | Ethyl acetate | 50 | 4.54±0.83 (5.54) | 82 |
| 22 | *M. doumeri* | Ethyl acetate | 200 | 3.11±0.60 (3.82) | 81 |
| 23 | Hui Dou Ba | Petroleum ether | 200 | 2.73±0.33 (3.48) | 78 |
| 24 | *Pouzolzia zeylanica* | Petroleum ether | 400 | 2.88±0.36 (3.48) | 78 |
| 25 | *A. artemisiifolia* | Ethyl acetate | 12.5 | 3.97±0.32 (5.07) | 78 |
| 26 | *Cyelcobalanpsis glauca* | The supernatant of water | 200 | 3.26±0.36 (4.257) | 77 |
| 27 | *G. pentaphyllum* | Ethyl acetate | 12.5 | 2.69±0.32 (3.65) | 74 |
| 28 | *R. glutinosa* | Petroleum ether | 400 | 2.90±0.71 (4.25) | 68 |
| 29 | Root bark of *M. alba* | Ethyl acetate | 400 | 2.19±0.32 (3.20) | 68 |
| 30 | *S. miltiorrhiza* | Ethyl acetate | 50 | 3.02±0.66 (4.54) | 66 |
| 31 | *C. pinnatifida* | Ethyl acetate | 50 | 3.08±0.35 (4.68) | 66 |
| 32 | *Clematis chinensis* | Ethyl acetate | 200 | 3.23±0.67 (4.94) | 65 |
| 33 | *R. glutinosa* | Ethyl acetate | 12.5 | 2.23±0.03 (3.43) | 65 |
| 34 | Hui Dou Ba | Ethyl acetate | 50 | 2.26±0.22 (3.48) | 65 |
| 35 | *O. japonicas* | Petroleum ether | 100 | 2.08±0.68 (3.32) | 63 |
| 36 | *P. ginseng* | Ethyl acetate | 200 | 3.20±0.36 (5.10) | 63 |
| 37 | *M. oleifera* | Ethyl acetate | 200 | 2.54±0.43 (4.10) | 62 |
| 38 | Rhizome of *P. australis* | Petroleum ether | 25 | 2.56±0.43 (4.16) | 61 |
| 39 | *G. pentaphyllum* | Petroleum ether | 50 | 2.23±0.25 (3.65) | 61 |
| 40 | *M. oleifera* | Petroleum ether | 400 | 2.42±0.16 (4.10) | 59 |
| 41 | *A. membranaceus* | Ethyl acetate | 6.25 | 2.17±0.34 (3.73) | 58 |
| 42 | *O. japonicas* | Ethyl acetate | 50 | 1.90±0.14 (3.32) | 57 |
| 43 | *A. lancea* | Ethyl acetate | 50 | 2.84±0.56 (5.01) | 57 |
| 44 | Fruit of *G. sinensis* | Petroleum ether | 100 | 2.69±0.26 (4.78) | 56 |
| 45 | *D. moniliforme* | Ethyl acetate | 100 | 2.60±0.35 (4.70) | 55 |
| 46 | *Anemarrhena asphodeloides* | Ethyl acetate | 200 | 2.53±0.59 (4.67) | 54 |
| 47 | *A. lancea* | Petroleum ether | 50 | 2.71±0.53 (5.01) | 54 |
| 48 | *S. miltiorrhiza* | The supernatant of water | 25 | 2.43±0.27 (4.54) | 54 |
| 49 | *M. doumeri* | The supernatant of water | 200 | 1.74±0.14 (3.82) | 52 |
| 50 | *Polygonatum odoratum* | Petroleum ether | 400 | 2.31±0.46 (4.60) | 50 |
| 51 | Fruit of *L. barbarum* | Petroleum ether | 400 | 2.02±0.51 (4.03) | 50 |
| 52 | *P. ginseng* | Petroleum ether | 200 | 2.46±0.36 (5.10) | 48 |
| 53 | Hui Dou Ba | The residual of ethanol | 400 | 1.63±0.14 (3.48) | 47 |
| 54 | *M. doumeri* | Petroleum ether | 200 | 1.74±0.14 (3.82) | 46 |
| 55 | Fruit of *L. barbarum* | Ethyl acetate | 400 | 1.83±0.25 (4.03) | 45 |
| 56 | *S. miltiorrhiza* | Petroleum ether | 2.5 | 2.02±0.17 (4.54) | 44 |
| 57 | *Cuscuta chinensis* | Ethyl acetate | 200 | 2.16±0.54 (4.91) | 44 |
| 58 | *C. pinnatifida* | Petroleum ether | 100 | 2.03±0.17 (4.68) | 43 |
| 59 | *Momordica charantia* | Petroleum ether | 100 | 2.30±0.46 (5.34) | 43 |
| 60 | *E. brevicornu* | The residual of ethanol | 100 | 2.35±0.67 (5.54) | 42 |
| 61 | *Ligustrum lucidum* | Petroleum ether | 200 | 1.69±0.22 (4.52) | 37 |
| 62 | Fruit of *G. sinensis* | The supernatant of water | 100 | 1.72±0.28 (4.78) | 36 |
| 63 | *D. moniliforme* | Petroleum ether | 50 | 1.67±0.33 (4.70) | 35 |
| 64 | *D. moniliforme* | The supernatant of water | 200 | 1.60±0.31 (4.70) | 34 |

[a]Only the extracts inducing transactivation of PPARγ have been included in this table. PPARγ: Peroxisome proliferator-activated receptor gamma; SD: Standard deviation; *D. moniliforme*: *Dendrobium moniliforme*; *G. sinensis*: *Gleditsia sinensis*; *E. brevicornu*: *Epimedium brevicornu*; *C. pinnatifida*: *Crataegus pinnatifida*; *S. miltiorrhiza*: *Salvia miltiorrhiza*; *L. barbarum*: *Lycium barbarum*; *M. doumeri*: *Malus doumeri*; *P. ginseng*: *Panax ginseng*; *A. lancea*: *Atractylodes lancea*; *O. japonicas*: *Ophiopogon japonicas*; *A. membranaceus*: *Astragalus membranaceus*; *M. oleifera*: *Moringa oleifera*; *G. pentaphyllum*: *Gynostemma pentaphyllum*; *P. australis*: *Phragmites australis*; *R. glutinosa*: *Rehmannia glutinosa*; *M. alba*: *Morus alba*; *A. artemisiifolia*: *Ampelocissus artemisiifolia*; *P. lobata*: *Pueraria lobata*; *P. sibiricum*: *Polygonatum sibiricum*; *P. hookeri*: *Pterocephalus hookeri*

fold to activate PPARγ by *A. sroxburghii* was 5.15 fold, about 160% compared to 0.5 μg/mL rosiglitazone [Figure 3a]. The maximum fold to activate PPARγ by *P. hookeri* was 4.06 fold, 121% of positive control [Figure 3b]. The maximum folds on the activation of PPARγ by *P. sibiricum* and *E. brevicornu* were 4.67 fold and 5.76 fold, which equaled to 117% and 104% of 0.5 μg/mL rosiglitazone [Figure 3c and d].

In addition, the residual of ethanol and the supernatant of water extracts of *P. hookeri* also had remarkable activation effects on PPARγ [Figure 4a and b].

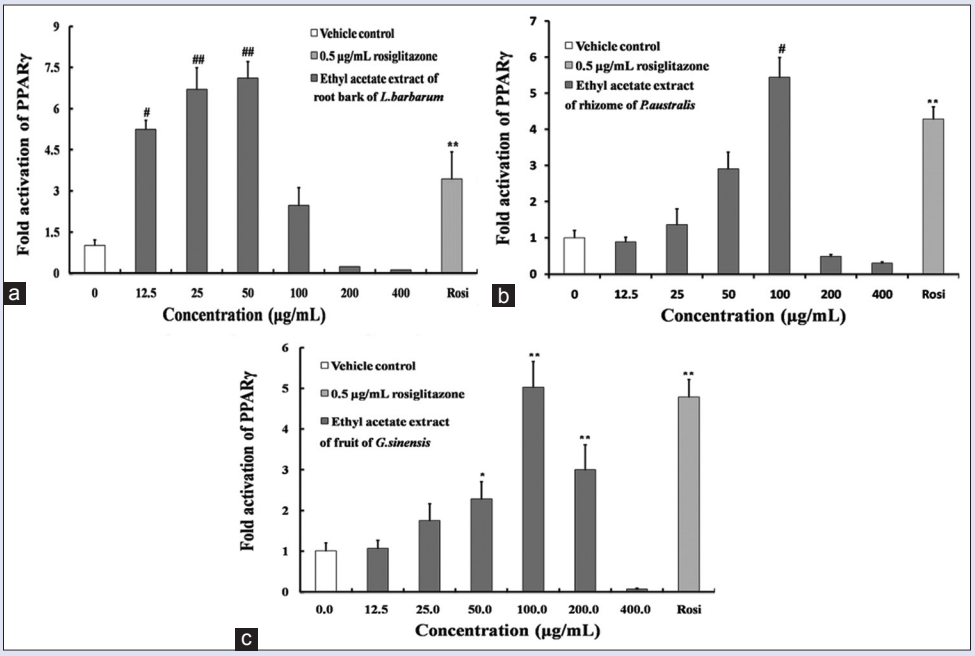


**Figure 2:** Fold activation of PPARγ by ethyl acetate extracts of Root bark of *Lycium barbarum*, (a) rhizome of *Phragmites australis,* (b) fruit of *Gleditsia sinensis.* (c) The data are expressed as the means and standard deviations of three independent experiments with triplicate well. **P* < 0.05 versus vehicle control dimethyl sulfoxide, ***P* < 0.01 versus vehicle control dimethyl sulfoxide (set to 1.0), #*P* < 0.05 versus 0.5 μg/mL rosiglitazone, ##*P* < 0.01 versus 0.5 μg/mL rosiglitazone

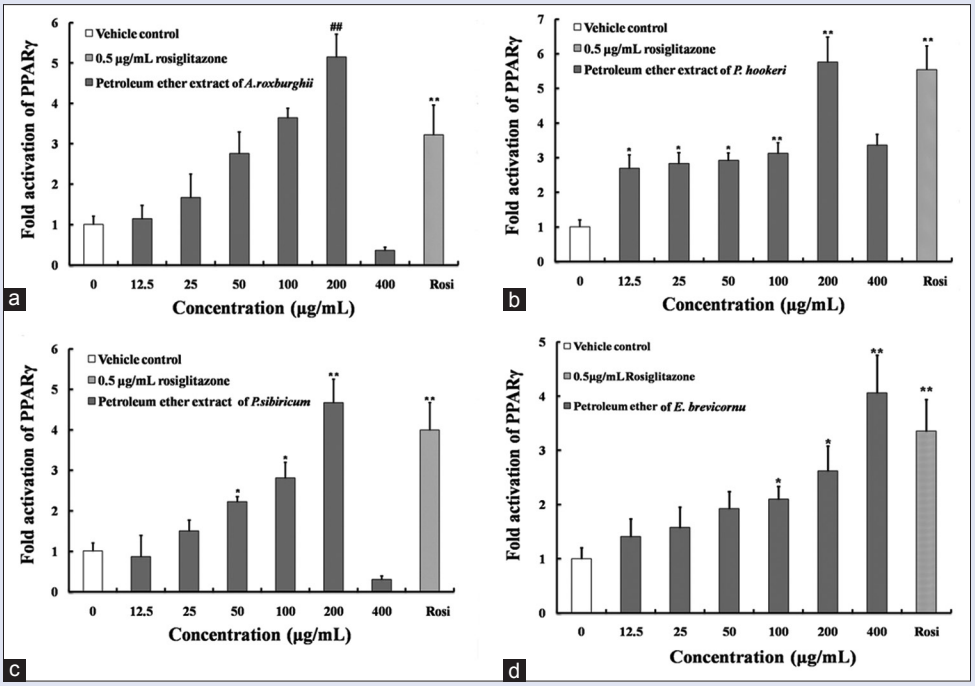


**Figure 3:** Fold activation of peroxisome proliferator-activated receptors-γ by n-hexane extracts of *Anoectochilu sroxburghii,* (a) *Pterocephalus hookeri,* (b) *Polygonatum sibiricum,* (c) *Epimedium brevicornu* (d). The data are expressed as the means and standard deviations of three independent experiments with triplicate well. **P* < 0.05 versus vehicle control dimethyl sulfoxide, ***P* < 0.01 versus vehicle control dimethyl sulfoxide (set to 1.0), #*P* < 0.05 versus 0.5 μg/mL rosiglitazone, ##*P* < 0.01 versus 0.5 μg/mL rosiglitazone

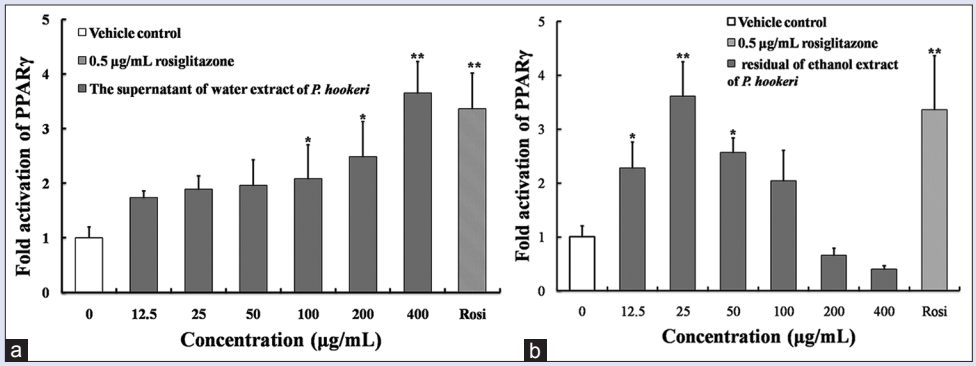


**Figure 4:** Fold activation of peroxisome proliferator-activated receptors-γ of the supernatant of water extract (a) and residual of ethanol extract (b) of *Pterocephalus hookeri*. The data are expressed as the means and standard deviations of three independent experiments with triplicate well. *$P < 0.05$ versus vehicle control dimethyl sulfoxide, **$P < 0.01$ versus vehicle control dimethyl sulfoxide (set to 1.0)

The maximum folds on the activation of PPARγ by them were similar to positive drug. The maximum folds to activate PPARγ by the residual of ethanol and the supernatant of water extracts were 3.65 fold and 3.61 fold, which were 107% and 104% that of positive control, respectively.

However, traditionally anti-diabetic medicine including *Schisandra chinensis*, the fruit of *Calendula officinalis*, *Alisma plantago-aquatica* and the root of *Trichosanthes kirilowii*, had no effects on the activation of PPARγ.

## DISCUSSION

Nuclear receptors are large family of receptors which allow an access to the control of gene regulation. The family contains steroid receptors, metabolic receptors, retinoid receptors, Vitamin D receptor and *et al*.[22,23] They play important roles in normal development, reproduction, and metabolism.[24-26] Most nuclear receptors, share a similar basic structure that contains two important domains named LBD and DNA-binding domain. The two domains are crucial for the regulation of the transcription of nuclear receptors. Especially, the LBD fulfills the functions of ligand binding, dimerization, and recruitment of co-regulators.[27-29] Ligand binding and activation of nuclear receptor induces a conformational change of it and participates in the regulation of transcription via many approaches such as activation of phosphorylation of the receptor, replacement of corepressors by coactivators and *et al*.[30,31]

Considering that nuclear receptor family binds to a wide range of lipophilic ligands derived from daily life, targeting nuclear receptors have been a major source for the development of new drugs.[32] It is noteworthy that researchers have found some active compounds from traditional medicines which are the ligands for some of these nuclear receptors.[23,33] For example, honoliol, a compound isolated from magnolia bark, is a partial PPARγ agonist which binds directly to PPARγ LBD.[20] On accounting that honokiol can prevent hyperglycemia and weight gain in mice, it becomes a clinically interesting compound which has opportunity to replace TZDs.

In the past few decades, the prevalence of diabetes mellitus has increased, especially type 2 diabetes. Type 2 diabetes mellitus are closely related to unhealthy diet, sedentary lifestyle, as well as the rise of obesity in the population, which in turn impel the search for new preventive and treatment strategies.[34,35] In recent years, metabolic receptors from nuclear receptor family get more and more attention due to their regulation of metabolic homeostasis.[36,37] It is noteworthy that PPARγ, which belongs to PPAR family, is widely used as an important target for the treatment of diabetes mellitus. Ligand binding of PPARγ can induce the expression of a lot of genes which in turns changes the lipids and glucose metabolism.[38,39] However, common agonists of PPARγ such as TZDs, have many serious side-effects such as cardiovascular risks, liver damage, weight gain, and peripheral edema.[40] Hence, significant research efforts have recently been undertaken to explore the potential drugs of activating PPARγ with less adverse effects.[40-43] TCMs seem to be an ideal replacement of TZDs for the treatment of hyperglycemia, insulin resistance, and the diabetic complications with less adverse effects.[44-46]

Based on the cell-based PPARs screening model.[47] In this study, thirty-seven traditionally used anti-diabetic TCMs were selected and to evaluate their activation activities of PPARγ. The activations of PPARγ-LBD by different polarity extracts from 37 TCMs were evaluated. The results indicated that the ethyl acetate extracts of root bark of *L. barbarum*, rhizome of *P. saustralis* and fruit of *G. sinensis* could significantly activate PPARγ. The activities of them were higher than that of 0.5 μg/mL rosiglitazone. Besides, petroleum ether extracts of *A. sroxburghii*, *P. hookeri*, *P. sibiricum*, *E. brevicornu* also can significantly activate PPARγ. The activities of them were also higher than that of 0.5 μg/mL rosiglitazone. Furthermore, the residual of ethanol and the supernatant of water extracts of *P. hookeri* had remarkable activation effects on PPARγ. Thus, three extracts (petroleum ether, the residual of ethanol and the supernatant of water) of *P. hookeri* were found to have a significant effect on the activation of PPARγ compared to 0.5 μg/mL rosiglitazone. On the other hand, previous studies showed that *P. hookeri* had good anti-inflammatory effects, and it was used to treat inflammation and analgesic in Tibet of China.[48] Other evidence also demonstrated that the ligands of PPARγ can treat inflammation in the development of diabetes mellitus.[49,50] Therefore, it is speculated that the significant activation of PPARγ by *P. hookeri* might be related to its remarkable anti-inflammatory activity.

On the contrary, we found that all extracts of *S. chinensis*, *C. officinalis*, *A. plantago-aquatica,* and the root of *T. kirilowii* could not activate PPARγ. Previous studies showed that extracts of *S. chinensis* were effective when used as aldosereductase inhibitors for the treatment of diabetes mellitus.[51] The petroleum ether extract of it had PTP1B and alpha-glucosidase inhibitory activities. Moreover, schisandrols A and B, schisandrins A and B from *S. chinensis* were the ligands of pregnane X receptor, a xenobiotic receptor from nuclear receptor family.[23] Thus, it was speculated that the anti-diabetic effect of *S. chinensis* was through activating mulitiple targets except for PPARγ. Similar to *S. chinensis*, the anti-diabetic effects of *C. officinalis*, *A. plantago-aquatica*and the root of *T. kirilowii* were not through activating PPARγ.

*Momordicac harantia* also named "bitter gourd" had been found to be able to activate PPARα and PPARγ *in vitro*.[52] In our study, we also found

it can activate PPARγ. Furthermore, extracts of *Panax ginseng*, *Salvia miltiorrhiza,* and *Gynostemma pentaphyllum* also can activate PPARγ.[23,53] The results were consistent with the previous study. However, among these chosen TCMs, only a few of them had been studied with regard to the PPARγ activation through a PPARγ transactivation assay *in vitro*. Our study firstly evaluated the PPARγ activation by different polarity extracts from 37 TCMs with a PPARγ transactivation assay, providing the basis of clarifying the specific mechanism, looking for other targets of activating extracts and screening the active compounds. Further studies will be focused on studying the specific mechanisms of active extracts on activating PPARγ and screening of the active compounds through bio-guided separation.

## CONCLUSION

In summary, we have established that petroleum ether extracts of *A. sroxburghii*, *P. hookeri*, *P. sibiricum*, *E. brevicornu*, ethyl acetate extracts of root bark of *L. barbarum*, rhizome of *P. saustralis* and fruit of *G. sinensis* can significantly activate PPARγ as shown by specific activation of a PPARγ-LBD luciferase receptor assay. Hence, the anti-diabetic activity of them could in part be mediated by this nuclear receptor. Additional research will be necessary to further identify the effects of active extracts such as ethyl acetate extract of root bark of *L. barbarum* on PPARγ-mediated gene expression and biological responses. Moreover, further studies also will be done to identify the active compounds of PPARγ-LBD.

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### Conflicts of interest

There are no conflicts of interest.

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Zhining Xia

## ABOUT AUTHOR

**Zhining Xia,** obtained his Ph. D, degree in University of Copenhagen. He is professor in the College of Chemistry and Chemical Engineering Chongqing University. Chongqing, China. His work mainly in: Medicinal plants, capillary electrophoresis, pharmaceutical analysis of Chinese medicines and nano-drug.