ABS-3801

Antioxidant activities of underexplored Chinese medicinal plant parts and their effect against high glucose-induced modulation of fibronectin expression

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Abstract

In this study, we have chosen some of the underexplored plant parts of Chinese medicinal herbs and analyzed their antioxidant activity and ability to modulate the expression of fibronectin during high glucose conditions. Extraction of the plant materials with different solvent led to 17 extracts and among which, 3 extracts (2, 12 & 17) were observed to render more than 50 µg/ml Vitamin C equivalents of DPPH free radical scavenging ability and 2 of them (2 & 17) showed more than 25 µg/ml of Vitamin C equivalents of ferric ion reducing power. Based on the antioxidant activity and comparison of their total phenolic content, we used extracts 2 & 17 to check their effect on fibronectin expression in MES-13 cells under high sugar conditions. We observed that both extracts showed a significant reduction of fibronectin expression compared to untreated cells with high glucose levels. The expression was much lesser than the normal untreated, normal sugar supplemented cells and this was not observed in vitamin C supplemented cells. In conclusion, crude extracts containing a group of phenolic compounds have shown significant effects against fibronectin expression leading to reduced ECM deposition and tissue fibrosis. Further exploration might provide insights into the exact mechanism and checkpoints of the extract that can successfully reduce diabetes-induced renal complications.

Background and study aim

Diabetes

Diabetes mellitus is a metabolic disease characterized by patient's blood sugar which will be higher than the standard value for a prolonged period of time. All diabetes-mediated complications are due to high glucose content and excessive glycosylation of functional proteins, leading to cellular conditions like mitochondrial dysfunction, Reactive oxygen species (ROS) generation, apoptosis, and Extracellular matrix (ECM) deposition leading to fibrosis

Chinese herbal medicines

Most of our ancestors relied on extracts obtained from medicinal herbs that contain a group of bioactive compounds that can act together to render optimal activity against disease progressing mechanisms

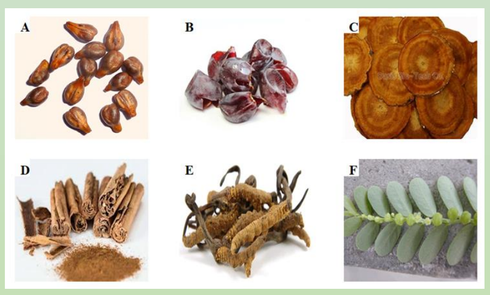


Figure 1: Different plant materials used in this study.

A. Grape seeds (https://www.feedipedia.org/node/692)
B. Grape skins (https://www.shutterstock.com/)
C. *Antrodia Campharata* (https://www.taiwantrade.com/)
D. *Cinnamomum osmophloeum* Kaneh (https://lovelytaiwan.org/)
E. *Cordyceps Sinensis* (E; https://hifasdaterra.co.uk/)
F. *Phyllanthus urinaria* L. (F; https://plants.ces.ncsu.edu/)

Our aim was to explore the antioxidant activities of some selected, under-explored plant parts (Figure 1) and their ability to inhibit fibronectin expression during high sugar condition in MES-13 renal mesangial cells

Methods

DPPH assay

We check the DPPH radical scavenging ability of PE extract (0 -300 µg/ml) with Trolox as Control

↓

In a 96-well plate, add 50µL sample + 50µL DPPH in Methanol to each well, mix well and perform three replicates, with water as the background value

↓

Incubate for 20 minutes in the dark, and measure its absorbance at 517nm

FRAP assay

FRAP working solution:
0.3M Acetate buffer + 10 mM TPTZ + 20 mM $FeCl_3.6H_2O$, at a ratio of 10: 1: 1

↓

In a 96-well plate, add 6µL sample + 180µL FRAP working solution to each well, mix well and perform three replicates, with water as the background value

↓

Incubate for 5 minutes in the dark, and measure its absorbance at 593nm

Total Phenolic content

Plant extract (80 µl; 100 µg/ml) + 720 µl of deionized water and 80 µl of Folin-Ciocalteu's reagent and mixed evenly and 800 µl of 7% Na2CO3 was added and incubated for 5 minutes

↓

Volume was adjusted to 2 ml using deionized water and absorbance was measured at 750 nm after 90 minutes

↓

The results were compared with the standard curve of L-ascorbic acid and total phenolic contents were expressed relative to the ascorbic acid equivalents

Western blotting

In a 24 well plate, 1×10^4 cells were seeded per well and incubated for 1 day at 37℃ and 5% CO2

↓

Change to serum-free medium and culture for 2 days

↓

Cells were grouped as Normal glucose supplemented, High glucose supplemented, with or with out extracts

↓

Cell proteins were extracted from all the groups, run on 10% SDS-PAGE and transferred to PVDF membrane on which the change in expression of Fibronectin was observed by Enhanced Chemiluminiscence

Summary

- Chinese medicinal herbs were explored and we found that among various crude extract, butanol extract of grape seed and methanolic extract of Phyllanthus urinaria showed significant DPPH free radical scavenging ability and ferric reducing power
- We mimicked the diabetic condition, in vitro by supplementing a high amount of glucose to renal mesangial cells (MES-13) and added crude extracts. We observed that expression of fibronectin at high glucose environment was significantly reduced by the extracts and the activity was higher compared to the ability of Vitamin C
- We conclude that Chinese medicinal plants and their parts possess high amounts of polyphenolic compounds and they work synergistically to inhibit vital pathological processes in diseases such as diabetes.

Results

Extract No	Crude extract (100 µg/ml)		Antioxidant activities Sample concentration (100 µg/ml)		
			DPPH radical scavenging activity[a]	FRAP assay[a]	Total phenolic content[b]
1	Grape seed	Chloroform	25.5	20.0	0.0
2		n-Butanol	96.3	53.6	7.6
3		Water	18.5	2.1	0.0
4	Grape skin	n-Hexane	5.1	0.9	0.0
5		n-Butanol	6.3	12.3	0.0
6		Dichloromethane	2.5	2.3	0.0
7		Ethyl acetate	23.1	14.4	0.8
8	*Antrodia Campharata*	n-Hexane	5.7	2.9	0.0
9		Chloroform	3.4	0.3	0.0
10		Methanol	5.6	3.5	0.0
11	*Cinnamomum osmophloeum Kaneh.*	Ethyl acetate	15.2	8.9	0.0
12		Methanol	50.6	16.1	0.4
13		n-Hexane	0.0	2.3	0.0
14	*Cordyceps Sinensis*	Chloroform	5.7	0.9	0.0
15	*Phyllanthus urinaria L.*	n-Hexane	3.7	1.4	0.0
16		Chloroform	3.9	2.0	0.0
17		Methanol	85.8	25.8	0.4

Table 1: Antioxidant activities of crude extracts obtained from all the plant materials.
a Relative antioxidant activity of the extract with respect to vitamin C (µg/ml)
b Relative phenolic content in the extract with respect to vitamin C (µg/ml)

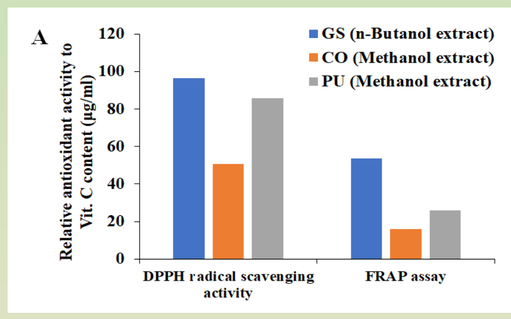


Figure 2: Comparison of antioxidant activities (A) and Total phenolic contents (B)

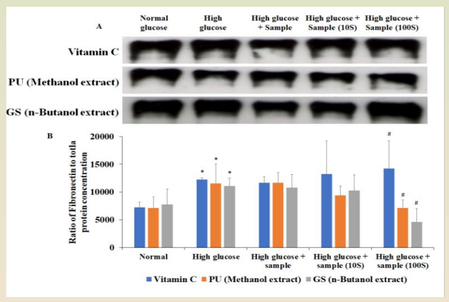
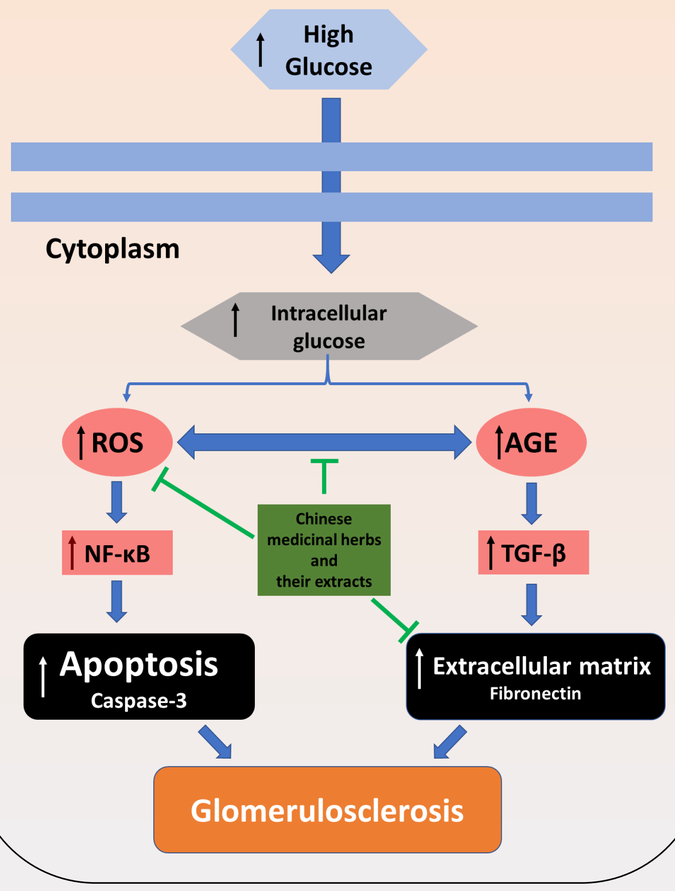


Figure 3: Western blotting analyses of Fibronectin. Protein bands observed after treatment against samples (A); Graph showing the difference in intensity of the bands observed in ImageJ software (B). PU indicates Phyllanthus urinaria and GS indicates grape seed. Concentration of the samples: Vitamin C – S – 0.086 µg/ml; 10S – 0.86 µg/ml; 100S – 8.6 µg/ml. PU – S – 0.1 µg/ml; 10S – 1 µg/ml and 100S – 10 µg/ml. GS – S – 0.089 µg/ml; 10S – 0.89 µg/ml; 100S – 8.9 µg/ml. The results were statistically analyzed by Student t-test (n=3) and comparisons were made as *High glucose treatment Vs Normal glucose treatment (* - p<0.05); # High glucose + Sample (100S) treatment Vs High glucose treatment (# - p<0.05).

Conclusion



Acknowledgement

Ministry of education (Grant number: 109F00908)

Yushing Biotech Co. Ltd., Taichung (Grant number 109B205)

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