

Original Research Article

Bergenin from *Cissus javana* DC. (Vitaceae) root extract enhances glucose uptake by rat L6 myotubes

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Abstract

Purpose: To examine the glucose uptake stimulatory activity of the root extract of *Cissus javana* DC. (Vitaceae) in L6 myotubes of rat, and also to identify the extract's active principles.

Methods: The methanol extract was prepared from *Cissus javana* tuberous roots and evaluated for glucose uptake stimulatory effects on L6 rat muscle cells and inhibitory activity against α -glucosidase. The chemical components were isolated using several chromatographic techniques, and their structures characterized by spectroscopic methods. Each isolate was then assayed for glucose uptake stimulatory and α -glucosidase inhibitory activities.

Results: The extract (100 μ g/ml) exhibited glucose uptake stimulatory effect (70.9 % enhancement) and α -glucosidase enzyme inhibitory activity (100 % inhibition). Through chromatographic separation, bergenin, stigmast-4-en-3-one and β -sitosterol were isolated and identified. Bergenin, at 100 μ g/ml (0.3046 mM), increased glucose uptake by L6 myotubes by 50.5 % without toxicity. At the same concentration, bergenin showed no inhibition on α -glucosidase enzyme, while stigmast-4-en-3-one and β -sitosterol exhibited 98.6 and 40.6 %, inhibition, respectively.

Conclusion: This study is the first report on the chemical constituents, and the glucose uptake stimulatory and α -glucosidase inhibitory activities of *Cissus javana* DC. roots. The findings reveal the antidiabetic potential of the plant and the glucose-uptake enhancing activity of bergenin.

Keywords: *Cissus javana*, α -Glucosidase, Antidiabetes, Rat skeletal muscle cells, Bergenin

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by elevated blood glucose level. Severe complications associated with DM, such as cardiovascular disease and damage to nerves, eyes and kidneys, frequently occur and

often lead to premature death [1]. Currently, several classes of oral antidiabetic agents are available. Biguanides, such as metformin, are anti-DM drugs that act by stimulating glucose uptake by muscle cells and reducing insulin resistance. However, the long-term use of these drugs can lead to vitamin B₁₂ and folic acid

deficiency, lactic acidosis and renal insufficiency [2]. In recent years, glucose-uptake enhancers of plant origin have been the subject of interest [3,4].

The genus *Cissus* (Vitaceae), also known as *Vitis*, consists of 350 species, widely distributed in China, India, Bangladesh, Nepal, and South-east Asia [5]. Several species of *Cissus*, including *C. cornifolia*, *C. barbeyana*, *C. repens*, *C. vinifera* and *C. verticillata*, have been reported for antidiabetic potential [6-10].

Cissus javana DC. has several synonyms, including *Vitis discolor* (Blume) Dalzell, *Cissus discolor* Blume, *Cissus javana* var. *pubescens* C. L. Li, and *Cissus sicyoides* Klein ex Steud. [11]. The plant is an herbaceous climber with distinct leaves (Figure 1). The upper surface contains white patches, whereas the lower surface is purple. Leaves are mostly ovate shaped with a heart-shaped base and an apiculate apex [12]. In Myanmar, the tuberous roots are used as a folk medicine for treating ovarian cancer [13], while in India, the leaves are known for anti-DM activity [14]. Prior to the present work, no studies on the anticancer or anti-DM constituents of *C. javana* roots have been described.



Figure 1: *Cissus javana*: (a) whole plant; (b) branch; (c) leaves; (d) tuberous roots

In this investigation, the MeOH extract, and fractions A and B (prepared from the roots of *Cissus javana*; see the experimental) were screened for cytotoxicity against human cancer

cells, but none of them showed activity ($IC_{50} > 50$ $\mu\text{g/ml}$; data not shown). However, the MeOH extract, when tested at 100 $\mu\text{g/ml}$, exhibited 70 % stimulation of glucose uptake by L6 rat skeletal muscle cells and 100 % inhibition of α -glucosidase enzyme. These results prompted us to further our study to identify the constituents responsible for the anti-DM potential of this plant.

EXPERIMENTAL

General

Mass spectra were obtained on a Bruker micro TOF mass spectrometer. NMR experiments were conducted using a Bruker Avance DPX-300 NMR spectrometer. Column chromatography was performed on silica gel (Merck). Gel filtration was carried out on Sephadex LH-20 (GE Healthcare). A Biochom EZ Read 400 microplate reader was used to measure the absorbance of samples in microtiter plates.

Plant materials

Cissus javana DC. roots were collected from Shan State, Myanmar in July 2017. The plant was authenticated by comparison with herbarium specimens at University of Pharmacy, Yangon, Myanmar.

Chemicals

Fetal bovine serum (FBS), alpha minimal essential medium (α -MEM), and penicillin-streptomycin (10000 IU/ml) were obtained from Thermo Fisher Scientific (Grand Island, NY, USA). Acarbose, glucose oxidase (GO) assay kit, α -glucosidase (from *Saccharomyces cerevisiae*), 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), *p*-nitrophenyl-D-glucopyranoside (*p*NPG) and sodium dodecyl sulfate (SDS) were purchased from Sigma Aldrich (St. Louis, MO, USA). Insulin (100 IU/ml) was from Biocon (Bangalore, India).

Cell lines and culture media

L6 cell culture (Rat skeletal muscle, ATCC[®]CRL-1458) was purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in growth medium (α -MEM with 10 % FBS and 1 % penicillin-streptomycin) and maintained at 37° C under 5 % CO₂ atmosphere.

Extraction and isolation

The dried powder of tuberous roots of *Cissus javana* DC. (190 g) were macerated with MeOH)

(1L × 3). The dried MeOH extract (8.8 g) was then treated with EtOAc to give EtOAc insoluble (fraction A, 6.36 g) and soluble (fraction B, 2.38 g) fractions after drying. Fraction A was separated by vacuum liquid chromatography (silica gel, hexane-EtOAc-MeOH) to give five fractions (AI to AV). Compound **1** (245 mg) was collected as colorless crystals from fraction AI after drying.

Fraction B was further separated on a silica gel column (hexane-EtOAc) to seven subfractions (BI to BVII). BII was further fractioned on Sephadex LH-20 (CH₂Cl₂) to give fifteen fractions (BII1-BII15). Purification of BII5 on a Sephadex LH-20 (MeOH) column yielded compound **2** (12 mg). BIV gave white precipitates upon standing at room temperature overnight. The precipitates were collected, washed with EtOAc and dried to give compound **3** (50 mg). Compound **2** (12 mg) was also obtained from fraction BVI as colorless crystals after evaporation of the solvent.

Assay for cytotoxicity against cancer cells

Assays for cytotoxic activity against human breast cancer (MDA-MB-231) cells were kindly carried out by Dr P Rojsitthisak, using established protocols [15].

α-glucosidase inhibition assay

The α-glucosidase inhibition assay was carried out following previously reported protocols [3,4]. The inhibitory activity of each test compound was based on the ability to reduce the release of *p*-nitrophenol from the substrate *p*NPG, in comparison with the positive control acarbose.

Glucose uptake stimulation assay

The glucose uptake stimulation assay was performed on L6 skeletal muscle cells according to reported methods [3,4]. Positive controls were metformin and insulin.

Cytotoxicity against I6 cells

Cytotoxicity of each test compound is evaluated by the MTT [3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide] assay method [3,4].

Statistical analysis

The data are presented as mean ± SD, and were analyzed by analysis of variance (ANOVS) using GraphPad, version 7.00 for Windows. *P* < 0.05) was considered statistically significant.

RESULTS

The MeOH extract and fractions A and B were evaluated for toxicity against L6 cells and glucose-uptake stimulatory activity at 1, 10 and 100 µg/ml. None of them showed cytotoxicity (cell viability > 80 %) (Figure 2).

The MeOH extract at 10 and 100 µg/ml could stimulate glucose uptake by 41.8 and 70.9 %, respectively. Fraction A appeared to have stronger activity, with 52.0, 72.4 and 75.3 % enhancement at 1, 10 and 100 µg/ml, respectively, but fraction B displayed much less activity, showing stimulatory activity only at 100 µg/ml (57.8 %) (Figure 2 and Table 1).

Table 1: Glucose uptake stimulatory activity of *Cissus javana*

Sample	Glucose uptake (%)	Enhancement (%)
DMSO	100	0
Metformin (2 mM)	218.9 ± 4.4*	118.9 ± 3.6
Insulin (500 nM)	192.7 ± 13.1*	92.7 ± 10.7
MeOH extract		
1 µg/ml	102.5 ± 25.6	NA
10 µg/ml	141.8 ± 35.3*	41.8 ± 28.8
100 µg/ml	170.9 ± 15.1*	70.9 ± 12.3
Fraction A		
1 µg/ml	152.0 ± 10.9*	52.0 ± 9.0
10 µg/ml	172.4 ± 12.6*	72.4 ± 10.3
100 µg/ml	175.3 ± 23.1*	75.3 ± 18.9
Fraction B		
1 µg/ml	82.2 ± 11.0	NA
10 µg/ml	114.2 ± 24.3	NA
100 µg/ml	157.8 ± 24.3*	57.8 ± 19.8
Bergenin		
1 µg/ml (0.003 mM)	79.3 ± 19.0	NA
10 µg/ml (0.0304 mM)	124.3 ± 36.3	NA
100 µg/ml (0.304 mM)	150.5 ± 9.1*	50.5 ± 7.4

**P* < 0.05; significantly different when compared to the control (DMSO); NA = not applicable

Chromatographic separation of fractions A and B gave compounds **1**, **2** and **3** (Figure 3). They were identified as bergenin [16], stigmast-4-en-3-one [17], and β-sitosterol [18], respectively, based on their NMR and MS spectral data.

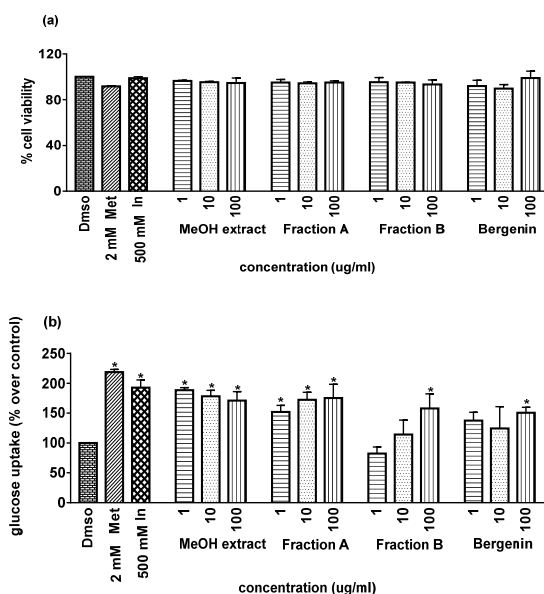


Figure 2: Cytotoxicity (a) and glucose uptake stimulation (b) of MeOH extract, fractions and bergenin; (* $p < 0.05$) Significantly different when compared to the control (DMSO). DMSO = control; Met = metformin, In = insulin (positive control)

DISCUSSION

Bergenin (**1**) has been previously isolated from several species of *Cissus* [19-20]. Compound **2** has been earlier found in *Cissus repens* [7], whereas **3** has been reported from *C. polyantha* [21]. Compounds **1 - 3** were subjected to assays for glucose uptake stimulatory potential. Unfortunately, the results for **2** and **3** could not be obtained because of their poor solubility in the test system, and this may partly account for the low activity of fraction B.

Bergenin (**1**) did not show activity at the concentrations of 1 and 10 $\mu\text{g/ml}$ but enhanced the glucose uptake by 50.5 % at 100 $\mu\text{g/ml}$ (0.3046 mM). This result was quite impressive

when compared with the percent enhancement by insulin (92.7 % at 500 nM) or metformin (118.9 % at 2 mM) (Figure 2). Interestingly, Kumar et al. investigated the hypoglycemic action of bergenin using streptozotocin-nicotinamide-induced type-2 diabetic rats and found that the compound could reduce the fasting blood glucose level in rats without effects on liver glycogen [22]. The findings in our study may suggest the mechanism of action of bergenin although more detailed studies are still needed. It should also be noted that **1** can be considered as structurally related to hydrolyzable tannins. Earlier studies revealed that the tannins present in plants could stimulate glucose uptake and inhibit adipogenesis [23].

In the assays for *in-vitro* α -glucosidase inhibitory activity, bergenin (**1**) showed no activity (2.2 % inhibition at 100 $\mu\text{g/ml}$), which was in agreement with a previous report [24]. The phytosterols **2** and **3** exhibited significant inhibitory effects (98.6 % and 40.6 % inhibition, respectively) in comparison with acarbose (21.9 % inhibition). These findings were consistent with earlier reported values [25].

CONCLUSION

The findings of this study indicate that a MeOH extract prepared from the tuberous roots of *Cissus javana* showed glucose uptake stimulatory activity and α -glucosidase inhibitory effect. Separation of the extract led to the isolation of 3 compounds, namely bergenin (**1**), stigmaster-4-en-3-one (**2**) and β -sitosterol (**3**). Bergenin (**1**) displayed remarkable glucose-uptake stimulatory activity on L6 rat skeletal muscle cells, but no α -glucosidase inhibitory potential. Steroids **2** and **3** showed recognizable α -glucosidase effects. However, they were not examined for the ability to enhance glucose uptake due to their poor solubility in the test

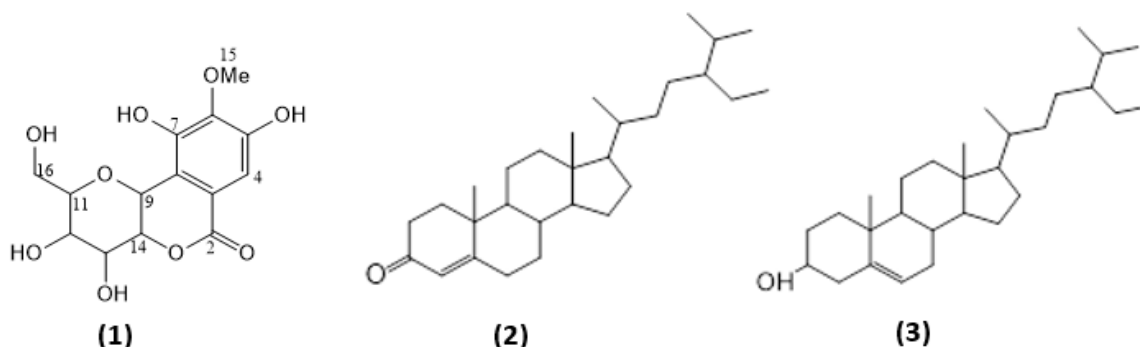


Figure 3: Structures of bergenin (**1**), stigmaster-4-en-3-one (**2**) and β -sitosterol (**3**)

system. These results suggest that the tuberous roots of *Cissus javana* DC. could be a potential source for the development of new antidiabetic drugs. To the best of our knowledge, this study is the first report of the chemical constituents and antidiabetic potential of the roots of *Cissus javana*.

DECLARATIONS

Acknowledgement

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Conflicts of interest

No conflicts of interest associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. HTS was responsible for the plant collection, isolation of compounds and α -glucosidase inhibition assays, as well as the preparation of the manuscript. PB and WP contributed to the cell-based assays for L6 glucose uptake and cytotoxicity. KL and BS supervised the project, reviewed the results and did critical reading and editing of the manuscript. All the authors have read the final manuscript and approved the submission.

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