

Evaluation of the Antidiabetic and Antibacterial Activity of *Cissus sicyoides*

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ABSTRACT

In this work we investigated the antidiabetic and antibacterial effect of *Cissus sicyoides* (CS) from Brazil. Diabetic rats that received water (A group) or extracts from the aerial parts of the plant (Cs group) during four weeks were employed. After this period, serum levels of glucose, cholesterol and triglycerides were measured. Glycemia was not affected by treatment with CS. However, there was an increased cholesterol and triglyceride level in Cs group. In addition, bioassay-guided fractionation of methanolic extract from aerial parts of CS was performed for isolation of antibacterial compounds. **b**Sitosterol and sitosterol-**bD**-glucopyranoside isolated showed antibacterial activity against *Bacillus subtilis* with minimal inhibitory concentrations (MICs) of 50 μ g/ml and 100 μ g/ml, respectively. In spite of popular belief, CS did not show antidiabetic activity. However, two compounds isolated from aerial parts of the plant (**b**sitosterol and sitosterol-**bD**-glucopyranoside) showed antibacterial activity.

Key words: *Cissus sicyoides*, Vitaceae, experimental diabetes mellitus, antibiosis, steroids

INTRODUCTION

Cissus sicyoides L. (Vitaceae) is a tropical plant widely used in the Brazilian folk medicine to treat diabetes mellitus. In Mexico, aqueous infusion of the plant is used in traditional medicine for relieving pain and inflammation. Additionally, aqueous extract of *Cissus sicyoides* showed vasoconstrictor effect on guinea pig aortic rings (Garcia *et al.*, 1997). Moreover, anthocyanins present in the fruit may have potential as a food colorant (Toledo *et al.*, 1983).

Investigations of various species from genus *Cissus* have been described. The leaves of *C. rheifolia* contain quinolizidine alkaloids, flavonoids, terpenoids, and allenic ketone (Saifah *et al.* 1983). The stem wood of *C. pallida* showed presence of stilbene, triterpenoids and steroids (Khan *et al.*, 1986). Lipids have been isolated from

aerial parts of *C. quadrangularis* and used in the treatment of bone fractures (Gupta *et al.*, 1991; Bhutani *et al.*, 1984). In addition, *C. populnea* from Benin is reputed as diuretic (Souza & Hounnon, 1985).

In this work, the antidiabetic and antibacterial activity of CS were evaluated.

MATERIALS AND METHODS

General experimental procedures: ¹H and ¹³C NMR at 300 and 75.5 MHz respectively, using TMS as standard, was registred in a Varian Gemini 300. GC/MS: Gas-liquid chromatography-spectrometry was performed on a Shimadzu Model CG-14A equipped with a Shimadzu Model QP 2000A mass spectrometer. The chromatograms were obtained under the following conditions: 1)

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Carbowax[®] column CG-FI-547 (50 m, 0.25 mm id.); 2) Carrier gas: Helium (1 ml/min); 3) Temperature: Injection port-220°C; 4) Programming: 80°C at 5 min then 80-230°C at 10°C/min.

Plant materials: The aerial parts of CS were collected in Maringá, Paraná, Brazil, in July 1997 and a Voucher, (N° HUM 4708) was deposited at the Department of Biology, University of Maringá, Maringá, PR, Brazil.

Preparations of aqueous extract: The aerial parts of CS were collected, immediately dried at 45°C, ground to a moderately fine powder (800 g). The aqueous extract (15%) was prepared by adding 150 g of dried plant to 1000 ml of distilled water and heated to about 80°C for 30 min.

Experimental diabetes: Adults male Albino Wistar rats weighing about 250g were employed. Experimental diabetes was obtained with an injection of alloxan (40 mg/kg) after an overnight fast. Diabetes was confirmed by serum glucose (Bergmeyer & Bernt, 1974) evaluation of a blood sample collected from the tail. Thus, diabetic rats with glycemia greater than 2 g/L were divided into two groups: animals treated orally during four weeks with aqueous extract (15%) aerial parts of Cs.(Cs group) and control rats, which received water (A group). The dose and period test was based on Brazilian folk medicine to treat of diabetes mellitus. After this period all animals were killed by decapitation and serum was obtained for determination of glucose (Bergmeyer & Bernt, 1974), cholesterol (Allain *et al.*, 1974) and triglycerides (McGowan *et al.*, 1983).

Microorganisms and growth conditions: The following microorganisms were used for antimicrobial activity: *Staphylococcus aureus* ATCC, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442 and *Bacillus subtilis* ATCC 6623. The microorganisms were grown in nutrient broth (Difco Laboratories, Detroit, MI) at 37 °C and maintained on nutrient agar slants at 4 °C.

Antibacterial activity assay: Methanol extracts and fractions of aerial parts of SC were tested for antibacterial activity using the diffusion technique on solid media. The results were recorded by

measuring the zones of growth inhibition. Chloramphenicol, vancomycin, tetracycline, and penicillin from Sigma Chemical Co (St. Louis, Mo) were used as reference antibiotics (controls) in the assay.

Susceptibility testing: To determine the minimal inhibitory concentration (MIC), twofold serial dilutions of the all samples and reference antibiotics were prepared using the Mueller-Hinton broth (Merck). Inocula were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard (10^8 colony-forming units [CFU]/ml) and diluted 1:10 for the broth microdilution procedure. Portion (100 µl) of each bacteria suspension were added to all wells of microdilution plate which contained 100 µl of the sample or reference antibiotics (control). The plates were incubated at 37°C for 24 h. The MIC was considered the lowest concentration of the sample that prevents visible growth. Minimal bactericidal concentrations (MBCs) were determined by subculturing, 10 µl from each negative well and from the positive growth control. MBCs were defined as the lowest concentration yielding negative subcultures or only one colony.

Isolation of active components: The dried and ground aerial part of CS (300g) was extracted with methanol at room temperature in the dark. The extract was evaporated under reduced pressure in a rotatory apparatus yielding 131 g of dry methanol extract. The methanol extract (10 g) was suspended in methanol/water (95%/5%; 800 ml) and successively partitioned with hexane and ethyl acetate (each 800 ml) and fractions were taken to dryness to yield fractions 7 and 3 g, respectively. Result on diffusion technique on solid media showed that ethyl acetate fraction was active against *B. subtilis*. The ethyl acetate fraction (3 g) was chromatographed on silica gel 60 (230-400 mesh) (250 g) eluated with hexane, hexane-EtOAc and EtOAc and gave 262 fractions. The fraction 68 (234 mg) was chromatographed again to give 1 (15 mg). The fraction 252 (43 mg) was submitted to gel chromatography on Sephadex LH-20 using CHCl_3 -MeOH (1:1) and to give 2 (3 mg).

RESULTS

The effect of aqueous extract (15%) of aerial parts of *CS* on serum levels of glucose, cholesterol and triglyceride is shown in Figure 1. *CS* did not affect blood glucose levels. However, *Cs* group showed increased cholesterol and triglyceride levels.

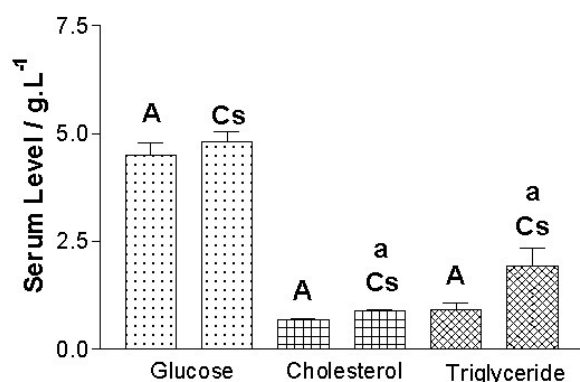


Figure 1 - Serum level of glucose, cholesterol and triglyceride from diabetic fed rats treated with water (A) or aqueous extract (15%) of *Cissus Sicyoides* (Cs) during 4 weeks. Each data represents the media \pm standard error of 10 animals. ^aP < 0.05 vs A group. Statistical analyses of the differences between the means were performed by student t test using a computer program (Primer biostatistics: the program).

A preliminary antibacterial assay with different fractions of methanol extracts of the aerial parts from *CS* showed that ethyl acetate fraction was potent against *B. subtilis* with an inhibition zone range of 14-17 mm, at a concentration of 0.1 mg/ml (not shown). The vancomycin standard showed an inhibitory zone range of 18-26 mm at a concentration of 1 mg/ml against the same bacteria. Ethyl acetate fraction obtained from aerial parts of *CS* revealed the presence of two antibacterial compounds against *B. subtilis*.

The phytochemical investigation with aerial parts of *Cs* led to isolation and identification of β -sitosterol (1) and sitosterol- β -D-glucopyranoside (2). The structural elucidation was based on the comparison of physical and spectral data (m.p. IR, CG-MS, ¹H- and ¹³C-NMR) with those reported in the literature (Goular et al. 1993; Pei-Wu et al. 1988).

Both β -sitosterol and sitosterol- β -D-glucopyranoside inhibited *B. subtilis* at a concentration of 50 and 100 μ g/ml, respectively (Table 1). The MBCs of the two compounds were one twofold dilution of the MICs for this organism. The endpoints were not reached for *S. aureus*, *E. coli*, and *P. aeruginosa* at 100 μ g/ml.

Table 1 - Minimal inhibitory concentration (MIC) of fractions 1 and 2 of *Cissus sicyoides* and reference antibiotics.

	MIC (μ g/ml)			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Sitosterol (1)	>100	50	>100	>100
Sitosterol- β -D-glucopyranoside (2)	>100	100	>100	>100
Chloramphenicol	4	NT	NT	NT
Vancomycin	2	0.75	NT	NT
Tetracycline	NT	NT	0.5	NT
Penicillin	0.12	NT	NT	NT

NT: not tested.

DISCUSSION

Although many traditional plant medicines ascribed antidiabetic action only a little number of active principles have been isolated and have been investigated for possible use in diabetes (Bailey & Day 1989). Our results did not support the popular belief that *CS* is an antidiabetic plant.

The increased serum cholesterol and triglycerides were consistent with the high quantity of lipid constituents in the genus *Cissus* isolated from

aerial parts of this plant (Gupta *et al.*, 1991). But it must be considered that the effect of *CS* on serum lipids was reversible, since after 24 hr of fasting the differences showed in Fig. 1 (fed rats) for cholesterol and triglyceride disappear (not showed).

It is well known that intensive use of an antibiotic is often followed by appearance of multiply resistant strains. The increasing occurrence of drug-resistant microorganisms has stimulated the search for new antimicrobial agents in higher

plants. In the present work, the antibacterial activity of CS was tested against four species of bacteria. Screening tests of the different fractions of methanol extracts of the aerial parts from CS showed that the ethyl acetate fraction was potent against *B. subtilis*. The compounds 1 and 2 isolated by using bioassay-guided procedure and with antibacterial activity against *B. subtilis* were identified as β -sitosterol (1) and sitosterol- β -D-glucopyranoside (2)

As a result of this finding the two compounds were subjected to MIC and MBC assay against *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*. For both compounds, the antibacterial activity was only observed against *B. subtilis*. The MIC values were more active for the compound Sitosterol (Table 1). The two compounds were inactive against *S. aureus*, *E. coli* and *P. aeruginosa*. The results of the reference drugs used in this study were similar to those presented in other report (Goyal & Gupta, 1988).

Hess *et al.* (1995) studied the antibacterial properties of β -sitosterol isolated from the aerial parts from *Vochysia divergens*. According to these authors, *E. coli* and *S. aureus* were resistant at doses ≤ 5 mg/ml

Our present study showed that the endpoints were not reached for *S. aureus*, *E. coli*, and *P. aeruginosa* at 100 μ g/ml. Thus, both compounds were virtually inactive to *S. aureus*, *E. coli* and *P. aeruginosa*.

In spite of popular belief, CS did not show antidiabetic activity. However, two compounds isolated from aerial parts of the plant (β -sitosterol and sitosterol- β -D-glucopyranoside) showed antibacterial activity.

RESUMO

No presente trabalho foram investigados os efeitos antibacteriano e antidiabético da planta *Cissus sicyoides* (CS) coletada no Brasil. Ratos diabéticos receberam água (grupo A) ou extratos da parte aérea da planta (grupo CS) durante 4 semanas. Após este período, os níveis séricos de glicose, colesterol e triglicérides dos ratos foram determinados. A glicemia não foi afetada pelo tratamento com CS. Entretanto, houve aumento nos níveis de colesterol e triglicérides nos ratos do grupo CS. Em adição, fracionamento bio-monitorado foi realizado para o isolamento de compostos com atividade antibacteriana. β -Sitosterol e sitosterol- β -D-glucopiranosídeo

isolados mostram atividade antibacteriana contra *Bacillus subtilis* com concentrações mínimas inibitórias (MICs) de 50 μ g/ml e 100 μ g/ml, respectivamente. Apesar da crença popular, CS não mostrou atividade antidiabética. Entretanto, dois compostos isolados da parte aérea da planta (β -sitosterol e sitosterol- β -D-glucopiranosídeo) apresentaram fraca atividade antibacteriana.

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