## A PROTEIN FROM THE AERIAL PART OF *Arundo donax* AND ITS HYPOGLYCEMIC ACTIVITY

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*Arundo donax* L. is a perennial rhizomatic cereal plant (Poaceae, Gramineae). According to the literature, the plant has been used since antiquity in Eastern folk medicine as a sudorific and diuretic agent [1–3]. The aim of the present research was to determine the quantitative protein content from the aerial part of *A. donax* and its amino-acid composition and to study its biological activity.

The quantitative protein contents in the aerial part of *A. donax* and the pulp after EtOH extraction were determined by a colorimetric method using Nessler reagent on a Metash V-5000 spectrophotometer [4]. The protein content in the aerial part was 20.40%; in the pulp, 11.7%.

The protein was isolated and purified by alkaline extraction, centrifugation with cooling, precipitation by dry ammonium sulfate, dialysis, and lyophilization of the desalted protein solution [5].

IR spectra of the protein were taken on a PerkinElmer 2000 FTIR spectrophotometer. IR spectrum (KBr,  $v_{max}$ , cm<sup>-1</sup>): 3214 (NH), 2921 (CH<sub>2</sub>), 1646 (C=O), 1539, 1416 [(ArH)=O-C-], 1075 (C-O-C-).

The amino-acid composition of the protein isolated from *A. donax* was analyzed after hydrolysis by HCl (5.7 N) at 160°C for 70 min at reduced pressure to determine its quality. Phenylthiocarbamyl (PTC) derivatives of free amino acids were synthesized by the method of Cohen and Strydom [5] and identified on an Agilent Technologies 1200 chromatograph.

The protein composition of A. donax comprised 17 amino acids with a complete set of essential amino acids (Table 1).

The protein amino-acid composition was dominated by arginine, cysteine, histidine, and amino acids with branched carbon chains (valine, isoleucine, leucine). The results showed that the protein was also balanced in nonessential amino acids.

Nonessential amino acid	Concentration	Essential amino acid	Concentration
Asp	59.32	Thr	0
Glu	59.56	Val	64.10
Ser	46.84	Met	12.29
Gly	47.26	Ile	40.06
Cys	68.09	Leu	86.46
Arg	113.53	His	65.68
Ala	20.28	Phe	28.14
Pro	49.23	Lys HCl	26.12
Tyr	39.60	Total	322.85
Total	503.71		

TABLE 1. Amino-Acid Composition of Total Proteins from the Aerial Part of A. donax, mg/g

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TABLE 2. Hypoglycemic Effect of Total Proteins from A. donax with Glucose Loading After a Single Injection,  $M \pm m$ , n = 6

Sample	Blood glucose level, mM		Effect, %	
Sample	initial	after 2.5 h	Effect, 70	
Control	$5.1 \pm 0.12$	$13.8 \pm 0.10^{*}$		
Glucarum 50 mg/kg	$5.0 \pm 0.17$	$10.0 \pm 0.11^*$ , **	27.5	
Total proteins from A. donax 50 mg/kg	$5.2 \pm 0.12$	9.6 ± 0.11* <sup>,</sup> **	30.4	
Total proteins from A. donax 100 mg/kg	$5.0 \pm 0.18$	$8.5 \pm 0.09^{*,**}$	38.4	

\*Statistically significant vs. initial level; \*\*vs. the corresponding control (p < 0.05).

TABLE 3. Comparison of Hypoglycemic Activity of Total Proteins from *A. donax* with Glucose Loading After a Single Injection at a Dose of 100 mg/kg,  $M \pm m$ , n = 6

Sample	Glycemia level, mM%			
	initial	after 3 d	after 7 d	
Control	$5.3 \pm 0.12$	12.6 ± 0.22* (+137.7)	13.1±0.23* (+147.2)	
Total protein from A. donax	$5.4 \pm 0.11$	$8.6 \pm 0.12^{**} (-31.7)$	7.6 ± 0.12** (-41.9)	
Glucarum	$5.2 \pm 0.10$	8.9 ± 0.25** (-29.4)	8.3 ± 0.22** (-36.6)	

In parentheses, effect (%) vs. control; \*statistically significant values (p < 0.05) vs. initial; \*\*vs. corresponding control.

The amino-acid composition of *A. donax* protein, which included arginine, amino acids with branched chains, and phenylalanine, was beneficial to efficacious treatment and improved the condition of diabetes mellitus patients [6, 7].

The hypoglycemic activity of total proteins from the aerial part of *A. donax* was studied in hyperglycemic rats (180–200 g) fasted for 16–18 h that were divided into four groups: 1) control group; 2, 3, and 4) test groups. Animals in test groups were administered once perorally at doses of 50 and 100 mg/kg 2 h before inducing experimental hyperglycemia the total protein components of *A. donax* and the reference drug glucarum (Shreya Life Sciences, India) at a dose of 50 mg/kg. The control group received distilled  $H_2O$  (0.5 mL). After this, all animals were administered aqueous glucose solution (40%) at a dose of 5,000 mg/kg. The blood serum glucose content was determined. Blood for analysis was taken from the rats by abscission of the end of the tail before administering the studied drugs and 30 min after glucose loading.

The blood sugar level was also determined with multiple injections after its last administration considering the peak of the hypoglycemic activity.

The experimental results showed that total proteins from *A. donax* had a pronounced hypoglycemic effect with experimental hyperglycemia. Table 2 shows that the blood sugar level in control rats increased by 171.0% 30 min after glucose administration (peak activity) and by 100, 84.5, and 70.0% in the test groups, respectively, relative to the initial levels. The blood glucose content in animals that received total proteins from *A. donax* and the reference drug glucarum decreased by 27.5, 30.4, and 38.4%, respectively, as compared to the control group 30 min after inducing hyperglycemia.

Table 3 presents results from an evaluation of the sugar-reducing activity of total proteins from *A. donax* with their daily administration for 7 d.

An analysis of the experimental results in Table 3 indicated that the hypoglycemic effect of the studied proteins increased after multiple administrations. For example, the hypoglycemic effect of the studied proteins was 31.7% relative to the control on the 3<sup>rd</sup> day of administration and 41.9% relative to the control after 7 d of administration. Table 3 shows that the hypoglycemic effects of the reference drug glucarum relative to the control were 29.4% and 36.6%, respectively.

Thus, total proteins from *A. donax* in rats with hyperglycemia after a single administration and especially after multiple administrations had a distinct hypoglycemic effect that exceeded that of the imported drug glucarum.

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