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Original article

Identification of amino acids in *Securigera securidaca,* a popular medicinal herb in Iranian folk medicine

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Abstract

Securigera securidaca (L.) Degen & Dorfl grows in different parts of Iran. The seeds of the species are used in Iranian folk medicine as an anti-diabetic agent. Many studies have established hypoglycemic effects of amino acids and in the present investigation, amino acids of *Securigera securidaca* seeds have been evaluated. The ground seeds were extracted using petroleum ether, hot ethanol and ethanol 50%, respectively. ethanol 50% extract was chromatographed over cation exchanging resin and the resulting amino acid fraction was subjected to HPLC after OPA derivatization and the amino acids were identified by comparing to standards. The results evidenced the presence of 19 amino acids in the plant extract including alanine, arginine, asparagine, aspartic acid, citrulline, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine and valine. Considering the role of some amino acids in diabetes, the above amino acids could be noted as hypoglycemic agents of the plant seeds but further studies are necessary.

Keywords: Amino acid, HPLC, ion exchange chromatography, *Securigera securidaca*,

Introduction

Securigera securidaca which grows wide in Iran [1], is a popular hypoglycemic plant in Iranian folk medicine and also in traditional medicine of some other countries [2,3].

Diabetes is one of the most common metabolic disorders and many investigations have been performed to determine new drugs for treatment of the disease. In the last few years, there has been an exponential growth in the herbal medicine research and the out coming drugs are gaining popularity both in developing and developed counties because of natural origin and also less side effects.

Several studies have proved anti-diabetic properties of some medicinal herbs. *In vivo* experiments have established hypoglycemic

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properties of *S. securidaca* seeds. In an investigation on hydro-alcoholic extract of the plant seeds, hypoglycemic effects of the extract in streptozocin-induced diabetic rats have been established dose-dependently [4]. It has also been reported that the plant indicated protective effect against alloxan-induced hyperglycemia in rats [5]. This plant is considered as an herbal option for diabetes [6]. Different studies have established the role of amino acids in diabetes. Specific amino acids are now known to regulate insulin secretion from pancreatic β-cells, acutely and chronically [7]. Since *S. securidaca* seeds are used as anti-diabetic agents in Iranian folk medicine, the observed effect might be related to the presence of amino acids. Phytochemical analysis have revealed that the species is rich in flavonoids [8], but to the best of our knowledge, there is no report about amino acids of the plant seeds. Therefore, in the present investigation, amino acids of the plant seeds were determined using high performance liquid chromatography (HPLC).

Experimental

General experimental procedures

Ion exchange column chromatography was performed using amberlite cation exchange resin CG50 (Sigma, USA). HPLC was carried out with Spherimage 80 C_{18} column (4×250 mm, 5 µm) using a Knauer instrument and a RF-10AxL fluorescence detector. Paper chromatography was performed using Watmann 1 cellulose paper. Standard amino acids and other chemicals were purchased from Merck (Germany). The solvents were of analytical or HPLC grade (Merck, Germany).

Plant material

Securigera securidaca seeds were purchased from Khuzestan province market (Iran) and identified by Dr. G.R. Amin, Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Extraction and isolation of amino acids

Milled and powdered seeds of the plant (500g) were extracted with petroleum ether, hot EtOH 96% and EtOH 50%, respectively. In order to determine the presence of amino acids in each extract, spot test was performed using paper chromatography with *n*-butanol: glycial acetic acid: water 3:1:1 as the mobile phase and ninhydrin as the reagent. The presence of amino acids was confirmed by purple spots on the paper [9]. The results obtained from paper chromatography demonstrated that amino acids were present only in the EtOH 50% extract.

In order to separate amino acids from EtOH 50% extract, ion exchange chromatography was performed using amberlite cation exchanging resin. The column (2.5×24.5 cm) was successively washed with distilled water and HCl 2N, and after acidifying, with distilled water until the pH of the eluent became neutral. The concentrated EtOH 50% extract was then subjected to the column and eluted with distilled water and NH_4OH 1N, respectively. The fractions were collected when the pH of the eluent became alkaline. Spot test was performed on each fraction and the fractions containing amino acids were collected and mixed (fraction A).

Identification of amino acids

In order to identify the amino acids in fraction A, the extract was concentrated under reduced pressure. Amino acids in the dried extract were derivatized by *o*-phthalaldehyde (OPA) method. OPA reacts with primary amines in the presence of the thiol grouping to form highly fluorescent isoindole products [10,11]. The derivatized amino acids were then subjected to HPLC (Run time= 40 min, Flow rate= 1 mL/min, λ ex=330 nm, λ em=440 nm). A linear gradient from solvent A (sodium acetate 1M, 80 mL and methanol, 20 mL) to solvent B (sodium acetate 1M, 25 mL and methanol, 75 mL) as eluent. Amino acids were compared to standards.

Results and Discussion

Diabetes is a widespread disease. In our fast changing world, a number of natural treatments for diabetes are explored by clinicians and experts. Evidences show that amino acids play a key regulatory role in numerous metabolic processes. They can act as potent hormone secretors, stimulating the secretion of insulin, glucagon, cortisol, insulin-like growth factor I and/or growth hormone [7]. Regarding the role of amino acids in

control of diabetes, in the present investigation, amino acids of *S. securidaca* which is one the medicinal species used in treatment of diabetes in Iranian folk medicine have been studied.

The HPLC chromatogram of the plant seeds extract established the presence of 19 amino acids including alanine, arginine, asparagine, aspartic acid, citrulline, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine and valine (table 1 and figure 1). As it has been shown in figure 2, they belong to different classes of amino acids. The role of some amino acids in insulin secretion has been reported before [12].

Table 1. Amino acid composition of *Securigera securidaca* seeds

No.	Amino acid	Abbreviation
1	alanine	ALA
2	arginine	ARG
3	asparagine	ASN
4	aspartic acid	ASP
5	citrulline	CIT
6	glutamic acid	GLU
7	glutamine	GLN
8	glycine	GLY
9	histidine	HIS
10	isoleucine	ILEU
11	leucine	LEU
12	lysine	LYS
13	methionine	MET
14	ornithine	ORN
15	phenylalanine	PHE
16	serine	SER
17	threonine	THR
18	tyrosine	TYR
19	valine	VAL

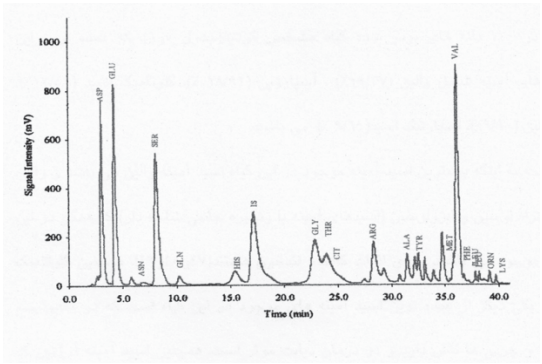

Figure 1. HPLC chromatogram of *Securigera securidaca* seeds extract

During an investigation, L-alanine has been shown to have insulinotropic properties both in cell lines and rat islets [13]. L-alanine could stimulate insulin secretion under specific conditions of nutrient availability [14]. Another amino acid which is thought to be involved in insulin secretion is glutamic acid. The role of L-glutamate in the stimulation of insulin secretion is still controversial. Intracellular generation of L-glutamate has been proposed to participate in nutrient-induced stimulus–secretion coupling, as an additive factor in the amplifying pathway of glucose stimulated insulin secretion [15]. Key

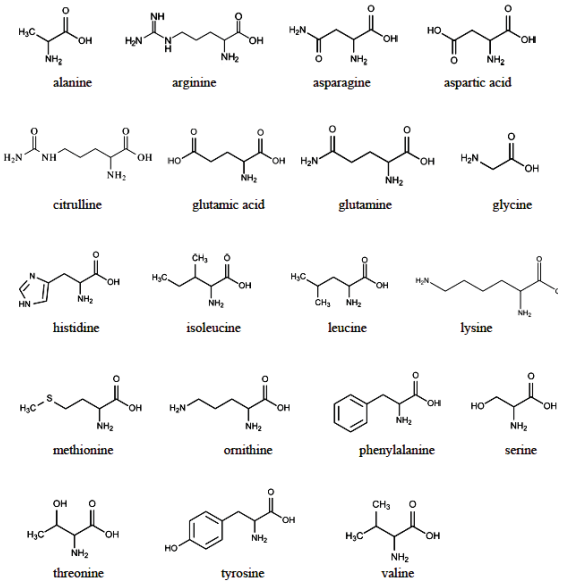

Figure 2. Structure of amino acids of *Securigera securidaca* seeds

amino acids such as alanine and glutamine can regulate β-cell function and insulin secretion. The mechanisms by which the mentioned amino acids confer their regulatory effects are complex and involve mitochondrial metabolism [12]. Other amino acids such as leucine or arginine may play a role in enhancing insulin secretion by allosteric activation of metabolism or membrane depolarization or a combination of these two possibilities [16,17]. It is concluded that the amino acids of *S. securidea* could be responsible for its blood sugar lowering effects which was the aim of the therapeutic usage of the seeds in Iranian folk medicine with different mechanisms. Moreover, the presence of 19 amino acids in this plant might be a potential for other various biologic properties.

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