Original Research Article

Determination of Amino Acids in Medicinal Plants from Southern Sonora, Mexico

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Abstract

Purpose: To analyze the amino acid contents of some plants used in traditional medicine in Southern Sonora, Mexico by using high performance liquid chromatography (HPLC).
Methods: The plant samples (Jutuki, Zizyphus obtusifolia A.Gray; Jito, Forchammeria watsonii Rose; Barchata, Lycium berlandieri Dunal; Citabaro, Vallesia glabra Link; Mangle Rojo, Rhizophora mangle L and Tatachinole, Tournefortia hartwegiana Steud) were dehydrated at room temperature and ground to a fine powder. Amino acid analysis was performed by reversed-phase HPLC. Elution was carried out with a gradient mobile phase of sodium acetate 0.1 M and methanol (9:1). Samples were derived with o-phthalaldehyde (OPA) and detected by fluorescence at 360(Ex)/455(Em) wavelengths.
Results: HPLC analysis resulted in a reliable detection and peak resolution. Tatachinole samples show the greater amino acid concentration (7.83 to 58.17 nM). Fifteen amino acids were detected in plant samples, with aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), alanine (Ala) and leucine (Leu) (43.55, 44.84, 29.60, 58.17, 43.05 and 38.73 nM, respectively) presenting the highest concentrations.
Conclusion: The amino acids - Asp, Glu, Ser, Gly, Ala and Leu - are naturally involved in osmolyte synthesis, cell metabolism, ammonia detoxification, antioxidant activity and alkaloid synthesis, suggesting that the therapeutic properties of these Southern Sonora plants may have some links to their amino acid composition.

Keywords: Amino acid profile, Medicinal plants, Osmolytes, Drought/saline stress

INTRODUCTION

Amino acids are essential in the synthesis of proteins and precursors in the formation of secondary metabolism molecules [1] that participate in cell signaling, gene expression and homeostasis regulation [2], protein phosphorylation, synthesis of hormones and antioxidant capacity [3]. Also, amino acids participate in various physiological processes such as skeletal muscle function, atrophic conditions, sarcopenia and cancer [4,5].

Drought stress and saline are conditions that favor the accumulation of products of primary metabolism as osmolytes, amino acids and

reducing sugars [3]. Also, the plants are adapted to several stress conditions such as high and low temperatures, high salt concentrations, exposure to heavy metals, ultraviolet radiation and drought stress as result of their continuous evolution [6]. The response of the plants results in the activation of mechanisms involved in the synthesis and accumulation of secondary metabolites responsible for the beneficial properties attributed to medicinal plants [7].

Traditional medicine practice entails the use of natural resources like plants, animals, water and minerals. The use of plants as an alternative for the treatment of renal, gastrointestinal, rheumatism, liver, anti-diabetes, amongst other diseases, has increased over the years [8]. The bioclimatic conditions of southern Sonora, Mexico allow the plants to accumulate compounds as an adaptive mechanism in response to stress [9]. The accumulation of primary metabolites for development of plants is well defined.

However, few studies focus on primary metabolites in plants naturally adapted to tolerate prolonged periods of drought stress/saline and the relationship with their therapeutic properties. To assess the relationship between the production of primary metabolites and their possible therapeutic properties, we analyzed the amino acid profile of plants from southern Sonora (Mexico) used in traditional medicine. The study of compounds generated by plants as a result of defense mechanisms permit understanding of the molecular mechanism involved in their medicinal properties.

EXPERIMENTAL

Chemicals

2-mercaptoethanol, *o*-phthalaldehyde (OPA) and α-aminobutyric acid were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Amino acid standard was purchased from PIERCE (no. 20089).

Plant materials

The root, leaf and bark of plants were collected from Southern Sonora region, northwest of Mexico, during May 2010 (Table 1). The plant samples were authenticated by Professor José Jesús Sánchez Escalante at Universidad de Sonora Herbarium, and voucher specimens were preserved in the laboratory for future reference. Plant samples were washed with distilled water

and dried at room temperature. Completely dehydrated samples were ground to a fine powder using an electric mill (Thomas Scientific Model 3383-L10), and used for amino acid analysis.

Table 1: List of plants from Southern Sonora Mexico used to amino acid screening

Plant	Common name	Voucher
Rhizophora mangle L	Mangle Rojo	20392
Tournefortia hartwegiana Steud	Tatachinole	20388
Zizyphus obtusifolia A.Gray	Jutuki	20394
Forchammeria watsonii Rose	Jito	20393
Lycium berlandieri Dunal	Barchata	20388
Vallesia glabra Link	Citabaro	20390

Amino acid analysis

Amino acid analysis of plant samples was based on methodology previously reported [10]. Powdered samples (3 mg) were hydrolyzed with HCl 6 M at 150°C during 6 h. After hydrolysis, the acid was removed by rotary evaporation (RE500 Yamato Scientific America Inc.). Sample was resuspended on 2 mL of sodium citrate buffer pH 2.2. Sample derivation was achieved adding *o*−phthalaldehyde (OPA) 7.5 mM to the sample on citrate buffer (OPA reagent contains β-mercaptoethanol and Brij 35). The HPLC method precision and accuracy was evaluated using external and internal standards. The amino acid reference standard consisted on fifteen amino acids (0.05 µmoles mL^{-1} each amino acid) and was utilized to determine the retention times for each amino acid. As well, internal standard α-aminobutyric (0.05 µmoles mL^{-1}) was added to amino acid reference standard and each plant sample to normalize and quantify the amino acid content.

A gradient mobile phase of sodium acetate 0.1 M pH 7.2 and methanol (9:1) elute sample for amino acid separation trough C18 column reversed-phase octadecyl dymethylsilane particles (100 x 4.6 mm x 1/4" Microsorb 100-3 C18). Fluorescence detection was realized using an excitation-emission wavelength of 360 and 455 nm respectively. Star Chromatography work station (Varian version 5.51) software was used to achieve amino acid peak integration.

Protein content

The protein content of each plant was measured to determine amino acid recovery by HPLC using Micro Kjeldahl method [11].

Statistical analysis

Origin 9.0 software (OriginLab Corporation, Northampton, MA 01060 USA) was used for descriptive statistical analysis.

RESULTS

The plant samples of Jutuki, Jito, Barchata, Citabaro, Mangle rojo and Tatachinole were taxonomical characterized on Herbarium of the University of Sonora (Fig.1).

The amino acid profiles of plants from southern Sonora were evaluated using HPLC (Fig 2). The OPA amino acid derivation method allowed identify and quantify fifteen amino acids in each sample, whereas HPLC amino acid analysis resulted in a reliable detection and high peak resolution (Fig 3).

Lycium berlandieri Dunal (Barchata)

Rhizophora mangle L (Mangle rojo)

Zizyphus obtusifolia A.Gray (Jutuki)

Vallesia glabra Link (Citabaro)

Forchammeria watsonii Rose (Jito)

Tournefortia hartwegiana Steud (Tatachinole)

Figure 1: Medicinal plants of southern Sonora used for amino acid analysis
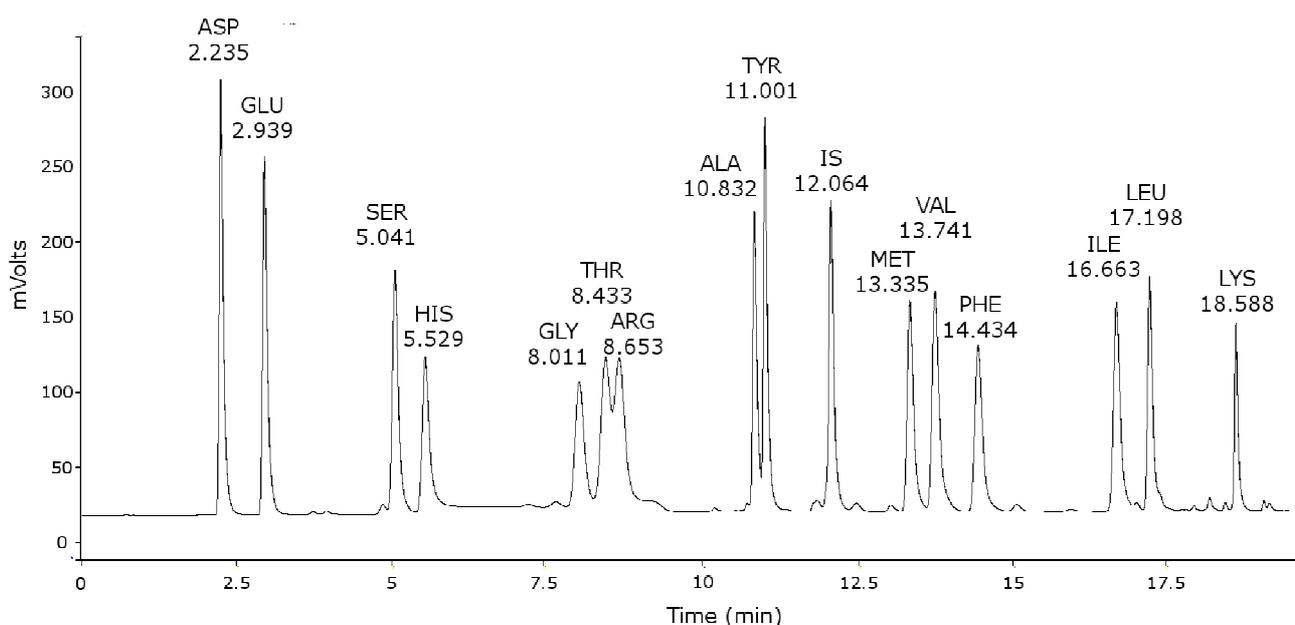

Figure 2: Amino acids standard chromatogram. *Note:* Each amino acid and internal standard (IS) is identified with the corresponding name and retention time (min)
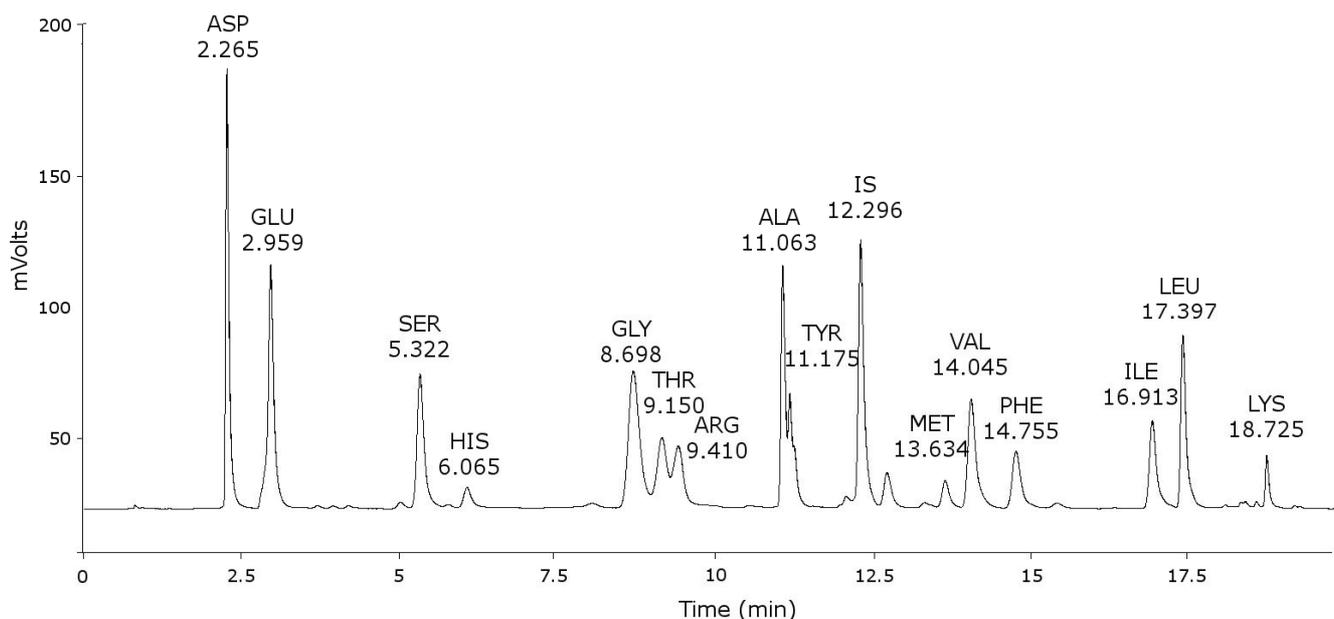
Figure 3: Tatachinole amino acid chromatogram. ***Note:*** Each amino acid and internal standard (IS) is identified with the corresponding name and retention time (min)

Table 2: Free amino acid concentration (nM) in medicinal plants of Southern Sonora

Amino acid	*Rhizophora mangle* L (Leaf)	*Rhizophora mangle* L (bark)	*Lycium berlandieri* Dunal	*Zizyphus obtusifolia* A. Gray	*Tournefortia hartwegiana* Steud	*Vallesia glabra* Link	*Forchammeria watsonii* Rose
ASP	22.40 ± 0.39	10.05 ± 0.99	14.45 ± 1.81	35.14 ± 1.16	43.55 ± 1.09	38.49± 1.34	17.25 ± 0.85
GLU	21.44 ± 0.11	10.63 ± 0.86	15.17± 0.63	9.82 ± 0.69	44.84 ± 1.05	26.53± 3.02	14.31 ± 1.11
SER	13.08 ± 0.62	7.51 ± 0.33	10.88 ± 0.78	13.00 ± 0.96	29.60 ± 1.27	21.61± 0.93	15.67 ± 1.83
HIS	4.15 ± 0.06	3.09 ± 0.28	2.61 ± 0.32	2.45 ± 0.11	5.96 ± 0.12	4.83 ± 0.27	3.35 ± 0.32
GLY	23.96 ± 2.34	12.63 ± 1.00	18.15 ± 0.94	14.58 ± 1.05	58.17 ± 2.84	36.37 ± 2.85	20.42 ± 3.68
THR	10.10 ± 0.17	4.69 ± 0.36	7.20 ± 0.59	5.87 ± 0.24	21.90 ± 0.29	14.13 ± 0.47	7.69 ± 0.59
ARG	10.06 ± 0.61	4.09 ± 0.21	6.18 ± 0.07	4.54 ± 0.12	19.21 ± 0.48	14.95 ± 0.39	8.20 ± 0.89
ALA	24.03 ± 1.62	11.03 ± 0.77	11.96 ± 0.49	11.74 ± 0.37	43.05 ± 2.14	28.89 ± 1.84	14.74 ± 1.90
TYR	10.79 ± 0.65	4.15 ± 0.29	3.20 ± 0.55	4.96 ± 0.28	14.53 ± 0.91	10.80 ± 0.27	8.26 ± 2.05
MET	4.01 ± 0.21	2.11 ± 0.10	2.86 ± 0.07	2.72 ± 0.34	7.83 ± 0.73	4.33 ± 0.35	2.57 ± 0.20
VAL	15.26 ± 0.38	7.34 ± 0.68	10.30 ± 0.97	7.99± 0.92	28.40 ± 1.57	18.55 ± 0.95	11.98 ± 0.33
PHE	9.64 ± 0.52	4.18 ± 0.33	5.37 ± 0.39	3.86 ± 0.37	18.51 ± 0.66	11.37 ± 0.56	5.55 ± 0.52
ILE	11.70 ± 0.72	5.72 ± 0.67	7.18 ± 0.48	6.10 ± 0.41	23.57 ± 0.86	14.73 ± 0.69	8.09 ± 0.15
LEU	20.76 ± 0.58	9.31 ± 0.85	10.08 ± 0.59	7.68 ± 0.41	38.73 ± 0.60	23.12 ± 0.93	10.57 ± 0.73
LYS	9.22 ± 0.69	4.82 ± 0.25	6.00 ± 0.45	5.31± 0.56	12.27 ± 0.76	11.43 ± 0.98	5.24 ± 0.18

Mean ± standard deviation (n = 3)

The amino acid peak area of standards and samples was calculated to determinate amino acid concentration (Table 2). The leaf of tatachinole plant presented higher amino acid concentrations with 29 to 58 nM and, glycine has the higher concentration, followed by glutamic and aspartic acid, alanine, leucine, serine and valine. The amino acid concentration in citabaro plant was 21 to 38 nM with high concentration of aspartic acid, followed by glycine, alanine, glutamic acid, leucine and serine. The leafs of mangle rojo presented amino acid concentrations of 15 to 24 nM with high concentrations of alanine, followed by glycine, aspartic and glutamic acid, leucine and valine.

The bark sample of jito and root of barchata presented an amino acid concentration of 10 to 20 nM with high concentration of glycine, aspartic acid, serine, alanine, glutamic acid, valine and leucine for bark of jito and glycine, glutamic and aspartic acid, alanine, serine, valine and leucine for the root of barchata. Root of jutuki and bark of mangle rojo presented the lowest amino acid profile concentration among all plants analyzed on this study, with concentrations of 7 to 13 nM. Aspartic acid in jutuki samples was the only

exception due high concentration presented. Amino acid profile concentration order was glycine, serine, alanine, glutamic acid, valine and leucine. For the bark of mangle rojo samples, the amino acid profile was in the concentration order glycine, alanine, glutamic and aspartic acid, leucine, serine and valine (Table 2).

The leaf of tatachinole presented the highest protein concentration (22.40 ± 0.42%) and recovery percent (83.89 ± 1.24%) by HPLC method compared to all plants analyzed. The citabaro plant shows the second higher protein concentration (19.90 ± 0.01%) but with a moderate percent of recovery (58.50 ± 1.66%) that is reflected on their amino acid profile. The bark of jito and root of barchata were third (12.60 ± 0.21%) and fourth (11.60 ± 0.14%) in terms of protein concentration, respectively, but also showed the lowest % recovery (39.01 ± 3.58 and 42.60 ± 2.54%, respectively). The mangle rojo (both bark and leaf) and jutuki plants presented the lowest protein concentration (4.27 ± 0.21and 9.45 ± 0.07; and 6.40 ± 0.42%, respectively) but have the higher percent of recovery (78.05 ± 5.75, 85.25 ± 1.85 and 75.71 ± 1.67%) compared to the other samples (Table 3).

Table 3: Protein concentration and recovery percent of HPLC free amino acid analysis in plant samples

Plant sample	% Protein content	Percent of recovery
Rhizophora mangle L (Leaf)	9.45 ± 0.07	85.25 ± 1.85
Rhizophora mangle L (bark)	4.27 ± 0.21	78.05 ± 5.75
Zizyphus obtusifolia A Gray (root)	6.40 ± 0.42	75.71 ± 1.67
Lycium berlandieri Dunal (root)	11.60 ± 0.14	42.60 ± 2.54
Tournefortia hartwegiana Steud (Leaf)	22.40 ± 0.42	83.89 ± 1.24
Vallesia glabra Link	19.90 ± 0.01	58.50 ± 1.66
Forchammeria watsonii Rose (bark)	12.60 ± 0.21	39.01 ± 3.58

Mean ± standard deviations (n = 3)

DISCUSSION

Secondary metabolism is key to plant adaptation to stress [12]. However, some molecules produced during primary metabolism can participate in stress response and provide plant defense properties. It has been demonstrated that amino acid concentration increases in salt-stressed plants [2]. The low precipitation and dry weather of Sonora region expose plants to high temperatures and saline soils [13]. The adaptation of southern Sonora is via the accumulation of molecules in stress response.

Tatachinole sheet and citabaro presented the highest concentration of amino acids. Although citabaro showed high protein concentration, its amino acid content was small to low recovery. Moreover, loss of some amino acids during sample hydrolysis decreased the detection limit of the HPLC method. Therefore, the amino acid content detected in barchata and citabaro, which presented the lowest recovery values (39 and 42%, respectively), might have been underestimated by the technique employed in this work.

Few studies have focused on quantitative amino acid analysis of these medicinal plants. Reports in the literature are related to results of intake or topical functions, which derive from their use in traditional medicine [14]. Fifteen amino acids were detected in each sample, but only aspartic acid (10.63 to 43.55 nM), glutamic acid (7.51 to 44.84 nM), serine (3.09 to 29.60 nM), glycine (4.69 to 58.17 nM), valine (4.18 to 28.40 nM), alanine (3.20 to 43.05 nM) and leucine (4.82 to 38.73 nM) are present in high concentrations. This is supported by the amino acid profile of Rizophora mangle L where protein/amino acid contents are dominated by acidic amino acids such as aspartic an glutamic acids, followed by neutral amino acids such as glycine [15].

Aspartic acid function is essential for purine, pyrimidine, asparagine and inositol synthesis. Glutamic acid and glycine participate in the synthesis of glutathione increasing the antioxidant capacity of the plant. Valine maintains the balance of branched chain amino acids, whereas alanine is involved on hepatic autophagy, gluconeogenesis and transamination. Leucine regulates the protein turnover (mTOR signaling) and gene expression [3,16].

Glycine, lysine, threonine and glutamate help to maintain intestinal integrity and health [17,18]. Jutuki plant is used to treat stomach infections [19] sample, and it showed low amino acid concentration, some of which are related to bowel function, which somehow may explain the medicinal properties of the plant.

Mangle rojo and tatachinole are used in the treatment of urinary tract infections [19]. Amino acids, such as aspartic, are related to detoxification and excretion of ammonia [17].

Aspartic acid is present at its highest concentration in tatachinole sheet, as well as in jutuki and citabaro samples.

Amino acids are precursors for the synthesis of secondary metabolites such as alkaloids, which provide chemical defense for plants that confer beneficial physiological effects in consumers. Alkaloids provide protection to plants from a variety of herbivores, and some of them possess significant pharmacological activity such as analgesic, antibacterial and antibiotic [12].

CONCLUSION

High concentrations of aspartic acid, glutamic acid, serine, glycine, valine, alanine and leucine predominate in all the plants analyzed. Specific metabolic processes in which these amino acids participate may be related to the therapeutic properties of plants as per their use in traditional medicine and, therefore may facilitate the understanding of their beneficial properties. However, further studies are needed to determine and isolate compounds responsible for the specific therapeutic properties of the plants.

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