Journal of Environmental Studies [JES] 2013. 12: 49-53

Original Paper

Chromatographic separation and identification of many amino acid compounds of *Leucaena glauca* L. growing in Iraq

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Rec. 15 Feb, 2013 Accept. 27 Mar, 2013

Abstract

The aim of the present study was to separation and investigation of many amino acids (glycine, aspargen, leucine, glutamic acid, proline, argenine, valine, alanine) from the leaves and seeds of *leucaena glauca* L. Amino acids were separated and investigated by high-performance liquid chromatography (HPLC) technique, which consisted of a UV-vis detector, C18, Mobile phase was Na_2SO_4. The result showed that the sample of chloroform extract of leaves was recorded of the highest quantity of aspargine (43.725%), leucine (8.905%) glutamic acid (4.300%), while leucine, proline, alanine were recorded the highest quantity in seeds (29.575%, 19.031%, 14.115%) respectively.

Key words: Amino acids, HPLC analysis, *Leucaena glauca* extracts.

Introduction

Leucaena is a genus of about 24 species of leguminous trees and shrubs, distributed from Texas U.S.A. to Peru, it belongs to the family leguminosae (Minari *et al.*, 2012). *Leucaena glauca* can be used for several purposes such as live stock folder green manure or as a source of firewood and timber, provide shade for other plants as well as its function in maintaining the fertility of the soil (Hassan and Radwan, 2010).

Leucaena requires warm temperatures (25-30)°C for optimum growth so it's can be found performing well in a wide range of rainfall environments from 650 to 3.000 mm, and grown in a wide variety of soil types including mildly acid soils PH>5.2 (Oluwasola and Ayobore, 2004).

Leuacena seeds are ovoid in shape and have brown hulls and yellow kernels.The kernels have an oil content of 11.9-15.3% and a protein content of 52.5-66.4% (Mohamed and Khadiga, 2009).

Although there are differences in amino acids concentration between legumes, there may be much variation between samples of one legume (Evans, 1985). The amino acids content of grain legumes varies according to cultivar and environment (Wiryawan, 1997).

Leuacena's seeds are fairly rich in the essential amino acids but some of it such as isoleucine, leucine, phenyl alanine, histidine, lysine and methionine are also present in moderate amounts (Miranda *et al.*, 2012).

The current study aimed to isolate and investigate some amino acid from leaves and seeds of *Leucaena glauca* by high-performance liquid chromatography (HPLC) technique.

Materials and Methods:

Plant Material:

During May 2013, leaves and seeds collected from tree *Leucaena glauca* at ten-year old which was growing in Mosul City.

The species was classified by Mr. Talal Taha that he is director of medicinal plant project of Mosul dam.

After air dried, the leaves and seeds were ground into fine powder and stored in a dark place until extraction was done.

Kingdom	:	Plantae-plants
Subkingdom	:	Tracheobionta-Vascular plants
Superdivision	:	Spermatophyta-Seed plants
Division	:	Magnoliophyta-Flowering plants
Class	:	Magnoliopsida-Dicotyledons
Subclass	:	Rosidae
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Mimosoideae
Tribe	:	Mimoseae
Genus	:	*Leucaena*
Species	:	*Leucaena glauca*

Figure (1): Classification of Leucaena plant according to APG system III, 2009.

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Preparation of amino acid extracts:

Leaves and seeds of *Leucaena glauca* were extracted with 70% methanol in a soxhlet apparatus until no more colour was observed.

The methanolic extract was concentrated under reduced pressure to give 20 ml oily material as crude extract with a yellowish-green.

10 ml of the crude extract was dissolved in 25 ml of aqueous methanol (1:1 v/v), the slurry is filtered and the extract was shaken with chloroform (2×50 ml). the aqueous layer was examined for amino acids after concentration (Gulfraz *et al.*, 2005).

HPLC Analysis:

According to (Schwarz, *et al*, 2005). the amino acids that containing in aqueous layer were confirmed by Shimadzu Lc-20AD (HPLC) equipped with a column (C18) and UV detector. Injection and detector temperature was 30°C. The mobile phase was 40 mM Na_2HPO_4 (PH 6.8) adjusted with methanesulfonic acid (Table 1).

At this condition, amino acids were identified by comparison of their retention time (R_t) with those of the standard compounds (Figure 3-10) and also quantified depending on peak area values.

Properties	Analytical conditions
Column	C18 (4.6×240) mm
Mobile phase	40 mM Na_2HPO_4, PH adjusted at 6.8
Temperature	30°C
Injection volume	100µL
Detection	UV-vis
Flow rate	1 mL/min
Wave length	210 nm

Table (1): The analytical condition of amino acids.

Results and Discussions:

The word proteins is derived from the (Greek) word proteins which means principal or prime, and amino acids are the monomeric units which derived of protein, the characteristics of amino acids are important to the structure and functions of the polymers (proteins), (Ahmed & Abdelati, 2008).

In this study, HPLC-technique used to separation and investigation of many amino acids (glycine, aspargen, leucine, glutamic acid, proline, argenine, valine, alanine) from the leaves and seeds of *Leucaena glauca* (Figure 2).

All the main amino acids were identified in leaves such as aspargine 43.705%; leucine 8.905% and glutamic acid 4.300% (Table 2), these values were superior to that obtained by Mohamed and Khadiga (2009) who showed that the leucaena seeds contained 1.80% leucine; 4.63% glutamic acid; 1.10% valine and alanine; 13.80% glycine; arginie 2.62%.

The continuation of above work we found the presence of eight free amino acids in leaves, five of them were known leucine, proline, alanine, glutamic acid and valine were identify in seeds by comparing their R_t-values and the authentic sample (Figure 11), (Table 2).

Generally, the seeds have a few of a main metabolism because the absence of evaluators of photosynthesis to conformation the proteins. Consequently the existence of proteins is reflected the redundancy of amino acids that formed as a result of denaturation of proteins, while the leaves have abundance of amino acids outcome of completion the assessors of photosynthesis and formation of proteins.

The same results was obtained by Oluwasola and Ayobore (2004) when amino acids analysis by using high-performance liquid chromatography (HPLC) at wave length of 325 nm and an emission wave length 465 nm, the column (150×4.6 mm) and the mobile phase was 1,4-dioxan and 2-propanol.

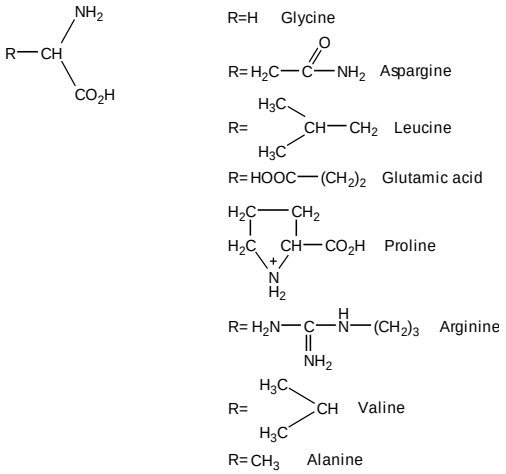

Figure (2): Formula of the amino acids that have been separated in the current study.

Amino acids compounds	Leaves		Seeds		Standard R_t(min.)
	Area %	Compounds R_t(min.)	Area %	Compounds R_t(min.)	
Glycine	0.009	1.92	**	**	2.90
Aspargine	43.725	2.93	**	**	2.83
Leucine	8.905	3.24	29.575	3.12	3.12
Glutamic acid	4.300	3.69	3.410	3.52	3.27
Proline	2.024	4.00	19.031	3.65	3.84
Arginine	0.441	4.57	**	**	4.07
Valine	0.899	5.38	0.093	5.29	5.18
Alanine	0.946	6.96	14.115	5.75	6.80

Table (2): Amino acids that have been separated from the leaves and seeds of *Leucaena glauca.*

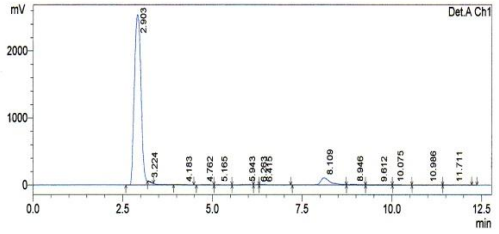

Figure (3): Chromatogram of glycine Standard, that appeared at 2.90 minutes by use 40 mM Na_2SO_4 phase in HPLC technique.

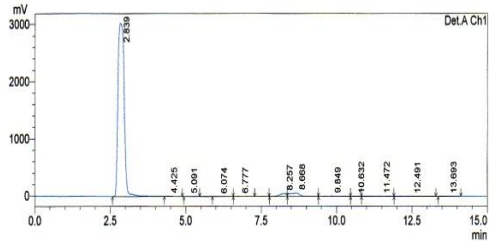

Figure (4): Chromatogram of aspargine standard, that appeared at 2.83 minutes by use 40 mM Na_2SO_4 phase in HPLC technique.

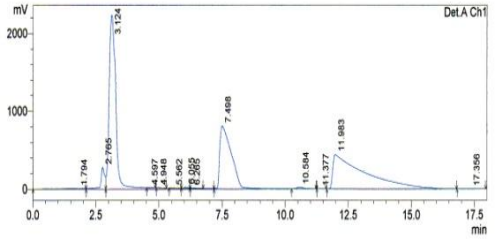

Figure (5): Chromatogram of leucine standard, that appeared at 3.12 minutes by use 40 mM Na_2SO_4 phase in HPLC technique.

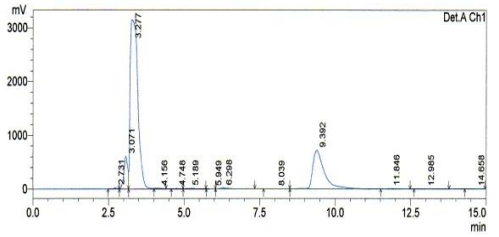

Figure (6): Chromatogram of glutamic acid standard, that appeared at 3.27 minutes by use 40 mM Na_2SO_4 phase in HPLC technique.

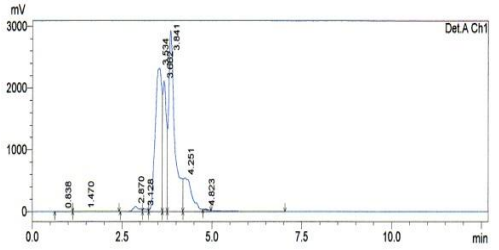

Figure (7): Chromatogram of proline standard, that appeared at 3.84 minutes by use 40 mM Na_2SO_4 phase in HPLC technique.

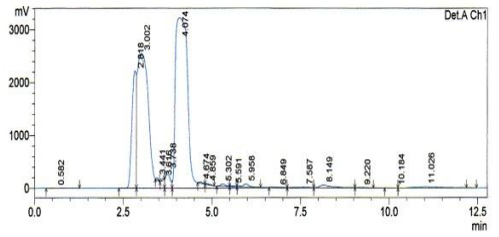

Figure (8): Chromatogram of arginine standard, that appeared at 4.07 minutes by use 40 mM Na_2SO_4 phase in HPLC technique.

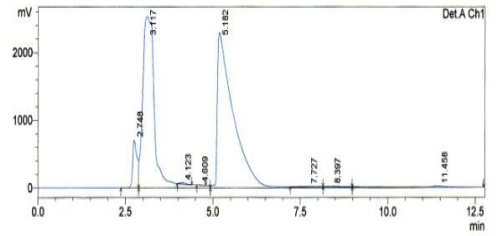

Figure (9): Chromatogram of valine standard, that appeared at 5.18 minutes by use 40 mM Na_2SO_4 phase in HPLC technique.

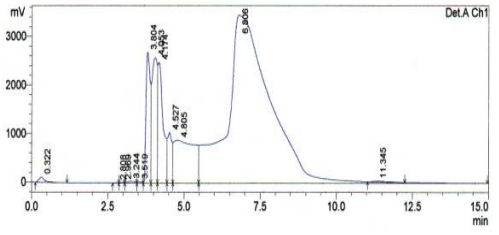

Figure (10): Chromatogram of alanine standard, that appeared at 6.80 minutes by use 40 mM Na_2SO_4 phase in HPLC technique.

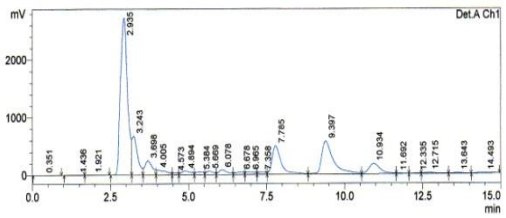

Figure (11): HPLC-Chromatograms of amino acids that have been extracted from Leaves of *Leucaena glauca* .

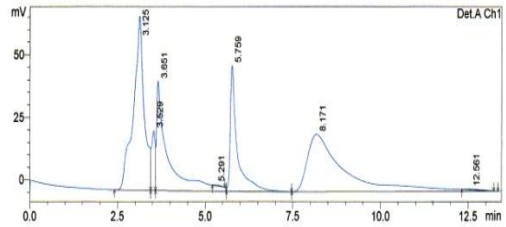

Figure (12): HPLC-Chromatograms of amino acids that have been extracted from Seeds of *Leucaena glauca*.

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الملخص العربى

الفصل والتشخيص الكروماتوغرافي لبعض مركبات الاحماض الامينية لنبات اللوسينا النامي في العراق

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الملخص
استخدمت تقنية كروماتوغرافيا السائل عالي الأداء (HPLC)، خلال الدراسة الحالية لفصل وتشخيص بعض الأحماض الامينية من أوراق وبذور نبات اللوسينا. وأظهرت النتائج أن مستخلصات الأوراق قد احتوت على ثمانية احماض امينية هي: الكلايسين، اسبرجين، لويسين، حامض الكلوتامك، برولين، ارجنين، فالين والالنين. من الناحية الكمية احتوى المستخلص الكلورفورمى للأوراق على أعلى كمية لمركب الاسبرجين (٤٣,٧٢٥%) واللويسين (٨,٩٠٥%) ثم حامض الكلوتامك (٤,٣٠٠%)، بينما سجل اللويسين، البرولين والالنين أعلى كمية في البذور إذ بلغت (٢٩,٥٧٥%، ١٩,٠٣١% و١٤,١١٥%) على التوالي.

الكلمات الدالة: الأحماض الامينية، التحليل بـHPLC، مستخلصات اللوسينا.