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Estimation the Total Protein Content of Iraqi *Melia azedarach* Leaves and Identification the Percent and Quantity of Amino acids in it using Amino Acid Analyses Device

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ABSTRACT

Melia azedarach is one from the most important medicinal plants because it contains many organic and inorganic compounds of medical and economic benefit. Plant trees are widely planted in Iraq, especially in Mosul city on side roads as shade plants in addition to their aesthetic value. The objective of this study, which is a continuation of earlier research on the identification of organic and inorganic compounds in plants, was to determine the total protein content and the proportion and quantity of amino acids in the leaves by using the keldahl method and the amino acid analysis device were employed. The results demonstrated that the protein content in 5 grams leaves was 13.2% also showed the protein contains 12 amino acids and that amino acids with nonpolar side chains were most abundant among all other amino acids, Leucine, on the other hand, was the least abundant amino acid (79.8 µg/g), whereas glutamic acid was the most abundant (1025.4 µg/g) among the identified amino acids.

Keywords: *Melia azedarach*, total protein, amino acid analyses device.

INTRODUCTION

Melia azedarach L. is a small to medium sized deciduous tree or shrub, close relative of neem (*Azadirachta* spp.), belonging to the family Meliaceae, widespread in tropical and subtropical countries and grows up to 50 feet in height (Kumar *et al*., 2003; Mukharjee, 2004), native to India, is extensively scattered in all countries (Alzohairy, 2016). It is widely cultivated in Iraq, especially in the city of Mosul and southern Iraq, in public parks as a shade tree along the outer roads (Mossa *et al*., 1987). The plant has many common names as chinaberry or white cedar (Hassanein, 2008). It is also called the Umbrella tree and Persian lilac in the world. For Arabs, it is called Sinahbah, Lailak, Zarur and Zanzalakht (Chakravarty, 1976).

The leaves of plants are glossy, two-lobed, dark green and alternate. As for the fruits, they grow on short axes and are fleshy white between March and the end of June, then they become solid yellow in the months of September until the beginning of winter, containing within them a single, striped, hardcore containing five smooth seeds (Mahato, 2005). The adult bark is brown and superficially streaked, while the juvenile bark is light gray, veining over time. The flowers are purple, with a distinct woody shape (Kellow, 1994). It has a typical sharp fragrance (Ramya *et al*., 2009).

Melia has old history of use as a folk remedy for several illnesses. Recent research on this plant has shown that it contains a variety of interesting bioactivities, including triterpenoids, limonoids, degraded limonoids, steroids, lignans, flavonoids, and phenolics. This feature gives the plant a variety of properties, including antioxidant, antimicrobial, antiviral, antifeedant, antidiabetic, antifungal, and cytotoxic activities, also such compounds are possible, making it a promising source of natural pesticides as repellent, inhibitory, or toxic to many insect pests. (Schmutterer, 1990; Al-Iraqi and Solyman, 2005; Hieu *et al*., 2022). Studies have also shown that nanoparticles extracted naturally from the leaves of the *Melia* have toxic and histological effects on the larvae and adults of houseflies (Idrees and Mustafa, 2022).

The current study aimed to estimate the percentage of protein found in the leaves and identify the quantity of amino acids in it, and is complementary to previous studies related to the extraction and identification of organic and inorganic compounds from this tree with multiple benefits, as it is considered the first study in Iraq concerned with this matter.

MATERIALS AND METHODS

Plant material

M. azedarach leaves were collected from the gardens of the Physics Department/ College of Science/ University of Mosul, dried in the shade at room temperature, and then ground into a powder using a Turkish-made electric grinder (CROUN type), then kept in the refrigerator at 4 °C for study purposes.

Total protein estimation

The Keldahl method was used to estimate the percentage of protein in the model under study, according to the steps mentioned by (Van Dijk and Houba, 2000), where 5 grams of the sample were taken and 5 ml of concentrated sulfuric acid was added to it with a mixture of potassium and copper sulfate. After that, the contents were heated up at temperature rises gradually until reaches 375 C° in order to perform the digestion process and until the mixture turned into a pale blue liquid, then the liquid was transferred to the distillation chamber in the Keldahl apparatus containing a concentrated solution of sodium hydroxide (40%), connected to a condenser that ends with a tube immersed in a beaker reception containing an appropriate volume of boric acid (20%), in addition to drops of methyl red and bromocresol blue dye. Then, the distillation flask is heated until an appropriate amount of distilled liquid (25 ml) is collected, it is then calibrated with hydrochloric acid (0.1 M), a control solution (Planck) was prepared from the above materials except the sample, the percentage of protein is calculated as follows:

$$\% \text{ Protein} = \frac{\text{HCl (V) x Molarity x 0.014 x 6.25}}{\text{Sample (W) X 100}}$$

Amino acid estimation

1-Amino acid extraction:

3 g of the sample was taken and 3 ml of hydrochloric acid (6 M) with 0.1% phenol was added to it. The mixture was placed in a 10 ml volumetric bottle and tightly closed. After that, it was placed in a convection oven at 45 °C for a day. Then 3 ml of sodium hydroxide and 0.1 mg of tartaric acid were added to it, and mixed well for 15 minutes. then the sample was filtered using a plastic filter 0.45um, and the injection process was performed (Dahl-Lassen *et al.*, 2018).

2- Derivation process

We followed the method used (Scriver *et al*., 2001) where 1 ml was taken from the extracted sample and 200 microliters of orthophthalene aldehyde (5%) (OPA) was added to it. The sample was shaken for 2 min, after which 100 microliters was taken from the last mixture and injected into the Amino acid analyses device (Korean origin) and according to the data: Acetonitrile, methanol, and formic acid make up the mobile phase 60: 20: 20, Derivatization with OPA is part of the injection program,100 µl is the injection volume, column: ZORBAX Eclipse-AAA; 3.5 m; 150 x 4.6 mm in length and width and Florescence detector (Ex = 445 nm, Em = 465 nm).

3- Preparation of the calibration curve

In order to prepare the calibration curve, 0.1 grams of high-purity amino acid mixture 99.9% was dissolved in nonionic water, transferred to a conical flask 250 ml, and the appropriate volume was added until its concentration became 250 ppm. Use the dilution law to prepare the calibration curve concentrations injected into the device.

RESULTS AND DISCUSSION

Results of the study showed that the percentage of total protein in 5 grams of the leaves sample was 13.2%. The results also showed in (Tables 1, 2 and 3) that the protein contains 12 amino acids that belong to the three amino acid classifications.

Six amino acids belonging to the nonpolar side chains amino acids were identified in the protein as shown in (Table 1) and in varying amounts, where the most abundant was the amino acid valine (402.5 µg/g), while the lowest abundant was the amino acid leucine (79.8 µg/g).

Table 1: Identified amino acids with nonpolar side chains. Chemical formula by https://pubchem.ncbi.nlm.nih.gov/.

No.	Amino acids	Reten. Tame (min)	Response	Amount (µg/g)	Chemical formula
1	Tryptophan	7.91	358.9	102.5	$C_{11}H_{12}N_2O_2$
2	leucine	9.93	123.6	79.8	$C_6H_{13}NO_2$
3	Glycine	13.34	212.5	315.4	$C_2H_5NO_2$
4	Alanine	15.13	654.1	225.8	$C_3H_7NO_2$
5	Valine	17.14	724.8	402.5	$C_5H_{11}NO_2$
6	Methionine	19.83	435.4	214.8	$C_5H_{11}NO_2S$

The amino acids in the (Table 2) represent amino acids with uncharged polar side chains and threonine was to be the most abundant in this group 720.3 µg/g followed by Cysteine 514.7 µg/g and Serine 114.8 µg/g. Three amino acids belonging to amino acids with charged polar side chains group (Table 3) were found in protein, and the highest percentage was glutamic acid 1025.4 µg/g, which is considered the most abundant amino acid in protein, while the lowest value was recorded for Arginine 203.6 µg/g.

Table 2: Identified amino acids with uncharged polar side chains. Chemical formula by https://pubchem.ncbi.nlm.nih.gov/.

No.	Amino acids	Reten. Tame (min)	Response	Amount (µg/g)	Chemical formula
1	Serine	9.12	314.8	114.8	$C_3H_7NO_3$
2	Threonine	11.82	347.8	720.3	$C_4H_9NO_3$
3	Cysteine	16.55	250.8	514.7	$C_3H_7NO_2S$

Table 3: Identified amino acids with charged polar side chains. Chemical formula by https://pubchem.ncbi.nlm.nih.gov/.

No.	Amino acids	Reten. Tame (min)	Response	Amount (µg/g)	Chemical formula
1	Glutamic acid	10.50	1252.5	1025.4	$C_5H_9NO_4$
2	Aspartic acid	22.15	333.5	241.3	$C_4H_7NO_4$
3	Arginine	13.92	356.5	203.6	$C_6H_{14}N_4O_2$

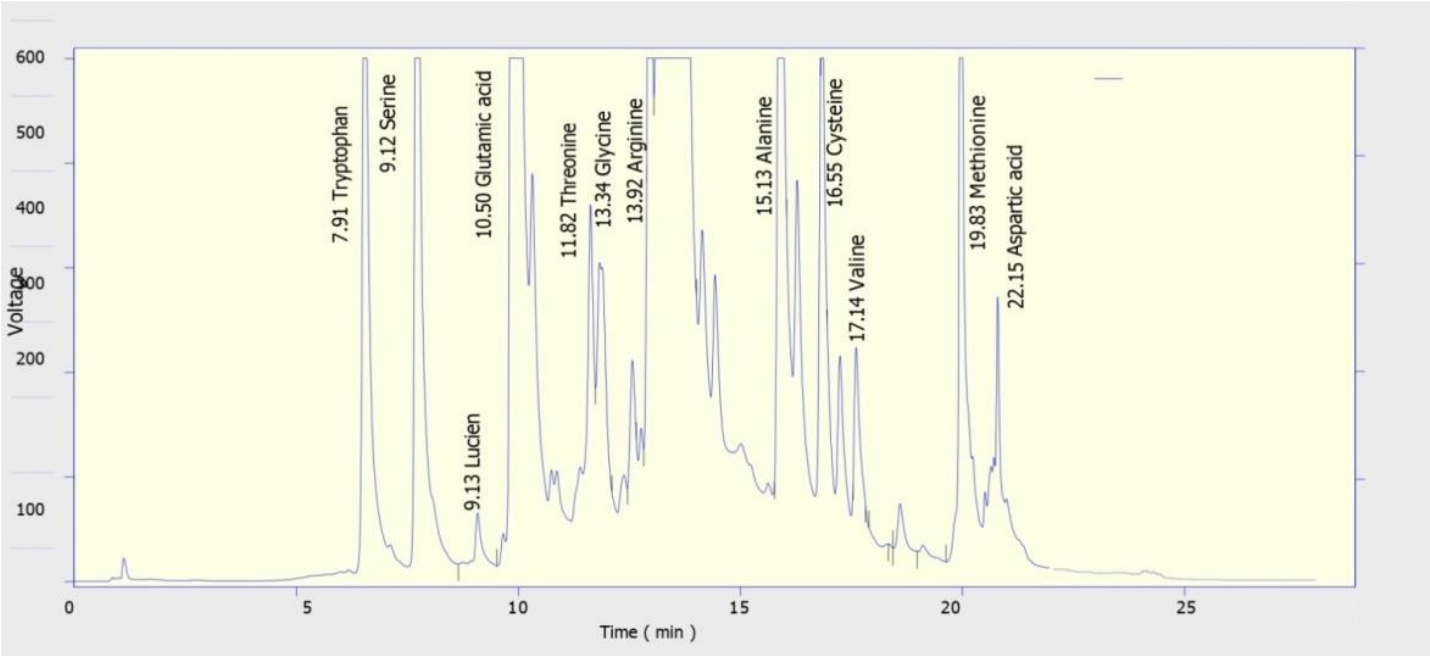

Fig.: Calibration curve for the identified amino acids.

According to our knowledge, this is the first study concerned with determining the total protein and amino acids it contains. Many previous studies were conducted to determine the organic and inorganic compounds in the leaves of *M. azedarach*, of which (Hemdan *et al*., 2023) that conducted a study on the most important vital compounds in the leaves of *A. indica* and *M. azedarach*, and found that *M. azedarach* contains phenolic acids: Gallic acid, protocatechuic acid , P-hydroxybenzoic acid, gentisic acid , Catechin , Chlorogenic acid, Caffeic acid, Syringic acid , Vanillic acid, Ferulic acid and Cinnamic acid, in addition to flavonoids: Rutin , Quercetin, Kaempferol and Chrysin. While (Dias *et al*., 2022) report the presence of Long-chain alkanes which are presented in high amounts: Tetracontane, Hexatriacontane, Dopentacontane; Sterols and Polyalcohol: β-Sitosterol, Stigmasterol, Campesterol, Myo-inositol; Amides and organic acids: Oleamide, Stearic acid, α-Linolenic acid, Linoleic acid, Palmitic acid, Succinic acid; in addition to carbohydrates: Sucrose, Melibiose, D-Glucose, β-D-Glucopyranose, D-(+)-Talofuranose, D-Psicofuranose, D-(+)-Fructofuranose in leaf of plants under well-watered (WW) and water-deficit (WD) conditions. Also leaves, rich in azidrachtin, which can be used as an insecticide and for fogging clothes (Ramya, 2009).

CONCLUSIONS

Access to the plant world, especially medicinal plants, which are an important source of many organic and inorganic compounds that may have multiple benefits for society, is in itself what the researchers sought, and from here came our study, which revealed the percentage of protein in the *M. azedarach* leaves and the amino acids that compose it. Therefore, our current study recommends detecting other biochemical compounds in the other medicinal plants and testing their effects as compounds with many medical, therapeutic or economic benefits, so that the picture will be clearer about the importance of this tree.

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تقدير المحتوى الكلي لبروتين اوراق نبات السبحبح العراقي وتشخيص نسبة وكمية الاحماض الامينية فيه باستخدام جهاز Amino Acid Analyses

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يعد نبات السبحبح *Melia azedarach* أحد أهم النباتات الطبية بسبب احتوائه على العديد من المركبات العضوية وغير العضوية ذات الفائدة الطبية والاقتصادية. يكثر زراعة أشجار النبات في العراق وخاصة في مدينة الموصل على الطرقات الجانبية كنباتات ظل إضافة إلى القيمة الجمالية لها. لذلك هدفت الدراسة الحالية، التي تعد استمراراً للدراسات التي تتعلق بتحديد وتشخيص المركبات العضوية وغير العضوية في النبات، إلى تحديد محتوى البروتين الكلي ونسبة وكمية الأحماض الأمينية في الاوراق. تم استخدام طريقة كيلدال وجهاز تحليل الأحماض الأمينية لهذا الغرض. أظهرت النتائج أن محتوى البروتين في 5.0 غرام من عينة الأوراق كان 13.2%. كما بينت نتائج دراستنا احتوائه على 12 حامضاً أمينياً، وأن الأحماض الأمينية ذات السلاسل الجانبية غير القطبية Amino acids with nonpolar side chains كانت الأكثر وفرة بين جميع الأحماض الأمينية الأخرى. من ناحية أخرى، كان الليوسين هو الحامض الأميني الأقل وفرة (µg/g79.8) في حين كان الجلوتاميك هو الأكثر وفرة (µg/g1025.4) من بين الاحماض الامينية المشخصة.

الكلمات الدالة: نبات السبحبح، البروتين الكلي، جهاز تحليل الاحماض الامينية.