



A RESEARCH ARTICLE

Qualitative and Quantitative Analysis of Amino Acids in the Leaves of Ashwagandha (*Withania somnifera* (L.)) from the Flora of Palestine: Exploring Their Relationship to the Plant's Therapeutic and Medicinal Properties

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Abstract

This research aims to study the qualitative and quantitative chemical composition of amino acids in the leaves of (*Withania somnifera* L.), which grows wild in Palestine, and to study the relationship between the presence of amino acids and the various medicinal effects of this plant.

Qualitative analytical chemistry was conducted on amino acids in various aqueous and alcoholic extracts of the leaves using multiple types of specific chemical reactions and various chromatographic techniques. The quantitative analysis of Amino acids was carried out by spectrophotometry technique. As a result of the qualitative phytochemical analysis of amino acids, ten amino acids were identified in the plant's leaves, including two essential amino acids (Valine, Tryptophan), two semi-essential amino acids (Cysteine, Cystine), and six non-essential amino acids (Alanine, Tyrosine, Aspartic, Glutamic, Glycine, Proline). As a result of the quantitative phytochemical analysis of amino acids, it was found that it contains 0.551% in the leaves. These results are the first to be presented for the plant (*Withania somnifera* L.), which is considered a plant of Palestine. This study explored the relationship between the presence of amino acids in the leaves of the Ashwagandha (*Withania somnifera* L.) and its medicinal properties. We hypothesize that the plant's ability to treat obsessive-compulsive disorder is linked to the presence of the amino acid valine, while its effectiveness in strengthening bones and muscles is associated with the amino acids valine and proline. Its ability to lower blood sugar levels is linked to the amino acid alanine. Its effects on enhancing alertness, improving mood, and reducing anxiety are associated with the amino acids valine and tyrosine, while improved sleep is linked to the amino acids tryptophan and glycine.

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1. Introduction

Various amino acids are essential for many physiological processes and functions in all plants and animals. Amino acids are also present in many medical and nutritional preparations derived from medicinal plants [1]. Recent research and studies have proven that amino acids are essential to the bodies of living organisms, especially humans. They are considered the building blocks of many proteins essential to humans, and amino acids play multiple roles in bodily functions. Furthermore, the body needs these amino acids to support metabolic processes such as breakdown and construction, and they are essential for building and producing essential hormones and neurotransmitters [2].

Amino acids can be classified into [3]:

1. Essential amino acids: These are the acids that the human body cannot produce on its own and must obtain from sources.
2. Non-essential amino acids: These are the acids that the body can produce on its own.
3. Semi-essential amino acids: These are the acids that the body can produce when similar amino acids are available.

Because amino acids are molecules that the bodies of all living organisms use to build proteins, and because the body needs approximately 20 different amino acids to function properly, essential amino acids and non-essential must be obtained through food or dietary supplements [2-4], as shown in Table 1.

Table 1. Essential and non-essential amino acids and their importance to the human body

No.	Amino acid	Medicinal effects
1	Valine	supports nerves and muscles and reduces feelings of fatigue.
2	alanine	contributes to energy production and blood sugar regulation.
3	Tryptophan	production of serotonin and melatonin and for improving sleep.
4	Glycine	Improves sleep and collagen formation
5	Cysteine	An important antioxidant and glutathione precursor
6	Tyrosine	Supports focus, memory, and hormone production
7	Proline	Supports joint and skin health

Research and studies conducted on plants that have a history and record of continuous use in folk and traditional medicine and have minimal side effects have become of interest to researchers. Among these plants is the plant ashwagandha (*Withania somnifera* L.), which is a plant rich in active chemical substances with diverse biological and pharmaceutical effects [4].

Ashwagandha (*Withania somnifera* L.) is a medicinal plant used in traditional folk medicine for a variety of ailments, including fever, cancer, diabetes, ulcers, hepatitis, eye inflammation, arthritis, heart problems, hemorrhoids, lower back pain, and to promote physique. Various parts of the plant have been credited with a wide range of benefits, including heart protection, anticancer properties, antifungal and antiviral effects, and antidepressant properties. It has also been used to lower blood sugar levels. Different parts of the plant have been evaluated over many years for their potential to treat erectile dysfunction in men, obsessive-compulsive disorder, anxiety, bone and muscle strengthening, weight loss, and diabetes [5].

Studies have shown that the plant (*Withania somnifera* L.) has broad pharmacological effects, due to the presence of a group of active ingredients. Many important medical preparations and nutritional supplements have been manufactured in many countries and in different pharmaceutical forms by various pharmaceutical manufacturing companies [4], as shown in Table 2.

Table 2. Herbal pharmaceutical preparations prepared from (*Withania somnifera* L.)

Herbal pharmaceutical preparations	Applications	Reference
Kapiva	Vital - Immunity booster	5
Ashwagandha		
Ashwagandha (<i>Withania somnifera</i>)	Combat cancer	6
Organic Ashwagandha	Immunesupport	7
Ashwagandha Gummies	Promotes immunity	8

Therefore, the aim of our study was to determine the qualitative and quantitative content of amino acids in the leaves of *Withania somnifera* L. (Figure 1) and to study the relationship between the presence of amino acids and the various medicinal effects of this plant.

**Figure 1.** *Withania somnifera*, L.

2. Materials and Methods

To prepare the aqueous and hydro-alcoholic extracts, the leaves of *Withania somnifera* L. were washed with distilled water and left to air dry at 30°C in the shade. The leaves were then crushed using an electric grinder. The powder was then stored in transparent plastic containers until needed for the study.

2.1. Plant Material

In mid-July 2025, leaves of *Withania somnifera* L. were harvested from the valleys of Ezbet Shofa village, filled with wild flowering plants, in the Tulkarm Governorate of the West Bank in Palestine.

2.2. Methods

2.2.1. Extraction

For qualitative analysis of amino acids, aqueous extracts were prepared from the leaves of the plant (*Withania somnifera* L.) at a ratio of 1:5 (herbal material: extract). The extracts were prepared and heated in a water bath with a reflux condenser for 30 minutes. After the extraction process, the different extracts were filtered [6].

2.2.2. Phytochemical examination- Qualitative analysis

Initially, the preliminary chemical study was carried out using different types of specific chemical reactions to detect the presence of amino acids in the leaves of *Withania somnifera* L. [7-9].

2.2.3. Phytochemical examination - Quantitative analysis

The quantitative chemical analysis of amino acids in *Withania somnifera* L. leaves was performed using spectrophotometric analysis of aqueous extracts [10].

2.2.4. Phytochemical examination- Chromatographic techniques

Amino acids from *Withania somnifera* L. leaves were detected by phytochemical screening using thin layer chromatography (TLC) on silica gel and paper chromatography in different systems to confirm the presence of major amino acids in *Withania somnifera* L. leaves.

2.2.5. Phytochemical examination

The results of the chemical tests were validated available references [11-14].

3. Results and Discussion

In this study, to detect the types of amino acids in the leaves extracts of *Withania somnifera* L., a variety of specific chemical processes were used, such as

1. comparing the R_f values and stain colors with the values of reference samples.
2. comparing the color change of the dye before and after treatment with specific reagents.

And at the end of the study, the amino acids in the different extracts from leaves of *Withania somnifera* L., were determined (Table 3).

3.1. Phytochemical examination- Qualitative analysis

Extracts from the leaves (*Withania somnifera* L.) were studied by performing qualitative analysis of amino acids using a variety of specific chemical reactions.

Amino acids screening

- **Biuret test:** A small amount of Biuret reagent is added to 2 ml of *Withania somnifera* L. leaf extract, the color changes from light blue to violet, indicating the presence of proteins or peptides.
- **Ninhydrin test:** Drops of 0.1-1% alcoholic solution of ninhydrin, with 2 ml of leaf extracts of *Withania somnifera* L. appear different colors, from yellow to purple, are evidence of the presence of amino acids.
- **Xanthoprote test:** Add 1 ml of concentrated nitric acid, with 2 ml of extracts of the leaves of the plant *Withania somnifera* L., a white precipitate appears, which changes to yellow after heating, further evidence of the presence of amino acids.

The results of the qualitative chemical analysis of amino acids in the leaves of *Withania somnifera* L., using various methods and methods of qualitative chemical reactions, are shown in Table 3.

3.2. Phytochemical examination - Quantitative analysis

For spectrophotometric analysis, aqueous extracts were prepared by placing 1.0 g of leaves in a 250 ml flask and adding 25 ml of distilled water, the mixture was then heated for 30 minutes in a boiling water bath equipped with a reflux condenser. The heated extract was then filtered through a small, folded filter paper into a 100 ml flask, the filter paper containing the remaining leaves was placed in another flask for repeated extraction using the same method and conditions, after cooling, the flask was refilled with the extract to a volume of 100 ml. For the analysis of the aqueous extracts, 2 mL of each extract or distilled water (to obtain a reference solution) was mixed with 4 mL of phosphate buffer (pH 6.4), 2 mL of 1% ninhydrin solution, and 2 mL of 0.05% ascorbic acid solution in a separate 100 mL flask.

The entire reaction mixture was then heated for 30 minutes and, after rapid cooling, returned to its original volume, the mixture was then analyzed spectrophotometrically.

The total amino acid content was estimated using spectrophotometry after the ninhydrin reaction at a wavelength of 568 nm in a 10 mm measuring cell. The total free amino acid content (%), expressed as the equivalent of glutamic acid and the dry, moisture-free leaf material (X), was calculated using the following formula:

$$\frac{A.100.100.B.2.100.100}{C.D.2.200.100.(100-W)} = \frac{A.B.50.100}{C.D.(100-W)}$$

Where A – Optical density of the test solution; C – Optical density of the standard glutamic acid solution; D-Weight of the sample (raw material), g; B – Weight of the standard sample (glutamic acid), g; W – Weight loss during drying of the raw material, %.

The result of the quantitative chemical analysis of amino acids in the leaves of *Withania somnifera* L., using spectrophotometric method are presented in [Table 3](#).

3.3. Phytochemical examination- Chromatographic techniques

Through qualitative phytochemical analysis, using thin-layer chromatography (TLC) and paper chromatography in various systems, the presence of amino acids in various extracts from the leaves of ashwagandha (*Withania somnifera* L.) was detected and confirmed, this confirms the validity of the results of previously conducted chemical tests.

Amino acids (chromatographic techniques)

To detect the presence of amino acids in the leaves of *Withania somnifera* L., qualitative chromatographic methods were used by thin layer chromatography (TLC) and by paper chromatography in different systems:

System A: n-butanol - acetic acid - water (4:1:2); **System B:** Ethanol- chloroform (1: 1)

After removing the chromatographic sheets from the different systems, they were air-dried and then treated with 0.2% ninhydrin alcoholic solution and placed in a drying chamber for 5 minutes at 100-105°C. Red, red-violet and brown spots appeared on the chromatogram, indicating the presence of amino acids. The results of the chromatographic studies are shown in [\(Table 3\)](#).

The results of phytochemical analyses (qualitative analysis - chromatographic techniques) and comparison with reference samples, revealed that ten free amino acids were found in the leaves of *Withania somnifera* L. , these acids included two essential amino acids, (Valine, Tryptophan)), two semi-essential amino acids, (Cysteine, Cystine), and six non-essential amino acids (Alanine ,Tyrosine ,Aspartic,Glutamic, Glycine, Proline). The result of the quantitative chemical analysis of amino acids in the leaves of *Withania somnifera* L., using spectrophotometric method are presented in [\(Table 3\)](#).

These amino acids were confirmed for the first time in the leaves of the plant (*Withania somnifera* L.) which grows in Palestine ([Table 3](#)).

The results of the current study showed that the leaves of the Ashwagandha plant (*Withania somnifera*) contain a wide range of amino acids, which scientific studies have proven to possess diverse medicinal effects ([Table 3](#)).

Table 3. Results of a qualitative and quantitative analysis of amino acids in the leaves of *Withania somnifera* L.

No	Amino acid	General formula	Rf system		Classification of amino acids			Spectrophotometric Results X, %
			A 4:1:2	B 1:1	Non-essential	Semi-essential	Essential	
1	Alanine	C ₃ H ₇ NO ₂	0.21	0.27	+			0.551 ±0.040
2	Valine	C ₅ H ₁₁ NO ₂	0.43	0.65			+	
3	Tyrosine	C ₉ H ₁₁ NO ₃	0.54	0.37	+			
4	Aspartic	C ₄ H ₇ NO ₄	0.16	0.07	+			
5	Glutamic	C ₅ H ₉ NO ₄	0.17	0.13	+			
6	Glycine	C ₂ H ₅ NO ₂	0.23	0.17	+			
7	Proline	C ₅ H ₉ NO ₂	0.24	0.36	+			
8	Cysteine	C ₃ H ₇ NO ₂ S	0.46	0.03		+		
9	Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	0.66	0.28			+	
10	Cystine	C ₆ H ₁₂ N ₂ O ₄ S ₂	0.44	0.04		+		

This study explored the relationship between the presence of amino acids in ashwagandha leaves and its medicinal properties. Its leaves are infused to reduce stress, anxiety, and ability to treat obsessive-compulsive disorder and we expect that this is linked to the presence of the amino acid valine, some plant extracts of it also improves sleep, and we expect that this is related to tryptophan, as this amino acid works to produce serotonin and melatonin.

while its effectiveness in strengthening bones and muscles and we expect that is linked to the amino acids valine and proline. Its ability to lower blood sugar levels is linked to the amino acid alanine. Its effects in promoting alertness, improving mood, and reducing anxiety are linked to the amino acids valine and tyrosine.

And its leaves have been used to boost the body's immunity, and we expect that this effect is related to the amino acid cysteine, which has antioxidant properties, this explains the widespread use of this plant in traditional folk medicine in many countries.

Therefore, the interest in studying this plant is justified, and we encourage researchers to study all its parts in order to find new plant medicines that may benefit humans and treat some of their diseases.

Studies have been conducted on the Ashwagandha plant (*Withania somnifera* L.) in Palestine [15-17], but they were about traditional and popular uses, and we did not find a study that discussed the phytochemical composition of the amino acids found in the plant's leaves.

At the end of our analytical chemical study on the plant (*Ashwagandha* -*Withania somnifera* L.), we emphasize that our study differs from previous research in the following aspects:

1. The researcher studied qualitative and quantitative Analysis of Amino Acids in the Leaves of Ashwagandha (*Withania somnifera* (L.)) flora of Palestine for the first time.
2. The presence of 10 amino acids in the leaves of (*Witania somnifera*, L.) was proven for the first time.
3. Through the results of the chemical study of the plant (*Witania somnifera*, L.), the researcher studied and explored the relationship between the plant and its chemical composition on the one hand, and its uses in folk and clinical medicine on the other hand, and concluded that there is a relationship between the presence of different amino acids in the leaves and the plant's possession of different therapeutic and medical properties.

4. Conclusions

This research aims to conduct a qualitative and quantitative analysis of the amino acids found in the leaves of *Withania somnifera* L., one of the most famous plants in Palestine. The researcher used various types of qualitative chemical reactions and various forms of chromatography to chemically study various aqueous and alcoholic extracts from the leaves of *Withania somnifera* L., Ten free amino acids were detected, including: two essential amino acids, (Valine, Tryptophan), two semi-essential amino acids, (Cysteine, Cystine), and six non-essential amino acids (Alanine, Tyrosine

,Aspartic ,Glutamic ,Glycine ,Proline), and the quantitative chemical analysis of amino acids in the leaves of *Withania somnifera* L. ,using spectrophotometric method was carried out .

The relationship between the presence of amino acids in *Withania somnifera* L., leaves and their uses in folk and clinical medicine was explored, revealing a clear connection between the presence of different amino acids and their relationship to the therapeutic and medicinal properties of the plant. For the first time, all of this was done on (ashwagandha (*Withania somnifera* L.)) that grows wild in Palestine.

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التحليل النوعي والكمي للأحماض الأمينية في أوراق نبات الأشواغاندا (*Withania somnifera* L.) من نباتات فلسطين: استكشاف علاقتها بالخصائص العلاجية والطبية للنبات

ملخص :

يهدف هذا البحث إلى دراسة التركيب الكيميائي النوعي والكمي للأحماض الأمينية في أوراق نبات الأشواغاندا (*Withania somnifera* L.)، الذي ينمو برّياً في فلسطين، ودراسة العلاقة بين وجود الأحماض الأمينية والفوائد الطبية المتعددة لهذا النبات.

أُجري تحليل كيميائي نوعي للأحماض الأمينية في مستخلصات مائية وكحولية مختلفة من الأوراق باستخدام أنواع متعددة من التفاعلات الكيميائية النوعية وتقنيات كروماتوغرافية متنوعة وأُجري التحليل الكمي للأحماض الأمينية باستخدام تقنية قياس الطيف الضوئي.

نتيجةً للتحليل الكيميائي النباتي النوعي للأحماض الأمينية، تم تحديد عشرة أحماض أمينية في أوراق النبات، من بينها حمضان أمينيان أساسيان (الفالين والتربتوفان)، وحمضان أمينيان شبه أساسيان (السيستين والسيستئين)، وستة أحماض أمينية غير أساسية (الالانين، التيروسين، الأسبارتيك، الجلوتاميك، الجليسين والبرولين). أظهر التحليل الكيميائي النباتي الكمي للأحماض الأمينية أن أوراق نبات الأشواغاندا (*Withania somnifera* L) تحتوي على 0.551% منها. وتُعد هذه النتائج الأولى من نوعها التي تُقدم لنبات الأشواغاندا، الذي يُعتبر من النباتات الفلسطينية.

استكشفت هذه الدراسة العلاقة بين وجود الأحماض الأمينية في أوراق الأشواغاندا وخصائصها الطبية. نفترض أن قدرة النبات على علاج الوسواس القهري مرتبطة بوجود حمض الفالين الأميني، بينما ترتبط فعاليته في تقوية العظام والعضلات بوجود حمضي الفالين والبرولين الأميين. أما قدرته على خفض مستويات السكر في الدم فترتبط بحمض الالانين الأميني. وترتبط تأثيراته في تعزيز اليقظة وتحسين المزاج وتخفيف القلق بوجود حمضي الفالين والتيروسين الأميين، في حين يرتبط تحسين النوم بوجود حمضي التربتوفان والجليسين الأميين.

الكلمات المفتاحية: التحليل الكيميائي النباتي، الأحماض الأمينية، الخصائص الطبية، نبات الأشواغاندا (*Withania somnifera* L)، نباتات فلسطين.