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Phytochemical Characterization of *Withania somnifera* (L.) Dunal flora Palestine: Correlating Bioactive Constituents with Traditional Uses and Modern Pharmacological Activities

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Abstract:

Ashwagandha preparations are used to treat various diseases and widely available in global herbal medicine markets. This article studies the phytochemical analysis of extracts from leaves, fruits, and roots of the Ashwagandha plant (*Withania somnifera* L.)- Solanaceae family that is prevalent in the lands of Palestine. Chemical analysis of all extracts from Ashwagandha was conducted using chemical reactions, and paper and thin-layer chromatography. The results of study revealed that the extracts of Ashwagandha contain alkaloid derivatives, flavonoid derivatives, tannins, amino acids, organic acids, steroids, and other chemical compounds.

The significance of this study lies in the fact that it is the first to provide a comprehensive phytochemical characterization of (*Withania somnifera* L.), which prevalent in Palestine. It presents substantial scientific evidence regarding the chemical composition of the Ashwagandha plant. These results support the plant's traditional medicinal use and highlight its potential as a valuable natural source of compounds which have pharmacologically active, opening up future possibilities for its application in the pharmaceutical drugs, nutritional, and antimicrobial therapy sectors.

Keywords: Phytochemical study, *Withania somnifera*, L., Flora of Palestine.

1. Introduction

In recent years, there has been increasing interest in the plant) *Withania somnifera*, L.), as evidenced by the increasing number of reports describing its health and medicinal benefits. Current research encompasses various aspects of human health and the treatment of certain diseases, such as diabetes and heart disease, including its neuroprotective, calming, and adaptogenic effects, as well as its impact on sleep. Other studies have also reported on its anti-inflammatory and antimicrobial properties (1).

The plant has traditionally been used for certain forms of sexual dysfunction and has been investigated for potential effects on erectile function, libido, and fertility (2-3).

Withania somnifera, L. (Ashwagandha) is an herb used in folk and traditional medicine in many countries, particularly India. The plant is known to occur in hot, dry, and semi-arid regions, including parts of the southern Mediterranean, North Africa, Iran, Jordan, Sudan, Palestine, Afghanistan, and Egypt (4-5) (6). Figure 1.



Figure 1. *Withania somnifera*, L.

Numerous studies have indicated its various uses, such as its use as an anti-anxiety, anti-inflammatory, antioxidant, and memory enhancer. It also has uses in neurological disorders (7). In the latest studies and research conducted on it, its new medical effects were identified, as it was shown to have antibacterial, antiviral, antitumor, and sexual stimulant effects (8).

The plant (*Withania somnifera*, L.) has a wide range of pharmacological effects, and many important medicinal preparations have been manufactured in different pharmaceutical forms such as capsules, tablets and powders by different pharmaceutical manufacturing companies (9), as shown in Table 1.

Table 1. Pharmaceutical preparations made from *Withania somnifera*, L.

Product Name	Form	Organ of plant	Medical Applications	Reference
Kapiva Ashwagandha	Capsules	Ashwagandha extract (root)	Vital Immunity booster	10
Ashwagandha (<i>Withania somnifera</i>)	Tablet	Ashwagandha Root powder	Combat cancer	11
Organic Ashwagandha	Powder	Root powder	Immune support	12
Ashwagandha Gummies	Gummy	Ashwagandha, Vitamin D	Promotes immunity	13

Given the chemical, biological, and pharmacological importance of *Withania somnifera*, L. and as part of the ongoing research on natural products and bioactive phytochemicals, the aim of our study was to qualitatively study the chemical composition of its leaves, fruits, and roots.

2. Materials and Methods:

2.1 Collection of plant material

In the months of July, September and October 2025, the leaves, fruits, and roots of *Withania somnifera*, L. were harvested from the wild plants that were growing in the hamlet of Kaffa in the Tulkarm Governorate (32.28°N, 35.03°E) in the West Bank, Palestine.

2.2 Preparation of plant extract

To prepare various extracts from the leaves, fruits and roots of *Withania somnifera* L., the above parts were cleaned with distilled water, left to dry in the air, then ground into powder using a mortar and kept in containers for qualitative chemical analyses.

To remove the fatty substances and chlorophyll from the leaves, fruits and roots of *Withania somnifera* L., they were soaked overnight at a temperature of 60-80°C using petroleum ether.

After removing the fatty substances, the leaves, fruits and roots of the plant (*Withania somnifera* L.) were extracted using a variety of solvents, hexane, chloroform, acetone and alcohol at varying concentrations, by using a Soxhlet apparatus at a temperature ranging between 60°C and 80°C. The various extracts obtained from the leaves, fruits and roots of the plant (*Withania somnifera* L.) using a variety of solvents were preserved at low temperatures until needed, after being concentrated in a rotary evaporator under vacuum.

2.3 Methods

Preliminary phytochemical screening

At the beginning of the phytochemical analyses, all extracts extracted from the leaves, fruits and roots of *Withania somnifera*, L. were examined using a variety of solvents (hexane, chloroform, acetone and alcohol in different concentrations) using the Soxhlet apparatus, by a set of special procedures to identify the main characteristic chemical components of this plant (14-15) (Table 2).

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

- butanol-acetic acid-water (4:1:2)
- ethanol-chloroform (1:1).

3. Results and Discussion:

To conduct qualitative phytochemical analyses of extracts from the leaves, fruits, and roots of the plant (*Withania somnifera*, L.), several types of qualitative chemical reactions were used to detect biologically active chemical compounds. The presence of specific phytochemical groups was assessed based on color changes following treatment with specific reagents. (Table 2).

After removing the chromatographic sheets from the two systems, they were air-dried, then treated with a 0.2% alcoholic ninhydrin solution and placed in a drying chamber for 5 minutes at 100–105°C. Red, reddish-purple, and brown spots appeared on the chromatograms, indicating the presence of compounds reacting with ninhydrin, consistent with amino acids.

Table.2. Initial qualitative analysis of active substances from *Withania somnifera*, L. Parts.

N.	Chemical composition	Qualitative interaction
1	Alkaloids	Dragendorff's reaction, Wagner's reaction, Hager's reaction
2	Flavonoids	Shibata's reaction (Shinoda reaction)
3	Tannins	Ferric chloride reaction
4	Amino acids	Biuret reaction- Ninhydrin reaction
5	Phenols	Lead acetate reaction
6	Steroid	Salkowski Test

The results of phytochemical analysis of extracts of leaves, fruits, and roots of *Withania somnifera* are presented in (Table 3). A wide range of chemical compounds were found, including alkaloids, flavonoids, tannins, amino acids, phenols, and steroids

Table 3: Chemical composition of the extracts from different organs of *Withania somnifera*, L.

Organs	Leaves	Fruits	Roots
Active substance	Result		
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Amino acids	+	+	+
Phenols	+	+	-
Steroid	+	+	-

(-): Denotes the lack of an active ingredient.

(+): shows that there is an active ingredient present.

Phytochemical analysis of Ashwagandha (*Withania somnifera* (L.) Dunal), collected in Palestine revealed several classes of secondary metabolites distributed among its leaves, fruits, and roots. Qualitative chemical analysis, supported by paper chromatography (PC), thin-layer chromatography (TLC), and comparison with established reference standards, confirmed the presence of several important phytochemical groups, including alkaloid derivatives, tannins, flavonoid derivatives, amino acids, steroids, and organic acids. Relationship between the identified phytochemical groups and their reported biological activities according to the scientific literature (16-19). See (Table 4).

Table 4: Potential Biological Activities Associated with the Detected Phytochemicals

N	Phytochemicals compounds	Potential Biological Activities
1	Alkaloids	Antimicrobial, neuroprotective, analgesic
2	Flavonoids	Antioxidant, anti-inflammatory, antimicrobial
3	Tannins	Antimicrobial, astringent, antioxidant
4	Steroids	Anti-inflammatory, immunomodulatory
5	Organic acids	Antioxidant, antimicrobial
6	Amino acids	Nutritional and metabolic functions

Among the identified components, alkaloid derivatives are of pharmacological interest. Ashwagandha is known to contain bioactive withanolides and other nitrogen-containing compounds, which contribute to its adaptogenic, anti-inflammatory, antimicrobial, and neuroprotective properties. The identification of flavonoids and tannins also points to a strong antioxidant capacity, as these phenolic compounds are known for their ability to scavenge free radicals, inhibit microbial growth, and reduce oxidative stress (1) (16-19).

The discovery of steroidal compounds is consistent with previous reports describing *Ashwagandha* (*Withania somnifera*) as a rich source of steroidal lactones (withanolides), which are considered the main bioactive components responsible for many of the plant's therapeutic effects. Similarly, the presence of amino acids and organic acids reflects the plant's nutritional and metabolic importance and may contribute to its medicinal value through synergistic interactions with other phytochemicals (2) (16-19).

The observed phytochemical composition suggests that the Ashwagandha plant found in Palestine has a similar chemical composition to that of plants cultivated in other geographical areas, while environmental conditions, such as climate, soil characteristics, altitude, and ecological factors, may influence the relative abundance of some metabolites. This geographical variation is well-documented in medicinal plants and can significantly affect their biological activity and therapeutic potential (1) (16-19).

More importantly, this study represents the first comprehensive phytochemical characterization of Ashwagandha native to Palestine, providing essential scientific evidence for its chemical composition. These findings support the traditional medicinal use of this plant species and highlight its potential as a valuable natural source of pharmacologically active compounds that could be used in future pharmaceutical, food, and antimicrobial applications (3) (16-19).

Overall, the results demonstrate that Palestinian Ashwagandha (*Withania somnifera*) is a promising medicinal plant, rich in a wide range of bioactive phytochemicals. This warrants further quantitative phytochemical analyses, advanced chromatographic techniques (HPLC, LC-MS/MS, GC-MS, and NMR), and pharmacological studies to isolate and evaluate its active constituents and their biological activity.

Based on the results of our study, and in accordance with multiple studies and research, it has been shown that the herb *Withania somnifera* (L.) is widely used in traditional and modern medicine to treat a variety of conditions, including immune diseases, as the plant is a vital immune booster and can treat some cancers (Table 1).

4. The value of study:

This study is considered important because it shows the results of studying different extracts of the plant (*Withania somnifera*, L.) that were obtained with different chemical liquids, and a group of different chemical compounds were discovered in the leaves, fruits and roots, in preparation for studying this plant in terms of the qualitative chemical composition, which will lead to knowing its medical effects.

5. Conclusions

The results of phytochemical analysis of leaves, fruits and roots of Ashwagandha (*Withania somnifera*, L.) of the Solanaceae family, which grows in Palestine.

As a result of the chemical study of various extracts from the leaves, roots and fruits of the (*Withania somnifera*, L.) using specific chemical reactions and various types of chromatography, the presence of alkaloids, tannins, flavonoids, amino acids, steroids, organic acids, and other phytochemical constituents was detected, for the first time in *Withania somnifera* growing in Palestine.



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