

Morpho-Phytochemical, Antibacterial and immunity boosting Studies of Ashwagandha (*Withania somnifera*) in different soils.

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Abstract

The study focusing on how soil and climatic conditions in Agra affect their immune-boosting properties. Morphological studies showed that vermicompost improved plant height, flowering, fruiting, seed development, and root size/shape. Phytochemical analysis of methanol root extracts revealed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, and tannins. Antibacterial tests demonstrated effectiveness against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. These results support the traditional medicinal use of these plants and highlight their potential as plant-based antimicrobial agents. The methanol extracts of plant showed significant antioxidant activity, exhibiting notable DPPH radical scavenging, which increased with higher extract concentrations, indicating its strong antioxidant capacity.

Keywords: : *Withania somnifera* plant, Morphological, Phytochemical, and Antibacterial and immunity booster.

1. Introduction

Morphological, Phytochemical, antibacterial and Immunity boosting studies of Ashwagandha (*Withania somnifera*) in different soils. Many types of medicinal properties are found in ashwagandha. This plant is in the form of an evergreen herb, it smells like a horse, that is way it is named Ashwagandha. It is also known as the prince of herbs in the Indian system of Ayurveda, because it has amazing ability to cure various types of diseases. It is a free gift given by nature to cure different types diseases. This plant belongs to the solanaceae family. Medicinal property are found in the root, stem and leaves of this plant. Root of *W. somnifera* has many biochemical activity and amazing medicinal properties.

Withania somnifera, commonly known as ashwagandha or winter cherry, which has been a staple in traditional medicine for centuries. The name "ashwagandha" is derived from the Sanskrit words "ashva" (horse) and "gandha" (smell), referring to the root's odor, which is said to resemble a sweating horse. Every part of *Withania somnifera* roots, leaves, flowers, bark, and stem is credited with medicinal properties. Research interest in this plant is growing globally, driven by its potential to treat various diseases and the increasing demand for its parts and derived chemicals. *Withania somnifera*'s morphological and medicinal properties can vary depending on its source. Typically cultivated as a late rainy season crop, it thrives in semitropical areas and is grown extensively using various cultivation practices. Propagation is usually carried out by seeds.

The use of plants and their products for medicinal purposes has been integral to human civilization since its beginnings. One of the earliest references to medicinal plants in Hindu culture is found in the "Rig Veda," which dates back to between 4500 and 1600 B.C. and is considered one of the oldest sources of

human knowledge. Approximately 80% of the global population relies on traditional medicine for primary healthcare, much of which involves plant extracts. In India, nearly 95% of prescriptions in traditional systems like Unani, Ayurveda, Homeopathy, and Siddha are plant-based.

Despite significant advancements in technology, science, and medicine, controlling the spread of infectious diseases remains a challenge. Microbes, among the oldest life forms on Earth, have existed for millions of years, evolving and adapting to survive changing conditions. In contrast, many larger ancient species have gone extinct, highlighting microbes' remarkable resilience and adaptability.

A healthy individual typically has a robust immune system capable of fending off infections caused by bacteria, fungi, viruses, and other pathogens. The immune system acts as the body's natural defense, producing various cells and molecules to combat a wide range of infections and harmful agents (1). It plays a pivotal role in protecting against external threats through helper and suppressor cells, as well as various soluble products (2). However, a weakened immune system can lead to illness or even death, as demonstrated during the recent coronavirus pandemic (3). To bolster immune defenses, experts recommend consuming foods or medications that can enhance immune function (4).

Plant-derived antimicrobials are gaining recognition for their potential therapeutic benefits. They can effectively treat infections while often minimizing the side effects associated with synthetic antimicrobials. The medicinal properties of plant materials largely stem from their diverse secondary metabolites, which include alkaloids, steroids, tannins, phenolic compounds, flavonoids, resins, fatty acids, and gums, each of which can have specific physiological effects.

W. somnifera listed in the Indian Materia Medica and has been used in the Ayurveda Siddha & Unani system of medicine and indigenous herbal medicine for over 3000 years (Anonymous, [5]).

2. MATERIALS AND METHODS

The current study was conducted at R.B.S. College's Botanical Garden in Agra to examine the effects of different sowing media under various soil conditions. The following media were used:

2.1 Ordinary Soil: Garden soil was scraped from a 25 cm thick layer of a cultivated field and was finely sieved before use.

2.2 Farm Yard Manure (F.Y.M.): F.Y.M. was mixed with the cultivated field soil in a 1:1 ratio, and this mixture was used to fill the pots.

2.3 Vermicompost: Freshly prepared vermicompost was obtained from a local manufacturer. It was mixed in small amounts with garden soil.

A-Morphological Studies: Ten plants were chosen and tagged for morphological research. Data was collected over the course of a year on various aspects, including plant morphology, leaf morphology, flower morphology, fruit morphology, and seed morphology.

B- Phytochemical Studies: The powdered root sample was subjected to qualitative screening for major phytochemical groups using standard procedures.

C- The antibacterial studies were carried out in various solvent extracts of Roots of Ashwagandha (*Withania somnifera*).

Present investigation was carried out with four strains of human pathogenic bacteria. The bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. All microorganisms were clinical isolates, obtained from the Microbiology Laboratory.

D. Evaluation of the immune booster (antioxidant activity) studies

The free radicals scavenging potential of the immune booster formulation was evaluated using the following assays: DPPH assay’s radical scavenging.

The DPPH scavenging activity was calculated in percentage by following formula:

$$\% \text{ inhibition of DPPH} = \{(AB-AS)/AB\} \times 100$$

Where, AB was the absorbance of the blank and AS was the absorbance of sample.

Result and Discussion-

W. somnifera (Ashwagandha) is a little evergreen, tiny, woody shrub plant , that grows naturally across 1.5 m height in damage garden, road side, rough and dry place, arid soil and waste area of Braj region of western Uttar Pradesh. The entire Ashwagandha plant is covered with short and silver branched. Leave are about 6-8 cm long, alternate, broadly ovate, simple, glabrous on upper surface, hairless and green. Stem is much branched, woody, silver-green, upright but sometime prostrate. Flowers are small, green, axillary, organized cymes, bisexual appear at the leaf nodes, 5-lobes calyx, sphere-shaped, stellately tomentose and acute, 5-lobes corolla, narrowly and yellow green. Stamens 5 and yellow-orange in color, the flower produce red fruit in midpoint. Fruit is red-yellow, soft, small, circular berry, globose and hairless. Seeds are numerous, kidney-shaped, pale-brown or yellow in colour, scurfy. Root is thick, heavy, long tuberous, branched, fleshy and whitish-brown in colour .



Fig- 1 (Morphology of *Withania somnifera*) Fig- 2



Fig-3 Fruits



Fig-4 Root

Plant Height of *W. Somnifera*- The height of *Withania somnifera* plant ranges from 50 – 70 cm. (fig-1 -2)

Fruit colour- The fruit are round in shape and red in colour. (Fig 3)

Root- Length of Root – 15-16 cm., colour of root is brown. (Fig 4)

1. Morphological Observations of *Withania somnifera* in Various Soil Conditions:

(i) Plant Morphology: *Withania somnifera* is a small to medium-sized, erect annual herb that reaches a height of 51-57 cm. in ordinary soil as well as farm yard manure. The plant has a branched stem, with the branches being ligneous and corky at the base, while the upper portions are covered with hairs. Plants grown in ordinary soil reach a height of approximately 62 cm, whereas those grown in vermicompost can attain up to 62-68 cm.

(ii) Leaf Morphology: The leaves are simple, glabrous, and alternate on the main stem. They are broadly ovate, with a petiole, and are green in color. The dimensions of the leaves range from 3-5 cm in length and 3-7 cm in width. The leaf size is fairly consistent across different soil conditions.

(iii) Flower Morphology: The flowers of *Withania somnifera* are small (0.5–0.7 cm), sub-sessile, complete, pentamerous, actinomorphic, hermaphrodite, and hypogynous. They have five persistent, gamosepalous sepals (0.4–0.7 cm), which are light green, boat-shaped, and enlarge with fruit development to cover the fr

The stamens are attached at the base of the corolla. Flowers are greenish or yellowish, with sub-included anthers and filaments 1-2.5 mm long.

The stigma and ovary are well-developed, with the ovary being bilocular and containing numerous ovules in axile placentation. The size of the flower is about 0.5 cm in plants grown in ordinary soil and farmyard manure, while plants grown in vermicompost produce flowers up to 0.7 cm.

(iv) Fruit Morphology: Fruiting occurs predominantly between April and July. The fruiting calyx is elongated and prominently ribbed. The fruits are berry-like, round, and measure 0.5-0.9 cm in diameter. They are light orange to dark orange in colour with a shiny epicarp.

(V) Seed Morphology: The seeds are small, sub-pyriform to reniform, and minutely reticulate-foveolate, with a yellowish-brown color. Each fruit contains 33–38 seeds. The seeds have a dry texture.

(vi) Root Morphology: The roots of *W.somnifera* are thick, fleshy, and whitish-brown in color. Generally, roots are straight and cylindrical, tapering down gradually, measuring about 15-17 cm in length and thick. The main roots are brownish on the outside with a creamy interior and have fibers similar to those of secondary roots. The size of the roots increases in plants grown in vermicompost.

Withania somnifera (L.) Dunal, commonly known as Ashwagandha or Winter Cherry, belongs to the Solanaceae family and is renowned for its therapeutic uses in both traditional and modern medicine due to the restorative properties of its roots^[6,7].

The flowers of this plant are sessile and appear in axillary clusters, each about 0.7cm long. They bloom almost year-round. The corolla can be greenish, yellow, or white-yellowish, with lobes that are lanceolate, acute, and thin, measuring approximately 5 mm in length. The calyx expands noticeably around the fruit.

The flowers can be bright yellow or greenish, and they develop into small berries that turn orange-red. The fruit, which is orange-red and contains numerous seeds, is present primarily in the spring^[8]. The seeds are numerous, yellow-white, reniform, and laterally compressed, poisonous, as noted by^[9].

2. **Phytochemical Analysis:** The methanol extracts from Ashwagandha (*Withania somnifera*), grown in different soil conditions, show a consistently rich phytochemical profile. Across all soil types, the extracts contain high levels of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, and tannins, indicating that soil variation does not significantly diminish the plant's ability to produce these key bioactive compounds.

3. ANTIBACTERIAL STUDIES

The observations on antibacterial activities of different concentration solvent methanol extracts of roots of *Withania somnifera* plant against four human pathogenic bacteria *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. are described in the following paragraphs.

Withania somnifera The observations on antibacterial activities of different concentration (20, 40, 60 and 80 µl) methanol solvent extracts of roots of *Withania somnifera* against human pathogenic bacteria *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are presented in Table -1

Table 1: Antibacterial activity of methanol Root extract of *Withania somnifera* against some human pathogenic Bacteria in various soil conditions

Sample		Inhibition zone (mm)				
	Name of Bacteria	<i>E.coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>	Standard
	Concentration	40 µl	40 µl	40 µl	40 µl	(40 µl)
1	Ordinary Soil	12.00	13.00	13.50	14.00	37
2	Farm yard	13.00	13.50	14.50	14.50	37
3	Vermicompost	15.00	15.60	16.50	17.00	37

These results support the traditional medicinal use of *Withania somnifera* for treating microbial infections, reinforcing the value of plant-based products as sources of antimicrobial agents. Additionally, other studies have confirmed the antimicrobial activity of *Withania somnifera* against different bacterial strains^[10-11].

4.EVALUATION OF THE IMMUNE-BOOSTER (ANTIOXIDANT ACTIVITY) STUDIES

The plants' antioxidant activities are evaluated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging assay.

4.1 DPPH Assay: This method measures the ability of antioxidants in the plant extracts to scavenge free radicals. The methanol extracts of plant showed significant antioxidant activity, exhibiting notable DPPH radical scavenging, which increased with higher extract concentrations, indicating its strong antioxidant capacity.

CONCLUSION

Focusing on Morphological and floral aspects, particularly the increase in root length. Plants grown with farmyard manure and vermicompost showed significant root growth. Phytochemical analysis of methanol extracts from the roots of *Withania somnifera* revealed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, and tannins. The methanolic root extracts exhibited antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. This suggests that the roots of *Withania somnifera* have defensive properties against bacterial infections. The study indicates that methanol is an effective solvent for extracting a range of active compounds, which could be valuable for developing traditional medicines. DPPH radical scavenging, which increased with higher extract concentrations, indicating its strong antioxidant capacity.

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