International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 14-12-2021, Accepted: 29-12-2021, Published: 14-01-2022
Volume 7, Issue 1, 2022, Page No. 743-746

Morpho-phytochemical and antibacterial studies of *Withania somnifera* in different soils

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Abstract
This study aims to investigate the Morpho-phytochemical and antibacterial properties of *Withania somnifera*, particularly focusing on different soil conditions and climatic factors in Agra influence its immune-boosting capabilities. Morphological studies showed improvements in plant height, flower, fruit, and seed parameters when grown in vermicompost. Notably, the shape and size of the roots were significantly enhanced in the vermicompost mixture. Phytochemical analysis of methanol extracts from the roots of *Withania somnifera*, cultivated under different soil conditions, confirmed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, and tannins. Antibacterial testing of methanolic root extracts revealed effectiveness against pathogens such as *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis*. These findings corroborate the traditional medicinal use of *Withania somnifera* for combating microbial infections, underscoring the potential of plant-based products as sources of antimicrobial.

Keywords: *Withania somnifera*, root extract of withania, morpho-phytochemical immune-boosting, antibacterial

Introduction

According to the latest survey of the World Health Organization, around 80% of the world's population uses herbal based medicines for primary health care. Recently, several reports of medicinal research proved that large numbers of herbal plants have anti-inflammatory properties and they also help to boost up the natural immunity of the human body. However, no side effects similar to the allopathic medicines or antibiotics have been observed.

The large numbers of researchers have revealed that the medicinal plants and their parts contain bioactive principles compounds which are known as secondary metabolites. The secondary metabolites are steroids, terpenoids, glycosides, alkaloids, phenyl propanoid, flavonoids and phenolics. These secondary metabolites are a source of medicine. These secondary metabolites are obtained from trees, shrubs and herbs. They are derived from various parts of plants like stems, leaves, roots flowers and fruits. However, primary important metabolites are carbohydrates, proteins, enzymes, chlorophyll and lipids which are very much valuable to the life cycle of the plant. Most of the plant species are used for treatment of human diseases and veterinary diseases in India.

Morphological studies of medicinal plants are closely related to both biological and medical sciences, focusing on the examination of plant shapes, sizes, textures, colors, structures, and other morphological features. Higher plants may have various modified organs such as underground rhizomes, tubers, bulbs, corms, spines, and bulbils. Understanding these morphological and anatomical aspects is crucial for identifying medicinal and herbal plants. This knowledge is fundamental for the pharmaceutical industry, as the quality of herbal preparations is directly linked to the characteristics of the roots, stems, leaves, flowers, and fruits used in medicines [1].

Medicinal plants have been essential sources of medicine since ancient times. Across various Indian cultures, plants and their parts have played a vital role in treating human diseases. Ancient texts such as the Vedas, the Bible, the Upanishads, and the Charak Samhita document the use of Indian herbs and medicinal plants, highlighting their significant medicinal properties [2].

India is home to a vast array of plant species used to treat both human and veterinary diseases. In addition to their use in traditional systems of medicine such as Ayurveda, Siddha, and Unani, these plants and their various parts are also integral to modern pharmaceuticals and products designed to boost immunity. A robust immune system is essential for maintaining good health. If our immune system is compromised, our bodies are less able to defend against microorganisms such as bacteria, viruses, and parasites. Human immunity is the body's natural defense mechanism against numerous infectious diseases [3]. Key factors influencing immunity include past infections, immunization, and environmental stimuli. To support and strengthen the immune system, it's important to consume a balanced diet rich in nutrients

Medicinal plants play a crucial role in protecting humans from various pathogenic microorganisms and diseases. Numerous plants are known for their ability to modulate the immune system, promoting overall health and enhancing the body's natural resistance to infections by restoring balance and conditioning bodily tissues. It is often speculated that the rejuvenating effects of these herbal remedies may be linked to their influence on the immune system. Plant-derived compounds, including proteins, lectins, and polysaccharides, have been shown to stimulate immune responses. Traditional Indian medicine, including Ayurveda, has long recognized the value of these plants in treating various ailments. Various studies revealed that plants with documented immune-modulatory effects, based on both experimental and clinical studies [4].

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. Prpaogation is usually carried out by seeds. This paper focuses on the morpho-phytochemical and antibacterial properties of *Withania somnifera*, examining how different soil conditions and climatic factors in Agra affect its immune-boosting qualities.

Material 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:

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

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.

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

For the experiment, *Withania somnifera* seeds were planted in earthen pots. The pots, freshly removed from the water tank and dried, were filled with the potting mixture. Each pot was accurately filled with 4 kg of the mixture, leaving approximately 3.00 cm at the top for water application.

1. **I. 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.

2. **Plant Collection and Extract Preparation:** Roots of *Withania somnifera* were collected from the botanical garden at R.B.S. College, Agra, U.P. The roots of *Withania somnifera* were thoroughly washed under running tap water and dried on paper towels. The aerial parts were then blended, and the mixture was extracted with methanol by macerating at room temperature (30°C) for 72 hours.

3. **Phytochemical studies**: The powdered root was assessed for the qualitative presence of the following major phytochemicals.

3.1. Alkaloids

Mayer's Test: In a test tube, 2 ml of the plant extract was mixed with 5 ml of 1% aqueous HCl. Then, 100 µl (or 4-5 drops) of freshly prepared Mayer's reagent was added. The appearance of a buff-colored precipitate indicates the presence of alkaloids [5].

Mayer's Reagent*:* Prepared by dissolving 1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water.

3.2. Glycosides

Legal Test: A small amount of pyridine was added to the sample in a test tube, followed by a few drops of alkaline sodium nitroprusside solution. A blood-red color indicates the presence of glycosides.

3.3. Steroids

Salkowski Test: 0.5-1 ml of the test solution was mixed with chloroform in a test tube. After adding a few drops of concentrated sulfuric acid and shaking well, the appearance of a red color in the lower layer indicates the presence of steroids.

3.4. Flavonoids

2 ml of 10% aqueous sodium hydroxide was added to 2 ml of the filtrate. A yellow coloration that changes to colorless upon adding dilute hydrochloric acid indicates the presence of flavonoids [6].

3.5. Phenols

A few drops of 10% ferric chloride solution were added to 2 ml of the extract. The appearance of a bluish-green or black color indicates the presence of phenols [7].

3.6. Saponins

2 ml of plant extract was mixed with 2 ml of distilled water in a test tube and shaken vigorously. The formation of a frothy foam indicates the presence of saponins [5].

3.7. Tannins

Fe Cl3 Test: 2 ml of plant extract was mixed with a few drops of 0.1% FeCl3 solution. The appearance of blue-green or blackish-green color or precipitate indicates the presence of tannins (Trease and Evans [6, 7].

4. **Anti-Bacterial studies:** The antibacterial activity of the samples was assessed using the agar well-diffusion method as described by [8]. Mueller-Hinton Agar (MHA, Hi Media, India, no. 2) was used as the bacterial growth medium. Extracts were prepared in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml. A standardized inoculum, diluted in sterile 0.9% saline, was used to inoculate the agar. Wells of 6 mm in diameter were created in the MHA plates, and 40 µl of each extract at different concentrations was added to the wells. The plates were incubated at 37°C for 24 hours. The antibacterial activity of the methanol extracts was determined by measuring the diameter of the inhibition zones around each well. Ciprofloxacin (40 µl) was used as a control antibiotic. The experiment was conducted in triplicate to minimize errors, and mean values were calculated. The four human pathogenic bacteria: *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used for antibacterial activities.

The antibacterial activities of methanol extracts (40 µl) from the roots of *Withania somnifera*, grown under different soil conditions

Results

1. Morphology of Whithania Somnifera (Ashwagandha)

Table 1: Morphological Characters of Withania *somnifera* In Different Soil Conditions

	Characters	Soil conditions		
		Ordinary Soil	**Farm yard Manure**	**Vermicompost**
1	Plant height	50-59 cm	50-59.00 cm	60-68.00 cm
2	Leaf Length and width	3- 5 x 3-8cm	3-5 x 3-8cm	3-5 x 3-8cm
3	Flower	0.5.00 cm	0.5.00 cm	0.7.00 cm
4	Fruit (Diameter)	0.5-0.8 cm	0.5-0.9 cm	0.5-0.9 cm
5	Number of Seed/Fruit	33–36	33–36	34-38
	Root	15.00cm	16.00cm	17.00cm

2. Phytochemical Studies

Table 2. Phytochemical studies of root extract of *Withania somnifera* in various soil condition

S. No.	Soil conditions	Ordinary Soil	Farm yard Manure	vermicompost
1.	Phytochemicals			
	Alkaloids	+	+ +	+ +
2,	Glycoceroids	+	+	+
3	Flavonoid	+	+	+
4.	Phenols	+	+ +	+ +
5.	Saponin	+	+	+
6	Steroids	+	+	+
7.	Tanins	+	+	+
8.	Proteins	-	-	-

3. Antimicrobial Studies: The antibacterial activities of methanol extracts (40 µl) from the roots of *Withania somnifera*, grown under various soil conditions, were tested against four human pathogenic bacteria: *E. coli, B. subtilis, P. aeruginosa, and S. aureus.* The results are summarized in Table 3.

It is evident from Table 3 that the highest antibacterial activity was observed against *S. aureus*, with a maximum zone of inhibition of 17.50 mm when the roots of plants were grown in vermicompost. In contrast, the lowest activity was recorded against *E. coli*, with a maximum zone of inhibition of 12.50 mm. in roots of plants grown in ordinary soil. The disc diffusion method demonstrated that the root extract has significant antibacterial potential. For comparison, Ciprofloxacin (40 µl) was used as a control antibiotic.

Table 3: 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	***E. coli***	***P. aeruginosa***	***B. subtilis***	***S. aureus***	**Standard**
	Concentration	40 µl	40 µl	40 µl	40 µl	(40 µl)
1	Ordinary Soil	12.50	13.50	14.00	14.50	37
2	Farm yard	13.00	13.00	14.00	14.50	37
3	Vermicompost	15.00	15.50	16.00	17.50	37

In *Withania somnifera* under different soil conditions, 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,

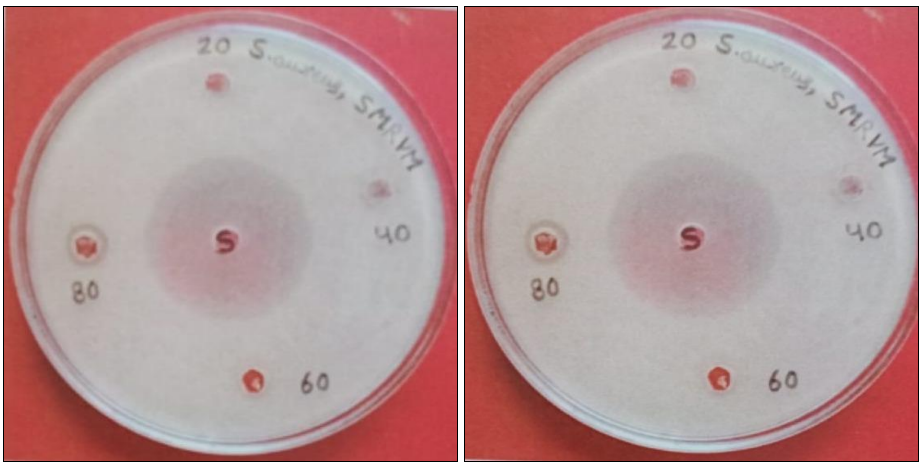

Fig 1: In E. coli Zone of Inhibition (12.50 mm.) **Fig 2:** In S. aureus Zone of Inhibition (17.50 mm.)

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.

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