

HPLC-based phytochemical characterization of ashwagandha Pratap [*Withania somnifera* (L.) Dunal] cultivated in the soils of Haridwar, Uttarakhand

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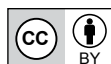
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Abstract. This study aimed to evaluate the phytochemical composition of *Withania somnifera* (L.) Dunal 'Pratap' variety cultivated in the soils of Haridwar, Uttarakhand, with a focus on quantifying key withanolides using High-Performance Liquid Chromatography (HPLC). Soil samples from the cultivation site at Teliwala nursery were collected and analyzed using standard APHA protocols and Atomic Absorption Spectroscopy to assess physicochemical and micronutrient profiles. Plant parts such as roots, stems, and leaves were subjected to HPLC-DAD analysis on a Shimadzu LC-2030C 3D system for quantification of six major withanolides, including withaferine A, withanoside IV & V, withanolide A & B, and withanone.

The soil was moderately alkaline (pH 8.37) with high organic carbon (1.43%), and low nitrogen (2.79 mg/kg), and phosphorus (7.0 mg/kg), though sulphur and zinc were deficient. The leaf tissues exhibited the highest total withanolide content (10.285 µg/mg), particularly withaferine A and withanone, followed by stems (1.358 µg/mg) and roots (0.886 µg/mg). The distinct withanolide profiles across plant parts highlight tissue specific accumulation trends. Notably, withanoside IV dominated in stems, while withanoside V peaked in leaves. The study demonstrates a clear association between favorable soil conditions and enhanced secondary metabolite synthesis in *W. somnifera*. While roots remain commercially important, the phytochemical richness of leaves suggests underutilized pharmacological potential. Future work should explore soil metabolite correlations across varied agro-ecological zones to optimize medicinal quality and cultivation strategies.

Keywords: *Withania somnifera*, phytochemical, withanolides, soil

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INTRODUCTION

Ashwagandha is a renowned medicinal herb used extensively in Ayurveda for its adaptogenic and rejuvenating properties and is prized for its ability to reduce stress, enhance immunity, improve brain function, and promote overall well-being. It is often called Indian ginseng or winter cherry (Majeed et al., 2023). The roots of ashwagandha contain active compounds like withanolides, which contribute to its therapeutic effects. It is particularly effective in lowering cortisol levels, thereby helping manage anxiety, depression, and fatigue (Arumugam et al., 2024). Additionally, ashwagandha supports heart health, hormone balance, muscle strength, and sleep quality, making it a versatile remedy (Sprengel et al., 2025). Given its wide-ranging benefits, it is commonly consumed in the form of powders, capsules, and herbal extracts in both traditional Indian system of medicine (as Ayurveda, Yoga, Naturopathy, Unani, Siddha, Sowa-Rigpa, and Homoeopathy) and modern nutraceutical formulations (Mukherjee et al., 2021). The quality of soil has a profound impact on the chemical composition and medicinal efficacy of ashwagandha (*Withania somnifera*) (Basak et al., 2020). It is a hardy, drought-resistant plant, thrives in dry and subtropical climates with well-drained sandy soil, and requires minimal irrigation, making it an excellent choice for sustainable farming (Dhruvi et al., 2025). It thrives best in sandy loam or light red soil with good drainage and a pH level between 7.5 and 8.02 (Pandey, 2025). These soil conditions allow optimal root development and enhance the concentration of medicinal compounds in the plant. Nutrient availability in the soil directly influences the concentration of withanolides, the bioactive compounds responsible for ashwagandha's therapeutic benefits. Organic fertilizers, such as farmyard manure and vermicompost, have been found to enhance root development and increase withanolide content more effectively than synthetic fertilizers. Additionally, soil moisture levels and environmental stressors, including drought, can trigger physiological responses that boost polyphenol content, enhancing the plant's antioxidant properties. The soil's pH and microbial activity also play a crucial role in optimizing nutrient uptake, leading to healthier plant growth and a higher concentration of medicinal compounds. By carefully managing soil conditions, balancing nutrients, organic matter, moisture, and microbial diversity, farmers can cultivate ashwagandha with superior potency and quality (Jat et al., 2021; Basak et al., 2020).

The important varieties of *Withania somnifera* are mainly Chetak, Nimithli, Poshita, Jawahar-20 and Pratap grown throughout the country for various purposes. The Pratap variety of ashwagandha differs from other varieties in several aspects, primarily in yield, withanolide content, cultivation adaptability, and medicinal potency. Pratap is known for its high dry root yield, making it a preferred choice for large-scale farming. It has a distinct profile of withanolide A and withaferine A, which are the primary bioactive compounds responsible for its medicinal benefits. Compared to other varieties like Chetak, Nimithli, Poshita, and Jawahar-20, Pratap offers consistent phytochemical composition, making it more suitable for diverse soil conditions. While some varieties, such as Poshita and Jawahar-20, may exhibit slightly higher concentrations of withanolides, Pratap remains popular due to its balanced medicinal potency and productivity (Singh et al., 2024). Its adaptability to different environmental conditions makes it a reliable choice for farmers seeking high-yield crops with robust therapeutic properties. Ashwagandha CIMAP Pratap (Figure 1), is highly promising with a high dry root yield of 0.3495 kg/m², high total withanolide content of 0.31%, high fresh and dry leaf yields of 0.0539 and 0.0087 kg/m² and high withaferine A content of 0.720% in the dry leaves. (Lal et al., 2012)

Ashwagandha, also known by its vernacular names such as Turangi-gandha, Indian Winter Cherry, and Indian Ginseng, is a medicinally important plant widely recognized in traditional systems of medicine. It is naturally distributed across South East Asia and extends from the Mediterranean region to South Africa, thriving at altitudes up to 1500 meters (Verma, Kumar, 2011; Umadevi et al., 2012). Taxonomically, the plant belongs to the family Solanaceae, which is characterized by diverse species of medicinal and economically significant plants.

Plant description: small, woody shrub, up to 1.5 m tall. Long, brown, tuberous root, 15–25 cm in length, stout and fleshy. Central stem with branches extend radially in a star pattern (stellate) and covered with wooly hairs (tomentose). Leaves simple, exstipulate, oblique, petiolate, broadly ovate to oblong, entire, shiny smooth, dull green in color, globose, and elliptical 30–80 × 20–50 mm, narrowed into 5–20 mm long petioles. Flowers in axillary umbellate cymes, small, green, inconspicuous, solitary, 4–6 mm in diameter. Fruits orange-red berries. Seed very light, small, smooth, lens-shaped, or kidney-shaped, 2–2.5 mm length × 1.5–2 mm width × 0.5 mm height (Verma, Kumar, 2011; Umadevi et al., 2012; Gaurav et al., 2023).

The plant parts of ashwagandha that are commonly utilized for medicinal and therapeutic purposes include its roots, leaves, bark, fruits, and flowers, each contributing distinct phytochemical properties and pharmacological activities (Gupta, Rana, 2007; Verma, Kumar, 2011; Umadevi et al., 2012) (Table 1).



Figure 1. Whole plant of *Withania somnifera* (L.) Dunal 'Pratap'.

Table 1. Medicinal properties in the roots, leaves and stems of ashwagandha.

Part Used	Medicinal Properties	Source
Roots	Hiccup, cold, cough, female disorders, senile debility, ulcers, schizophrenia, chronic stress, insomnia, anxiety, memory/cognitive enhancement, obsessive-compulsive disorder, rheumatoid arthritis, type-2 diabetes, male infertility	Verma, Kumar, 2011; Umadevi et al., 2012; Tandon, Yadav, 2020; Gaurav et al., 2023
Leaves	Carbuncles, conjunctivitis, inflammation, swellings, tumors	Jayaprakasam et al., 2003; Umadevi et al., 2012; Gaurav et al., 2023
Bark	Asthma, bed sores	Umadevi et al., 2012
Stem	Tumors, nocturnal leg cramps, diarrhea, psychiatric palpitation	Gaurav et al., 2023
Flowers	Respiratory problems	Gaurav et al., 2023
Fruit	Respiratory problems	Verma, Kumar, 2011; Umadevi et al., 2012; Gaurav et al., 2023

Ashwagandha is used in many forms for the treatment of various ailments as well as for other purposes. More commonly Ashwagandha Churna (Powder) is used for stress relief, energy enhancement, and immune support. It is often mixed with milk or honey for better absorption (Sultana, 2023). Ashwagandha Rasayana is a specialized formulation that boosts longevity, strengthens the nervous system, and improves overall vitality. Ashwagandha Lehyam (Herbal Paste) used for rejuvenation and muscle strength, particularly beneficial for athletes and those recovering from illness (Sachin et al., 2017). Ashwagandha Capsules & Tablets are convenient forms for daily supplementation, supporting mental health, hormonal balance, and anti-inflammatory benefits. All parts of the plant are used medicinally for the treatment of various ailments specially in Ayurveda.

According to Ayurvedic literature, ashwagandha possesses distinct properties that define its therapeutic role. Its Rasa (taste) is described as bitter, pungent, and astringent, while its Guna (qualities) are light and unctuous, making it suitable for balancing bodily functions. The plant exhibits a Virya (potency) that is hot in nature, and its Vipaka (post-digestive effect) is pungent. In terms of its influence on the doshas, ashwagandha is known to balance Vata and Kapha, while mildly increasing Pitta, thereby contributing to its wide application in Ayurvedic formulations (Joshi, Joshi, 2021).

The purpose of the study was to evaluate the productivity of the Pratap variety, developed by the Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India. This variety demonstrates greater biomass and root size compared to Nagori and other cultivars.

Moreover, Pratap thrives in regions with moderates to average rainfall and exhibits resistance to fungal infections that commonly affect the roots of the other varieties (Khabiya et al., 2024).

MATERIALS AND METHODS

The present study was carried out at the plant nursery established at Teliwala (Project No. NGO/Ashwagandha-Campaign/UK-01/2023-24) located within latitude 29.98° N and longitude 77.98° E, district Haridwar, Uttarakhand, India at an elevation of 271 m above mean sea level. Haridwar experiences a mild climate with temperatures ranging from 15 °C to 35 °C during the spring (March to May) and 25 °C to 35 °C during the monsoon (June to September). The mean annual rainfall ranges between 2136.7 mm during June to September (Pirasteh et al., 2025).

Soil analyses

Soil sampling was conducted following standard protocols to account for spatial heterogeneity within the nursery. Fifteen subsamples were collected at random locations from a depth of 0–15 cm and subsequently homogenized to form a composite sample. The composite soil was air-dried under natural sunlight, sieved through a 2 mm mesh, and stored for subsequent physicochemical analysis.

Mineral content estimation

The analysis of various mineral constituents along with other physicochemical parameters was conducted by following the standard methods provided as per APHA (American Public Health Association). 12.5 g of soil sample was taken in a 100 mL and to it 25 mL of DTPA (Diethylenetriaminepentaacetic acid) solution was mixed. The contents were shaken for 2 hours at 70–80 oscillations per minute on a shaker and then filtered

through acid washed distilled water rinsed Whatman filter No. 1 filter paper. The filtrate was stored in plastic bottles and used accordingly. The micronutrients were determined by using Atomic Absorption Spectroscopy (AAS). The standard stock solutions were prepared by dissolving 1 g of metal in a minimum volume of 1:1 nitric acid and then diluted to 1000 mL with distilled water to obtain 1000 µg/mL solution of micronutrient. Then 50 mg/L working solution was prepared using standard. Then, for each metal, a series of standard solution of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L prepared (Black, 1965) and used for analysis.

Plant analyses

The plant materials were collected from the same site from where the soil samples were collected. The random collection of plant samples resulted in obtaining 1 kilogram of plant material of *W. somnifera* var Pratap.

Preparation of Pratap ashwagandha extracts

Crushed dry powdered raw materials (RM) of Pratap ashwagandha were weighed as follows: roots – 2.07 g, leaves – 2.02 g, and stems – 2.02 g. Each weighed sample was transferred into a 10 mL volumetric flask, and 7 mL of methanol was added. The mixtures were sonicated for 30 minutes, cooled to room temperature, and the volumes were adjusted with methanol. The resulting solutions were filtered through a 0.45 µm nylon filter, and the filtrates were used for subsequent analysis.

Reagents and Standard preparation

All the reagents used during the analysis were of HPLC grade. Acetonitrile (Cat. No.-40030LC250) was procured from Finar (Gujarat, India), methanol (Cat. No.M0276) from Rankem (Maharashtra, India), acetic acid (Cat. No.D14D741529) from Merck (Maharashtra, India). Deionized water was obtained from a Milli Q system (Millipore, Billerica, MA, USA). The various standards withanoside IV (potency – 96.2%), withanoside V (potency – 97.6%), withaferine A (potency – 98.0%), withanolide A (potency – 97.5%), withanone (potency – 96.7%) and withanolide B (potency – 95%) were dissolved in methanol to prepare 1000 mg/L standard stock solution individually. The working solution was prepared taking 0.05 mL of 1000 mg/L from each withanolide standard stock solution and then was diluted to 1 mL of methanol to make 50 mg/L.

Procedure

The quantification of withanolides in ashwagandha Pratap plant parts were analyzed by HPLC-DAD using an in-house protocol developed at Patanjali research Foundation. The analysis was performed using Prominence-i LC-2030C 3D plus HPLC system. Separation was achieved using a VD Spher PUR-120 C18-U (5 µm, 4.6*250 mm) column subjected to binary gradient elution. Two solvents used for the analysis consisted of water containing 0.00014 kg potassium hydrogen phosphate in 900 mL water, added with 0.5 mL ortho-phosphoric acid and made up to 1000 mL with water (solvent A) and acetonitrile (solvent B). Column temperature was kept 27 °C and flow was set 1.5 mL/min during the analysis. 20 µL of standard and test solution were injected and wavelength was set at 227 nm. The limit of detection (LOD) for this method was determined to be 0.02 µg/mg, while the limit of quantification (LOQ) was established at 0.05 µg/mg.

RESULTS

The soil at the study site exhibited a moderately alkaline pH of 8.37 (Table 2), a range considered favorable for the cultivation of *Withania somnifera*. High levels of organic carbon (1.43%) and organic matter (2.45%) suggest a well-structured soil environment with enhanced microbial activity and good nutrient retention. The presence of essential macronutrients such as nitrogen (2.79 mg/kg), phosphorus (7.0 mg/kg), and potassium (35.55 mg/kg) which was low. Nutrients analysis revealed deficiencies in sulphur (2.37 mg/kg) and zinc (0.56 mg/kg), both critical for enzymatic function and secondary metabolism. In contrast, iron (15.1 mg/kg), manganese (8.84 mg/kg), and cop-

Table 2. Soil analysis report of study site.

Soil health indicator	Value	Unit	Critical limit / Range	Reference category	Used method	Reference
pH in water solution	8.37		< 6.5 (acidic) 6.5–7.5 (neutral) > 7.5 (alkaline)	Alkaline	Potentiometer	ICAR (2011); Soil Health Card Scheme (2015)
Electric conductivity	150.53	µS/cm	< 400 (normal) 400–800 (critical) > 800 (saline)	Normal	Conductometric method	ICAR (2011)
Organic carbon	1.43	%	< 0.5 (low) 0.5–0.75 (medium) > 0.75 (high)	High	Walkley, Blacks (1934)	ICAR (2011)
Organic matter	2.45	%	< 0.5% (low) 0.5–1.5% (medium) > 1.5% (high)	High	Walkley, Blacks (1934)	ICAR (2011)
Available nitrogen	2.79	mg/kg	< 280 (low) 280–560 (medium) > 560 (high)	Low	Subbiah, Asii (1965)	Subbiah, Asii (1965)
Phosphorus	7.00	mg/kg	< 10 (low) 10–25 (medium) > 25 (high)	Low	Olsen et al. (1954)	Olsen et al. (1954)
Potassium	35.55	mg/kg	< 120 (low) 120–280 (medium) > 280 (high)	Low	Merwin, Peech (1950) Flame photometer	Merwin, Peech (1950)
Sulphur	2.37	mg/kg	≤ 10 (deficient) > 10 (sufficient)	Deficient	Chesin, Yien (1950)	Chesin, Yien (1950)
Zinc	0.56	mg/kg	≤ 0.6 (deficient) > 0.6 (sufficient)	Deficient	Atomic Absorption Spectroscopy Method	Lindsay, Norvell (1978)
Iron	15.1	mg/kg	≤ 4.5 (deficient) > 4.5 (sufficient)	Sufficient	Atomic Absorption Spectroscopy Method	Lindsay, Norvell (1978)
Copper	0.32	mg/kg	≤ 0.2 (deficient) > 0.2 (sufficient)	Sufficient	Atomic Absorption Spectroscopy Method	Lindsay, Norvell (1978)
Manganese	8.84	mg/kg	≤ 1.0 (deficient) > 1.0 (sufficient)	Sufficient	Atomic Absorption Spectroscopy Method	Lindsay, Norvell (1978)

per (0.32 mg/kg) were detected at sufficient levels, further affirming the soil's overall suitability for medicinal plant cultivation with scope for targeted micronutrient amendments.

High Performance Liquid Chromatography (HPLC) analysis, performed using a binary gradient elution system at 227 nm, quantified the distribution of withanolides in root, stem, and leaf tissues of the Pratap variety of *W. somnifera*. Among the three plant parts analyzed, leaves demonstrated the highest total withanolide content at 10.285 µg/mg, followed by stems at 1.358 µg/mg and roots at 0.886 µg/mg. This gradient highlights a prominent accumulation of bioactive constituents in aerial tissues, particularly leaves, suggesting underutilized therapeutic potential and implications for more sustainable plant harvesting strategies.

The HPLC based quantification of six key withanolides across different plant tissues of *Withania somnifera* (Pratap) revealed distinct patterns of metabolite accumulation. Withanoside IV was consistently present in all parts, with the highest concentration in stems (0.412 µg/mg), followed by leaves (0.379 µg/mg) and roots (0.337 µg/mg). Withanoside V was exclusively detected in leaves (0.384 µg/mg), remaining below quantifiable levels in roots and stems. A particularly striking observation was the markedly higher concentration of withaferine A in leaves (5.226 µg/mg), compared to its trace presence in roots (<0.05 µg/mg) and moderate levels in stems (0.131 µg/mg). Withanolide A showed a contrasting pattern, being most abundant in roots (0.381 µg/mg), present in stems (0.172 µg/mg), but not detected in leaves. Withanone also exhibited significant tissue variation, with leaves accumulating the highest amount (4.297 µg/mg), followed by stems (0.642 µg/mg) and roots (0.168 µg/mg). Withanolide B remained undetectable (<0.05 µg/mg) across all examined tissues. These findings underscore the tissue specific biosynthesis and storage of pharmacologically important withanolides in *W. somnifera* and suggest that the aerial parts, particularly the leaves, may offer potent therapeutic potential beyond traditional root based applications.

The concentration of total withanoides in the roots, leaves and stems were found to be 0.886 µg/mg, 10.285 µg/mg and 1.358 µg/mg respectively. The concentration of withanoside IV was found maximum in stems however withanoside V was maximum in leaves. Withaferine A, withanone were maximum in leaves (Table 3). Figure 2, 3, 4 represents the overlay HPLC-DAD chromatogram of standard mix and Pratap ashwagandha roots, leaves and stems respectively at 227 nm.

Table 3. Phytochemicals present in the roots, leaves and stems of Pratap ashwagandha.

Withanolides	Pratap ashwagandha roots [µg/mg]	Pratap ashwagandha leaves [µg/mg]	Pratap ashwagandha stems [µg/mg]
Withanoside IV	0.337	0.379	0.412
Withanoside V	<0.05	0.384	<0.05
Withaferine A	<0.05	5.226	0.131
Withanolide A	0.381	<0.05	0.172
Withanone	0.168	4.297	0.642
Withanolide B	<0.05	<0.05	<0.05
Total withanolides [µg/mg]	0.886	10.285	1.358

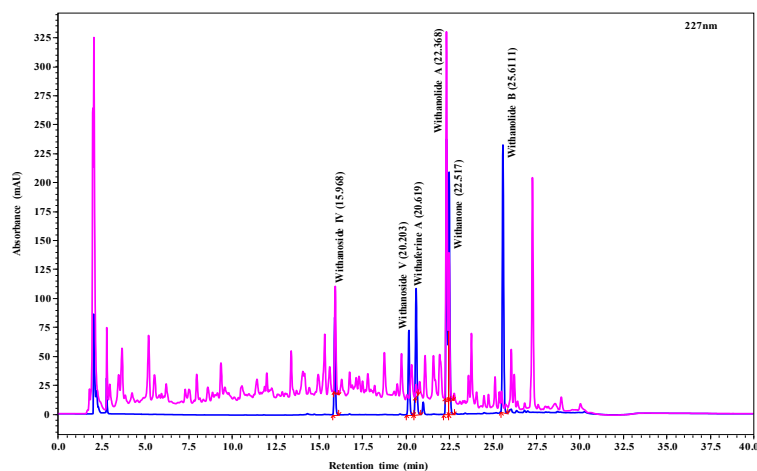


Figure 2. Overlay HPLC-DAD chromatogram of **standard mix** and **Pratap Ashwagandha roots** (PRF/CHI/0225/0087) at 227 nm.

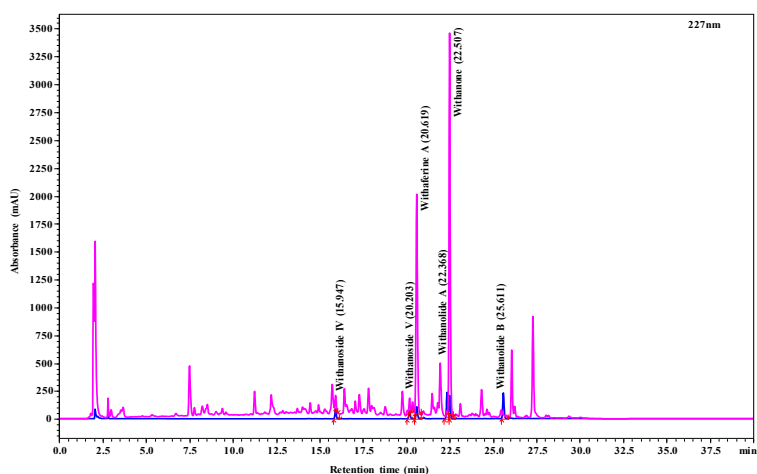


Figure 3. Overlay HPLC-DAD chromatogram of **standard mix** and **Pratap Ashwagandha leaves** (PRF/CHI/0225/0088) at 227 nm.

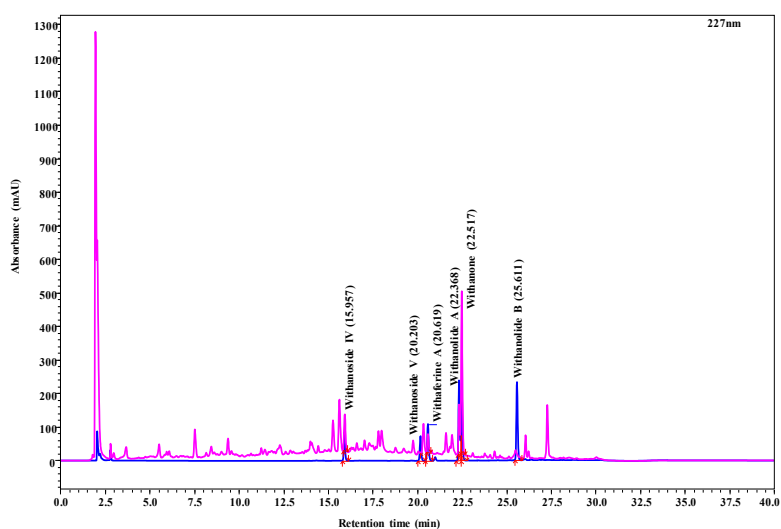


Figure 4. Overlay HPLC-DAD chromatogram of **standard mix** and **Pratap Ashwagandha stems** (PRF/CHI/0225/0089) at 227 nm.

DISCUSSION

The present study offers compelling evidence for the impact of soil conditions on withanolide biosynthesis in *W. somnifera* cultivated in Haridwar. The moderately alkaline soil, coupled with high organic carbon and adequate macronutrient levels, appears to foster robust phytochemical accumulation particularly in leaf tissues.

The soil nutrients are regarded as one of the factors affecting the growth and yield of ashwagandha plants. However, the information of the nutritional contents of soil correlating with the growth and yield parameters of different varieties of ashwagandha is very scanty. In a study the response of *W. somnifera* to prolonged soil moisture deficit during late growth stages was evaluated. The dry matter production, chlorophyll fluorescence, and physiological functions in *W. somnifera* were affected adversely by soil moisture deficit, although the dry root matter was found to be more in more stressed conditions as compared to control. The quality of root was found to be poor in well-watered control due to branching of roots as compared to others. The experimented result concluded that under moderate soil moisture stress there was increased root biomass, higher root dry weight with increased root quality (Shah et al., 2010).

Rajak (2013) reported that the application of 40:50:40 kg NPK per hectare has been found to significantly enhance both dry root and seed yield in the Poshita variety under the Northern Dry Zone of Karnataka. Similarly, supplementing the soil with 6 tonnes of farmyard manure (FYM) combined with 1.5 tonnes of vermicompost per hectare contributes to improved dry root yield, seed production, and an increase in alkaloid content within the roots. Notably, the integrated use of organic and inorganic inputs, comprising 6 t FYM, 1.5 t vermicompost, and 40:50:40 kg NPK per hectare, proves to be the most effective strategy for maximizing root yield and achieving superior quality roots in Poshita cultivated under these agro-climatic conditions.

The roots, stems and leaves of ashwagandha are widely used, however there is much demand of its roots. Ashwagandha, also known as Indian ginseng, has various uses for both its leaves and stems. Ashwagandha leaves are traditionally used in Ayurvedic medicine for treating skin diseases, bronchitis, and ulcers, and as a paste for tubercular glands. In some regions, warm leaves are used for eye issues and as a fermentation for sore eyes, boils, and swellings. The leaves are also reported to possess anthelmintic (anti-worm) and febrifuge (fever-reducing) properties. Ashwagandha stems are less commonly discussed in traditional uses, but they may be incorporated into the preparation of ashwagandha products alongside the roots and leaves (Paul et al., 2021).

The elevated total withanolide content in leaves (10.285 µg/mg), is similar the total of 10.601 µg with minor difference (Balkrishna et al., 2024) surpassing even roots, contrasts with traditional pharmacognosy that primarily emphasizes root usage. High concentrations of withaferine A and withanone in leaves corroborate earlier findings suggesting aerial parts may possess significant therapeutic value. The deficiency in sulphur and zinc, both pivotal in enzymatic and secondary metabolic pathways, suggests targeted supplementation could further enhance phytoconstituent yields. Prior research (Jat et al., 2021; Basak et al., 2020) aligns with these findings, advocating integrated nutrient management to optimize both yield and bioactive compound content.

Interestingly, the variation in withanolide distribution across plant tissues such as the prominence of withanoside IV in stems and withanoside V in leaves, highlights potential biochemical compartmentalization and differential gene expression in metabolite biosynthesis pathways. Moreover, references to lunar influence on growth traits (Tavhare et al., 2015) and the responsiveness of ashwagandha to organic amendments reinforce

the significance of spatiotemporal factors and nutrient bioavailability in shaping plant secondary metabolism. In light of these findings, leaves emerge as underutilized reservoirs of bioactivity, meriting further pharmacological and agronomic exploration. This shift could not only diversify therapeutic applications but also enhance the sustainability of ashwagandha harvesting by reducing root dependence.

FUTURE SCOPE

Building upon the findings of this study, future research can focus on soil metabolite modeling through the application of advanced multivariate analysis and chemometric tools to predict how specific edaphic parameters influence withanolide synthesis, thereby supporting precision agriculture in medicinal plant cultivation. Temporal and phenological investigations across diverse seasons and developmental stages will be crucial to understanding how withanolide profiles shift in response to abiotic and biotic stressors, such as drought or lunar cycles. Given the unexpectedly high concentrations of key withanolides in aerial parts, particularly leaves, there is significant scope for pharmacological and formulation research exploring their utility in standardized extracts, potentially reducing the ecological footprint of root harvesting. Additionally, on farm trials integrating organic inputs like vermicompost and FYM alongside balanced inorganic fertilizers across agroclimatic gradients could help develop optimized, sustainable nutrient management frameworks. Expanding this research to include other highyielding cultivars such as Poshita, Chetak, and Jawahar-20 under comparable soil conditions may also validate the phytochemical supremacy of the Pratap variety or reveal new candidates for regional cultivation.

CONCLUSION

The present study underscores the strong interplay between soil health parameters and phytochemical accumulation in *Withania somnifera* (Pratap variety) cultivated in the fertile alluvial plains of Haridwar. The moderately alkaline pH and high organic carbon, and low nitrogen, phosphorus and potassium levels provided a conducive environment for robust biomass development and metabolite biosynthesis. Notably, the aerial parts especially the leaves, exhibited a remarkably higher concentration of key withanolides such as withaferine A and withanone compared to the traditionally valued roots. This suggests a broader pharmacological potential within the plant beyond root-based formulations, which may open new avenues for therapeutic applications and sustainable harvesting practices.

Deficiencies in sulphur and zinc highlight the need for customized soil nutrient management strategies to further optimize plant health and bioactive yield. The study also contributes a reference database for the Pratap variety, supporting region specific cultivation and quality assurance frameworks for medicinal plant industries.

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REFERENCES

- APHA, 2005. Standard Methods for the Examination of Water and Wastewater, Washington DC: American Public Health Association.
- Arumugam V., Venugopal V., Balakrishnan A., Bhandari R., Boopalan D., et al., 2024.** Effects of ashwagandha (*Withania Somnifera*) on stress and anxiety: A systematic review and meta-analysis. *EXPLORE*, 103062.
- Balkrishna A., Kumari P., Singh P., Pathak N., Verma S., et al., 2024.** Withanolides-enriched leaf extract of *Withania somnifera* exert anti-obesity effects by inducing brown adipocyte-like phenotype via tuning MAP-kinase signaling axis. *International Journal of Biological Macromolecules*, 2024 Dec;282(Pt 2):136883, doi: 10.1016/j.ijbiomac.2024.136883.
- Basak B.B., Saha A., Gajbhiye N.A., Manivel P., 2020.** Potential of organic nutrient sources for improving yield and bioactive principle of ashwagandha (*Withania somnifera*) through enhanced soil fertility and biological functions. *Communications in Soil Science and Plant Analysis*, 51(6): 779-793.
- Black C.A., 1965.** Methods of soil analysis. Part 1. American Society of Agronomy, Inc. Publ. Madison Wisconsin USA.
- Dhruvi S., Honey K., Rahul P., 2025.** A systemic review of treatment of rheumatoid arthritis using herbal plants. *International Journal of Scientific Research and Technology*, 2(3): 345-374.
- Chesnin L., Yien C.H., 1950.** Turbidimetric determination of available sulphates. *Soil Science Society of America Proceedings*, 15: 149-151.
- Gaurav H., Yadav D., Maurya A., Yadav H., Yadav R., et al., 2023.** Biodiversity, biochemical profiling, and pharmaco-commercial applications of *Withania somnifera*: A review. *Molecules*, 28(3), 1208.
- Gupta G.L., Rana A.C., 2007.** *Withania somnifera* (Ashwagandha): a review. *Pharmacognosy Reviews*, 1(1): 129-136.
- ICAR - Indian Institute of Soil Science, 2011. Soil test based fertilizer recommendations for targeted yields of crops. Bhopal: ICAR.
- Jat R.S., Basak B.B., Gajbhiye N.A., 2021.** Organic manures and biostimulants fostered soil health and increased the harvest quality of the medicinal herb ashwagandha. *Agronomy Journal*, 113(1): 504-514.
- Jayaprakasam B., Zhang Y., Seeram N.P., Nair M.G., 2003.** Growth inhibition of human tumor cell lines by withanolides from *Withania somnifera* leaves. *Life sciences*, 74(1): 125-132.
- Joshi V.K., Joshi A., 2021.** Rational use of Ashwagandha in Ayurveda (Traditional Indian Medicine) for health and healing. *Journal of Ethnopharmacology*, 276, 114101.
- Khabiya, R., Choudhary, G.P., Jnanesha, A.C., Kumar, A., & Lal, R.K., 2024.** An insight into the potential varieties of Ashwagandha (Indian ginseng) for better therapeutic efficacy. *Ecological Frontiers*, 44(3), 444-450.
- Lal R.K., Chandra R., Chauhan H.S., Misra H.O., Sangwan R.S., et al., 2012.** Registration of a new high yielding variety "CIMAP-Pratap" of Ashwagandha [*Withania somnifera* (L.) Dunal] suitable for cultivation in drought prone areas of India. *J. Med. Aromat. Plant Sci.*, 34(3&4): 178-182.
- Lindsay W.L., Norvell W.A., 1978.** Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, 42(3): 421-428.
- Majeed M., Nagabhushanam K., Mundkur L., 2023.** A standardized Ashwagandha root extract alleviates stress, anxiety, and improves quality of life in healthy adults by modulating stress hormones: Results from a randomized, double-blind, placebo-controlled study. *Medicine*, 102(41), e35521.
- Merwin H.D., Peech M., 1950.** Exchangeability of soil potassium in relation to the uptake of potassium by plants. *Soil Science Society of America Proceedings*, 15: 125-128.

- Mukherjee P.K., Banerjee S., Biswas S., Das B., Kar A., Katiyar C.K., 2021.** *Withania somnifera* (L.) Dunal - Modern perspectives of an ancient Rasayana from Ayurveda. *Journal of Ethnopharmacology*, 264, 113157.
- Olsen S.R., Cole C.V., Watanabe F.S., Dean L.A., 1954.** Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular No. 939.
- Pandey A.K., 2025.** Good agricultural and collection practices of important medicinal plants. pp. 549-569. In: *Textbook of Forest Science*; Singapore: Springer Nature Singapore.
- Paul S., Chakraborty S., Anand U., Dey S., Nandy S., et al., 2021.** *Withania somnifera* (L.) Dunal (Ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedical and toxicological aspects. *Biomedicine & Pharmacotherapy*, 143, 112175.
- Pirasteh S., Samad A., Ahmad R., Thakural L.N., Khan, H. H., et al., 2025.** Geospatial and AHP based identification of potential zones for groundwater recharge in Haridwar District of India. *Frontiers in Environmental Science*, 13, 1421918.
- Rajak R., 2013.** Integrated nutrient management in ashwagandha (*Withania somnifera* Dunal). Doctoral dissertation, KRC College of Horticulture College of Arabhavi, University of horticultural sciences, Bagalkot.
- Sachin S., Dogra P., Thanoch M., Singh N.P., 2017.** Ashawgandha (*Withania somnifera*) A Rasayna in Ayurveda and benefits of its use than other performance enhancing substances in sports medicine–A review article. *International Journal of Physical Education and Sports*, 2(12): 16–24.
- Singh M., Bhutani S., Dinkar N., Suyal D.C., Perveen K., et al., 2024.** Assessment of pharmacological activities of specialized metabolites of *Withania somnifera* (L.). *South African Journal of Botany*, 166: 259–271.
- Shah S., Saravanan R., Gajbhiye N.A., 2010.** Phytochemical and physiological changes in Ashwagandha (*Withania somnifera* Dunal) under soil moisture stress. *Brazilian Journal of Plant Physiology*, 22(4), 255-261.
- Soil Health Card Scheme Guidelines, 2015. Ministry of Agriculture & Farmers Welfare, Government of India, New Delhi.
- Sprenkel M., Laskowski R., Jost Z., 2025.** *Withania somnifera* (Ashwagandha) supplementation: a review of its mechanisms, health benefits, and role in sports performance. *Nutrition & Metabolism*, 22(1), 9.
- Subbiah B.V., Asija G.L., 1965.** A rapid procedure for the estimation of available nitrogen in soils. *Current Science*, 25: 259–260.
- Sultana R., 2023.** The traditional immune boosting recipes. pp. 28-48. In: *Alternative Remedies and Natural Products for Cancer Therapy: An Integrative Approach*; Bentham Science Publishers.
- Tandon N., Yadav S.S., 2020.** Safety and clinical effectiveness of *Withania Somnifera* (Linn.) Dunal root in human ailments. *Journal of Ethnopharmacology*, 255, 112768.
- Tavhare S.D., Nishteswar K., Shukla V.J., 2015.** Influence of lunar cycles on growth of Ashwagandha (*Withania somnifera* [L.] Dunal). *AYU (An International Quarterly Journal of Research in Ayurveda)*, 36(3): 258–264.
- Umadevi M., Rajeswari R., Rahale C.S., Selvavenkadesh S., Pushpa R., Kumar K.S., Bhowmik D., 2012.** Traditional And Medicinal Uses of *Withania Somnifera*. *The Pharma Innovation*, 1(9, Part A), 102–110.
- Verma S.K., Kumar A., 2011.** Therapeutic uses of *Withania somnifera* (Ashwagandha) with a note on withanolides and its pharmacological actions. *Asian Journal of Pharmaceutical and Clinical Research*, 4(1): 1–4.
- Walkley A., Black I.A., 1934.** An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, 37(1): 29–38.