

Quality Evaluation of Ashwagandha (*Withania somnifera*) Capsules: A Comprehensive Assessment of Physicochemical, Phytochemical, and Safety Parameters

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Ashwagandha (*Withania somnifera*) is a revered adaptogenic herb in Ayurvedic medicine, valued for its neuroprotective, anti-inflammatory, and stress-modulating properties. This study evaluates the quality of hydroalcoholic root extract-based capsules through comprehensive physicochemical, phytochemical, and safety assessments to ensure compliance with AYUSH standards. Methods included organoleptic evaluation, physicochemical testing (e.g., weight variation, disintegration), qualitative phytochemical screening, and quantitative analyses for pesticides (GC-MS), aflatoxins (HPLC), heavy metals (ICP-MS), microbial load, Withaferin A (HPTLC), and total withanolides (gravimetric). Results confirmed stable organoleptic & physicochemical parameters (700 mg $\pm$ 7.5% weight, <15 min disintegration time, <7% moisture content); presence of bioactive compounds like glycosides, flavonoids, and saponins; undetectable pesticide and aflatoxin residues; low heavy metals, absent pathogens in microbial analysis, 0.09% Withaferin A; and 0.81% total withanolides. These findings affirm the capsule's safety, efficacy, and standardization, supporting their therapeutic potential in managing neurodegenerative disorders and stress-related conditions.

Keywords: Ashwagandha, Physicochemical, neuroprotective, withanolides

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Introduction

Ashwagandha (*Withania somnifera*), widely revered in Ayurveda as a *Rasayana*, is known for its adaptogenic, neuroprotective, & immunomodulatory properties.[1] Globally recognized for its remarkable therapeutic potential, *Ashwagandha* has gained immense popularity worldwide, driving a surging market demand for its products in wellness and pharmaceutical sectors. Its broad spectrum of benefits includes enhancing cognitive health, exerting potent antioxidant effects, and bolstering immunity, making it a cornerstone in holistic health management. In regions like North America and Europe, *Ashwagandha* has become a staple in dietary supplements, with sales driven by rising awareness of its role in mental wellness and performance optimization. Extensive clinical trials and consumer adoption trends further affirm its cross-cultural validation, positioning it as a trusted adaptogen in contemporary integrative medicine. Owing to its therapeutic relevance in stress-induced disorders, neurodegeneration, fatigue, and immune dysfunction,[2] its standardized extract form is increasingly incorporated into modern phyto-pharmaceutical formulations. Quality validation of herbal supplements is crucial to ensure reproducibility, safety, and bio-efficacy, particularly given the variability arising from cultivation, extraction methodology, and processing conditions.

The major constituents of *Ashwagandha* are withanolides, a class of steroidal lactones central to its pharmacological efficacy. Among withanolides, especially Withaferin A, serve as key bioactive markers attributed to the neuroprotective and anti-inflammatory actions of *Ashwagandha*. [3] Present study aimed to develop and evaluate standardized *Ashwagandha* capsules using hydro-alcoholic extract and assess their organoleptic, physicochemical, phytochemical, safety, & bioactive marker profiles. These evaluations were designed to ensure regulatory compliance, product integrity, and therapeutic consistency, thereby supporting the formulation's scientific validation and clinical applicability.

Materials and Methods

1. Sourcing of Hydro alcoholic Extract

The hydro alcoholic extract of *Ashwagandha* roots was procured from a certified supplier compliant with ISO, GMP & FDA guidelines.

The extract was authenticated for botanical identity and standardized to contain withanolides, verified through Analytical methods.

2. Preparation of Ashwagandha Capsule

The hydro alcoholic extract was received as a dry, standardized powder. The process involved blending the extract with minimal excipients and sieved through 100 mesh size. Then it was encapsulated in vegan capsules (size 0) using an automated encapsulation machine, with each capsule containing 600 mg of extract. The process adhered to GMP protocols to ensure consistency and quality. Ingredient list is given in the Table 1:

Table 1: Composition of Ashwagandha Capsule

SN	Ingredient	Botanical Name	Part Used
1.	Ashwagandha extract	Withania somnifera	Root

3. Organoleptic Analysis

Colour, odour, and taste were assessed to confirm sensory characteristics. These qualitative evaluations provided initial insights into the product's consistency.[4]

4. Physicochemical Analysis

Physicochemical parameters, including average capsule weight, disintegration time, and moisture content, were measured to assess stability and bioavailability.[5] Tests followed pharmacopoeia standards, using calibrated equipment to ensure precision and reproducibility of results.

5. Phytochemical Screening

Qualitative phytochemical tests were performed on water and ethanolic extracts to identify key bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and glycosides. Tests such as Wagner's reagent test, NaOH, FeCl₃, foam, Salkowski test and were conducted following standard protocols to confirm the presence of constituents responsible for *Ashwagandha*'s therapeutic effects.[6]

6. Pesticide Residue Analysis

Pesticide residues were analyzed using gas chromatography-mass spectrometry (GC-MS) to detect organophosphates, organochlorines, and pyrethroids.[7] Samples were extracted with organic solvents and quantified against certified standards to ensure compliance with AYUSH safety limits for herbal products.

7. Aflatoxin Analysis

Aflatoxin levels (B1, B2, G1, G2, and total) were quantified using high-performance liquid chromatography (HPLC) with fluorescence detection and immunoaffinity column cleanup. This analysis ensured capsules were free from mycotoxin contamination, aligning with AYUSH safety standards.[4]

8. Heavy Metal Testing

Heavy metals (lead, cadmium, arsenic, mercury) were quantified ICP-MS after nitric acid digestion.[8] Concentrations were compared against AYUSH permissible limits to confirm the absence of toxic metals, ensuring consumer safety.

9. Microbial analysis

The microbial limit test was carried out following the standard protocol prescribed in the Ayurvedic Pharmacopoeia of India. Samples were collected under aseptic conditions. Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) media were prepared by dissolving the respective powders in double-distilled water, adjusting the pH with 1N NaOH and 1N HCl, followed by heating, autoclaving, and pouring into sterile Petri dishes. The media were allowed to solidify aseptically. For microbial analysis, 1 gram of each sample was serially diluted with 9 ml of sterile diluent. From suitable dilutions, 0.1 ml aliquots were inoculated on the media using the spread plate method. NA plates were incubated at 37°C for 24–48 hours, while SDA plates were incubated at room temperature for 3–5 days. The resulting microbial colonies were examined using microscopic and biochemical techniques. Colony counts were then compared with the microbial limit standards specified in the Ayurvedic Pharmacopoeia of India.[5]

10. Withaferin A Analysis

Withaferin A in *Withania somnifera* root extract was quantified by HPTLC densitometry using an HPTLC system, silica gel 60 F₂₅₄ plates (0.2 mm, E. Merck), Withaferin A standard (≥98% purity), and AR-grade solvents (methanol, ethyl acetate, toluene, acetic acid) with anisaldehyde-sulphuric acid reagent. Standard solution (1000 ppm) was prepared by dissolving 10 mg Withaferin A in 10 mL methanol. Test solution was made by refluxing 5 g powdered root with methanol (25 mL × 4) for 10–15 min, filtering, concentrating, dissolving 250 mg residue in methanol, and diluting to 25 mL.

Chromatographic conditions: mobile phase, ethyl acetate:toluene:acetic acid (9:1:0.6 v/v/v); chamber saturation, 20 min; application, 2 µL standard, 5 µL sample; development, 8 cm; air-dried; derivatized with anisaldehyde-sulphuric acid, heated at 105°C for 5 min; scanned at 530 nm (tungsten lamp, absorbance-reflectance mode).

Quantification was based on peak area comparison with a standard calibration curve.[9]

11. Total withanolides Analysis

Total withanolide content in *Withania somnifera* root was quantified gravimetrically by extracting 10 g powdered root with methanol (100 mL, then 50 mL × 2) using a Soxhlet extractor for 3–4 h, combining filtrates, and evaporating to dryness.

The residue was dissolved in 50 mL water, partitioned with chloroform (3 × 30 mL), washed with 10 mL water, dried over anhydrous sodium sulfate, filtered, and evaporated to a semi-solid.

Withanolides were precipitated with petroleum ether, left for 12 h, filtered through pre-weighed paper, washed with cold petroleum ether, dried at 105°C for 1 h, cooled, and weighed.[10]

Results

1. Organoleptic Tests

The sensory evaluation confirmed consistency with expected *Ashwagandha* properties. These findings are presented in Table 2.

Table 2: Organoleptic analysis of Ashwagandha Capsule

SN	Parameters	Result
1.	Colour	Brown
2.	Odour	Characteristic
3.	Taste	Astringent

2. Physicochemical Analysis

The capsules met pharmacopoeia standards, ensuring stability and bioavailability. Detailed results are shown in Table 3.

Table 3: Physicochemical Analysis of Ashwagandha Capsule

SN	Parameters	Result
1.	Average Weight of Capsule	700 mg ± 7.5%
2.	Disintegration Time	NMT 15 min
3.	Moisture	NMT 7%

3. Phytochemical Screening

Phytochemical tests confirmed the presence of key bioactive compounds, with variations between water and ethanolic extracts. These findings are summarized in Table 4.

Table 4: Phytochemical Analysis of Ashwagandha Capsule

SN	Organic Phytochemical constituents	Name of the test conducted	Water Extract	Alcoholic Extract
1.	Carbohydrate	Molisch's test	+	+
2.	Sugar	Benedict's test	+	+
3.	Reducing Sugar	Fehling's test	+	—
4.	Ketose	Seliwanoff's test	-	—
5.	Protein	Biuret test	+	+
6.	Starch	K I test	+	—
7.	Glycoside	Salkowski test	+	+
8.	Steroid		-	—
9.	Terpenoid		-	—
10.	Flavonoid	Alkaline reagent	+	—
11.	Phenol	Phenol reagent test	+	+
12.	Saponin	Foam test	+	+
13.	Alkaloid	Wagner's reagent	+	—
14.	Tannin	Ferric chloride test	—	—
15.	Coumarin	NaOH test	—	+
16.	Aminoacids	Ninhydrin test	—	—
17.	Quinone	H2SO4 test	—	—

4. Pesticide Residue Analysis

The analytical results demonstrated undetectable levels of pesticides in sample, thereby confirming compliance with safety standards established by AYUSH. The complete pesticide residue profile is provided in Table 5.

Table 5: Pesticide Residue Analysis of Ashwagandha Capsule

SN	Parameters	Unit	Result	Specification
1.	Fenitrothion	mg/kg	Not Detected	0.5
2.	Fenvalarate	mg/kg	Not Detected	1.5
3.	Methidathion	mg/kg	Not Detected	0.2
4.	Phosalone	mg/kg	Not Detected	0.1
5.	Azinphos methyl	mg/kg	Not Detected	1.0
6.	Chlorpyrifos	mg/kg	Not Detected	0.2
7.	Chlorpyrifos methyl	mg/kg	Not Detected	0.1
8.	Deltamethrin	mg/kg	Not Detected	0.5
9.	Dichlorvos	mg/kg	Not Detected	1.0
10.	Dithiocarbamates (as CS ₂)	mg/kg	Not Detected	2.0
11.	Endrin	mg/kg	Not Detected	0.05
12.	Ethion	mg/kg	Not Detected	2.0
13.	Fonofos	mg/kg	Not Detected	0.05
14.	Malathion	mg/kg	Not Detected	1.0
15.	Parathion	mg/kg	Not Detected	0.5
16.	Parathion methyl	mg/kg	Not Detected	0.2
17.	Quintozene (sum of quintozone, pentachloroaniline and methyl pentachlorophenyl sulphide)	mg/kg	Not Detected	1.0
18.	Pyrethrins (sum of)	mg/kg	Not Detected	3.0
19.	Bromopropylate	mg/kg	Not Detected	3.0
20.	Chlordane (Sum of G S and trans)	mg/kg	Not Detected	0.05
21.	Diazinon	mg/kg	Not Detected	0.5
22.	Primiphos methyl	mg/kg	Not Detected	4.0
23.	Chlorfenvinphos	mg/kg	Not Detected	0.5
24.	Alachlor	mg/kg	Not Detected	0.02
25.	Aldrin and Dieldrin (Sum of)	mg/kg	Not Detected	0.05
26.	Cypermethrin (and ISO[ROE9])	mg/kg	Not Detected	1.0
27.	DDT (Sum of p,p- 'DDT, o,p- 'DDT, p,p-'DDE)	mg/kg	Not Detected	1.0
28.	Endosulfan (Sum of Isomers and Endosulfan Sulphate)	mg/kg	Not Detected	3.0
29.	Heptachlor (Sum of Heptachlor and Heptachlorepoxide)	mg/kg	Not Detected	0.05
30.	Hexachloro Benzene	mg/kg	Not Detected	0.1
31.	Hexachlorocyclohexane Isomers (Other Than gamma)	mg/kg	Not Detected	0.3
32.	Lindane (gamma- Hexachlorocyclohexane)	mg/kg	Not Detected	0.6
33.	Permethrin	mg/kg	Not Detected	1.0
34.	Piperonyl butoxide	mg/kg	Not Detected	3.0

5. Aflatoxin Analysis

Aflatoxin concentrations were below the limits of detection, ensuring the absence of mycotoxin contamination in accordance with AYUSH guidelines. Results are detailed in Table 6.

Table 6: Aflatoxin Analysis of Ashwagandha Capsule

SN	Parameters	Unit	Result
1.	Aflatoxin B1	ppb	BDL
2.	Aflatoxin B2	ppb	BDL
3.	Aflatoxin G1	ppb	BDL
4.	Aflatoxin G2	ppb	BDL
5.	Total Aflatoxin	ppb	BDL

6. Heavy Metal Testing

The heavy metal analysis indicated concentrations within the permissible limits established by AYUSH, thereby confirming the safety of the sample. These results are presented in Table 7.

Table 7: Heavy Metal Analysis of Ashwagandha Capsule

SN	Metal	Concentration (mg/kg)	Specification
1.	Lead (Pb)	0.25	NMT 10.0
2.	Cadmium (Cd)	BDL	NMT 0.3
3.	Arsenic (As)	0.17	NMT 3.0
4.	Mercury (Hg)	BDL	NMT 1.0

7. Microbial Analysis

Microbial tests confirmed the absence of pathogens and counts within acceptable limits. The microbial safety profile is shown in Table 8.

Table 8: Microbial Analysis of Ashwagandha Capsule

SN	Parameters Tested	Specification	Unit	Result
/Range				
1.	Total Yeast and Mold Count	10 ³	CFU/ml	Absent
2.	Total Plate Count for Bacteria	10 ⁵	CFU/ml	Absent
3.	E.coli	Absent	Present or Absent/ml	Absent
4.	Pseudomonas aeruginosa	Absent	Present or Absent/ml	Absent
5.	Salmonella spp.	Absent	Present or Absent/ml	Absent
6.	Staphylococcus aureus	Absent	Present or Absent/ml	Absent

8. Withaferin A Analysis

HPTLC analysis confirmed the presence of Withaferin A, a key bioactive marker compound in *Ashwagandha*. The results are shown in the Table 9, Figure 1 & Figure 2.

Table 9: HPTLC Analysis of Ashwagandha Capsule

SN	Parameters	Result
1.	Withaferin A	0.09 %

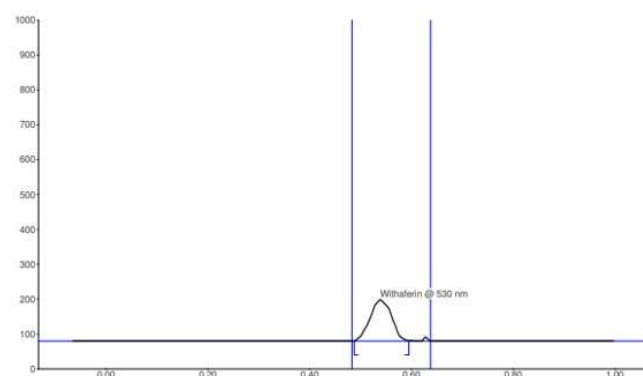


Figure 1: Chromatogram of Ashwagandha Capsule



Figure 2: Chromatogram of Ashwagandha Capsule at 366 nm

9. Total Withanolides Analysis

Table 10. gives Gravimetric analysis results of total withanolides, validating the standardization of the extract.

Table 10: Gravimetric Analysis of Ashwagandha Capsule

SN	Parameters	Result
1.	Withanolides	0.81%

Discussion

The results demonstrate that the Ashwagandha capsules meet AYUSH quality and safety standards across multiple parameters. Organoleptic properties showed a brown colour, characteristic odour, and astringent taste, aligning with expected sensory profiles for *Ashwagandha*. Physicochemical evaluations revealed an average capsule weight of 700 mg ($\pm 7.5\%$) (100 mg vegan capsule shell weight), disintegration time not exceeding 15 minutes, and moisture content below 7%, which support product stability and bioavailability. The phytochemical profile confirmed the presence of carbohydrates, sugars, reducing sugars, proteins, starch, glycosides, flavonoids, phenols, saponins, alkaloids, and coumarins in varying extracts, underscoring the therapeutic potential. Carbohydrates, particularly in the form of natural bioactive polysaccharides, hold significant promise for preventing and managing neurodegenerative diseases due to their multifaceted advantages, such as broad-spectrum targeting & minimal toxicity.[11] These polysaccharides can enhance cognitive performance by counteracting the harmful effects of aberrant amyloid-beta and tau proteins—hallmarks of Alzheimer's disease. Due to their potential in managing Alzheimer's disease, these polysaccharides can also benefit other memory disorders by reducing oxidative damage and restoring neural adaptability.[12] Proteins are reservoirs of antioxidant activity, as each large protein structure can liberate multiple bioactive peptides and free amino acids that collectively deliver a markedly elevated capacity to neutralize free radicals and stabilize cellular environments.[13] Glycosides, valued for their robust antioxidant and anti-inflammatory properties, mitigate oxidative stress and reduce inflammatory responses in the brain, thereby offering multifaceted neuroprotection.[14] In cerebral infarction specific glycosides showed marked efficacy by inhibiting neuronal apoptosis and suppressing key pro-inflammatory cytokines, including IL-6 and TNF- α , which collectively enhance neuronal viability.[15] Flavonoids exhibit substantial neuroprotective potential, primarily attributable to their pronounced anti-apoptotic, and antioxidant attributes, which collectively mitigate oxidative stress, attenuate neuroinflammatory pathways, and sustain neuronal integrity.[16]

The antioxidant properties of phenolic compounds hold particular promise for neurodegenerative diseases, whose pathophysiological mechanisms are predominantly driven by oxidative stress within the brain. Furthermore, these compounds confer additional benefits, such as their capacity to cross the blood-brain barrier (BBB), thereby enabling targeted neuroprotection and enhanced therapeutic efficacy.[17] Saponins enhance the proliferation and maturation of immune tissues *via* diverse molecular signalling cascades, modulate the function of various immune cell populations, and elevate the release of pro-immune cytokines alongside antigen-targeted immunoglobulins, collectively amplifying overall immunological efficacy.[18] Alkaloids, as key phytochemicals, unleash powerful antioxidant responses that position them as viable candidates for combating neurodegenerative disorders by directly addressing oxidative stress pathways at their core.[19] Natural coumarins distinguish themselves through a vast pharmacological spectrum, prominently featuring anti-Alzheimer's and neuroprotective effects.[20] Along with that Coumarins exhibits antioxidant activity.[21]

Pesticide residue analysis detected no residues for compounds like fenitrothion, chlorpyrifos, DDT, and others, all below specified limits such as 0.5 mg/kg for fenitrothion and 1.0 mg/kg for DDT. Aflatoxins B1, B2, G1, G2, and total were below detection limits, indicating no mycotoxin risk. Heavy metals were low, with Lead at 0.25 mg/kg (NMT 10.0), Arsenic at 0.17 mg/kg (NMT 3.0), and Cadmium/Mercury below detection levels (NMT 0.3 and 1.0, respectively). Microbial analysis showed absence of *E. coli*, *Staphylococcus sp.*, *Pseudomonas sp.*, and *Salmonella sp.*, bacterial count and yeast/mold count confirming safety.

Withanolides exert inhibitory effects on multiple facets of inflammatory pathways, thereby mitigating neuroinflammation. Beyond this modulatory role, various withanolides exhibited significant promotion of neurite outgrowth and regeneration, supporting neuronal repair and plasticity.[3] Notably, withaferin A demonstrates dual antioxidant and anti-inflammatory attributes, enhancing cellular resilience against oxidative stress and inflammatory cascades. Furthermore, withaferin A engages directly with glucocorticoid receptors in the brain, influencing cortisol dynamics and attenuating stress responses.

Complementary mechanisms may involve the GABAergic modulatory effects of *Ashwagandha*, coupled with its enhancement of sleep quality, both of which contribute to overall stress reduction.[22]

Conclusion

This comprehensive evaluation of hydro-alcoholic root extract-based *Ashwagandha* (*Withania somnifera*) capsules underscores their adherence to stringent AYUSH and pharmacopoeial standards, affirming their quality, safety, and standardization for therapeutic applications. The organoleptic features conform to authentic *Ashwagandha* standards, while all physicochemical attributes fall within acceptable ranges, affirming product stability, uniformity, and bioavailability. The qualitative phytochemical screening revealed a rich range of bioactive constituents, including carbohydrates, proteins, starch, glycosides, flavonoids, phenols, saponins, alkaloids, and coumarins, predominantly in ethanolic extracts, which collectively contribute to the herb's adaptogenic, antioxidant, and neuroprotective efficacy. Notably, these compounds target oxidative stress, neuroinflammation, Immune response positioning *Ashwagandha* as a promising adjunct in managing neurodegenerative disorders like Alzheimer's disease and stress-related conditions.

Quantitative analyses further validate the capsules' potency and safety: Withaferin A content at 0.09% and total withanolides at 0.81% confirm standardization of key markers, while the absence of pesticide residues, aflatoxins, and pathogens, coupled with low heavy metal levels mitigates contamination risks and supports consumer safety. Microbial counts were absent reinforcing microbiological integrity.

In summary, these findings establish the evaluated capsules as a reliable, efficacious formulation, bridging traditional Ayurvedic wisdom with modern quality assurance. By mitigating variability inherent in herbal products, this standardization enhances therapeutic confidence in *Ashwagandha*'s role against chronic stress, cognitive decline, oxidative stress & general fatigue. Future research could explore clinical efficacy in human cohorts, long-term stability under varied storage conditions, and synergistic formulations with other adaptogens to broaden neuroprotective applications.

Ultimately, this study advocates for widespread adoption of such rigorously vetted herbal supplements in integrative healthcare paradigms.

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