American Herbal Pharmacopoeia™
and Therapeutic Compendium

Ashwagandha Root

Withania somnifera

Analytical, Quality Control,
and Therapeutic Monograph

April 2000

A H P

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The reporting on the use of proprietary products reflects studies conducted with these preparations and is not meant to be an endorsement of these products.

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Cover Photograph
Ashwagandha *Withania somnifera*

Photograph courtesy of Martin Wall Photography, Pleasant Garden, NC

NOMENCLATURE

Botanical Nomenclature

Withania somnifera (L.) Dunal.

Botanical Family

Solanaceae

Definition

Ashwagandha consists of the dried roots of *Withania somnifera* (L.) Dunal. conforming to the methods of identification provided.

Common Names

United States:	Ashwagandha (*Herbs of Commerce*).
Bengali:	As'vagandha, ashvagandha.
Gujarati:	Asgandha, asan, asoda, ghoda asoda.
Hindi:	Asgandh, A sh a ga n dha.
Sanskrit:	Ashwagandha (syn.: ashvagandha, asvagandha, hayagandha, vajigandha).
Unani:	Asgand.

Figure 1 Ashwagandha *Withania somnifera* (L.) Dunal.

Art courtesy of Sabinsa Corporation, Piscataway, NJ

HISTORY

The use of ashwagandha in ayurvedic medicine dates back 3000-4000 years to the teachings of the famed ayurvedic scholar Punarvasu Atreya. Subsequently, it was included in the writings of Charaka, Sushruta, and many other ayurvedic scholars throughout the centuries (Atal and Schwarting 1961). Ashwagandha, derived from the Sanskrit *ashva* meaning "horse" and *gandha* meaning "smell", describes the strong aroma of the root which is considered to be reminiscent of a horse's skin, sweat, or urine, depending upon to which authority one refers. The species name *somnifera* refers to the Latin *somnus* meaning "to sleep", apparently alluding to the use of ashwagandha as a nervine and sedative.

Historically, ashwagandha was widely used throughout India as a tonic, especially for emaciation in people of all ages, including infants, and for enhancing reproductive function in both men and women. In one text, it was stated that ashwagandha taken for a fortnight with milk, ghee, oil, or warm water promotes development in an emaciated body "as rains do for younger crops". It is classed among the "rasayanas" (rejuvenative tonics), the most highly regarded of all medicinal substances in ayurveda. The ayurvedic scholar Charaka (100 BC) wrote of rasayanas, "One obtains longevity, regains youth, gets a sharp memory and intellect and freedom from diseases, gets a lustrous complexion, and strength of a horse". Charaka described various uses for ashwagandha, including its effectiveness for treating hiccups and female disorders.

Ashwagandha historically was used for inflammation, to reduce abdominal swelling, as a mild purgative, and for the treatment of swollen glands. In the Unani tradition, the root was considered as a tonic, aphrodisiac, and emmenagogue; was used to treat asthma, inflammations, leukoderma, bronchitis, lumbago, and arthritis; and also promoted conception. In the Punjab region, it was similarly used as an aphrodisiac and for lower back pain.

Ashwagandha has been used in traditional herbal healing practices of Africa. The Southern Sotho prepared a decoction of the roots for colds and chills. The Transvaal Sotho used the root to tone the uterus in women who habitually miscarry, a use commonly employed in India as well. It has also been used to facilitate expulsion of the afterbirth. An infusion of the root bark has been used for asthma, a use also common to traditional herbal practices in India (Watt and Breyer-Brandwijk 1962). In the United States herbal market, ashwagandha is currently most often used for its tonifying properties while ayurvedic practitioners continue to use it for its myriad of reported activities. In India it is highly regarded as a tonic and is used in formulas for a wide range of imbalances.

Ashwagandha is included in the *Ayurvedic Pharmacopoeia of India* in which it is cited as a strengthening tonic, aphrodisiac, and for the treatment for arthritis. It is the primary component of numerous traditional ayurvedic tonic and anti-aging compounds (Ayurvedic Pharmacopoeia of India 1989; Tripathi and others 1996).

IDENTIFICATION

Botanical Identification

Withania somnifera (L.) Dunal. Woody herb or shrub, 3-15 dm tall, growing from a long, tuberous taproot; stellate-tomentose. Leaf: Simple, exstipulate, petiole 6-20 mm long; blade elliptic to ovate-lanceolate, apex acute or rounded, base acute to long-decurrent, on vegetative shoots 8-10 cm long and alternate, on reproductive shoots 3-8 cm long and opposite, arranged in pairs of one large and one smaller leaf; margin entire or wavy. Inflorescence: Axillary, umbellate cyme of 2-25 yellow-green, short-pedicellate flowers. Flower: Perfect, radially symmetric, campanulate; calyx with 5 acute triangular lobes; corolla twice the length of the calyx, 7-8 mm long, with 5 lanceolate lobes, spreading or reflexed; stamens 5, slightly exserted, filaments alternate to petal lobes, partially fused to corolla; ovary superior, glabrous, stigma shallowly bifid. Fruit: Berry; globose; 5-6 mm diameter; orange-red; enclosed in green, membranous, inflated calyx approximately 2.5 cm diameter and slightly 5-angled. Seeds: Many, discoid, 2.5 mm across, pale yellow (Atal 1958; Atal and Schwarting 1961; Dunal 1852 [original citation]; Hutchinson and Dalziel 1963; Kiritikar and others 1935).

Distribution: Semi-arid habitats. Flowers year-round. Africa and Mediterranean, east into India. Found both wild and cultivated. Because of the many morpho- and chemotypes found in this species, further infraspecific taxonomic work is warranted. To date, at least five different morphological and geographic forms have been identified, though names have not been assigned to them (Atal and Schwarting 1961; Dunal 1852 [original citation]; Hutchinson and Dalziel 1963).

Figure 2a Botanical characteristics of ashwagandha *Withania somnifera* (L.) Dunal.

Kirtikar KR, Basu BD, An ICS. 1935. Indian Medicinal Plants. Lalit Mohan Basu. Allahabad, India

Figure 2b **Ashwagandha *Withania somnifera* (L.) Dunal.**

Photograph courtesy of Amsar Private Ltd, Indore, India

Figure 2c **Ashwagandha *Withania somnifera* (L.) Dunal.**

Photograph courtesy of Martin Wall Photography, Pleasant Garden, NC

Figure 2d **Ashwagandha *Withania somnifera* (L.) Dunal (note mature capsules)**

Photograph courtesy of Richo Cech, Horizon Herbs, Williams, OR

Figure 2e **Ashwagandha *Withania somnifera* (L.) Dunal (note red seeds)**

Photograph © 2000 Roy Upton, Soquel, CA

Macroscopic Identification

The fresh roots are white to yellow-brown with a creamy interior. The dried roots are straight and unbranched, the thickness varying with age. The main roots bear fiber-like secondary roots. The outer surface of the root is buff to gray-yellow and has longitudinal wrinkles. The base of the stem is green, variously thickened, cylindrical, and longitudinally wrinkled. The roots break with a short uneven fracture. Aroma: Characteristic, horse-like; strongly pungent when fresh. Taste: Sweetish, bitter, and astringent; slightly mucilaginous.

Powder: Dusty white or gray to yellow-brown (Ayurvedic Pharmacopoeia of India 1989; Chadha 1976).

Figure 3a Freshly harvested ashwagandha root. Mt Madonna Retreat Center, Watsonville, CA

Photograph © 2000 Roy Upton, Soquel, CA

Figure 3b Freshly dried ashwagandha root

Photograph © 2000 Roy Upton, Soquel, CA

Figure 3c Dried ashwagandha root

Photograph courtesy of Amsar Private Ltd, Indore, India

Figure 3d Freshly harvested ashwagandha root (dried; graded smaller to larger, left to right)

Photograph © 2000 Roy Upton, Soquel, CA

Figure 3e Ashwagandha root and root powder

Photograph © 2000 Roy Upton, Soquel, CA

Microscopic Identification

Whole Root: The transverse section shows a narrow band of yellowish cork; a narrow cortex packed with starch grains; and a wide central woody region radiating many long, distinct, medullary rays.

Powder: Cork thin-walled; lignified, cubical, or elongated cells, often indistinct and collapsed, with yellowish-brown contents; 2-3 cells deep in smaller roots, up to 16 in larger primary roots. Parenchyma of the cortex large thin-walled cells, packed with starch granules, and occasionally containing microsphenoidal crystals of calcium oxalate. Xylem elements are either tracheidal and bordered-pitted or, more rarely, reticulately thickened vessels. Fibers from xylem have thickened lignified walls and simple pits. Starch abundant, simple or 2-4 compound, with a pronounced irregularly shaped hilum.

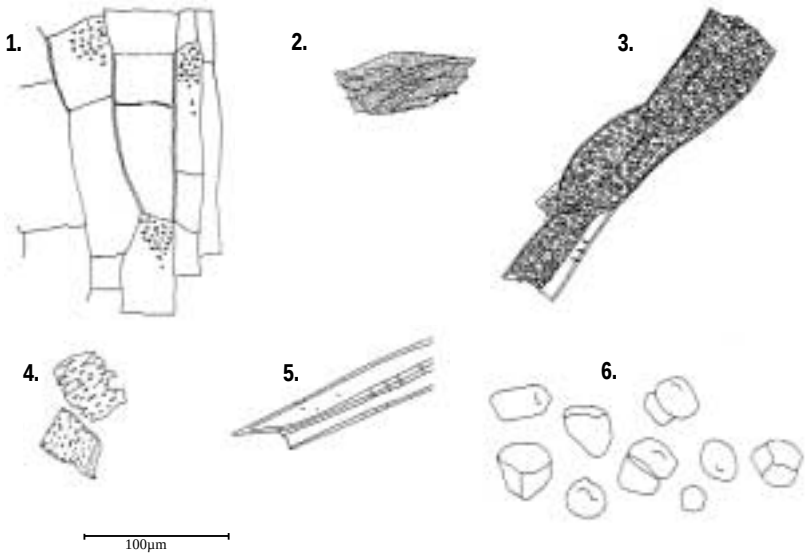


Figure 4 Microscopic characteristics of ashwagandha

1. Parenchyma from cortex with microsphenoidal crystals of calcium oxalate.
2. Cork cells collapsed and indistinct.
3. Bordered-pitted tracheids.
4. Fragments of reticulate xylem vessels.
5. Fibers from xylem.
6. Starch.

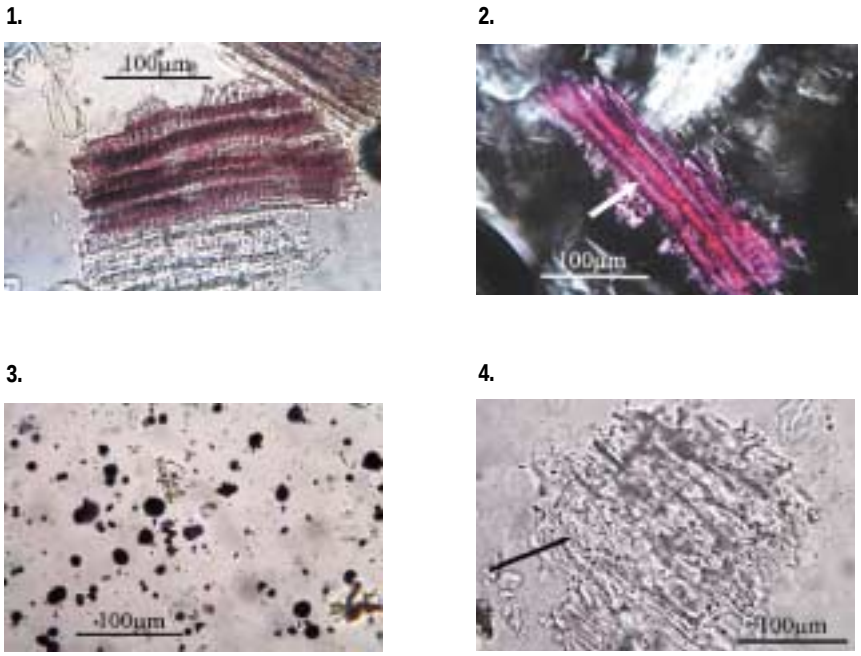


Figure 5 Microscopic images of ashwagandha

1. Fibers from xylem.
2. Fragment of reticulate xylem vessels.
3. Starch granules.
4. Parenchyma from cortex with microsphenoidal crystals of calcium oxalate.

Microscopic images courtesy of Alkemists Pharmaceuticals, Costa Mesa, CA

Prepared with phloroglucinol + HCl (1 & 2) and glycerine + chlorol hydrate-zinc-iodide solution; objective 40x; magnification 400x.

Commercial Sources and Handling

Commercial supplies of ashwagandha are obtained from both wild and cultivated sources. Wild supplies are prevalent throughout India though are more abundant in the warmer parts. Large amounts of material are also cultivated on plantations predominantly in the regions of Madhya Pradesh (Manasa) and Rajasthan (Ayurvedic Pharmacopoeia of India 1989). There are five primary chemotypes that are reported to be traded and originate from different areas. These are simply categorized as Form I to Form V. Form I, cultivated exclusively in Madhya Pradesh, is reportedly the primary source of commercial material in India and, presumably, the primary material imported into the United States. The name ashwagandha Nagore (ashwagandha of Nagore), in reference to a nearby area where wild ashwagandha occurred naturally, is sometimes applied to material cultivated in this area. Form II originates from the sandy desert regions of Pilani, Marwar, and some other parts of Rajasthan. Form III grows in Chandigarh and other mountainous areas of Punjab and Uttar Pradesh. Form IV grows near Delhi. Form V grows near Delhi and Ahmedabad. The characteristics of the various roots are similar and are difficult to differentiate.

There are several sources of domestically cultivated ashwagandha. However, little investigation has been conducted to determine the forms under cultivation. It is clear from a thin layer chromatography (TLC) analysis that some of this material is the same as material cultivated in Madhya Pradesh while other samples are different. In India, botanical and chemical investigations have been conducted regarding the different forms but little of these data were readily available. All samples tested by American Herbal Pharmacopoeia™ collaborating laboratories contained withaferin A.

Collection

In India, the fresh roots of one-year-old plants are harvested between January and March. The stem is cut 1-2 cm above the crown as soon after collection as possible and the lateral roots are removed. The main tap root is either cut transversely 4-8 cm long or dried in the sun whole. The roots are further cleaned, trimmed, and graded according to quality (size) before reaching the market (see Qualitative Differentiation) (Atal and Schwarting 1961). The primary alkaloids of both wild and cultivated ashwagandha have been reported to be the same (Chadha 1976).

Drying

Generally, whole roots are sun-dried.

Qualitative Differentiation

The dried roots are graded according to thickness and uniformity with the thickest ones considered to be the highest quality.

Grade I: The roots are generally cylindrical, uniform in size, and 1-1.25 cm in diameter. The external surface of the root is white, starchy, and reveals a nonwoody xylem.

Grade II: The roots are thinner than grade I, approximately 0.5-1.0 cm in diameter, and occasionally dark and rough on the exterior.

Grade III: The roots vary in diameter and have a pale brown, wrinkled surface. A hard lignified xylem is present.

Grade IV: The roots are long and thin, rarely exceeding 3 mm in diameter. They are considered relatively useless for therapeutic purposes. Some sources mix the grades together while other sources will include the crown and/or stem remains (Atal and Schwarting 1961).

Note: TLC analysis of smaller to larger roots, root bark, whole root, and only wood showed no apparent qualitative or quantitative differences.

Cultivation

In India, it has been reported that approximately 5 pounds of seed per acre are sown. Sowing is done by broadcasting. The plants are easily established and thrive without fertilizer, irrigation, or weeding. Excessive rain damages crops. The yield per acre is approximated at 500 pounds of fresh root which, when dry, reduces to about 150 pounds. In exceptional cases, yields of up to 300 pounds per acre have been reported (Atal and Schwarting 1961). According to the Central Council for Research in Indian Medicine and Homeopathy, cultivation of ashwagandha is relatively easy since the crop does not require much attention or labor, it grows in poor soil, and it requires no irrigation during its growing season.

Ashwagandha root is also cultivated in America and is used in the production of some domestic herbal products. From American Herbal Pharmacopoeia™ analysis, it is clear there are two chemotypes under cultivation each with different HPTLC chromatograms (see Figures 7-9). One sample corresponds to authenticated material cultivated in Madhya Pradesh while the other corresponds to material that is also traded in India. β-sitosterol and withaferin A were present in all samples.

Storage

Follow general guidelines for proper storage of botanical medicines. Protect from light, moisture, air, heat, and insect infestation.

Adulterants

It has been reported that ashwagandha is often substituted or adulterated with *W. coagulans*.

Preparations

Crude ashwagandha is used singly and in many formulas (see Traditional Ayurvedic Medicine Supplement). In ayurvedic medicine it is traditionally used as a powder, decoction, medicated wine, ghee paste, medicated jam, and

medicated oil (Frawley and Lad 1986; Nadkarni 1993). Domestically, ashwagandha is generally available in the following forms: whole roots, powders, liquid extracts, capsules, tablets, standardized extracts, and classic ayurvedic formulas.

CONSTITUENTS

The primary constituents of interest in ashwagandha are alkaloids and steroidal lactones, the latter of which are known as withanolides.

The constituent profile of various chemotypes of *Withania* spp. varies significantly and the chemical differences have not been well articulated. Chemotype I is reported to be particularly rich in withaferin A (0.2%). Chemotype II reportedly contains a compound similar to that of withaferin A. Chemotype III contains a wide variety of withanolides. Beyond these generalizations, little data regarding the constituent profiles of the various chemotypes are available. Some of the constituent differences can be attributed to environmental conditions and/or to differences in analytical methodology (Schwarting and others 1963).

Withanolides (Steroidal Lactones)

Several withanolides are identified in ashwagandha, including withanolide A-Y (Besalle and others 1987; Tripathi and others 1996), withaferin A (Subramanian 1982), 5-dehydroxywithanolide-R, withasomniferin-A (Rahman and others 1991), withasomidienone (Rahman and others 1993), and withasomniferols A-C (Anjaneyulu and Rao 1997). There are as many as 40 similar structures synonymously identified in the literature as withaferin A (Chadha 1976; Kapoor 1990; Lockley and others 1976; Schwarting and others 1963).

Alkaloids (0.13% to 4.3%)

Ashwagandhine, ashwagandhinine, somniferiene, somniferinine, withanine, withaninine, pseudowithanine, withasomnine, isopelletierine, cuscohygrine, dl-isopelletierine, anapherine, anahygrine, and visamine. Many authors cite nicotine as a constituent of ashwagandha based on a report by Majumdar and co-workers in 1955. However, research conducted by Atal (1958) found that neither the roots nor leaves contain nicotine (Atal 1958; Chadha 1976; Das and others 1964; Kapoor 1990; Schwarting and others 1963).

Additional Constituents

Sitoindosides VII, VIII (acylsteryl glucosides), IX and X (C-27-glycowithanolides) (Bhattacharya and others 1987; Ghosal and others 1988; Wagner and others 1994), saccharose, β-sitosterol, hentriacontane, scopoletin, dulcitol, chlorogenic acid (Anjaneyulu and Rao 1997; Majeed 1992), glycosides, fatty oils, essential oils, choline, aspartic acid, glycine, tyrosine, alanine, proline, tryptophane, glutamic acid, cysteine, cystine (Kapoor 1990), arginine, and ornithine (Subramanian 1982).

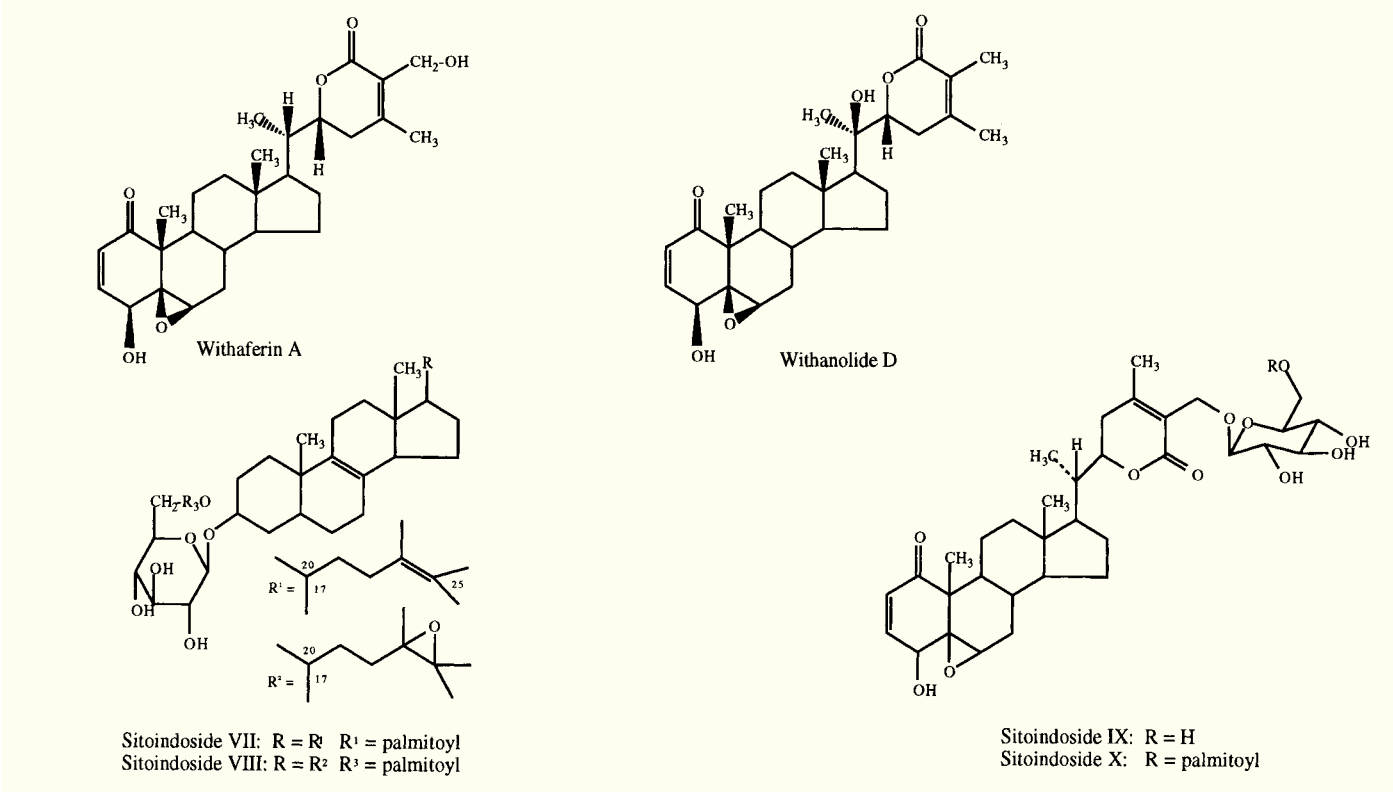


Figure 6 **Representative withanolides and sitoindosides of ashwagandha**

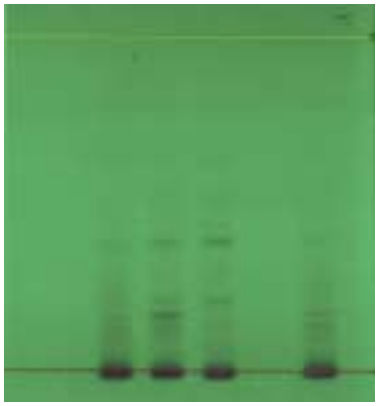


Figure 7a **UV 254 nm**

Figure 7b **Sulfuric acid reagent, visible light**

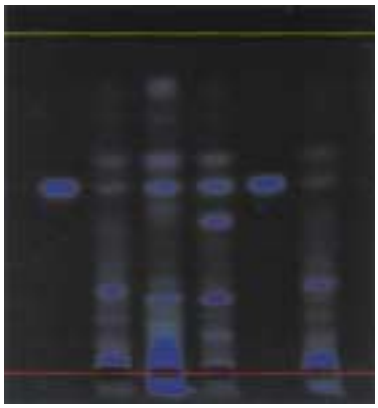


Figure 7c **Sulfuric acid reagent, UV 366 nm**

Figures 7a-c **Characteristic HPTLC fingerprints of three types of ashwagandha using β-sitosterol as a reference standard**

Lanes 1, 5: β-sitosterol.

Lanes 2, 6: Ashwagandha (authentic sample from Madhya Pradesh: Type I).

Lane 3: Ashwagandha (domestically cultivated material: Type II).

Lane 4: Ashwagandha (commercial Indian sample of unknown origin: Type III).

ANALYTICAL

Various qualitative and quantitative methods are provided in the literature. However, the various chemotypes to which each method should be applied was not disclosed. This, along with the lack of available reference standards and authenticated botanical specimens, makes substantiation difficult. The following TLC method was substantiated using withaferin A and β-sitosterol as internal standards and may be used for quality control purposes.

Thin Layer Chromatography (TLC/HPTLC)

Sample Preparation

In a 100 mL Erlenmeyer flask, add 1 mL ammonia (25% to 27% in water) to 100 mg of powdered herb and shake well. Add 10 mL methanol and sonicate for 10 seconds. In a water bath, heat mixture to boiling for 3 minutes, then filter. Evaporate filtrate to dryness and reconstitute with 1 mL methanol. Transfer solution to a small sample vial. This is the test solution.

Standard Preparation (optional)

Dissolve 1 mg of β-sitosterol in 1 mL of chloroform and transfer this solution into a small sample vial. If available, 1 mg of withaferin A is dissolved in 1 mL methanol. This is the reference solution.

Reagent Preparation

Sulfuric acid reagent: While cooling with ice, carefully add 5 mL of sulfuric acid to 95 mL of cold methanol.

Chromatographic Conditions

Stationary Phase:

HPTLC plates 10 cm x 10 cm or 20 cm x 10 cm silica gel 60 with fluorescence indicator (EM Science; Whatman, Macherey, and Nagel; or equivalent).

Note: HPTLC plates allow for better separation, sharper zones, reduced development time, and require less solvent consumption per plate than standard TLC plates. The method can also be run with standard TLC plates.

Mobile Phase:

Toluene:ethyl acetate:formic acid (50:15:5).

Sample Application:

10 µl volumes of the test solution and 2 µl of the reference standard(s) are applied each as a 6 mm band. The application position should be 8 mm from lower edge of plate.

Development:

10 x 10 cm or 20 x 10 cm Twin Trough Chamber (or equivalent), saturated for 10 minutes, 5-10 mL developing solvent per trough (or enough solvent to have a level of 5 mm in chamber), developing distance 80 mm from lower edge of plate. Dry plate in stream of cold air for 5 minutes.

Detection:

a) UV 254 nm.

b) Sulfuric acid reagent: Immerse plate in reagent for 1 second, dry in stream of cold air, heat to 110 ˚C for 2 minutes. Examine plate in visible light.

c) Examine derivatized plate under UV 366 nm.

R_f Values:

Compare to the chromatograms provided.

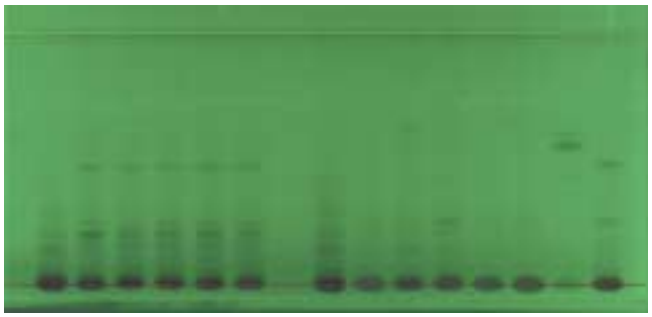


Figure 8a **UV 254 nm**

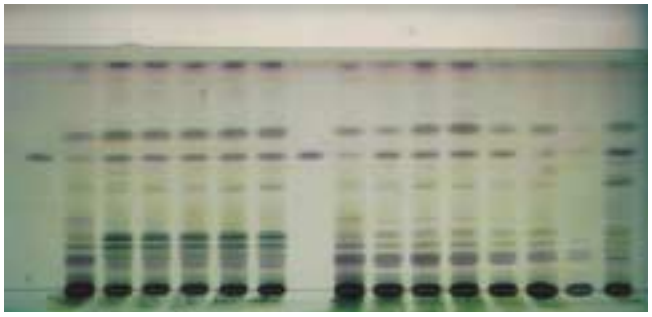


Figure 8b **Sulfuric acid reagent, visible light**

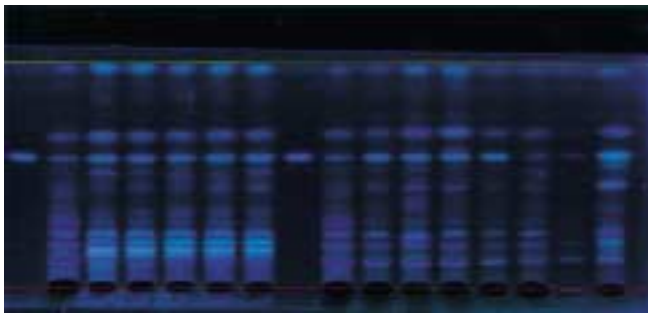


Figure 8c **Sulfuric acid reagent, UV 366 nm**

Figures 8a-c **Characteristic HPTLC fingerprints of various commercial samples of ashwagandha using β-sitosterol as a reference standard**

Lane 1: β-sitosterol.

Lane 2: Ashwagandha (authentic sample from Madhya Pradesh: Type I).

Lane 3: Ashwagandha (domestically cultivated; root bark only: Type II).

Lane 4: Ashwagandha (domestically cultivated; wood only: Type II).

Lane 5: Ashwagandha (domestically cultivated; smallest portion of root: Type II).

Lane 6: Ashwagandha (domestically cultivated; medium portion of root: Type II).

Lane 7: Ashwagandha (domestically cultivated; largest portion of root: Type II).

Lane 8: β-sitosterol.

Lane 9: Ashwagandha (authentic sample from Madhya Pradesh: Type I).

Lane 10: Commercial sample from India (Type II).

Lane 11: Commercial sample from India (Type II).

Lane 12: Domestically cultivated (Type II).

Lane 13: Commercial sample from India (Type II).

Lane 14: Commercial sample from India (Type II).

Lane 15: Ashwagandha (authentic sample from Madhya Pradesh: Type I).

Lane 16: Commercial sample from India (Type III).

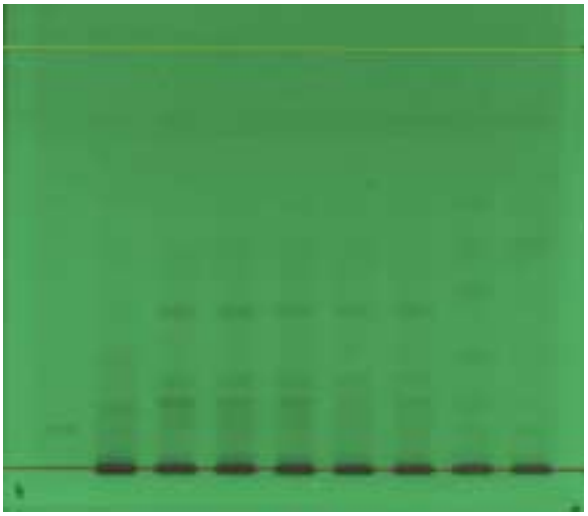


Figure 9a **UV 254 nm**

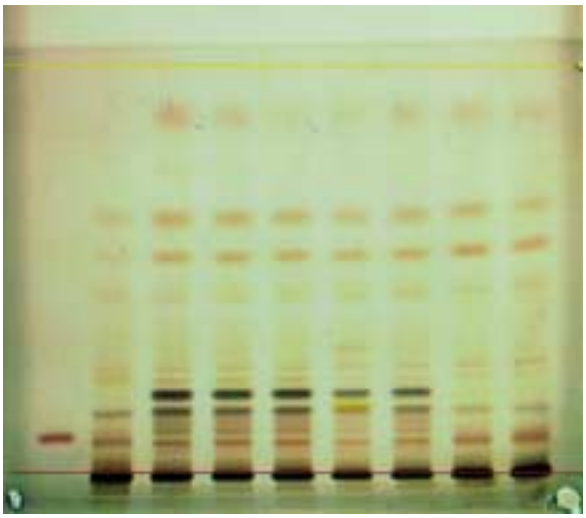


Figure 9b **Sulfuric acid reagent, visible light**

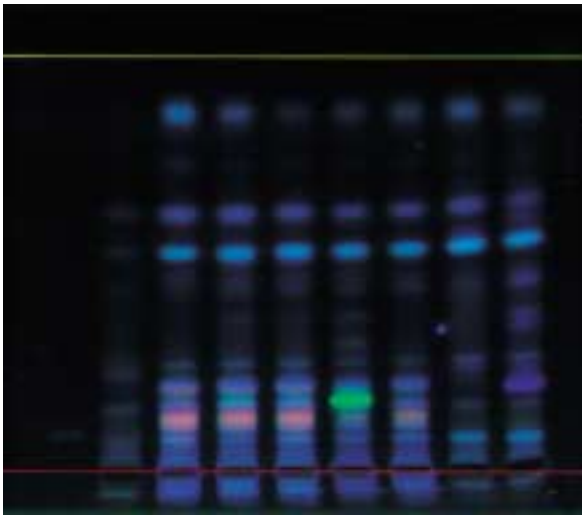


Figure 9c **Sulfuric acid reagent, UV 366 nm**

Figures 9a-c **Characteristic HPTLC fingerprints of three types of ashwagandha using withaferin A as a reference standard**

Lane 1: Withaferin A.

Lane 2: Ashwagandha (authentic sample from Madhya Pradesh: Type I).

Lane 3: Ashwagandha (domestically cultivated; root bark only: Type II).

Lane 4: Ashwagandha (domestically cultivated; wood only: Type II).

Lane 5: Ashwagandha (domestically cultivated; smallest portion of root: Type II).

Lane 6: Ashwagandha (domestically cultivated; medium portion of root: Type II).

Lane 7: Ashwagandha (domestically cultivated; largest portion of root: Type II).

Lane 8: Commercial sample from India (Type I)

Lane 9: Ashwagandha (domestically cultivated: Type I).

Discussion of Chromatograms

Three different fingerprints were obtained from the various samples collected. Type I represents the fingerprint obtained from authenticated *W. somnifera* (L.) Dunal. cultivated in Madhya Pradesh, India. The other samples tested include two different sources of domestically cultivated ashwagandha and commercial samples from India of unknown origin. For purposes of simplicity, these samples are identified as Type I, Type II, and Type III.

a) UV 254 nm: The reference solution of β-sitosterol does not show any band. The test solution of Type I and II shows a prominent band at R_f = 0.2 and two weaker bands just below. Samples of Type II and III show a band at R_f = 0.46. Type III shows an additional band at R_f = 0.26. A faint band corresponding to withaferin A is seen at R_f = 0.18 in all samples.

b) Sulfuric acid reagent examined in visible light: The reference solution of β-sitosterol shows a gray-violet band at R_f = 0.58. The test solutions of all samples show a band similar in color and R_f to β-sitosterol. The intensity of these bands differ. Below, at R_f = 0.46, is a strong and a weak gray band. There is a gray-violet band at R_f = 0.68 and one in the solvent front. In the lower R_f region, all types show a pair of dark gray bands at R_f = 0.2. In Type I, the upper band of that pair is weak. In Type II, both bands of the pair are strong. In Type III, both bands are weak. Directly above this pair is another dark band in Types II and III. At R_f = 0.14 in Type I, there are two or three pink to violet bands close together. In Type II, the first and third band are darker in color. In Type III, the middle of the three bands dominates. A reddish-brown band of different intensities, corresponding to withaferin A, is seen at R_f = 0.18 in all samples.

c) Sulfuric acid reagent examined under 366 nm: The reference solution of β-sitosterol shows a light blue fluorescent band at R_f = 0.58. The test solutions of all samples show a band similar in color and R_f to β-sitosterol. Below, at R_f = 0.46, is a medium to strong white band. There is a white band at R_f = 0.68 and one in the solvent front. In the lower R_f region, all types show a pair of blue-violet bands at R_f = 0.2. In Type I, the upper band of that pair is weaker. In Type II, both bands of the pair are equally strong. In Type III, the lower band of the pair is greenish, the upper band is blue. Directly above this pair is another blue band in Type I. Between R_f = 0.14 and R_f = 0.2, close together are two to three bands. In Type I, one of the bands is blue and the other is brown. In Type II, all three bands are light violet. In Type III, the middle of the three bands is strong. A band ranging in color and intensity from pink to reddish-brown or light blue, corresponding to withaferin A, is seen at R_f = 0.18 in all samples. In Figure 9c, a fluorescent green band is seen in one sample. This is an anomaly and is likely due to a contaminant on the plate.

Discussion of Various Samples

The ashwagandha samples compared represent the predominant supplies in American trade. Most, including those which are domestically cultivated, are consistent with Type I and Type II. Both types are under cultivation in the United States. All samples tested contained β-sitosterol and withaferin A at varying concentrations. The constituent profile of the various sizes of roots, root bark, and root wood appeared to be identical qualitatively and quantitatively. There are only slight differences between Types I and III.

Herb samples provided by Amsar Private Ltd, India; Bazaar of India, Berkeley, CA; Dabur Research Center, India; Frontier Herb Cooperative, Norway, IA; Horizon Herbs, Williams, OR; and Zandu Pharmaceuticals, India. Withaferin A provided by Zandu Pharmaceuticals, India.

Qualitative Standards

Foreign Matter:

Not more than 2% (Ayurvedic Pharmacopoeia of India 1989).

Moisture Content:

Not more than 10% (dry at 105 ˚C for 5 hours to constant weight) (Ayurvedic Pharmacopoeia of India 1989).

Total Ash:

Not more than 7% (determined with 2-3 g of powdered root incinerated at a temperature not exceeding 450 ˚C) (Ayurvedic Pharmacopoeia of India 1989).

Acid Insoluble Ash:

Not more than 1% (determined by boiling the ash for 5 minutes in dilute hydrochloric acid; collect, wash with hot water, ignite to constant weight. Calculate as a percentage in reference to the dried root) (Ayurvedic Pharmacopoeia of India 1989).

Alcohol (25%):

Not less than 15% soluble extractive (Ayurvedic Pharmacopoeia of India 1989).

Alkaloids:

Not less than 0.2% (Ayurvedic Pharmacopoeia of India 1989).

Microbial Contamination:

Negative to limits of detection for pathogens *Salmonella* spp., *Escherichia coliform,* and *Staphylococcus aureus.*

THERAPEUTICS

Pharmacokinetics

Data on the metabolism, distribution, and excretion of ashwagandha preparations are not available.

Pharmacodynamics

Ashwagandha has primarily been used as an adaptogenic tonic and anti-inflammatory agent and secondarily has been researched for its potential as an anticancer and immunomodulatory agent. While in vitro and animal research confirm many of the traditional uses of ashwagandha, well-controlled human trials are lacking.

Adaptogenic Effects

Human Clinical Studies

Ashwagandha has been researched for its potential as an adaptogen. Russian pharmacognosist Lazarev coined this term to define a class of pharmacologically active substances that, in a nonspecific manner, enhance the resistance of an organism to adapt to various stressors. Adaptogens were further defined as follows: 1) they must show a nonspecific activity, for example an increase in power of resistance against physical, chemical, or biological noxious agents; 2) they must have a normalizing influence independent of the nature of the pathological state; 3) they must be innocuous and must not influence normal body functions more than required (Brekhman 1980, cited in Wagner and others 1994). A significant amount of research regarding the adaptogenic activity of certain botanicals such as ginseng *Panax ginseng*, schisandra *Schisandra chinensis*, and Siberian ginseng *Eleutherococcus senticosus* has been conducted. Numerous studies report that adaptogens assist the organism in maintaining homeostasis during stress and help to regain homeostasis after stress.

In one double-blind clinical study of healthy females (n = 15) and males (n = 15), the effects of ashwagandha on psychomotor performance were compared against a placebo control and Asian ginseng *Panax ginseng*. Test parameters included tapping, cancellation test, mental mathematic calculations, logical deduction, choice reaction times, and auditory reaction. The performance of both treatment groups was reported to be superior to placebo. Ashwagandha (250 mg capsules twice daily) in turn was reported to be superior to ginseng (undefined standardized extract; dose not disclosed). The duration of the study was 40 days. No qualifying data regarding level of efficacy were provided (Karnick 1991).

Animal Studies

Numerous animal studies suggest that ashwagandha does possess adaptogenic activity. One group of researchers reported adaptogenic effects in mice and rats subjected to various stresses. Administration of 100 mg/kg intraperitoneally (ip) of ashwagandha (preparation undefined) resulted in an almost doubling of swimming time from 385 ± 26 minutes to 740 ± 33 minutes in the forced swimming test with mice (n = 20 each of control and verum) ($P < 0.001$). The weight of the adrenals and concentration of corticosterone and ascorbic acid content of adrenals before and after swimming was assessed as was anabolic activity (Table 1). Adrenal weight of swimming animals was significantly greater ($P < 0.001$) than the nonswimming group. Ascorbic acid and corticosterone content of the adrenals in the swimming animals were significantly reduced as compared to the nonswimming group. When pretreated with ashwagandha, no significant differences in adrenal weight ($P > 0.05$) or changes in ascorbic acid and corticosterone levels of treated ($P > 0.05$) and nonswimming animals were observed. In the same study, ashwagandha significantly reduced the incidence of cold-, restraint-, and aspirin-induced gastric ulcers ($P < 0.001$, $P < 0.001$, $P < 0.01$, respectively; Table 2) (Singh and others 1982). Sitoindosides have similarly been shown to reduce the incidence and severity of stress-induced gastric ulcers in rats (Table 3) (Bhattacharya and others 1987).

Antistress activity of ashwagandha was investigated using adult rats and a variety of stress tests including a cold water swimming test. Cold water swimming stress increases plasma corticosterone levels. Rats subjected to cold swimming were administered an aqueous suspension of 100 mg/kg (by gastric intubation) of powdered ashwagandha root, a dose reportedly equivalent to a standard human dose. Pretreatment with ashwagandha significantly increased

Table 1 Effects of ashwagandha on the weight and ascorbic acid and cortisol content of adrenals of mice after physical stress (5 hr swimming)

Group	Number of animals	Dose mg/kg ip	Physical stress	Adrenal mg/100 g ± SE	Ascorbic acid content mg/100 g of adrenals ± SE	Cortisol content mg/100 g of adrenals ± SE
Normal (saline)	20	0.25 mL	None	20 ± 1.2	271 ± 21	2.37 ± 0.4
Control	20	0.25 mL	Swimming	30 ± 1.6*	137 ± 14*	1.28 ± 0.21*
Withania somnifera	20	100	Swimming	22 ± 1.1**	245 ± 18**	2.30 ± 0.18**

*$P < 0.001$

**$P > 0.05$ as compared to nonswimming animals

Source: Modified from Singh and others (1982).

Table 2 Effects of ashwagandha on stress-induced gastric ulcers in rats

Group	Number of animals	Dose (mg/kg ip)	Number of animals showing ulcers	Mean ulcer index	P < value
Cold (4 ˚C, 2 hours)					
Control (saline)	10	0.25 mL	10	36	—
Ashwagandha	10	100	2	8.7	< 0.001
Restraint (18 hours)					
Control (saline)	10	0.25 mL	10	34.6	—
Ashwagandha	10	100	2	9.8	< 0.001
Aspirin (200 mg/kg ip)					
Control (saline)	10	0.25 mL	10	37	—
Ashwagandha	10	100	3	9.5	< 0.01

P values calculated by student's *t*-test

Source: Modified from Singh and others (1982).

Table 3 Effects of sitoindosides (SG-1, SG-2) on restraint-induced gastric ulcers in rats

	Incidence of ulcers (%)	Number of ulcers	Severity mean	Group score
Control (saline)	100[d]	10.6 ± 1.9[e]	18.2 ± 5.6[e]	46
SG-1				
50 mg/kg	50[a]	3.4 ± 1.2[a]	3.9 ± 0.8[c]	16.2
100 mg/kg	30[b]	1.2 ± 0.9[b]	2.0 ± 0.8[c]	11.6
SG-2				
50 mg/kg	40[a]	2.8 ± 0.6[b]	2.8 ± 1.1[c]	14.0
100 mg/kg	20[b]	0.8 ± 0.2[c]	1.6 ± 0.9[c]	10.6

n = 10 in each group

a, b, and c denote statistical significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, in comparison to the respective saline tested group

d chi-square test

e student's *t*-test

Values are expressed as means ± SEM

Source: Modified from Bhattacharya and others (1987).

swimming times ($P < 0.05$) and maintained corticosterone levels that were only slightly above those of the controls (Table 4). The researchers concluded that ashwagandha appears to induce a state of nonspecifically increased resistance during stress (Archana and Namasivayam 1999).

In a study comparing the effects of ashwagandha to ginseng *Panax ginseng*, 1 g/kg of an aqueous suspension (100 mg/mL; 2.8% total steroids) was administered orally for 7 days to one-month-old albino mice. In the forced swimming test, the swimming time of controls was 163.3 ± 34.34 minutes. Swimming times for ashwagandha and ginseng were 474.1 ± 82.14 minutes and 536.6 ± 62.55 minutes, respectively. Anabolic activity was also measured. Both botanicals stimulated weight gain, but this effect was greater with ashwagandha. An increase of 9.00 ± 0.36 g was observed with ashwagandha as compared to 8.66 ± 1.33 g for ginseng and 7.33 ± 0.80 g for the controls ($P < 0.001$: comparison between groups). An increase in lean muscle mass was also reported (Grandhi and others 1994).

Other groups of researchers reported that an ashwagandha extract and/or sitoindosides administered to mice and rats improved memory-related performance in passive avoidance tasks and protected against stress-induced responses ranging from anxiety, depression, thermic changes, gastric ulcers, convulsions, tribulin* activity, and adrenocortical activation. In one study, administration of an ashwagandha extract (1:1 aqueous-methanolic extract: doses of 20, 50, 100 mg/kg ip; $P < 0.01$, $P < 0.05$, $P < 0.001$, respectively) resulted in a reduction of stress responses. This was accompanied by a preservation of adrenal ascorbic acid and corticosterone levels suggesting, as did previous reports (Archana and Namasivayam 1999; Singh and others 1982), that a corticosterone-sparing effect is one mechanism of action of adaptogens. In this study, withaferin A was found to be ineffective (Bhattacharya and others 1987). In another study, sitoindosides IX and X, each administered to rats at 50 mg/kg periorally (po) for 3 days, augmented learning acquisition and improved short-term memory as determined by passive avoidance test. As in the previous study, withaferin A administered at the same dosage was ineffective (Ghosal and others 1989).

*Tribulin is an endogenously produced monoamine oxidase inhibitor present in the urine of humans and rats especially under stressful conditions.

Based on findings that there is a severe loss of cholinergic neurons in the basal forebrain of Alzheimer's patients and on the traditional use of ashwagandha for enhancing cognitive functions, investigations were made to determine if ashwagandha and its constituents have an effect on cholinergic transduction. Bhattacharya and others showed that sitoindosides from ashwagandha reversed cholinergic deficiency and cognitive deficits induced by ibotenic acid (Bhattacharya and others 1995). A study by Schliebs and others provides additional information. An extract of ashwagandha (mixture of sitoindosides VII-X and withaferin A; 40 mg/kg ip; 7 days) was administered to rats. The following findings were reported: in comparison with controls, acetylcholinesterase (AChE) activity in the vertical diagonal band was reduced (12%) while AChE activity was slightly enhanced in the lateral septum (20%) and globus pallidus (12%); enhanced M_1 muscarinic acetylcholine receptor (M_1-mAChR) binding in the lateral (43%) and medial septum (48%) and frontal cortices (20%); and increased M_2-mAChR binding in various cortical regions, including cingulate (23%), frontal (22%), piriform (17%), parietal (17%), and retrosplenial (12%) cortices ($P < 0.05$, respectively). No activity was noted on γ-aminobutyric acid ($GABA_a$), benzodiazepine binding, and NMDA and AMPA glutamate receptor subtypes. The researchers concluded that increased cortical mAChR binding may be partially responsible for the improvements in cognitive functions traditionally associated with use of ashwagandha (Schliebs and others 1997).

In a poster by researchers Saksena and others it was reported that part of the adaptogenic action of sitoindosides VII and VIII is their ability to suppress stress-induced increases in *Corpus striatum* dopamine receptors in rats. According to this research, there was a significant increase in *Corpus striatum* dopamine receptor population in animals subjected to immobilization stress for 5 hours. This increase was reportedly inhibited by administration of ashwagandha (preparation and dose undefined) (Saksena and others 1989).

Table 4 Effects of ashwagandha on plasma cortisone levels and swimming times in the forced swimming test

Parameters	Controls (n = 10)	Ashwagandha 100 mg/kg po for 7 days (n = 10)	Stress (cold water swimming test) (n = 6)	Stress plus ashwagandha 100 mg/kg po for 7 days (n = 6)
Plasma cortisone (µg/dl)	98.65 ± 0.51	98.95 ± 0.27	107.20 ± 0.38*	99.77 ± 0.14
Total swimming time (minutes)	—	—	5.30 ± 0.24	8.9 ± 0.5*
	Controls (saline) (n = 20)	**Ashwagandha 100 mg/kg ip (n = 20)**	**—**	**—**
Total swimming time (minutes)	385 ± 26**	740 ± 33**	—	—

Values are expressed as means ± SEM*P < 0.05 **P < 0.001 student's t-test

Source: Modified from Archana and Namasivayam (1999) and Singh and others (1982).

Anti-inflammatory Effects

Animal Studies

Ashwagandha root powder (1 g/kg), when tested against acute/subacute models of inflammation such as adjuvant-, ovalbumin-, or formalin-induced inflammation in mice and rats, exhibited significant anti-arthritic and anti-inflammatory activity (Agarwal and others 1999; Anbalagan and Sadique 1981a, 1981b, 1985; Begum and Sadique 1988). Researchers Begum and Sadique observed that body weight loss typically associated with artificially induced arthritic conditions was reversed after treatment with ashwagandha (1 g/kg ip) after 15 days. A marked reduction of approximately 72% in swelling of the rat paw was noted. Radiographic observation showed that ashwagandha treatment additionally prevented bony degeneration. The anti-inflammatory activity was reported to be superior to 15 mg/kg hydrocortisone which resulted in an approximately 48% reduction of inflammation after 15 days (Begum and Sadique 1988; Handa and others 1992). A dose-dependent suppression of α2-macroglobulin serum levels (an indicator useful for diagnostic and prognostic assessment of arthritic and inflammatory conditions) was observed in the animals treated with ashwagandha. A maximum reduction of 75% in α2-macroglobulin levels was observed at a dose of 100 mg/100 g (Anbalagan and Sadique 1981a, 1981b, 1985).

Budhiraja and co-workers reported that the withanolides 3-f-hydroxy-2-3-dihydrowithanolide F and withanolide E (isolated from *W. coagulans*) showed significant activity in inflammatory models when administered in 10 mg/kg ip dose levels to rats. In addition, 3-β-hydroxy-2-3-dihydrowithanolide F at 10 mg/kg ip protected against CCl_4-induced hepatotoxicity. In the earlier of these two studies, the anti-inflammatory activity of withanolide F was approximately five times more active than phenylbutazone and equal in activity to hydrocortisone (Budhiraja and others 1984; Budhiraja and Sudhir 1987).

In a recent study, the anti-inflammatory effect of an ashwagandha preparation (characterized as a total extract, 100 mg/mL) was noted using different hypersensitivity animal models (type-I IgE-mediated active paw anaphylaxis; cell mediated delayed type hypersensitivity; n = 6 per test). In the active paw anaphylaxis model, disodium chromoglycate (DSCG; 5 mg/kg), a mast cell stabilizer, was used as a comparison. In comparison with the control, DSCG alone significantly inhibited inflammation (64% versus 0.0%; P < 0.01). The total extract of ashwagandha (100 and 1000 mg/kg) reduced inflammation (32% and 37%, respectively) as compared to the control. This effect was insignificant in comparison with DSCG (P < 0.01). The unidentified ashwagandha fraction (150 and 300 mg/kg) significantly inhibited the inflammatory response (41% and 44%, respectively) when compared to both the control and DSCG (P < 0.05) (Agarwal and others 1999).

Antioxidant Effects

Animal Studies

Antioxidant activity has been reported for crude ashwagandha extract, sitoindosides VII-X, withanolides collectively, and withaferin A specifically. In one study, significant increases in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities in the frontal cortex and striatum of rat brains were observed at doses of 10 and 20 mg/kg ip of sitoindosides VII-X. The effects were reported to be comparable to deprenyl (2 mg/kg ip) (Bhattacharya and others 1997). Similar findings were reported by another group of researchers. In this study, ashwagandha root powder ($0.7\text{-}1.4^{-1}$ g/kg by gastric intubation for 30 days) decreased $CdCl_2$-induced lipid peroxidation and increased SOD and CAT activities in liver homogenates of mice. Effects observed at 15 days were not significant (Panda and others 1997). In another study, an aqueous suspension of an ashwagandha root extract (100 mg/kg) reduced the level of lipopolysaccharide-induced lipid peroxidation in rabbits and mice (Dhuley 1998a). One last study reported a significant reduction in lipid peroxidation in mice (n = 10) after administration of an aqueous ashwagandha extract (1.75% withanolides; $1.4g^{-1}/kg^{-1}$; $P < 0.05$). In this study, antioxidant activity was also associated with increased activity of hepatic glucose-6-phosphatase ($P < 0.01$), SOD ($P < 0.001$), and catalase ($P < 0.01$) (Panda and Kar 1998) (Table 5).

Central Nervous System (CNS) Effects

Animal and In Vitro Studies

The effects of ashwagandha on the central nervous system (CNS) and on smooth muscles was investigated. Administration of a 70% methanolic ashwagandha extract to rats (n = 144) and mice (n = 220) resulted in a lack of spontaneous movement and response to stimuli, sluggishness, diminution of muscle tone, an inability to maintain equilibrium, and delayed righting reflex. Onset of effect was immediate with intravenous (iv) administration, within 15 minutes with ip administration, and within 1 hour after oral administration (Malhotra and others 1960) (Table 6).

A later study by the same researchers, using papaverine as a comparison, reported on the effects of a 4% total alkaloids solution derived from ashwagandha root on various smooth muscles. Variable effects were observed. At relatively low concentrations, the alkaloid preparation induced spasm of isolated rabbit and rat ileum while at larger doses, it had a relaxant action. The spasmodic action was markedly reduced by atropine sulfate and was abolished by pentolinium tartrate. The pattern of relaxant and spasmolytic activity of the preparation was similar to that of papaverine, suggesting a direct musculotropic action. In the same study, CNS depression was observed in mice, rats, dogs, and monkeys. The ashwagandha preparation was approximately 7-12 times weaker, depending on the tissue, than that of papaverine. No anticonvulsant activity was observed (Malhotra and others 1965).

Crude drug and isolated compounds of ashwagandha have been reported to possess anxiolytic and CNS-inhibitory effects (Bhattacharya and others 1987; Dandiya and Chopra 1970). Mehta and co-workers (1991) tested the ability of ashwagandha extract to interact with GABAergic transmission. These researchers reported an inhibition of $[^3H]$GABA and $[^{35}S]$TBPS receptor binding and enhanced receptor binding of $[^3H]$flunitrazepam at various dosages (Table 7). Additionally, a 35% increase in ^{36}Cl-influx in the absence of GABA was observed as compared to basal values. This effect was blocked by bicuculline and picrotoxin and enhanced by diazepam.

When the ashwagandha extract and GABA were tested in combination on $[^3H]$flunitrazepam binding, the effect was not additive. The effect of combination treatment indicated that the extract decreased the enhancing effect of GABA. These results suggest that the anxiolytic properties of ashwagandha are related to GABA mimetic activity and are in agreement with those reported previously by Atal and Schwarting (1961). These results further suggest that ashwagandha may potentiate the effects of barbiturates (Mehta and others 1991).

In a series of other studies, an ashwagandha root extract was shown to elicit anticonvulsant activity primarily through GABA receptor binding. In one study, at 30 and 100 mg/kg, a delay in the onset of the extensor phase of convulsion was observed; at 200 mg/kg the tonic extensor phase was inhibited (Kulkarni and others 1993). In follow-up studies, it was reported that ashwagandha root extract (100 mg/kg) significantly inhibited pentylenetetrazol-induced convulsions with an effect that was comparable to diazepam (100 mg/kg) (Kulkarni and George 1996). Similar, though not significant, findings were reported in a follow-up study which noted that the anticonvulsant effects were mediated via $GABA_A$ receptor binding (Kulkarni and others 1998). In an earlier anticonvulsant study, it was reported that an alcohol extract (100 mg/100 kg) exerted a greater anticonvulsant effect (45%) than either powdered form (1 kg/100 kg; 30%) or decoction (1:4; 2 mL/100 kg; 25%). The effect observed was significant but was not as marked as that of phenobarbitone (95%) (Ral and others 1983).

Table 5 Effects of ashwagandha root extract (1.4 g^{-1}/kg^{-1}) daily for 20 days on hepatic lipid peroxidation, superoxide dismutase, catalase, and glucose-6-phosphatase activity in the mouse

Group	Lipid peroxidation (nmol malondialdehyde h^{-1} (mg protein)$^{-1}$)	Superoxide dismutase (units (mg protein)$^{-1}$)	Catalase (µmol H_2O_2 decomposed h^{-1} (mg protein)$^{-1}$)
Control	0.409 ± 0.022	5.55 ± 0.17	53.15 ± 1.92
Treated	0.208 ± 0.046*	8.29 ± 0.25**	63.86 ± 3.079*

Data are means ± SEM (n = 10)

*P < 0.01, **P < 0.001, significantly different from respective control values

Source: Panda and Kar 1998.

Table 6 Sedative and analgesic action of ashwagandha extract

Model	Dosage range mg/100 g	Number of animals	Action	ED_{50} (19/20 confidence limits) mg/100 g
Mice	20-140	107	Sedation	32.0
Mice	10-70	113	Sedation	20.9
Rats	75-200	84	Sedation	94.3
Rats	150-450	60	Sedation	396.1

Source: Modified from Malhotra and others (1960).

Table 7 Effects of ashwagandha methanol extract on the binding of [^{3}H]GABA, [^{35}S]TBPS, and [^{3}H]flunitrazepam to rat brain cerebral cortex membranes*

Ashwagandha extract at various concentrations	% inhibition of specific binding [^{3}H]GABA	[^{35}S]TBPS	% inhibition of specific binding [^{3}H]flunitrazepam
a) Extract (1 mg/mL)			
2 µl	0	—	—
5 µl	20 ± 6	—	20 ± 4
10 µl	35 ± 7	—	31 ± 6
b) Extract (10 mg/mL)			
1 µl	55 ± 5	9 ± 3	—
2 µl	—	20 ± 4	45 ± 8
5 µl	80 ± 10	51 ± 5	66 ± 12
10 µl	87 ± 12	82 ± 12	91 ± 16
c) Extract (100 mg/mL)			
10 µl	100	92 ± 6	—
d) Extract 10^{-4} *M* GABA	—	—	96.7
+ 5 µl ashwagandha extract (10 mg/mL)	—	—	60 ± 8*
e) 5 x 10^{-4} *M* pentobarbital	—	—	66 ± 16*
+ 5 µl ashwagandha extract (10 mg/mL)	—	—	118 ± 16*

Data represent a mean ± standard deviation of four experiments each done in triplicate

* P < 0.05 as compared to respective control

Source: Modified from Mehta and others (1991).

Cardioactive Effects

Animal and In Vitro Studies

Withanolide F has been shown to produce a moderate decrease in the blood pressure of dogs and a myocardial depressant effect in rabbit Langendorff preparation. This compound also increased contractility of the cardiac muscle (positively inotropic) and increased contraction rate of the heart (positively chronotropic) in frog heart preparation (Budhiraja and Sudhir 1987). The clinical relevance of this finding must be questioned since the amphibian cardiovascular system is considerably different than the mammalian cardiovascular system. Cardiotonic activity of two alkaloids, ashwagandhine and ashwagandhinine, reportedly from material cultivated in Madhya Pradesh was reported. The two compounds were administered to various animal models at dosages ranging from 0.1 to 8.0 mg. In the perfused frog heart, ashwagandhine elicited slight to moderate positive inotropic (and occasionally negative inotropic) activity and negative chronotropic effects. In this model, ashwagandhinine exhibited only negative inotropic effects. In the isolated rabbit heart, ashwagandhine (0.2-1.6 mg) produced a slight to moderate positive inotropic and a slight bradycardic effect. The cardioactive effect of ashwagandhinine (0.8-3.0 mg) was inconclusive since at times it produced either a slight positive or negative inotropic effect or elicited no action at all. In the anesthetized dog, ashwagandhine (1-3 mg/kg iv) produced a slight to moderate increase in ventricular contraction. Ashwagandhinine (1-5 mg/kg iv) had no effect (Das and others 1964).

Immunomodulatory Effects

Animal and In Vitro Studies

A number of studies have focused on the use of ashwagandha as an immunomodulatory agent, in general, and, specifically, its potential as an anticancer agent.

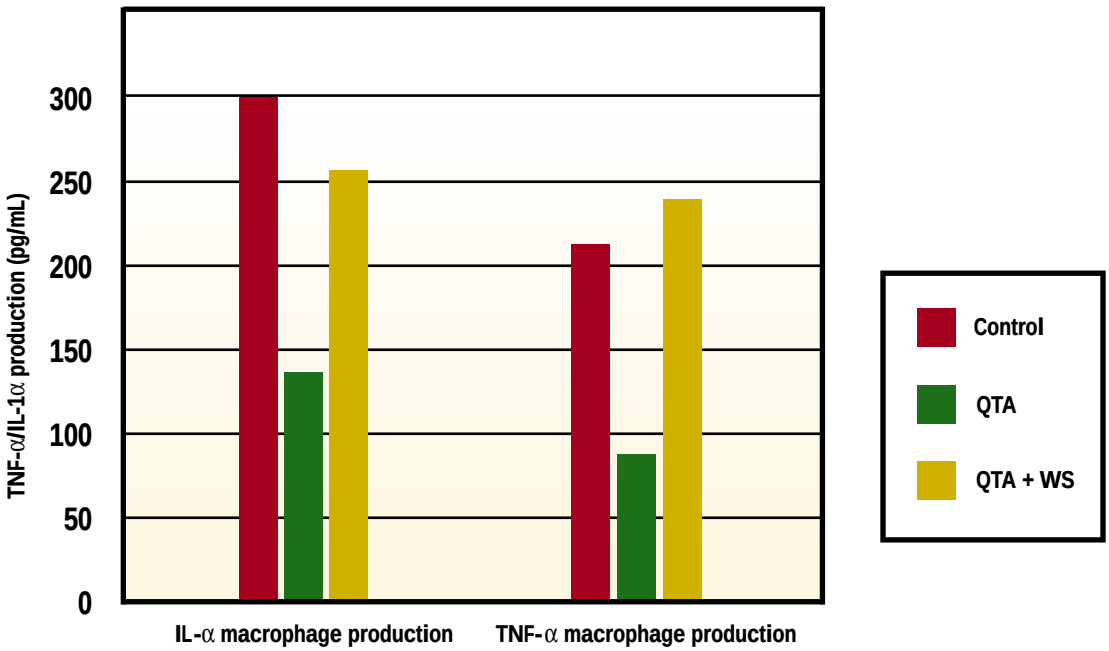
Withanolides have been shown to possess both immunosuppressive and immunopotentiating actions. Withaferin A has been found to possess immunosuppressive actions in graft-versus-host reactions and chemically induced arthritis while conversely exhibiting immunoactivating properties through a significant proliferation of macrophages in mice with Ehrlich ascites carcinoma (25-60 mg/kg ip) (Budhiraja and Sudhir 1987). Immunosuppression of B and T lymphocytes and thymocytes in mice was also reported for withaferin A and withanolide E at very low doses (undefined). The experiment reportedly demonstrated a specific action of the compounds on antigen recognition as well as B lymphocytes (Shohat and others 1978).

Sitoindosides IX and X (100-400 µg/mouse ip) have been reported to enhance the mobilization and activation of peritoneal macrophages, enhance phagocytosis, and increase the activity of lysosomal enzymes secreted by macrophages in mice (Ghosal and others 1989). Similar macrophage-stimulating activity was reported in ochratoxin A (OTA)-induced immune suppression in mice. Administration of a methanol extract (1:10) significantly inhibited OTA-induced immune suppression as measured by chemotactic activity, interleukin-1α (IL-1α) production, and tumor necrosis factor-α (TNF-α) ($P < 0.005$ when compared with the control group; $P < 0.02$ when compared with the OTA-treated group). In this study, ashwagandha administered with OTA significantly increased chemotaxis activity and macrophage IL-1α and TNF-α production as compared to the OTA group. In addition, TNF-α and chemotaxis activity were slightly higher in the ashwagandha-treated animals as compared to the controls (Table 8) (Dhuley 1997). Another study reported that administration of ashwagandha (100 mg/kg po; 7 days) improved the survival time of *Aspergillus*-infected mice. The researcher similarly postulated that antifungal activity was associated with increased macrophage activation (Dhuley 1998b).

Direct antitumor activity of injected crude ashwagandha root extract and withaferin A has been demonstrated in animal models both in vitro and in vivo (Bhatia and others 1987; Budhiraja and Sudhir 1987; Devi and others 1992). The antitumor activity of an alcohol extract was reported to be similar to both vincristine and podophyllin. In this study, an aquaeous extract was found to be ineffective and the dosages used were excessively high for normal human consumption (Devi and others 1992). Inhibition of urethane-induced lung adenomas was reported by another group of researchers. Animals treated with 125 mg/kg subcutaneously (sc) of urethane biweekly for 7 months showed signs of weight loss, increased mortality, leukopenia, and a decrease in lymphocyte percentage as compared to untreated controls. Simultaneous oral administration of 200 mg/kg daily of ashwagandha completely reversed the hematological changes and tumor formation (Singh and others 1986).

Ashwagandha has also been shown to inhibit chemotherapeutic- and radiation-induced immunosuppression. Specifically, reversal of cyclophosphamide-induced leukopenia has been demonstrated in at least 4 studies (Agarwal and others 1999; Davis and Kuttan 1998; Kuttan 1996; Ziauddin and others 1996). In one of the studies, one group of mice was treated with 10 nonlethal doses of cyclophosphamide (25 mg/kg) on consecutive days. Another group was treated with 10 consecutive doses of 20 mg/mouse of a 70% methanolic ashwagandha extract. Various hematologic parameters were monitored, including total white blood cell (WBC) count, hematopoietic activity, and hepatic enzymes. In the cyclophosphamide group, total WBC count dropped significantly. For the first 5 days an equal reduction in WBCs was observed in both the cyclophosphamide- and ashwagandha-treated groups (Table 9). At approximately day 5, the data appear to show that WBCs began to increase in ashwagandha-treated mice and were almost normalized by day 30 (the authors reported normalization at day 15). In comparison, WBCs in the cyclophosphamide group remained low at day 30. After 11 days of treatment, bone marrow cellularity was increased in the ashwagandha group (10.9×10^6) as compared to the cyclophosphamide alone group (5.6×10^6 cells/femur). After day 15, the ashwagandha group showed normal bone marrow cellularity (16.7×10^6 cells/femur) as compared to the cyclophosphamide group (13.1×10^6 cells/femur). Similar

Table 8 Effects of ashwagandha on interleukin-1α (IL-1α) and tumor necrosis factor (TNF-α) macrophage production



Source: Modified from Dhuley (1997).

Table 9 Effects of ashwagandha on total white blood cell count

Day	Total white blood cells (mm^3) cyclophosphamide group (approximates)	Total white blood cells (mm^3) ashwagandha (20 mg/mouse) group (approximates)
0	8500	8500
5	4300	4800
10	4000	5800
15	4800	7000
20	4200	7500
25	4900	8000
30	5000	8100

Source: Davis and Kuttan 1998.

increases in α-esterase activity were reported for the ashwagandha group (1800/4000 cells; $P < 0.001$) as compared to the control (normal animals; 1189/4000) and the cyclophosphamide group (687/4000). No significant changes in liver panels were observed. Other improvements in body weight and health of the intestinal villi were reported in the ashwagandha group (Davis and Kuttan 1998).

In the study by Agarwal and others (1999), cyclophosphamide-induced immunosuppression (20 mg/kg; categorized as a B-cell depleting dose) was suppressed by concomitant treatment with an unidentified fraction of ashwagandha (WS2; 300 mg/kg of extract). In the same study, cyclophosphamide-induced delayed hypersensitivity resulted in a significant reduction in WBCs and platelets. Pretreatment with one of the ashwagandha fractions (WST) inhibited this reduction but not significantly ($P < 0.1$). Both fractions (WST and WS2), when given with cyclophosphamide, increased WBCs ($P < 0.001$ and $P < 0.02$, respectively) and platelets ($P < 0.05$). The researchers concluded that ashwagandha preparations, most especially the WS2 fraction, counteract cyclophosphamide myelosuppression and have immunomodulatory activity with regard to IgE-mediated and cell-mediated hyper-reactivity (Agarwal and others 1999).

In the study of Ziauddin and others, reversal of chemotherapeutic-induced immunosuppression was similarly demonstrated (Ziauddin and others 1996). In this study, an unidentified ashwagandha preparation significantly inhibited cyclophosphamide-, azathioprine-, or prednisolone-induced myelosuppression in mice. A significant increase in hemoglobin, red and white blood cells, platelets, and body weight was reported for the treated mice as compared to the untreated animals. Another study demonstrated that ashwagandha was useful for supporting immune functioning during administration of radiation therapy. In this study, a 75% methanolic extract (10 mg) significantly increased total leukocyte count in normal Balb/c mice and reduced leukopenia induced by a single sublethal dose of γ-radiation (4 Gy). The ashwagandha extract also increased bone marrow cellularity (146.3%) and normalized the ratio of normochromatic erythrocytes and polychromatic erythrocytes in mice after radiation exposure. The researcher suggested that the primary effect was through stimulation of stem cell proliferation (Kuttan 1996).

In addition to these findings, ashwagandha has been shown to potentiate the effects of radiation therapy. Pretreatment of Chinese hamster V79 cells with withaferin A (2.1 µg/mL) prior to irradiation significantly enhanced the sensitivity of the cells to the radiation treatment resulting in an increased killing of the cells (sensitizer enhancement ratio of 1.5) (Devi and others 1996). According to the findings of another group of researchers, the effect of withaferin A in conjunction with radiation therapy was considered to be equivalent to the radio-potentiating effects of cyclophosphamide against experimental mouse tumors though, when administered alone, withaferin A was less cytotoxic than cyclophosphamide (Ganasoundari and others 1997).

In one final study, an aqueous extract of ashwagandha (100 mg/kg po) reversed cyclophosphamide-induced myelosuppression. Pretreatment with ashwagandha induced a significant leukocytosis ($P < 0.001$ as compared to controls; n = 10 each) with predominant neutrophilia ($P < 0.001$) and abolished neutropenia induced by a single dose of cyclophosphamide (Dahanukar and Thatte 1997).

Other Effects

In a study of an orally administered commercial ashwagandha root extract (100 mg/kg), researchers reported that the extract inhibited the development of tolerance to the analgesic response of morphine (10 mg/kg) and its physical dependence. The effects were observed only after 10 days of treatment. Inhibition of the development of withdrawal jumps was assessed by the naloxone test. It was concluded that ashwagandha root extract is safe in the treatment of opiate addiction (Kulkarni and Ninan 1997). Analgesic activity of ashwagandha has been reported by others (Vohora and Dandiya 1992).

Ashwagandha has been shown to stimulate the thyroid to secrete and/or synthesize thyroxine. Administration of ashwagandha root extract (1.4 g/kg by gastric intubation; 20 days) to mice increased serum 3,3',5-triiodothyronine (T3) (18%; $P < 0.05$) and tetraiodothyronine (T4) (approximately 111%; $P < 0.001$) concentrations. Iodothyronine 5'-monodeiodinase activity did not change significantly (Panda and Kar 1998).

Conclusion

Ashwagandha is one of the most valued botanicals in ayurvedic medicine. Its popular use in the United States is as an adaptogenic tonic. While it is often used singly as a tonic in India, in traditional ayurvedic medicine, it is most often used in combination with other botanicals primarily as a reproductive and rejuvenative tonic, for memory enhancement, and as an anti-inflammatory. These uses are consistent with its use by modern ayurvedic practitioners. While there are a significant number of animal and in vitro studies supporting these uses, adequate human clinical trials are lacking and are needed to accurately assess the clinical effectiveness of crude ashwagandha preparations. Similarly, the quality of the design of many of the available studies is varied, thereby making it difficult to extrapolate the findings presented to human clinical use.

The most recent investigations have focused on the potential of ashwagandha as an anticancer and immunomodulating agent with numerous activities reported. Though human clinical data are lacking, these data suggest that concomitant use of ashwagandha and chemotherapeutic drugs, such as cyclophosphamide, may prevent some of the side effects associated with conventional anticancer agents and potentiate the effects of radiation therapy. The dosages used in the majority of these studies greatly exceed the typical human dose and, at these high levels, may result in toxicity, thereby limiting the use of ashwagandha as a direct antitumor agent. Clinical investigation of its potential as an immunosupporting agent in conjunction with conventional anticancer therapies is merited.

In addition to the lack of human clinical trials, a significant limitation in evaluating the pharmacological findings reported is the lack of detailed characterization of the preparations used in the various studies. Attention has been made by most researchers to obtain authenticated plant material. However, the majority of the papers do not address either chemical or therapeutic differences between the various chemotypes that are commonly found in trade. Further research of well-characterized preparations is needed.

Actions

Adaptogen, anti-inflammatory, anti-arthritic, anxiolytic, immunomodulator, sedative, tonic.

Indications

Arthritis, anxiety, insomnia, physical debility, stress.

Substantiated Structure and Function Claim

Ashwagandha possesses general tonic and adaptogenic activity and can help to enhance the ability of an organism to adapt to various stressors (Grandhi and others 1994; Wagner and others 1994).

Dosages

Powder:

3-6 g daily (Ayurvedic Pharmacopoeia of India 1989).

Decoction:

20-30 g added to heated cow's milk (Sharma 1956).

Medicated Wine:

2 tablespoons, 2-4 times daily (Sharma 1956).

Medicated Ghee:

1 teaspoon, 2 times daily (Sharma 1956).

Medicated Oil:

Internally, 3-10 drops; or apply externally (Nadkarni 1993).

Tincture:

No standardized dosage information available.

SAFETY PROFILE

Classification of the American Herbal Products Association

Class 2b: Not to be used during pregnancy; 2d: May potentiate the effects of barbiturates (McGuffin and others 1997).

Side Effects

When used within the recommended dosage range, ashwagandha is well tolerated.

Large doses have been reported to cause irritation to mucous and serous membranes resulting in gastrointestinal upset, diarrhea, and vomiting (Chadha 1976).

Contraindications

Not to be used if experiencing stomach ulcers (Atal and Schwarting 1961).

Interactions

The chemotherapeutic drug methylthiodeacetyl colchicine has been shown to potentiate the antitumor effect of withaferin A (Kupchan and others 1965). Ashwagandha may potentiate the sedative effects of barbiturates (Atal and Schwarting 1961; Malhotra and others 1960) (Table 10). Various investigations give a variety of results. According to one report, ashwagandha produced an equivocal potentiation of barbiturates at a dose of 1 g/kg (Singh and others 1979). In another study, barbiturate potentiation was reported at doses of 300 mg/kg (Rao and Karanth 1990). Yet another study reported a decrease in barbiturate-induced sleeping time at the same dose of 300 mg/kg (Singh and others 1978).

In one study of rats, ashwagandha was shown to significantly reduce the effective dose of diazepam and clonazepam. The authors reported $GABA_a$ receptor binding (Kulkarni and others 1998).

Pregnancy, Mutagenicity, and Reproductive Toxicity

There are conflicting reports regarding the use of ashwagandha in pregnancy. Large but undefined doses have been reported to possess abortifacient activity (Chadha 1976; Svoboda 1992). Of several ayurvedic practitioners consulted, none reported having observed an abortifacient activity clinically. Conversely, ashwagandha has, traditionally and in modern ayurvedic practice, been used to prevent miscarriage and stabilize the fetus (Tirtha 1998). Specific data regarding potential mutagenicity and reproductive effects are lacking. In erring on the side of caution, it is recommended that ashwagandha not be used during pregnancy unless under the direct supervision of a qualified health professional.

Lactation

No data available.

Carcinogenicity

No data available.

Influence on Driving

No data available. Based on a review of the available literature and the experience of modern herbal practitioners, no negative effects are to be anticipated.

Precautions

Not be used during pregnancy unless under the direct supervision of a qualified health professional (see Pregnancy, Mutagenicity, and Reproductive Toxicity).

Overdose

No data available.

Treatment of Overdose

No data available.

Toxicology

Ashwagandha and its constituents have a very low toxicity. In a single human study in which 500 mg daily of an undefined ashwagandha powder extract was administered for 40 days, no adverse effects were reported (Karnick 1991).

Animal toxicity studies suggest that ashwagandha and its constituents are safe even when administered in high doses. In one acute toxicity study, the approximate LD_{50} was reported as 1750 ± 41 mg po in albino mice (weighing 20-25 g) (Singh and others 1982). In a similar study, graded doses of from 500 to 5000 mg/kg po of sitoindosides showed a relatively low level of acute toxicity with LD_{50}s of sitoindosides VII and VIII at 1076 ± 78 mg/kg and 1564 ± 92 mg/kg ip, respectively (Bhattacharya and others 1987). Another study reported no deaths of albino mice administered 1000 mg/kg po of sitoindosides IX and X. In this study, LD_{50}s of ip administrations of these compounds were reported as 518 ± 34 mg/kg and 808 ± 68 mg/kg for sitoindosides

IX and X (Ghosal and others 1989).

When the entire plant was administered as 25% of the diet of mice, researchers observed microscopic lesions as follows: centrilobular hydropic degeneration in the liver and peribronchial; perivenous edema in the lungs; and marked intertubular vascular congestion, tubular casts, and tubular degeneration in the kidneys. Although caution was recommended, these researchers concluded that the common use of ashwagandha in combination with other herbs may reduce any possible toxicity (Arseculeratne 1985).

Crude alcohol extracts of the root powder were screened for their acute (24 hours) toxicity in Swiss albino mice and subacute (30 days) toxicity in Wistar rats. Single ip injections of 1100 mg/kg of the extract were not lethal in any of the mice, whereas 100 mg incremental increases in dose produced a large increase in death rate. No animals survived after injections of 1500 mg/kg. The LD_{50} of the extract was calculated to be 1.26 g/kg body weight. A significant reduction in the weights of spleen, thymus, and adrenal glands was observed in the male rats at the end of the subacute experiment after having been administered repeated injections of ashwagandha extract at a dose of 100 mg/kg body weight. No deaths or changes in peripheral blood constituents were observed (Sharada and others 1993) (Table 11).

International Status

United States

Regulated as a dietary supplement.

India

Official in the *Ayurvedic Pharmacopoeia of India*. Required to contain not less than 0.2 % of total alkaloids (Ayurvedic Pharmacopoeia of India 1989). Listed in the *Ayurvedic Formulary of India* (Namjoshi 1972).

Table 10 **Potentiation of sedative effects of pentobarbital sodium in mice treated with ashwagandha extract**

Pentobarbital mg/100 g ip	Ashwagandha 100 g ip	Number of mice	Sedative effect % mice X^3 test	Latent period (minutes) *t* test	Sleeping time (minutes) *t* test
1.5	—	35	22.9	12.1 ± 1.0*	1.8 ± 1.7
1.5	50	12	25.0*	11.6 ± 1.7*	2.2 ± 2.1*
1.5	60	12	41.7	12.6 ± 1.4*	2.9 ± 2.4*
1.5	70	12	50.0	12.2 ± 1.6*	3.9 ± 4.9*
1.5	80	24	100.0	8.2 ± 2.2**	29.4 ± 15.0**
2.0	—	11	63.6	11.6 ± 2.5**	14.5 ± 12.2**
2.0	70	12	100.0*	7.8 ± 0.6**	30.2 ± 8.4**
2.5	—	18	88.9	9.5 ± 2.8**	29.7 ± 17.5**
2.5	70	18	100.0*	5.4 ± 0.7**	59.0 ± 30.8**
5.0	—	6	100.0	4.5 ± 2.5**	81.7 ± 26.7**
5.0	70	12	100.0	2.2 ± 0.3**	135.0 ± 30.6**

P values calculated in relation to sleep induced by pentobarbital alone; *$P < 0.05$ ** $P < 0.01$

Values are expressed as means ± SEM

Source: Modified from Malhotra and others (1960).

Table 11 **Acute toxicity (24 h mortality) of ashwagandha in Swiss albino mice**

Group	Dose mg/kg	Log dose	Dead/total	Dead %	Corrected %	Prohibit value
1	1100	3.04	0/10	0.00	2.5	3.04
2	1200	3.08	5/12	41.66	41.66	4.79
3	1300	3.11	11/15	73.33	73.33	5.63
4	1400	3.15	10/12	83.33	83.33	5.97
5	1500	3.18	10/10	100.00	97.50	6.96

Corrected formula: for the 0% dead: 100 (0.25/n); for the 100% dead: 100 (n-0.25/n)

Source: Sharada and others 1993.

Ashwagandha Root

Withania somnifera

अश्वगन्धा

THERAPEUTICS

Tastes and Properties:

Sweet, astringent, bitter; warm, heavy, moist (Kapoor 1990; Neginhal 1988).

Actions

Alterative, anti-inflammatory, antiseptic, antitussive, aphrodisiac, astringent, bitter, deobstruent, diuretic, nervine, sedative, tonic (Nadkarni 1993). Reduces vata and kapha.

Indications

Rejuvenative tonic, respiratory disorders, asthma, coughs, insomnia, nervous disorders, general debility, gynecological disorders, and male infertility.

Internal: Classically, ashwagandha is described as reducing kapha and vata and increasing pitta. It is predominantly used as a strengthening and rejuvenative tonic for all forms of weakness, fatigue, convalescing, and wasting.

For children, ghee cooked with 1/4 the amount of paste of ashwagandha and pepper taken with 10 times the amount of milk is specifically indicated for emaciation and malnourishment. For women, milk cooked with a decoction of ashwagandha and added ghee is used as a remedy for gynecological disorders, predominantly as a fertility tonic and to reduce the chance of miscarriage (Atal and Schwarting 1961; Chadha 1976). For men, ashwagandha is used as a tonic to enhance sexual vitality, treat spermatorrhea, and enhance sperm production (Atal and Schwarting 1961; Nadkarni 1993). For these purposes, the powdered root is typically administered with ghee and honey and followed with milk. Other primary uses of ashwagandha include its use for respiratory disorders such as coughs and asthma (Chadha 1976; Frawley and Lad 1986; Nadkarni 1993), arthritis, and edema.

Ashwagandha is said to nurture and clear the mind, calm and strengthen the nerves, promote a sound and restful sleep, and rejuvenate the seven bodily tissues, namely chyle; blood; flesh, muscles, and tendons; fat; bone; bone marrow and nervous tissue; and semen. It is a specific tonic for the elderly and those convalescing from chronic illness.

External: An oil is prepared and applied externally for the treatment of a sore back. The oil can also be used topically as a general external body treatment for hemiplegia. The fresh green root is reportedly reduced to a paste with cow's urine or water and applied to glandular swellings.

Ashwagandha *Withania somnifera* (L.) Dunal.

Art courtesy of Sabinsa Corporation, Piscataway, NJ

Preparations

Powder [churna]:
Mix with equal parts of ghee and honey (Nadkarni 1993) or with milk.

Decoction [kwatha]:
1 part fresh herb per 16 parts water or 1 part dried herb per 8 parts water boiled slowly until reduced to 1/4 and 1/16, respectively (Nadkarni 1993).

Medicated Wine [arishta]:
950 mL of decoction, 350 g of cane sugar (jaggery), 190 mL honey per 35 g of herb. Allow to ferment for 7-15 days (Nadkarni 1993).

Medicated Ghee [ghrita]:
1 part decoction of herbs, 10 parts milk, 1 part ghee, simmer slowly to paste (to be taken with meals) (Nadkarni 1993).

Medicated Oil [Narayana taila]:
Decoction of herbs. Add 40 parts sesame oil per 2 parts herb paste. Boil together 1 hour.

Standard Formulas

Formulas listed in the *Ayurvedic Pharmacopoeia of India* include Nyagrodhadi, Varnya, Samgrahi, Bhagnasandhanakara, Mutrasamgrahaniya (Ayurvedic Pharmacopoeia of India 1989). Ashwagandha is frequently cooked in milk with long pepper *Piper longum*, sugar or honey, and ghee.

SAFETY PROFILE

Precaution

In excess, ashwagandha may increase pitta or ama.

REFERENCES

Agarwal R, Diwanay S, Patki P, Patwardhan B. 1999. Studies on immunomodulatory activity of *Withania somnifera* (ashwagandha) extracts in experimental immune inflammation. *J Ethnopharmacol* 67:27-35.

Anbalagan K, Sadique J. 1981a. Response of α-1 globulins of serum during inflammation. *Curr Sci* 50(2):88-9.

Anbalagan K, Sadique J. 1981b. Influence of Indian medicine (ashwagandha) on acute-phase reactants in inflammation. *Indian J Exp Biol* 19:245-9.

Anbalagan K, Sadique J. 1985. *Withania somnifera* (ashwagandha), a rejuvenating herbal drug which controls α-2 macroglobulin synthesis during inflammation. *Int J Crude Drug Res* 23(4):177-83.

Anjaneyulu ASR, Rao DS. 1997. New withanolides from the roots of *Withania somnifera*. *Indian J Chem* 36B:424-33.

Archana R, Namasivayam A. 1999. Antistressor effect of *Withania somnifera*. *J Ethnopharmacol* 64:91-3.

Arseculeratne SN. 1985. Studies on medicinal plants of Sri Lanka. Part 14: toxicity of some traditional medicinal herbs. *J Ethnopharmacol* 13(3):323-35.

Atal CK. 1958. A pharmacognostic study of *Withania somnifera* Dunal. [dissertation]. Storrs (CT): Univ Connecticut. 75 p. Available from: University of Connecticut, Storrs.

Atal CK, Schwarting A. 1961. Ashwaganda: an ancient Indian drug. *Econom Bot* 15:256-63.

Ayurvedic Pharmacopoeia of India. 1989. Asvaganda. Volume 1. 1st ed. New Delhi: Government of India, Ministry of Health and Family Welfare. 233 p.

Begum V, Sadique J. 1988. Long term effect of herbal drug *Withania somnifera* on adjuvant induced arthritis in rats. *Indian J Exp Biol* 26:877-82.

Bessalle R, Lavie D, Frolow F. 1987. Withanolide Y, a withanolide from a hybrid of *Withania somnifera*. *Phytochemistry* 26(6):1797-1800.

Bhatia P, Rattan S, Cavallius J, Clark B. 1987. *Withania somnifera* (ashwagandha), a so-called rejuvenator, inhibits growth and macromolecular synthesis of human cells. *Med Sci Res* 15:515-6.

Bhattacharya SK, Goel R, Kaur R, Ghosal S. 1987. Anti-stress activity of sitoindosides VII and VIII, new acylsterylglucosides from *Withania somnifera*. *Phytother Res* 1(1):32-7.

Bhattacharya SK, Kumar A, Ghosal S. 1995. Effects of glycowithanolides from *Withania somnifera* on an animal model of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. *Phytother Res* 9(2):110-3.

Bhattacharya SK, Satyan KS, Ghosal S. 1997. Antioxidant activity of glycowithanolides from *Withania somnifera*. *Indian J Exp Biol* 35(3):236-9.

Brekhman II. 1980. Man and Biologically Active Substances, The Effect of Drugs, Diet and Pollution on Health. Oxford: Pergamon Pr.

Budhiraja RD, Sudhir S. 1987. Review of biological activity of withanolides. *J Sci Indian Res* 40:488-91.

Budhiraja RD, Sudhir S, Garg KN. 1984. Anti-inflammatory activity of 3 β-hydroxy-2,3-dihydro-withanolide F. *Planta Med* 50(2):134-6.

Chadha S, editor(s). 1976. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. Volume 10. New Delhi: Council of Scientific and Industrial Research. 591 p.

Dahanukar SA, Thatte UM. 1997. Current status of ayurveda in phytomedicine. *Phytomedicine* 4(4):359-68.

Dandiya PC, Chopra YM. 1970. CNS acting drugs from plants indigenous to India. *Indian J Pharmacol* 9(2):127.

Das PK, Malhotra CL, Prasad K. 1964. Cardiotonic activity of ashwagandhine and ashwagandhinine, two alkaloids from *Withania ashwagandha*, Kaul. *Arch Int Pharmacodyn* 150(3-4):356-62.

Davis L, Kuttan G. 1998. Suppressive effect of cyclophosphamide-induced toxicity by *Withania somnifera* extract in mice. *J Ethnopharmacol* 62:209-14.

Devi PU, Akagi K, Ostapenko V, Tanaka Y, Sugahara T. 1996. Withaferin A: a new radiosensitizer from the Indian medicinal plant *Withania somnifera*. *Int J Radiat Biol* 69(2):193-7.

Devi PU, Sharada A, Solomon F, Kamath M. 1992. In vivo growth inhibitory effect of *Withania somnifera* (ashwagandha) on a transplantable mouse tumor, sarcoma 180. *Indian J Exp Biol* 30:169-72.

Dhuley JN. 1997. Effect of some Indian herbs on macrophage functions in ochratoxin A-treated mice. *J Ethnopharmacol* 58(1):15-20.

Dhuley JN. 1998a. Effect of ashwagandha on lipid peroxidation in stress-induced animals. *J Ethnopharmacol* 60(3):173-8.

Dhuley JN. 1998b. Therapeutic efficacy of ashwagandha against experimental *Aspergillosis* in mice. *Immunopharmacol Immunotoxicol* 20(1):191-8.

Dunal MF. 1852. *Withania somnifera*. In: De Candolle, editor(s). Prodromus Systematis Naturalis Regni Vegetabilis. Volume 13. [no place of publication.] 453 p.

Frawley D, Lad V. 1986. The Yoga of Herbs. Santa Fe: Lotus. 255 p.

Ganasoundari A, Zare SM, Devi PU. 1997. Modification of bone marrow radiosensitivity by medicinal plant extracts. *Brit J Radiol* 70(834):599-602.

Ghosal S, Kaur R, Srivastava R. 1988. Sitoindosides IX and X, new glycowithanolides from *Withania somnifera*. *Indian J Nat Prod* 4(1):12-3.

Ghosal S, Lal J, Srivastava R, Bhattacharya SK, Sachidananda U, Jaiswal A, Chattopadhyay U. 1989. Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycowithanolides from *Withania somnifera*. *Phytother Res* 3(5):201-6.

Grandhi A, Mujumdar AM, Patwardhan B. 1994. A comparative pharmacological investigation of ashwaganda and ginseng. *J Ethnopharmacol* 44:131-5.

Handa SS, Chawla AS, Sharma AK. 1992. Plants with anti-inflammatory activity. *Fitoterapia* 63(1):3-31.

Hutchinson J, Dalziel JM. 1963. Flora of West Tropical Africa. Volume 2. Millbank: Crown Agents for Overseas Governments and Administrations.

Kapoor LD. 1990. CRC Handbook of Ayurvedic Medicinal Plants. Boca Raton: CRC. 416 p.

Karnick CR. 1991. A double-blind, placebo-controlled clinical study on the effects of *Withania somnifera* and *Panax ginseng* extracts on psychomotor performance in healthy Indian volunteers. *Indian Med* 3(2):1-5.

Kiritikar KR, Basu BD, An ICS. 1935. Natural Order Solanaceae. In: Blatter E, Caius JF, Mhaskar KS, editor(s). Indian Medicinal Plants. Volume 3. 2nd ed. Allahabad, India: Lalit Mohan Basu. 2393 p. Reprinted 1995.

Kulkarni SK, George B. 1996. Anticonvulsant action of *Withania somnifera* (ashwagandha) root extract against pentylenetetrazol-induced kindling in mice. *Phytother Res* 10(5):447-9.

Kulkarni SK, George B, Mathur R. 1998. Protective effect of *Withania somnifera* root extract on electrographic activity in a lithium-pilocarpine model of status epilepticus. *Phytother Res* 12:451-3.

Kulkarni SK, Ninan I. 1997. Inhibition of morphine tolerance and dependence by *Withania somnifera* in mice. *J Ethnopharmacol* 57(3):213-7.

Kulkarni SK, Sharma A, Verma A, Ticku MK. 1993. GABA receptor-mediated anticonvulsive action of *Withania somnifera* root extract. *Indian Drugs* 30:305-12.

Kupchan SM, Doskotch RW, Bollinger P, McPhail AT, Sim GA, Renauld JAS. 1965. The isolation and structural elucidation of a novel steroidal tumor inhibitor from *Acnistus arborescens*. *J Am Chem Soc* 87:5805-6.

Kuttan G. 1996. Use of *Withania somnifera* Dunal. as an adjuvant during radiation therapy. *Indian J Exp Bio* 34(9):854-9.

Lockley JS, Rees HH, Goodwin T. 1976. Biosynthesis of steroidal withanolides in *Withania somnifera*. *Phytochemistry* 15:937-9.

Majeed M. 1992. Ashwaganda: a brief review of origin, distribution, pharmacognosy, chemistry and pharmacology. Piscataway (NJ): Nutrascience. 60 p. Available from Sabinsa Corp.

Malhotra CL, Das PK, Dhalla S. 1960. Studies on withania ashwagandha. Part 1: Effect of total extract on central nervous system and smooth muscles. *Indian J Physiol Pharmacol* 4:35-48.

Malhotra CL, Mehta VL, Prasad K, Das PK. 1965. Studies on withania ashwagandha, Kaul. Part IV: The effect of total alkaloids on the smooth muscles. *Indian J Physiol Pharmacol* 9(1):9-15.

McGuffin M, Hobbs C, Upton R, Goldberg A. 1997. Botanical Safety Handbook. Boca Raton: CRC. 231 p.

Mehta AK, Binkley P, Gandhi SS, Ticku MK. 1991. Pharmacological effects of *Withania somnifera* root extract on GABA receptor-complex. *Indian J Med Res* [B] 9:312-5.

Nadkarni AK. 1993. Indian Materia Medica. Volume 1. Bombay: Popular Prakashan. 1319 p.

Namjoshi AN. 1972. The Ayurvedic Formulary of India. Part I. New Delhi: Government of India, Department of Health.

Neginhal VG. 1988. Dravyaguna Vijnana Indian Medicinal Plants. Bangalore, India: Directorate of Indian Systems of Medicine and Homeopathy, Government of Karnataka. 22 p.

Panda S, Gupta P, Kar A. 1997. Protective role of ashwagandha in cadmium-induced hepatotoxicity and nephrotoxicity in male mouse. *Curr Sci* 72(8):546-7.

Panda S, Kar A. 1998. Changes in thyroid hormone concentrations after administration of ashwagandha root extract to adult male mice. *J Pharm Pharmacol* 50(9):1065-8.

Rahman AU, Abbas S, Shahwar DE, Jamal SA, Choudhary MI. 1993. New withanolides from *Withania* spp. *J Nat Prod* 56(7):1000-6.

Rahman AU, Jamal SA, Choudhary MI, Asif E. 1991. Two withanolides from *Withania somnifera*. *Phytochemistry* 30(11):3824-6.

Ral NP, Tiwari SK, Tomar GS. 1983. Experimental studies on an indigenous drug *Withania somnifera* Dunal. in electro-induced convulsions in albino rats. *Nagarjun* 206-8.

Rao A, Karanth KS. 1990. Neuropharmacological activity of *Withania somnifera*. *Fitoterapia* 61(3):237-40.

Saksena AK, Singh SP, Dixit KS, Singh N, Seth K, Seth PK, Gupta GP. 1989. Effect of *Withania somnifera* and *Panax ginseng* on dopaminergic receptors in rat brain during stress. *Planta Med* 55(1):95.

Schliebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V. 1997. Systemic administration of defined extracts from *Withania somnifera* (Indian ginseng) and Shilagit differentially affects cholinergic but not glutamatergic and gabaergic markers in rat brain. *Neurochem Int* 30(2):181-90.

Schwarting AE, Bobbitt JM, Rother A, Atal K, Khanna KL, Leary JD, Walter WG. 1963. The alkaloids of *Withania somnifera*. *Lloydia* 26(4):258-73.

Sharada AC, Solomon FE, Devi PU. 1993. Toxicity studies on the root extract of *Withania somnifera* in rats/mice. Manipal, India: Department of Radiology, Kasturba Medical College.

Sharma PV. 1956. Dravyaguna Vigyan. Varanasi, India: Chowkhumba Orientalis. 597 p.

Shohat B, Kirson I, Lavie D. 1978. Immunosuppressive activity of two plant steroidal lactones withaferin A and withanolide E. *Biomedicine* 28(1):18-24.

Singh N, Nath R, Lata A, Singh SP, Kohli RP, Bhargava KP. 1982. *Withania somnifera* (ashwaganda), a rejuvenating herbal drug which enhances survival during stress (an adaptogen). *Int J Crude Drug Res* 20(1):29-35.

Singh N, Nath R, Singh DR, Gupta ML, Kohli RP. 1978. An experimental evaluation of protective effects of some indigenous drugs on carbon tetrachloride-induced hepatotoxicity in mice and rats. *Int J Crude Drug Res* 16(1):8-16.

Singh N, Singh SP, Nath R, Singh DR, Gupta ML, Kohli RP, Bhargava KP. 1986. Prevention of urethane-induced lung adenomas by *Withania somnifera* (L.) Dunal. in albino mice. *Int J Crude Drug Res* 24(2):90-100.

Singh RH, Malviya PC, Sarkar FH, Udupa KN. 1979. Studies on the psychotropic effect of Indian indigenous drug, asvagandha [*Withania somnifera* Dunal.]. Part II: Experimental studies. *J Res Indian Med Yoga Homeop* 14(1):49-54.

Subramanian S. 1982. Ashwaganda: an ancient ayurvedic drug. *Arogya - J Health Sci* 8:135-9.

Svoboda RE. 1992. Ayurvedic: Life, Health and Longevity. London: Arkana Penguin. 240 p.

Tirtha SS. 1998. The Ayurveda Encyclopedia. Bayville (NY): Ayurvedic Holistic Center Pr. 669 p.

Tripathi AK, Shukla Y, Kumar S. 1996. Ashwagandha [*Withania somnifera* Dunal. (Solanaceae)]: a status report. *J Med Aromatic Plant Sci* 18:46-62.

Vohora SB, Dandiya PC. 1992. Herbal analgesic drugs. *Fitoterapia* 63(3):195-207.

Wagner H, Nörr H, Winterhoff H. 1994. Plant adaptogens. *Phytomedicine* 1(1):63-76.

Watt JM, Breyer-Brandwijk MJ. 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd ed. London: Livingstone 1457 p.

Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B. 1996. Studies on the immunomodulatory effects of ashwagandha. *J Ethnopharmacol* 50(2):69-76.

Table of Contents

Nomenclature 1
Botanical Nomenclature
Botanical Family
Definition
Common Names

History 1

Identification 2
Botanical Identification
Macroscopic Identification
Microscopic Identification

Commercial Sources and Handling 6
Collection
Drying
Qualitative Differentiation
Cultivation
Storage
Adulterants
Preparations

Constituents 7

Analytical 8
Thin Layer Chromatography (TLC/HPTLC)
Qualitative Standards

Therapeutics 12
Pharmacokinetics
Pharmacodynamics
- Adaptogenic Effects
- Anti-inflammatory Effects
- Antioxidant Effects
- Central Nervous System Effects
- Cardioactive Effects
- Immunomodulatory Effects
- Other Effects
- Conclusion

Actions
Indications
Substantiated Structure and Function Claim
Dosages

Safety Profile 21
Classification of the American Herbal Products Association
Side Effects
Contraindications
Interactions
Pregnancy, Mutagenicity, and Reproductive Toxicity
Lactation
Carcinogenicity
Influence on Driving
Precautions
Overdose
Treatment of Overdose
Toxicology

International Status 22

Traditional Ayurvedic Medicine Supplement 24

References 25

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