

Adulteration of Ashwagandha (*Withania somnifera*) Roots, and Extracts

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Goal: The goal of this bulletin is to provide information and/or updates on the issue of adulteration of ashwagandha (*Withania somnifera*, Solanaceae) root materials and their extracts to the international herbal products industry and extended natural products community in general. It is intended to complement the previously published work on *W. somnifera* root and extract adulteration, i.e., the American Herbal Pharmacopoeia monograph by Upton et al.¹ and the article by Mundkinajeddu et al.² by reporting new data on the occurrence of adulteration, the market situation, and its subsequent consequences on the industry and end users.

Scope: The focus of this bulletin is on the sale of ashwagandha root powder and/or root extracts that contain undeclared ashwagandha leaf and/or stem material for the

unethical financial gain of the seller. However, low amounts (i.e., below 2%) of leaf/aerial parts in roots or root powder may be acceptable since these represent permissible contents of foreign organic matter according to pharmacopeial standards.³ In addition, ingredients and products containing mixtures of ashwagandha leaf and root and/or their extracts, where the presence of both plant parts is appropriately listed on the material's certificate of analysis and/or finished product label, are not considered adulteration and are not within the scope of this document.

1. General Information

1.1 Common name: Ashwagandha⁴

1.2 Other common names:

English: Indian ginseng*,^{5,6} winter cherry⁷

Arabic: Bahman, ubad



Ashwagandha *Withania somnifera*
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*The ABC-AHP-NCNPR Botanical Adulterants Prevention Program does not recommend the use of the inappropriate common name "Indian ginseng" (or its translation into languages other than English as noted above) in commercial trade or in the scientific or popular literature. Ashwagandha has no botanical or chemical relationship or similarity to plants that are appropriately referred to as "ginseng" in the herb trade and/or in scientific and/or popular literature, i.e., plants from the genus *Panax* (family Araliaceae). The inappropriate common names using the term 'Indian ginseng', or translations into other languages where the term 'ginseng' is used, are provided simply as a means of assisting quality control personnel et al. in identifying plant material and/or extracts that contain *W. somnifera*.

Bengali: Ashvagandha, dhuppa
Chinese: *Cui mian shui qie* (催眠睡茄), *nan fei zui qie* (南非醉茄)
Danish: Withania, blærebaeger
French: Ashwagandha, cerise d'hiver, coqueret somnifère, ginseng indien*
German: Ashwagandha, Indischer Ginseng*, Schlafbeere, Winterkirsche
Gujarati: Ghodakun
Hindi: Asgandh
Italian: Ashwagandha, ciliegia d'inverno, ginseng indiano*
Kannada: Hiremaddina gadde
Malayalam: Amukkura
Marathi: Askandha
Nepalese: Aasoganda
Norwegian: Withania, indisk ginseng*
Persian: Meheman
Punjabi: Asgand
Pustu: Kutilad
Sanskrit: Ashvagandha
Sinhalese: Amukkara
Spanish: Ashwagandha, cerezo de invierno, ginseng indiano*, oroval
Swedish: Withania, indisk ginseng*
Tamil: Amukira, amukkara, asuragandi
Telugu: Panneru gaddu
Tibetan: Ba-dzi-gandha

1.3 Latin binomial: *Withania somnifera* (L.) Dunal⁸

1.4 Synonyms: *Physalis somnifera* L., *Withania kansuensis* Kuang & A.M. Lu, *Withania microphysalis* Suess⁸

1.5 Botanical family: Solanaceae

1.6 Plant part and form: The part used is the dried root, traditionally used as a powder. Much of the *W. somnifera* in the current market is being supplied to herbal products and dietary supplement manufacturers in the form of a dry extract. In most cases, the extract yield is approximately 10 times lower than the

initial weight of raw material; i.e., 1 kg of dried root yields 100 g of *W. somnifera* root extract. The extract typically contains steroidal lactones called withanolides in concentrations between 1.5-5.0% (w/w) in the extract.

1.7 General use(s):

In Ayurveda, ashwagandha is claimed to have aphrodisiac, sedative, anxiolytic, rejuvenating, and life-prolonging properties. According to *The Ayurvedic Pharmacopoeia of India*, the powdered dried root of ashwagandha is used to treat inflammatory disorders, phthisis (any wasting or atrophic disease, weakness, diseases associated with *vata dosha* [a body type, or constitution in Ayurveda]), and male impotence.⁹ It is one of the most important herbs of Ayurveda used for millennia as a *Rasayana* for its wide ranging health benefits. *Rasayana* is described as a preparation that promotes a youthful state of physical and mental health, and expands happiness. These types of remedies are given to small children as tonics, and are also taken by the middle-aged and elderly to increase longevity. The *Charaka Samhita*, a Sanskrit text on Ayurveda, classifies ashwagandha as *Balya* (promoter of strength).¹⁰ It is also used as a general energy-increasing tonic known as *Medharasayana* (promotes learning and memory function) and in geriatric problems.¹¹ The root of the plant has been traditionally used to promote youthful vigor, endurance, strength, health, and increasing the production of vital fluids, muscle fat, blood, lymph, semen, and cells.¹²

2. Market

2.1 Importance in the trade: In the US market, the vast majority of ashwagandha supplements are sold in the Natural Channel. Sales in the two major retail channels combined have steadily increased from an estimated US \$4.53 million in 2014 to an estimated \$12.24 million in 2017, corresponding to an annual sales increase of ca. 39% (Table 1).

2.2 Market dynamics: While ashwagandha has a long-standing history of use in traditional Indian medicine systems, interest in the benefits of ashwagandha roots and root extracts in Australia, Europe, and North America has only recently started to emerge. Sales of ashwagandha dietary supplements have seen double-digit growth over the

Table 1. Ashwagandha Dietary Supplement Sales in the US from 2012-2017

Channel	2014		2015		2016		2017	
	Rank	[US\$]	Rank	[US\$]	Rank	[US\$]	Rank	[US\$]
Natural ^a	22	3,765,921	12	5,722,569	8	8,732,489	6	10,625,382
MMO ^{b,c}	71	767,242	60	556,739	63	908,643	51	1,611,915

^a According to SPINS (SPINS does not track sales from Whole Foods Market.)

^b According to SPINS/IRI (The Mainstream Multi-Outlet [MMO] channel was formerly known as the Food, Drug, and Mass Market channel [FDM], exclusive of possible sales at Walmart, a major retailer in the United States and beyond.)

Sources: T. Smith (American Botanical Council) e-mail to S. Gafner, September 2, 2015, September 3, 2015, and June 19, 2018. K. Kawa (SPINS) e-mail to S. Gafner, July 11, 2016.

past years (Table 1). Current growth is said to be driven by the increased awareness of benefits, such as stress relief and increase in energy, and the increased support of benefits from published clinical studies.¹³ The increasing demand has created pressure for increased cultivation, which is lagging behind the demand, according to a 2015 review article on conservation and sustainability of the plant.¹⁴

2.3 Supply sources: *Withania somnifera* is native to India and the Mediterranean region in North Africa, and it is widely distributed in Pakistan, Sri Lanka, South Africa, Iraq, Iran, Syria, and Turkey. In India, ashwagandha is commercially cultivated in Madhya Pradesh, Gujarat, Maharashtra, Rajasthan, Haryana, Punjab, Karnataka, and Uttar Pradesh provinces. In the Neemuch and Mand-saur districts of Madhya Pradesh province alone the cultivated area exceeds 5000 hectares (ha) and, in India overall, approximately 10,770 ha of land are used to grow ashwagandha with an annual production of 8429 metric tons.^{14,15} In October 2018, costs for high quality dried ashwagandha roots varied between US \$2.46 – 3.56 on the Indian market (although roots considered as lower grades, known as *tar*, were sold for as little as US \$1.50), compared to US \$0.34 – 0.82 for dried ashwagandha leaves. (A. Agarwal personal knowledge)

2.4 Raw material forms: Most companies manufacture their own *W. somnifera* root extract from dried roots, which is in agreement with the use of ashwagandha in traditional Ayurvedic medicine. Consistent with this, ashwagandha use is described primarily for its root in national pharmacopeias, such as the *United States Pharmacopeia* (USP),³ the *British Pharmacopoeia*,¹⁶ the *Indian Pharmacopoeia*,¹⁷ the *Ayurvedic Pharmacopoeia*,⁹ and reference works like the World Health Organization monograph.¹⁸ In addition, a majority of the published clinical studies have been carried out with ashwagandha root materials. Some proprietary ashwagandha ingredients appropriately labeled to contain the extracts of aerial parts, leaves, or combinations of roots and other plant parts are available on the North American market, and elsewhere. Obviously, such materials, transparently and appropriately labeled, can be legally marketed as ashwagandha supplements in the context discussed in this publication.

3. Adulteration

3.1 Known adulterants: Undisclosed non-root parts of *W. somnifera*, such as leaves, stem, and aerial parts of ashwagandha, which are rich in withaferin A as well as other withanolides.

3.2 Information confirming adulteration: The quality evaluation of ashwagandha raw materials and finished products has been the subject of three papers.^{2,19,20} Sangwan et al. found concentrations between 0.02 and 2.34 mg withaferin A per gram of ashwagandha and highly variable chemical fingerprints in 10 commercial products provided by dietary supplement manufacturers in India. The authors commented that some of the results could be due in part to



Ashwagandha *Withania somnifera*
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“unregulated and often non-descript supplementation” of the root.¹⁹ Mundkinajeddu et al. used high-performance liquid chromatography with UV detection (HPLC-UV) to analyze authenticated samples of *W. somnifera* leaves (n = 5), aerial parts (n = 3), and roots (n = 17), which were obtained from India and Egypt.² In addition, 10 commercial extract samples labelled as “derived from the roots” were analyzed for the presence of flavonol glycosides (Figure 1), which are markers for adulteration with aerial parts. It was observed that only two of the commercial extract samples did not contain any of the marker compounds for aerial parts, indicating that aerial parts are sometimes used as adulterants in ashwagandha root extracts. However, verification of 28 samples of whole roots in the Indian state of Kerala did not find any evidence of adulteration. The authors reported that occurrence of mold on the root surface was a common problem.²⁰ Data on identity testing of 584 commercial raw material samples of ashwagandha root (Alkemist Laboratories; Costa Mesa, CA) by high-performance thin-layer chromatography (HPTLC) using the conditions outlined in Figure 2 showed that 119 samples (20.4%) were not composed solely of authentic root material. Sample rejection was due to the presence of leaf material in 84 samples (14.0%). (S. Sudberg [Alkemist Laboratories], email communication to S. Gafner, May 12, 2017) Finally, adulteration with ashwagandha leaf extracts was also mentioned by Shaheen Majeed (Sabinsa Corporation, East Windsor,

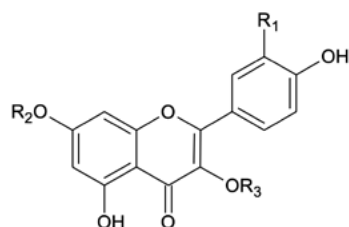


Figure 1: Principal flavonol glycosides in ashwagandha aerial parts

- 1: R₁ = OH, R₂ = glucose, R₃ = rhamnose-(1→6)-galactose
- 2: R₁ = OH, R₂ = glucose, R₃ = rhamnose-(1→6)-glucose
- 3: R₁ = H, R₂ = glucose, R₃ = rhamnose-(1→6)-galactose

NJ) who commented that “*Withania* harvested roots mixed with aerial parts and other plant parts have become a major concern.”¹³

3.3 Accidental or intentional adulteration: The motivation behind adulteration in commercial products is financial gain. Since ashwagandha has seen a steady increase in sales, there is more global demand for its roots. This has led to a considerable increase in costs of roots, compared to the lower-cost aerial parts, which, as noted above, also contain withanolides. Larger amounts of aerial parts

adulteration can happen at harvesting stage by the farmers as some may not be aware of differences in the constituents and the importance of using roots only rather than aerial parts. While cutting aerial parts during the root harvesting process, they may cut the roots too far aboveground, leading to raw materials that contain a small portion of aerial parts.

3.4 Frequency of occurrence: The limited data available do not provide clarity about the extent of adulteration. Ganzera et al. used HPLC-UV to analyze six commercial products purchased in the United States. Two products had higher contents of withaferin A, the compound found at high concentrations in ashwagandha leaves, but the authors gave “seasonal variations, a different extraction procedure applied by the manufacturer or different chemotypes of the plant” as possible reasons for the difference.²¹

Mundkinajeddu et al.² analyzed 10 commercial samples labeled as “derived from the roots” obtained from vendors and dietary ingredient manufacturers from India for the presence of flavonol glycosides (Figure 1) and concluded that eight products (80%) were found to be adulterated with aerial parts. Another study, where four herbal products (obtained from local stores in Coimbatore, India) labeled to contain crude ashwagandha were evaluated using DNA barcoding (*rbcL* and ITS2 gene regions were amplified), did not provide any evidence for adulteration with plant material from a plant in a genus other than *Withania*. Ashwagandha DNA sequences were obtained from three samples; the fourth sample

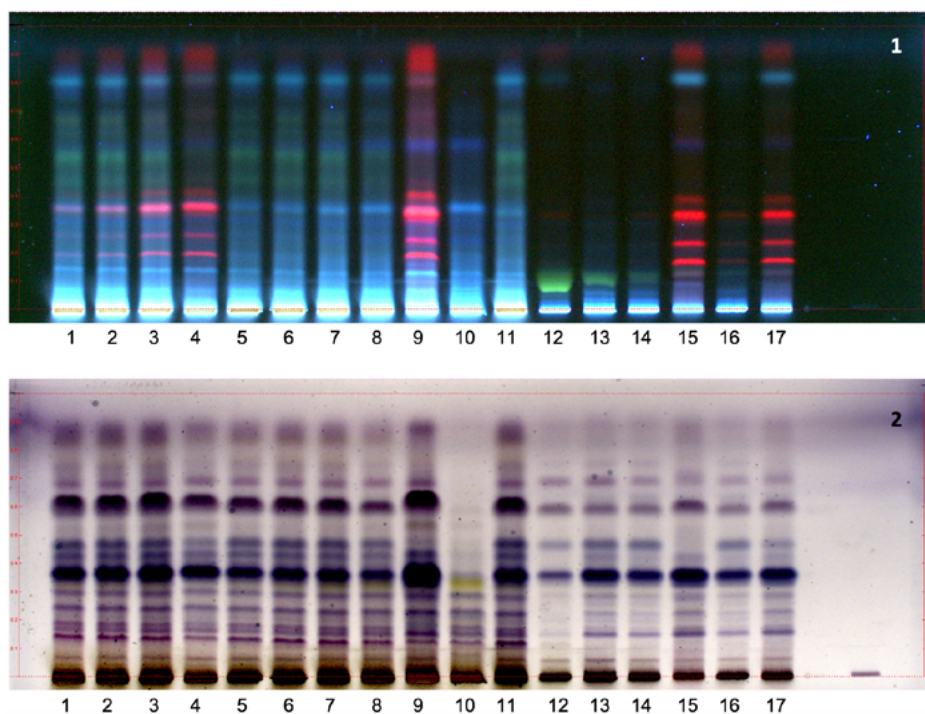


Figure 2. HPTLC analysis of ashwagandha root, aerial parts, and leaf extracts, and mixtures of root and leaf extracts.

Lanes 1-4: 80% aqueous methanol extracts of roots and aerial parts, mixed at 19:1, 9:1, 3:1, and 1:1 ratios, respectively; lanes 5-8: 80% aqueous methanol extract of roots and water extract of aerial parts, mixed at 19:1, 9:1, 3:1, and 1:1 ratios, respectively; lane 9: 80% aqueous methanol extract of the aerial parts; lane 10: water extract of the aerial parts; lanes 11-14: 80% aqueous methanol extracts of roots; lane 15: 80% aqueous methanol extract of leaves; lanes 16 and 17: 80% aqueous methanol extracts of roots and leaves, mixed at 19:1, and 1:1 ratios, respectively.

Stationary phase: Silica gel 60, F₂₅₄, 10 x 10 cm HPTLC plates.

Mobile phase: Methylene chloride-methanol-acetone-diethyl ether [100:6.5:6.5:6.5] (v/v/v/v)

Detection: (1) UV at 365 nm

(2) Vanillin/sulfuric acid reagent (after drying at 110°C for 5 min), observed under white light

yielded only a short sequence using the *rbcL* primer, and no sequence at all with ITS2.²² Since genetic methods are unable to differentiate plant parts, the data from the DNA barcoding study do not provide evidence that any of the four samples were made solely from ashwagandha root material. Based on the practical experience of the authors, occurrence of accidental adulteration appears to be low.

3.5 Possible therapeutic issues: The use of *Withania* leaf extracts appears to be safe. Data from studies with a product containing root and leaf extracts showed mild and transient adverse events similar to those observed with placebo.^{23,24}

3.6 Analytical methods to detect adulteration: Documentary standards on ashwagandha have been published by the American Herbal Pharmacopoeia,¹ *Ayurvedic Pharmacopoeia of India*,⁹ *Siddha Pharmacopoeia of India*,²⁵ *Unani Pharmacopoeia of India*,²⁶ the World Health Organization (WHO) Monographs,¹⁸ as well as in the *British Pharmacopoeia*,¹⁶ *Indian Pharmacopoeia*,¹⁷ and *United States Pharmacopoeia*.³ These standards cover microscopic, macroscopic, high-performance thin-layer chromatography (HPTLC), and high-performance liquid chromatography (HPLC) methods for identification of ashwagandha roots and quantification of withanolides. Parts of *Withania somnifera* other than roots (e.g., stems, leaves) which have been used as adulterants, can be identified when present in crude powdered form using microscopic analysis.²⁷

An HPTLC method for the detection of withanolides is listed also in the American Herbal Products Association's Botanical Identity References Compendium.²⁸ Higher relative concentrations of withaferin A have been reported in the leaves (typically >100 times than roots) and stems (typically >10 times than roots).²⁹ This could be used as an indicator of the presence of undeclared aerial parts of *W. somnifera* in roots. However, reliance on withanolides as chemical markers to distinguish plant parts is hampered by the occurrence of several ashwagandha chemotypes with differing withanolide patterns.^{30,31} Therefore, Mundkinajeddu et al. developed an HPLC method for simultaneous determination of the three flavonoid glycosides quercetin 3-*O*-robinobioside-7-*O*-glucoside (1), quercetin 3-*O*-rutinoside-7-*O*-glucoside (2), and kaempferol 3-*O*-robinobi-

oside-7-*O*-glucoside (3) which occur only in the aerial parts of *W. somnifera*.²

A limit test for flavonol glycosides has been included as an addition the USP monographs on Powdered Ashwagandha Root and Powdered Ashwagandha Root Extract, requiring contents to be no more than 0.01% for powdered root, and 0.04% for extracts. A clear distinction between leaf and root is also possible using ¹H NMR-based chemometric methods.^{32,33} The two main metabolites that separate root samples from other plant parts in 50% aqueous methanol extracts were identified as sucrose and γ -aminobutyric acid.³¹ DNA-based methods, such as those reported by Shanmughanandhan et al.,²² are not appropriate for the detection of root adulteration with various ashwagandha plant parts since there are no validated genetic methods available to distinguish plant parts at this time.

Adulteration with leaf material can also be detected by HPTLC. Figure 2 shows HPTLC profiles for authenticated *W. somnifera* root extracts, as well as root extract samples adulterated with methanol and water extracts of the aerial parts. Based on the data, admixture of as little as 5% methanol extracts of the aerial parts can be detected by the presence of red bands (due to chlorophyll pigments) at *R_f* = 0.2-35 and 0.9, but not for aqueous extracts of aerial parts. The same samples were analyzed by HPLC (Figure 3) using the method by Mundkinajeddu et al.,² where the presence of flavonol glycosides 1-3 in both the methanolic extract and the water extract of the aerial parts was observed, thus confirming the presence of the adulterant.

3.7 Perspectives: *Withania somnifera* extracts are often "standardized" on the basis of their withanolide content,

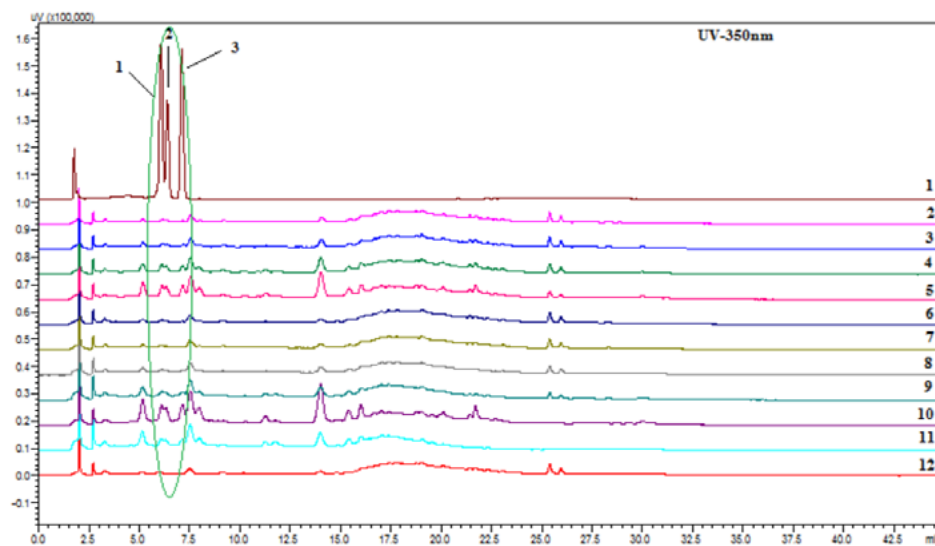


Figure 3: Representative HPLC at 350 nm chromatograms of a standard mixture of quercetin 3-*O*-robinobioside-7-*O*-glucoside (1), quercetin 3-*O*-rutinoside-7-*O*-glucoside (2), and kaempferol 3-*O*-robinobioside-7-*O*-glucoside (3, lane 1); mixtures of ashwagandha 80% aqueous methanol extracts of roots and aerial parts (lanes 2-5); mixtures of ashwagandha 80% aqueous methanol extract of roots and water extract of aerial parts (lanes 6-9); 80% aqueous methanol extract of the aerial parts (lane 10); water extract of the aerial parts (Lane 11); and 80% aqueous authentic root extract (lane 12).

which are present in aerial parts as well as roots. This has opened the possibility of undisclosed extracts from aerial parts being used for intentional adulteration of *W. somnifera* root extract. There are quality dietary supplements where the addition of ashwagandha leaves, stems, and aerial parts to *W. somnifera* root extracts is appropriately labeled. However, the undeclared addition of various plant parts other than the root with the sole intention of making a greater profit for the seller is considered unethical and fraudulent. The expected increase in the global demand for *W. somnifera* root extracts in the coming years may further exacerbate pressures on the supply chain and pricing, and hence, increase the risk of adulteration.

4. Conclusions

The adulteration of *W. somnifera* root extract by adding undeclared extracts from aerial parts of the plant to commercial products continues to provide potentially less value to the end user and impact the reputation of the botanicals and natural products industry. This practice has a considerable adverse impact on companies that sell genuine root material and extract, because of the availability of lower-cost adulterated materials and extracts. Validated analytical methods that enable detection of this type of adulteration are available and should be adopted in every quality control laboratory. The implementation of such validated methods by ethical suppliers and botanical product manufacturers will provide appropriate testing data, as opposed to non-specific spectrophotometric methods that often provide misleading results with regard to the proper identity and authenticity of *W. somnifera* root raw materials and extracts.

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REVISION SUMMARY

Version # , Author,	Date Revised	Section Revised	List of Changes
Version 1, V.K. Singh, D. Mundkinajeddu, A. Agarwal, J. Nguyen, S. Sudberg, S. Gafner, M. Blumenthal	n/a	n/a	none

Official Newsletter of the ABC-AHP-NCNPR Botanical Adulterants Program

Wide Range of Useful News on Botanical Adulteration:

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- New Science Publications
- New Analytical Methods
- Regulatory Actions
- Upcoming Conferences & Webinars

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