Ashwagandha (Withania Somnifera)

Aswagandha In Ayurveda

- In Ayurveda, Withania is widely claimed to have potent aphrodisiac, sedative, rejuvenative and life prolonging properties. It is also used as a general energy-enhancing tonic known as Medharasayana, which means 'that which promotes learning and a good memory' and in geriatric problems .

- The plant was traditionally used to promote youthful vigor, endurance, strength, and health, nurturing the time elements of the body and increasing the production roduction of vital fluids, muscle fat, blood, lymph, semen and cells.

- The similarity between these restorative properties and those of ginseng roots has led to Ashwagandha roots being called Indian ginseng .

- It also helps counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature ageing, emaciation, debility, and muscle tension.

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Aswagandha In Ayurveda

- It also helps counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature ageing, emaciation, debility, and muscle tension.
- The leaves of the plant are bitter in taste and used as an a Anthelmintics the infusion is given in fever. Bruised leaves and fruits are locally applied to tumors and tubercular glands, carbuncles and ulcers .
- The roots are used as a nutrient and health restorative in pregnant women and old people.
- The decoction of the root boiled with milk and ghee is recommended for curing sterility in women.
- The roots are also used in constipation, senile debility, rheumatism, general debility, nervous exhaustion, loss of memory, loss of muscular energy and spermatorrhoea.

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Active Constituents

- The plant contains a range of different classes of chemical constituents such as alkaloids, steroidal lactones, and flavonoids.

- All chemicals listed pertain to the root unless otherwise specified, as the root is the part used.

- Anaferine (Alkaloid), Anahygrine (Alkaloid), Beta-Sisterol, Chlorogenic acid (in leaf only), Cysteine (in fruit), Cuscohygrine (Alkaloid), Iron, Pseudotropine (Alkaloid), Scopoletin, Somniferinine (Alkaloid), Somniferiene (Alkaloid), Tropanol (Alkaloid), Withanine (Alkaloid), Withananine (Alkaloid) and Withanolides A-Y(Steroidal lactones).

- The main constituents of ashwagandha are **alkaloids** and **steroidal lactones**. Among the various alkaloids, **withanine** is the main constituent. The other alkaloids are somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropane, choline, cuscohygrine, isopelletierine, anaferine and anahydrine. Two acyl steryl glucoside viz. **Sitoindoside VII** and **sitoindoside VIII** have been isolated from **root**. The **leaves** contain **steroidal lactones**, which are commonly called **withanolides**. The withanolides have C28 steroidal nucleus with C9 side chain, having six membered lactone ring.

Accumulation Of Three Important Bioactive Compounds In Different Plant Parts Of Withania Somnifera And Its Determination By The LC-ESI-MS-MS (MRM) Method

- A comprehensive experiment was conducted to study the accumulation pattern and determination of three important bioactive compounds namely **withaferin-A (WA), 12-deoxywithastramonolide (WO) and withanolide-A (WD)** and its determination by the liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS) method in root, stem, fruits and leaves of Withania somnifera. A rapid and sensitive LC-ESI-MS-MS method was developed and validated for the determination of these three important bioactive compounds, having same molecular weight.

- The multiple reaction monitoring method was established by two transitions for each analyte and intense transition used for quantification. Separation of the three analytes was achieved within a run time of 5 min on an RP-18 column using a mobile phase consisting of acetonitrile and 0.1% acetic acid in water in an isocratic condition.

- The developed method was validated as per the ICH guidelines. The developed method was found to be suitable for identification and quantification of WA, WO and WD in different plant parts such as roots, stems, fruits and leaves of W. somnifera. The accumulation of WA was highest in leaves samples (8.84 ± 0.37 mg/g) and it was 2.23, 5.85 and 27.26 times higher than its concentration in fruits, stems and roots, respectively. WO and WD contents were highest (0.44 ± 0.016 and 0.72 ± 0.016 mg/g, respectively) in root.

Chemical Constituents

- The chemistry of Withania species has been extensively studied and several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids, tannin etc. have been identified, extracted, and isolated .At present, more than 12 alkaloids, 40 withanolides, and several sitoindosides (a withanolide containing a glucose molecule at carbon 27) have been isolated and reported from aerial parts, roots and berries of Withania species. The major chemical constituents of these plants, withanolides, are mainly localized in leaves, and their concentration usually ranges from 0.001 to 0.5% dry weight (DW) .

- The withanolides are a group of naturally occurring C28-steroidal lactones built on an intact or rearranged ergostane framework, in which C-22 and C-26 are appropriately oxidized to form a six-membered lactone ring. The basic structure) is designated as the withanolide skeleton.

- Withaferin A (4β,27-dihydroxy-1-oxo-5β,6β-epoxywitha-2-24-dienolide, was the first member of this group of compounds to be isolated. Withaferin A is totally absent in roots, stems, seeds and persistent calyx of fruits of intact plants but present in leaves . Today over 130 withanolides from Solanaceae genera are known, mostly occurring in free form, but in a few cases also as glycosides.

Other Compounds

- Examination of W. somnifera roots has resulted in the isolation of a new dimeric thiowithanolide, named ashwagandhanolide. A bioassay-guided purification of the methanolic extract of W. somnifera fruits yielded withanamides A-I .

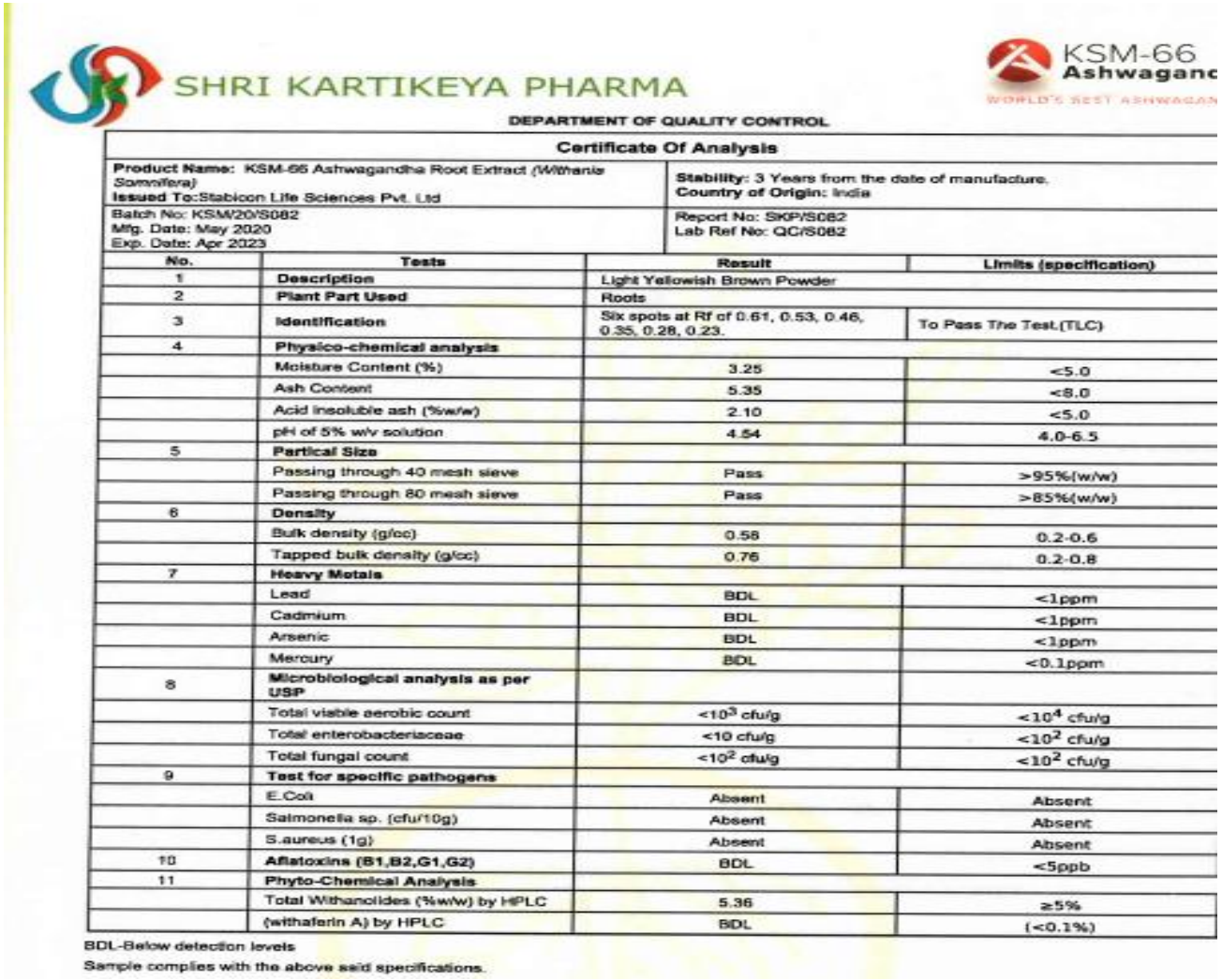
- In their quantitative analysis of Indian chemotypes of W. somnifera by TLC densitometry, Gupta et al. detected alkaloids in all the abovementioned plant parts, with the highest content found in leaves. This is in contrast to the general belief that tropane alkaloids are restricted to the roots of Withania spp. Extraction with 45% alcohol yields the highest percentage of alkaloids.

- The isolation of nicotine, somniferine, somniferinine, withanine, withananine, pseudowithanine, tropine, pseudotropine, 3α- tigloyloxytropane, choline, cuscohygrine, dl-isopelletierine and new alkaloids anaferine and anhygrine has been described. The reported total alkaloid content in the roots of Indian W. somnifera varies between 0.13 and 0.31%, though much higher yields (up to 4.3%) have been recorded in plants of other regions/countries. In addition to the alkaloids, the roots are reported to contain starch, reducing sugars, hentriacontane, glycosides, dulcitol, withanicil, an acid and a neutral compound. The leaves are reported to contain five unidentified alkaloids (yield 0.09%), chlorogenic acid, calystegines (nitrogen-containing polyhydroxylated heterocyclic compounds) withanone, condensed tannin and flavonoids. The berries have amino acids. Four types of peroxidases have been purified and characterized from W. somnifera roots .

https://www.mdpi.com/1420-3049/14/7/2373/htm

Market Sample-COA

Plant Part Used: Root



SHRI KARTIKEYA PHARMA

KSM-66 Ashwagand

DEPARTMENT OF QUALITY CONTROL

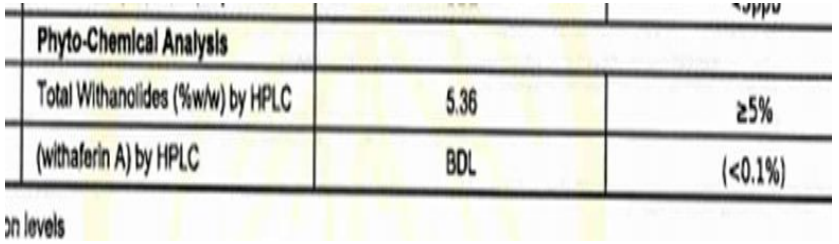
Certificate Of Analysis

Product Name: KSM-66 Ashwagandha Root Extract (*Withania Somnifera*) **Issued To:** Stabicon Life Sciences Pvt. Ltd	**Stability:** 3 Years from the date of manufacture. **Country of Origin:** India
Batch No: KSM/20/S082 Mfg. Date: May 2020 Exp. Date: Apr 2023	Report No: SKP/SC82 Lab Ref No: QC/S082

No.	Tests	Result	Limits (specification)
1	**Description**	Light Yellowish Brown Powder	
2	**Plant Part Used**	Roots	
3	**Identification**	Six spots at Rf of 0.61, 0.53, 0.46, 0.35, 0.28, 0.23.	To Pass The Test.(TLC)
4	**Physico-chemical analysis**		
	Moisture Content (%)	3.25	<5.0
	Ash Content	5.35	<8.0
	Acid insoluble ash (%w/w)	2.10	<5.0
	pH of 5% w/v solution	4.54	4.0-6.5
5	**Partical Size**		
	Passing through 40 mesh sieve	Pass	>95%(w/w)
	Passing through 80 mesh sieve	Pass	>85%(w/w)
6	**Density**		
	Bulk density (g/cc)	0.58	0.2-0.6
	Tapped bulk density (g/cc)	0.76	0.2-0.8
7	**Heavy Metals**		
	Lead	BDL	<1ppm
	Cadmium	BDL	<1ppm
	Arsenic	BDL	<1ppm
	Mercury	BDL	<0.1ppm
8	**Microbiological analysis as per USP**		
	Total viable aerobic count	$<10^3$ cfu/g	$<10^4$ cfu/g
	Total enterobacteriaceae	<10 cfu/g	$<10^2$ cfu/g
	Total fungal count	$<10^2$ cfu/g	$<10^2$ cfu/g
9	**Test for specific pathogens**		
	E.Coli	Absent	Absent
	Salmonella sp. (cfu/10g)	Absent	Absent
	S.aureus (1g)	Absent	Absent
10	**Aflatoxins (B1,B2,G1,G2)**	BDL	<5ppb
11	**Phyto-Chemical Analysis**		
	Total Withanolides (%w/w) by HPLC	5.36	≥5%
	(withaferin A) by HPLC	BDL	(<0.1%)

BDL-Below detection levels

Sample complies with the above said specifications.



Phyto-Chemical Analysis		
Total Withanolides (%w/w) by HPLC	5.36	≥5%
(withaferin A) by HPLC	BDL	(<0.1%)

on levels

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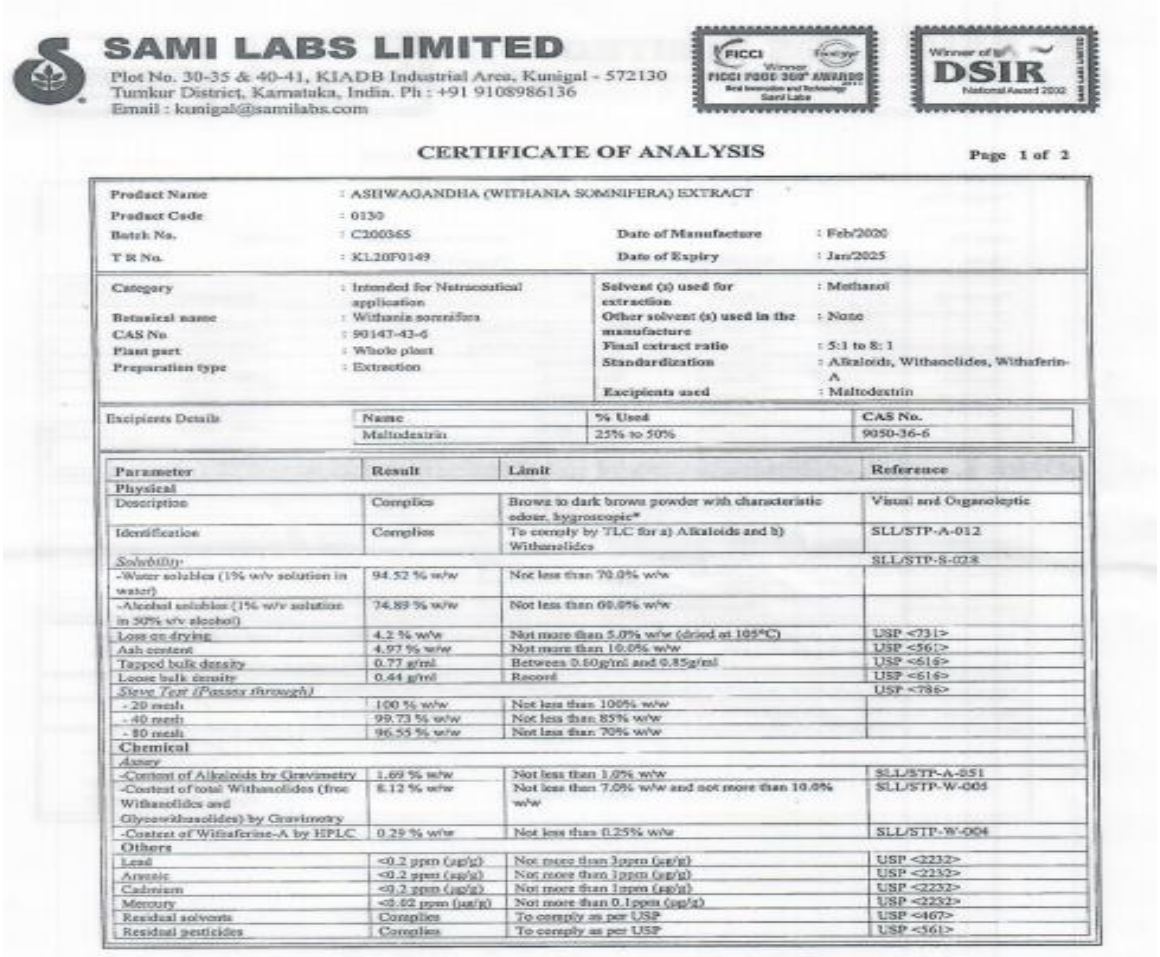
Plant Part: Whole plant



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CERTIFICATE OF ANALYSIS

Page 1 of 2

Product Name	: ASHWAGANDHA (WITHANIA SOMNIFERA) EXTRACT		
Product Code	: 0130		
Batch No.	: C200365	Date of Manufacture	: Feb/2020
T R No.	: KL20F0149	Date of Expiry	: Jan/2025
Category	: Intended for Nutraceutical application	Solvent (s) used for extraction	: Methanol
Botanical name	: Withania somnifera	Other solvent (s) used in the manufacture	: None
CAS No	: 90147-43-6	Final extract ratio	: 5:1 to 8: 1
Plant part	: Whole plant	Standardization	: Alkaloids, Withanolides, Withaferin-A
Preparation type	: Extraction	Excipients used	: Maltodextrin

Excipients Details	Name	% Used	CAS No.
	Maltodextrin	25% to 50%	9050-36-6

Parameter	Result	Limit	Reference
Physical			
Description	Complies	Brown to dark brown powder with characteristic odour, hygroscopic*	Visual and Organoleptic
Identification	Complies	To comply by TLC for a) Alkaloids and b) Withanolides	SLL/STP-A-012
Solubility			SLL/STP-S-028
-Water solubles (1% w/v solution in water)	94.52 % w/w	Not less than 70.0% w/w	
-Alcohol solubles (1% w/v solution in 90% v/v alcohol)	74.89 % w/w	Not less than 60.0% w/w	
Loss on drying	4.2 % w/w	Not more than 5.0% w/w (dried at 105°C)	USP <731>
Ash content	4.97 % w/w	Not more than 10.0% w/w	USP <561>
Tapped bulk density	0.77 g/ml	Between 0.60g/ml and 0.85g/ml	USP <616>
Loose bulk density	0.44 g/ml	Record	USP <616>
Sieve Test (Passes through)			USP <786>
- 20 mesh	100 % w/w	Not less than 100% w/w	
- 40 mesh	99.73 % w/w	Not less than 85% w/w	
- 80 mesh	96.55 % w/w	Not less than 70% w/w	
Chemical			
Assay			
-Content of Alkaloids by Gravimetry	1.69 % w/w	Not less than 1.0% w/w	SLL/STP-A-051
-Content of total Withanolides (free Withanolides and Glycowithanolides) by Gravimetry	8.12 % w/w	Not less than 7.0% w/w and not more than 10.0% w/w	SLL/STP-W-005
-Content of Withaferine-A by HPLC	0.29 % w/w	Not less than 0.25% w/w	SLL/STP-W-004
Others			
Lead	<0.2 ppm (µg/g)	Not more than 3ppm (µg/g)	USP <2232>
Arsenic	<0.2 ppm (µg/g)	Not more than 3ppm (µg/g)	USP <2232>
Cadmium	<0.2 ppm (µg/g)	Not more than 1ppm (µg/g)	USP <2232>
Mercury	<0.02 ppm (µg/g)	Not more than 0.1ppm (µg/g)	USP <2232>
Residual solvents	Complies	To comply as per USP	USP <467>
Residual pesticides	Complies	To comply as per USP	USP <561>

Assay		
-Content of Alkaloids by Gravimetry	1.69 % w/w	Not less than 1.0% w/w
-Content of total Withanolides (free Withanolides and Glycowithanolides) by Gravimetry	8.12 % w/w	Not less than 7.0% w/w and not more than 10.0% w/w
-Content of Withaferine-A by HPLC	0.29 % w/w	Not less than 0.25% w/w

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Withanolides

- About 35 withanolides have been isolated from the roots of *W. somnifera* of which withanoside V (WS V), withaferin A, withanolide A (WN A) and withanolide B (WN B) are the major components. Withaferin A has anti-tumor, apoptotic, anti-angiogenesis, radiosensitizing and anti-inflammatory activities. WN A is effective as a neurological, immunological and anti-stress agent. WS IV and V play an important neuro-regenerative role. Thus in spinal cord injury WS IV and V improve hindlimb function and increase the myelin layer in the peripheral nervous system. In the light of this, it is important to find out the bioavailability of the individual withanolide compounds to ascertain their therapeutic efficacy.

CONCLUSIONS

- Based on the present studies on the absorption characteristics of the tested withanolides it may be concluded that WN A, WNN, 1,2 DWM and WN B were highly permeable; whereas WS IV, and WSV showed low permeable. Surprisingly WF A, the highly biologically active withanolide was found to be either impermeable or metabolized on passing through the cell layer. It is likely that absorption of WFA in vivo is a complex process and possibly a system employing Caco-2 cells could provide better insight in the absorption characteristics of WFA.

Withanolides

- Withanolides have received significant attention due to their versatile biological activities demonstrated in vitro and/or in vivo. These activities have been described as antitumor, cytotoxic ,apoptotic, anti-inflammatory , immunomodulating , antimicrobial , antistress , antioxidant , anti-neurodegenerative , radiosensitizing , and insect antifeedant . Withaferin A, the most studied withanolide, possesses a wide array of the pharmacological activities described above and thus carries a great clinical potential for drug development .

- Most notably, the antitumor and associated anti-inflammatory activities of WA and other withanolides results from targeting multiple signaling pathways simultaneously, particularly the nuclear factor kappa B (NF- κB), signal transducer and activator of transcription (STAT), and ubiquitin proteasome pathways. The potent biological activities of withanolides such as WA and tubocapsenolide A, especially the antitumor and anti-inflammatory properties have been attributed to the presence of key structural features such as an α,β-unsaturated ketone in ring A, a 5β,6β-epoxide in ring B, and a lactone side chain [1, 4, 7, 13, 30, 58–62].

- Cysteine residues in the proteins are often implicated to react with these key electrophilic sites on the withanolide molecule .While other withanolides may possess α,β-unsaturated ketone and/or epoxide in some respect (e.g., paraminabeolides, capsisteroids, and chantriolides) and are bioactive, they are generally less potent than those withanolides possessing all three crucial functional groups.

Withanolides

- In addition to the inhibition of NF- κB activation, WA and several other withanolides have been shown to directly block the expression of LPS- or TNFα-induced NF-κB-regulated inflammatory genes such as iNOS, COX-1, COX-2, and NO .Nitric oxide is a small molecule that regulates MMPs(matrix metalloproteinases)and joints extracellular matrix, and is modulated through iNOS. COX-1 and COX-2 convert arachidonic acid to prostaglandins, which in turn cause a significant inflammatory response. COX-1 is constitutively expressed in most cell types, and is responsible for maintenance of normal physiologic function, whereas COX-2 is inducible in response to proinflammatory cytokines .

- Withaferin A, viscosalactone B, 2,3-dihydrowithaferin A, and 4-(2,2-dimethyl-3-oxocyclopropoxy)-2,3-dihydrowithaferin A were shown to inhibit COX-2 enzyme but not COX-1. Like other withanolides, withanolide sulfoxide, a sulfoxide dimer of WA was highly selective in inhibiting COX-2 compared to COX-1.

Withanolide sulfoxide from Aswagandha roots inhibits nuclear transcription factor-kappa-B, cyclooxygenase and tumor cell proliferation.

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Modulation Of The AP-1 Pathway By Withanolides

- The transcription factor AP-1, which plays a key role in the inflammatory response is implicated in several diseases such as cancer, psoriasis, inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and fibrosis . The AP-1 complex consists of homo and hetero dimers of Jun (JunD, C-Jun, and JunB) and the Fos (FosB, C-Fos, Fra-1 and Fra-2) family of proteins. Cytokines, chemokines, hormones, and growth factors as well as external stress factors are known to activate AP-1 signaling. The AP-1 complex translocates to the nucleus in response to stress signaling cascades, such MAPKs and c-Jun terminal kinases .

- This in turn leads to activation of AP-1 and regulates multiple functions such as differentiation, transformation, proliferation, and survival . The crude ethanol extract of WS has been shown to inhibit the nuclear localization of both AP-1 and NF- κB in LPS-activated peripheral blood mononuclear cells (PBMC) of both normal and RA patients, as well as synovial fluid mononuclear cells (SFMC) of RA patients. This in turn led to decreased downstream transcription target genes such as MMPs, COX-2, and iNOS, all of which are known mediators of RA.

Withania somnifera inhibits NF-kappaB and AP-1 transcription factors in human peripheral blood and synovial fluid mononuclear cells.

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Withanolides Can Modulate The PPARγ Pathway

- PPARγ plays a key role in inflammation through modulation of pro inflammatory transcription factors such as NF-κB and AP-1 . Treatment of 3T3-L1 adipocytes with WA resulted in phosphorylation of extracellular signal-regulated kinase (ERK), followed by decreased expressions of PPARγ leading to altered levels of Bcl2 and Bax expression, induction of apoptosis, and inhibition of adipogenesis .

Modulation Of The Hsp90 Pathway By Withanolides

- Heat shock proteins (Hsp) are ATP-dependent ubiquitously expressed molecular chaperones that are involved in the folding, assembly, maintenance, and transport of key regulatory proteins involved in numerous signaling pathways in the cell. Several environmental and physiological stimuli such as hypoxia, oxidative damage, inflammation, infection, and elevated temperature induce the expression of these highly conserved molecular chaperone family of proteins as a protein homeostasis and survival response . The Hsp90 family of proteins (Hsp90α, Hsp90β, GRP94, and TRAP1) form a large complex with other co-chaperones such as cdc37, HSP70-HSP90 organizing protein, p27, Hsp32, and Hsp70. This complex then stabilizes and maintains functional activity of proteins/kinases in many key signaling pathways, such as PI3K/ Akt/mTOR, p38/MAPK, and NF-κB, all of which play critical roles in inflammation, chronic inflammatory diseases, and oncogenesis. Through inhibition of Hsp90, and therefore inhibition of its oncogenic chaperone clients, cancer cells undergo apoptosis .

- Several studies have shown that withanolides such as WA, withalongolides A and B, tubocapsenolide A, and some of their synthetically modified analoges such as withalongolide A triacetate and withalongolide B diacetateare are able to target multiple cancers such as colon, prostate, brain, breast, head and neck, skin, adrenal, and thyroid both in *vitro* and in vivo. Withanolides such as WA and withalongolide A are known to block Hsp90 chaperone function through blocking the Hsp90/cdc37 complex, and induction of thiol-mediated oxidative stress.

Withanolide Modulation Of Nrf 2 Pathway

- Nuclear factor erythroid 2 related factor 2(Nrf2) is a transcription factor that regulates genes involved in redox homeostasis, inflammation, energy metabolism and cellular growth . Nrf2 is engaged in crosstalk with several signaling pathways that play a critical role in the pathogenesis and progression of chronic diseases, including NF-κB, PI3K, MAPK, glycogen synthase kinase-3β, and notch .

- Molecular docking studies have shown that both WA and withanone interact with the amino acids Ala 69, Gln 75, and Phe 71 of Nrf2 . In another study, WA induced reactive oxygen species (ROS) that activated JNK and stabilized Nrf2 that resulted in activation of NADPH quinone oxidoreductase and Tap73 transcriptional function leading to **apoptosis of cancer cells** . **WA was also shown to inhibit NFκB, AP-1, and Nrf2** in adriamycin-resistant human myelogenous erythroleukemic K562/Adr cells in a dose-dependent manner.

Modulation Of The HIF-1 Pathway By Withanolides

- Transcriptional activation of HIF-1 upregulates several genes that control glycolytic metabolism, angiogenesis, invasion, metastasis, and cell survival, such as VEGF, MMPs, stromal cell-derived factor-1, e-cadherin, chemokine receptor 4, EGF, and transforming growth factor beta (TGF-β) 3 . Crosstalk between **NF-κB and HIF pathways** has been shown to be associated with several chronic inflammatory diseases such as cancer, RA, asthma, and chronic obstructive pulmonary diseases .

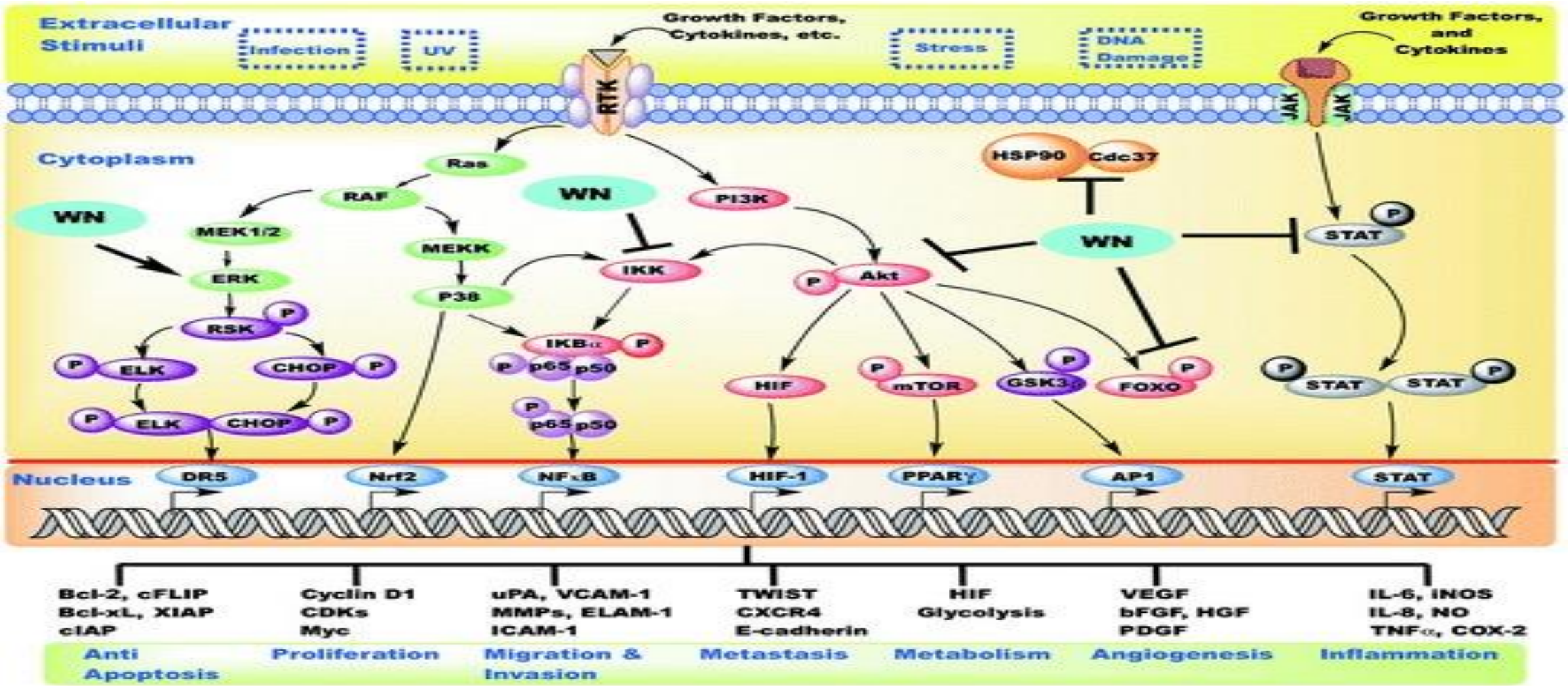
- In solid tumors, the availability of oxygen within the tumor decreases as distance from blood vessels increases resulting in the creation of hypoxic regions . This is known to be responsible in part for therapy resistance and metastatic spread . Although, no study thus far directly demonstrates inhibition of HIF-1 transcriptional activation by withanolides, a few note down regulation of migration-promoting HIF-mediated genes such as VEGF, heterogeneous nuclear ribonucleoprotein K (hnRNP-K) and MMPs, which lead to restriction of angiogenesis and metastasis .

Summary

- Studies show the ability of withanolides to target multiple interconnected signaling pathways such as PI3K/Akt/mTOR, JAK/STAT, AP-1, NF-κB, PPARγ, Nrf2 and MAPK. Withanolides target these pathways through multiple mechanism, such as blocking Hsp90-Cdc37 co-chaperone interaction, targeting Akt and its downstream pathways, and induction of thiol-mediated oxidative stress.

- Each of these mechanisms and pathway interactions play important roles in the development of chronic inflammatory diseases. Building on the studies identifying mechanisms of action of withanolides, we will discuss the clinical importance of withanolides on inflammatory mediated diseases including chronic inflammatory/autoimmune, cancer, and neurologic.

Schematic Diagram Representing Modulation Of Various Inflammatory Pathways By Withanolides



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7121644/

Anti-Stress

- This research confirms the theory that Ashwagandha has a significant anti-stress adaptogenic effect . Research conducted at the Department of Pharmacology, University of Texas Health Science Center indicated that extracts of **Ashwagandha produce GABA-like activity, which may account for the herb's anti-anxiety effects** .

- GABA (Gamma Amino-butyric acid) is an inhibitory neurotransmitter in the brain. Its function is to decrease neuron activity and inhibit nerve cells from over firing. This action produces a calming effect. Excessive neuronal activity can lead to restlessness and insomnia, but GABA inhibits the number of nerve cells that fire in the brain, and helps to induce sleep, uplift mood, and reduce anxiety.

- Ashwagandha has traditionally been used to stabilize mood in patients with behavioral disturbances. Research has revealed that the herb produces an anti-depressant and anti-anxiety effect in rodents comparable to the anti-depressant drug imipramine and the anti-anxiety drug lorazepam (Ativan) .

Anti-Stress

- In fact, Ashwagandha is one of the most widespread tranquillizers used in India, where it holds a position of importance similar to ginseng in China. It acts mainly on the reproductive and nervous systems, having a rejuvenative effect on the body, and is used to improve vitality and aid recovery after chronic illness .

- Research results showed that both Ashwagandha and Panax ginseng decreased the frequency and severity of stress-induced ulcers, reversed stress-induced inhibition of male sexual behavior, and inhibited the effects of chronic stress on retention of learned tasks. Both botanicals also reversed stress-induced immunosuppression, but only the Withania extract increased peritoneal macrophage activity.

- *Withania somnifera*, however, has an advantage over Panax ginseng in that it does not appear to result in .ginseng-abuse syndrome., a condition characterized by high blood pressure, water retention, muscle tension, and insomnia .

Antibiotic Activity

- The antibiotic activity of the roots as well as leaves has recently been shown experimentally. **Withaferin A** in concentration of 10µg/ml inhibited the growth of various Gram-positive bacteria, acid-fast and aerobic bacilli, and pathogenic fungi. It was active against Micrococcus pyogenes var aureus and partially inhibited the activity of Bacillus subtilis glucose-6-phosphatedehydrogenase.

- Withaferin A inhibited Ranikhet virus. Antibiotic activity of Withaferin A is due to the presence of the unsaturated lactone-ring. The lactone showed strong therapeutic activity in experimentally induced abscesses in rabbits, the being somewhat stronger than that of Penicillin. It substantiates the reputation of the leaves as a cure for ulcers and carbuncles in the indigenous system of medicine .

https://www.rroij.com/open-access/a-review-on-pharmacological-profile-of-withania-somnifera-ashwagandha.php?aid=33844

Antioxidant Effect

- Free radical damage of nervous tissue may be involved in normal aging and neurodegenerative diseases, e.g., epilepsy, schizophrenia, Parkinson's, Alzheimer's, and other diseases.

- The active principles of WS, sitoindosides VII-X and withaferin A (glycowithanolides), have been tested for antioxidant activity using the major free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain frontal cortex and striatum.

- Decreased activity of these enzymes leads to accumulation of toxic oxidative free radicals and resulting degenerative effects. An increase in these enzymes would represent increased antioxidant activity and a protective effect on neuronal tissue.

https://www.rroij.com/open-access/a-review-on-pharmacological-profile-of-withania-somnifera-ashwagandha.php?aid=33844

Anti-aging Activity

- Ashwagandha was tested for its anti-aging properties in a double-blind clinical trial.

https://www.rroij.com/open-access/a-review-on-pharmacological-profile-of-withania-somnifera-ashwagandha.php?aid=33844

Nootropic Effect

- Chronic WS administration significantly reversed reserpine-induced retention deficits. In different study with WS root extract improved retention of a passive avoidance task in a step-down paradigm in mice. WS also reversed the scopolamine-induced disruption of acquisition and retention and attenuated the amnesia produced by acute treatment with electroconvulsive shock (ECS), immediately after training.

- Chronic treatment with ECS, for 6 successive days at 24 h intervals, disrupted memory consolidation on day 7. Daily administration of WS for 6 days significantly improved memory consolidation in mice receiving chronic ECS treatment. WS, administered on day 7, also attenuated the disruption of memory consolidation produced by chronic treatment with ECS. On the elevated plus-maze, WS reversed the scopolamine-induced delay in transfer latency on day 1. On the basis of these findings, it is suggested that WS exhibits a nootropic-like effect in naïve and amnesic mice .

Nootropic Effect

- Effects of sitoindosides VII-X and withaferin isolated from aqueous methanol extract of roots of cultivated varieties of WS were studied on brain cholinergic, glutamatergic and GABAergic receptors in rats. The compounds slightly enhanced acetylcholinesterase (AChE) activity in the lateral septum and globus pallidus, and decreased AChE activity in the vertical diagonal band.

- Oral administration of withanoside IV significantly improved memory deficits in Abeta-injected mice and prevented loss of axons, dendrites, and synapses. Sominone, an aglycone of withanoside IV, was identified as the main metabolite after oral administration of withanoside IV.

- Withanoside IV may ameliorate neuronal dysfunction in Alzheimer's disease and that the active principle after metabolism is sominone. In another study reserpine treated animals also showed poor retention of memory in the elevated plus maze task paradigm.

Antiparkinsonian Properties

- Antiparkinsonian effects of WS extract has been reported due to potent antioxidant, antiperoxidative and free radical quenching properties in various diseased conditions. of WSG, rather than its GABA-mimetic action reported for the prevention of haloperidol-induced TD .

- WS significantly reversed the catalepsy, tardive dyskinesia and 6- Hydroxydopamine elicited toxic manifestations and may offer a new therapeutic approach to the treatment of Parkinson's disease.

https://www.rroij.com/open-access/a-review-on-pharmacological-profile-of-withania-somnifera-ashwagandha.php?aid=33844

Withanolides

- About 35 withanolides have been isolated from the roots of *W. somnifera* of which withanoside V (WS V), withaferin A, withanolide A (WN A) and withanolide B (WN B) are the major components. Withaferin A has anti-tumor, apoptotic, anti-angiogenesis, radiosensitizing and anti-inflammatory activities.

- WN A is effective as a neurological, immunological and anti-stress agent. WS IV and V play an important neuro-regenerative role. Thus in spinal cord injury WS IV and V improve hindlimb function and increase the myelin layer in the peripheral nervous system.In the light of this, it is important to find out the bioavailability of the individual withanolide compounds to ascertain their therapeutic efficacy.

Bioavailability

- Based on the present studies on the absorption characteristics of the tested withanolides it may be concluded that WN A, WNN, 1,2 DWM and WN B were highly permeable; whereas WS IV, and WS V showed low permeable. Surprisingly WF A, the highly biologically active withanolide was found to be either impermeable or metabolized on passing through the cell layer. It is likely that absorption of WFA in vivo is a complex process and possibly a system employing Caco-2 cells could provide better insight in the absorption characteristics of WFA.

Conclusions

- Based on the present studies on the absorption characteristics of the tested withanolides it may be concluded that WN A, WNN, 1,2 DWM and WN B were highly permeable; whereas WS IV, and WS V showed low permeable. Surprisingly WF A, the highly biologically active withanolide was found to be either impermeable or metabolized on passing through the cell layer. It is likely that absorption of WFA *in vivo* is a complex process and possibly a system employing Caco-2 cells could provide better insight in the absorption characteristics of WFA.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4630722/

Withanolide A

- Withanolide A is sparingly soluble in aqueous solutions. To enhance aqueous solubility , dilute the organic solvent solution into aqueous buffers or isotonic saline .

- If performing biological experiments, ensure the residual amount of organic solvent is insignificant , since organic solvents may have physiology effects at low concentrations. We do not recommend storing the aqueous solution for more than one day.

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Thank You

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