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A Study of Phytochemical Analysis and Pharmacological Activities of *Withania somnifera*

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

Ashwagandha roots (*Withania somnifera* L. Dunal) have a history of utilization in Ayurveda to address conditions such as fever, asthma, arthritis, rheumatism, inflammation, tuberculosis, mental disorders, and male sexual issues. Through phytochemical analyses, it has been determined that the plant predominantly contains alkaloids (such as *withanine*, *sominiferine*, *tropine*, *isopelletierine*, and *anaferine*) and steroidal lactones (including withanolides A-Y, withaferine A, *withasominiferols* A-C, *withanone*, and *sitoindosides*) as the active compounds. These constituents and extracts exhibit various pharmacological effects, encompassing antioxidant, antitumor, antimicrobial, antivenom, and anti-parkinsonian properties. This review provides an up-to-date overview of *ashwagandha*, focusing on its phytochemistry and pharmacology. The plant and numerous secondary metabolites have displayed efficacy in ameliorating diverse human ailments. Nonetheless, additional research is imperative to ascertain the precise mechanisms underlying their actions.

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1. INTRODUCTION

Ashwagandha (*Withania somnifera* L. Dunal), also known as Indian ginseng or winter cherry, originates from northwestern India [1]. Its name translates to "horse's smell" in Sanskrit, referring to the root's fragrance. It is found in India, Pakistan, Sri Lanka, Afghanistan, Egypt, South Africa, Morocco, and Jordan. In India, it's cultivated mainly in Uttar Pradesh, Madhya Pradesh, Punjab, Gujarat, and Rajasthan [2]. The Latin name "*somnifera*" means "sleep-inducing" [3]. Used in Asian medicine for 3000+ years, *ashwagandha* treats various health issues [5]. It's called Amukkira, Amukkiram, Punir, Asgandh, Akshan, Tilli in India [4]. Its powdered root, consumed daily, aims to delay aging, rejuvenate organs, and enhance fertility [6]. *Ashwagandha* leaves are used in Ayurveda and Unani for tumors [5]. It's part of 200+ Indian folk formulations, treating issues like asthma, inflammation, insomnia, anxiety, and more [7-13]. The root extracts (withanolides) possess antioxidant, immunomodulatory, anti-aging, and other properties [7-13]. Traditionally, boiled *ashwagandha* root powder with milk was used for female sterility [14]. It enhances vitality, strength, and bodily fluids [15]. *Ashwagandha* fruits aid digestion, and infant growth, and have sedative qualities [16]. Leaves address swelling, fever, and ophthalmitis [17]. The plant is useful for geriatric problems, stress, and arthritis [18,19]. In modern medicine, W. *somnifera* extracts enhance brain health, counteract aging, and help with conditions like weakness, epilepsy, memory loss, and neurodegenerative diseases [20-22]. *Ashwagandha* extracts are dietary supplements globally [21]. Studies confirm its aphrodisiac potential and impact on testosterone [23-28]. This review highlights *Ashwagandha's* phytochemical and therapeutic benefits against *various diseases.*

2. PHYTOCHEMICAL ANALYSIS

Ashwagandha roots contain diverse phytochemicals like amino acids, alkaloids, ergostane steroids, terpenes, and flavonoids, offering disease-mitigating potential [19, 26]. Essential bioactive compounds include withaferin A, withanone, and withanolides, with therapeutic effects such as antioxidant, antidiabetic, antimicrobial, anticancer, and more [29-41]. Withaferin A inhibits angiogenesis and counters cancer [42]. Sitoindoside-IX and -X enhance memory and reduce stress [43]. Nutritional composition (roots, leaves, fruits) is shown in Table 1, including minerals like calcium, copper, zinc, carotenoids, and vitamin C [44-46]. Geographic factors affect phytochemical composition [44]. Relevant metabolites include flavonoids [47]. Indian-grown *ashwagandha* roots contain alkaloids (0.13–0.31%) [48]. Alkaloids and steroidal lactones are major constituents. Alkaloids: *withanine*, *pseudowithanine*, etc. Steroidal lactones: *withanolides* A-Y, *withaferin* A, and more [49-52]. Molecular structures are in Table 2.

3. PHARMACOLOGICAL ACTIVITIES

The pharmacological effects of *W. somnifera* have been comprehensively evaluated in preceding articles [2,5,11,50,53-57], encompassing research conducted up until 2015. In recent times, two additional reports [58,59] have also addressed specific facets of *ashwagandha's* pharmacological activities, spanning up to 2020. To provide an updated perspective on this pivotal.

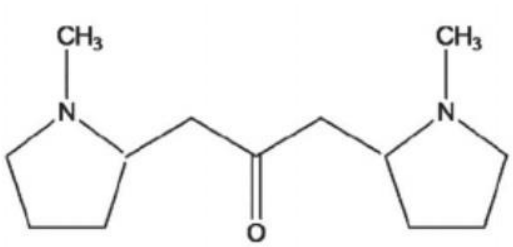
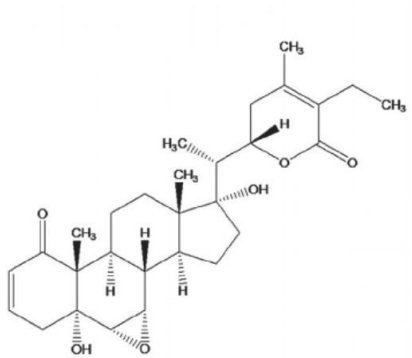
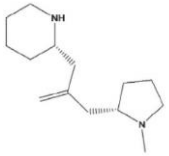
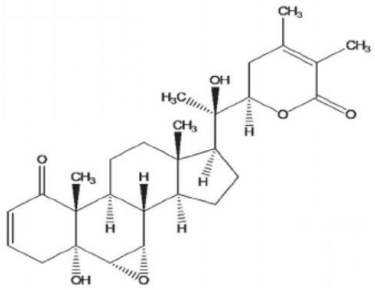
Ayurvedic herb, we have undertaken a review of pharmacological studies conducted between 2015 and 2020, which were not encompassed in earlier reviews. This review aims to analyze the advancements made during these years compared to the preceding ones. The extracts and bioactive components derived from *ashwagandha* exhibit a diverse array of pharmacological effects, including antioxidative, anticancer, and immunomodulatory activities. A compilation of the significant pharmacological activities attributed to *ashwagandha* is presented in Table 3.

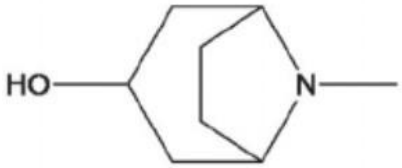
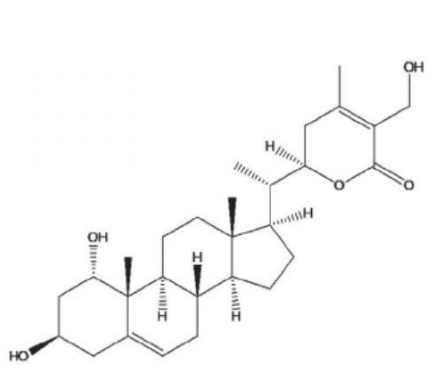
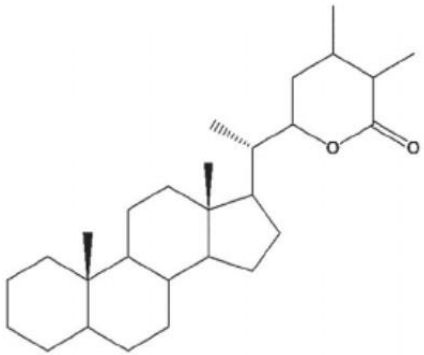
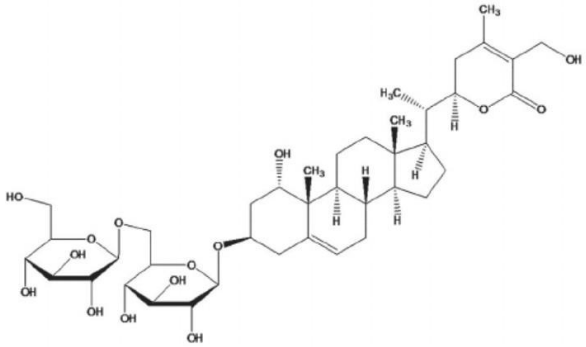
Neuroprotective activity: Elhadidy et al. [74] found *ashwagandha* countered aluminum-induced neurotoxicity (200 mg/kg p.o.). Dutta et al. [75] noted *ashwagandha* improved motor function in ALS mice and inhibited glial activation. *Ashwagandha* co-treatment with toxins mitigated Parkinson's markers [12,13]. Limited animal models studied *ashwagandha's* nootropic potential; human trials are needed.

Table 1. Proximate, mineral and metabolites concentration of *Withania somnifera*

Compositions	Proximate [%]			Reference
	Fruit	Roots	Leaves	
Protein	11.0	1.6	5.3	
Ash	9.1	3.7	8.6	
Carbohydrates	55.9	64.4	51.5	[44]
Crude Fiber	4.0	5.0	2.3	
Fat	2.9	2.4	1.1	
Minerals (mg/100g)				
Copper	4.2	0.8-3.3	3.5	
Calcium	-	23	-	[45,46]
Iron	60.2	74.0	94.5	
Carotenoids	-	7.6	-	
Vitamin C	-	3.7	-	
Manganese	3.7	1.2-5.9	3.4	
Zinc	4.0	1.6-4.4	3.6	
Metabolites (mg/100g)				
Alkaloids				
Berberine	-	0.41	-	
Harmine	-	0.26	-	
Caffeine	-	1.22	-	[47]
Papaverine	-	0.16	-	
Noscapine	-	0.32	-	
Theobromine	-	0.26	-	
Flavonoids				
Kaempferol	-	0.78	-	
Myricetin	-	0.22	-	
Rutin	-	4.21	-	
Quercetin	-	7.21	-	
Quercitrin	-	5.22	-	[47]
Rhamnetin	-	1.15	-	
Phenolic acids				
Coumaric acid	-	0.67	-	
Caeic acid	-	1.99	-	[47]
Chlorogenic acid	-	1.03	-	
Ferulic acid	-	0.55	-	
Gallic acid	-	4.02	-	

Table 2. Chemical structures of the major bioactive constituents of *Withania somnifera*

Bioactive constituents	Chemical structures	Bioactive constituents	Chemical structures
Cuscohygrine		Withanone	
Anahygrine		Withanolide A	

Bioactive constituents	Chemical structures	Bioactive constituents	Chemical structures
Trophine		Sominone	
Steroidal Lactone		Withanoside IV	

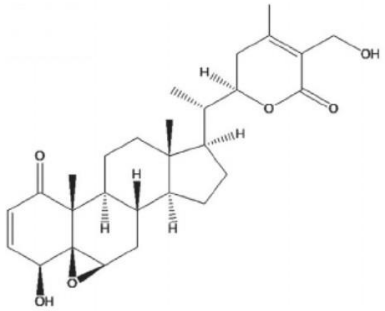
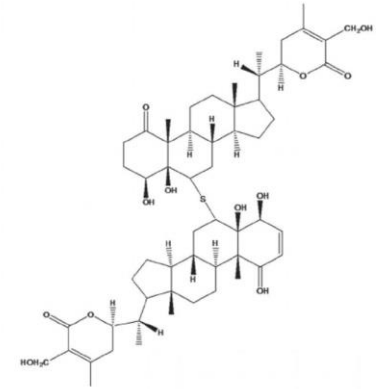
Bioactive constituents	Chemical structures	Bioactive constituents	Chemical structures
Withaferin A		Ashwagandhanide	

Table 3. Pharmacological activities of *W. somnifera* root extracts and alkaloids LC50 – lethal concentration; IC50 – inhibitory concentration

Activity	Extract Type	Methods Used	IC50/dosage	References
Adaptogenic and anxiolytic activity	Root extract	Fiy-eight male and female participants with a baseline perceived stress scale (PSS) score >20	250 and 600 mg/twice/day, 8 weeks	[69]
Anticancer	Methonol ectract	HepG2 hepatoma cell line	IC50:1.89 µg/ml	[65]
Anticancer	withaferin A	Transgenic adenocarcinoma of mouse prostate(TRAMP) model	5 mg/kg/day; 8 week	[64]
Activity	**Extract Type**	**Methods Used**	**IC_{50}/dosage**	**References**
Adaptogenic and anxiolytic activity	Root extract	Fiy-eight male and female participants with a baseline perceived stress scale (PSS) score >20	250 and 600 mg/twice/day, 8 weeks	[69]
Anticancer	Methonol ectract	HepG2 hepatoma cell line	IC_{50}:1.89 µg/ml	[65]
Anticancer	Methonol ectract	HCT 116 human colorectal cell line	IC_{50}:2.19 µg/ml	[65]
Anticancer	*W. somnifera* prote fraction	MDA-MB-231 human breast cancer cells	IC_{50}:92 µg/ml	[63]
Anti-neuro inflammatory	Leaf extract	*Ashwagandha* leaf water extract using β-amyloid and lipopolysaccharide (LPS)- stimulated primarymicroglial cells and BV-2 microglial cell line	IC_{50}:0.2%; 48h	[61]
Anti-neuro inflammatory	Chloroform fractio	Chloroform fraction IV (FIV) using β-amyloid anlipopolysaccharide (LPS)- stimulated primary microglial cells and BV-2 microglial cell line	IC_{50}:15 µg/ml; 48h	[61]
Antioxidant activity	Extract type rootextract	Lipid peroxides (LPO), superoxide dismutase (SOand glutathione (GSH) were tested in adult male Wistar rats	500 mg/kg, p.o once a day from day 14 to day21	[60]
Antiarthritic activity	Root extract	Assessed the level of inflammatory cytokines suchas Tumor Necrosis Factor (TNF)-α, IL-1β, IL-6 anIL-10 in collagen-induced arthritic (CIA) rats	300 mg/kg/day; 20 day	[62]
Body strength	aqueous extract (Sensoril®)	19 recreationally active men were randomized in adouble-blind fashion to placebo or Sensoril®	500 mg/day; 12 week	[67]
Cytotoxic activity	Root extract	The crude extract of Withania was tested for cytotoxicity against A375 cells by MTT assay	350, 250 and 200 µg/m for 24, 48 and 72 h,respectively	[68]
Insomnia and anxiet	Root extract	60 patients were randomly divided into two groupashwagandha (n=40) and placebo (n=20)	300 mg, 2 doses/ day;weeks	[72]
Increased testosterolevel	withanolideglycosides	43 people completed the 16-week period of trial	21 mg/day for 8 weeks	[71]
Insomnia	Root extract	150 subjects divided into two groups: ashwagandh(n=75) and placebo (n=75)	600 mg/once/ day; 42days	[73]
Nephroprotective	Root extract	Gentamycin-induced nephrotoxicity in adult maleWistar rats	500 mg/kg, p.o once a day from day 14 to day21	[60]

Ashwagandha inhibits various cancers [76-81]. Lee et al. [82] noted apoptotic effects on HNSCC cells. Daily *ashwagandha* (200 mg/kg) reduced lung adenomas [83]. Withaferin A impacted NSCLC cells [84]. It reduced Helicobacter pylori-induced IL-1 [85]. *Ashwagandha* protein fraction induced apoptosis in breast cancer cells [63]. Few studies explored *ashwagandha's* anticancer potential. Future research should rigorously evaluate its efficacy through clinical trials with appropriate controls and dosages.

Antimicrobial activity: *Ashwagandha* roots display potent antimicrobial properties. Methanolic leaf extracts inhibit gram-positive bacteria like Staphylococcus aureus, and Enterococcus sp. (zones of inhibition: 20.6 mm, 19.4 mm) [86]. A recent study found *ashwagandha* extract inhibiting S. aureus, and E. coli (17 mm to 24 mm zones) [87]. Leaf extracts (6.25 mg/ml, 12.5 mg/ml) hindered the growth of gram-negative bacteria (E. coli, S. typhi, K. *pneumonia*, P. *aeruginosa*, C. *freundii*) [88]. Extract (100 mg/ml) suppressed *Staphylococcus aureus, Streptococcus sobrinus, Streptococcus mutans, Salmonella paratyphi* B [89,90].

Most studies use disc diffusion, but limitations exist. Further Minimum Inhibitory Concentration (MIC) assays are necessary to enhance comprehension [91].

Anti-depressant activity: In a six-week trial, 120 mg/day *ashwagandha* root extract improved sleep quality significantly in 150 participants compared to placebo ($p<0.001$) [92]. The treatment group showed better sleep efficiency, total sleep time, latency, and wake after sleep onset.

Salve et al. [69] gave 250 mg/day, and 600 mg/day root extract for eight weeks. Perceived stress and cortisol levels were reduced in 58 participants vs. placebo. Another study using 240 mg/day for 60 days showed stress reduction [28].

Earlier studies demonstrated *ashwagandha* (20, 40, 50 mg/kg/once daily for five days) reduced anxiety in animal models [34, 93].

Anti-inflammatory activity: A 47% methanolic extract curbed LPS-induced nitric oxide and TNF-α production [94]. Aqueous *ashwagandha* root extract (300 mg/kg/wt.) enhanced IL-10 secretion and suppressed NF-κB [62]. Doses of 600, and 800 mg/kg mitigated arthritis severity, inhibiting inflammatory mediators [95]. Lower doses (100 mg/kg, 15 days) alleviated arthritis, surpassing hydrocortisone's effect [96]. *Ashwagandha* inhibited granuloma formation [97].

Spermatogenic Activity: *Ashwagandha* enhances semen quality by reducing oxidative stress, and elevating hormone levels [23,26,71,98]. 300 mg/kg for 8 weeks improved sperm count, and motility [99]. 100 mg/kg for 30 days increased sperm count, and motility [100]. 200 mg/kg reduced sperm abnormalities [101].

Cultivation of *Withania* somnifera: Field cultivation yields more fruits, and seeds than greenhouse. Early seedling preparation is crucial for ample fruit production. *Withaferin* A, and *withanolide* D were detected in both conditions [102]. Soil salinity affects growth [103]. Enhanced ZnSO4 leads to increased Withaferin A, and glutathione [104].

4. CONCLUSION

In conclusion, this review emphasizes *Ashwagandha's* ethnopharmacological applications, phytochemical composition, and potential therapeutic benefits for various human conditions. The literature supports its efficacy against ulcers, insomnia, memory impairment, anxiety, bronchitis, and neurological disorders. Bioactive compounds like *withaferin* and *sitoindosides* exhibit cellular protection and counteract illnesses. Delving into ashwagandha extracts and constituents reveals positive impacts. Yet, existing gaps in research need attention for validation and further exploration.

5. LIMITATIONS AND FUTURE DIRECTIONS

This review acknowledges limitations in some studies' experimental designs, emphasizing the need for proper control groups and clear dosages for accurate interpretation. Both organic and aqueous *ashwagandha* extracts deserve exploration to understand their potential fully. While animal and cellular models have shown *ashwagandha's* therapeutic effects, more human clinical trials are essential for translation. Research should expand to human trials involving extracts, alkaloids, and steroidal lactones. Further investigation into bioactive constituents, their bioavailability, and pharmacokinetics is needed to understand their roles. *Ashwagandha's* alkaloids and steroidal lactones hold pharmaceutical promise.

Ethnobotanical and modern medicinal uses should be explored comprehensively to maximize their potential benefits for human health.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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