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Withania somnifera (Linn.) Dunal: a review of chemical and pharmacological diversity

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Abstract *Withania somnifera* Dunal, is a commonly used herb in Indian Ayurvedic medicine system. Due to its pharmacological value and an inexhaustible source of novel biologically active compounds, it has been a great interest for researchers. The plant is known to possess anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory and hemopoetic properties. Various withanolides, steroidal lactones, have been isolated from *W. somnifera* and were known to have high therapeutic value. Based on the differences in the substitution patterns of withanolides the species has been classified into various chemotypes. So far, three different chemotypes have been identified, which have been further classified into ecotypes based on the contents of withanolides. Present review summarizes the phytochemical variability and pharmacological advances reported in literature.

Keywords Ashwagandha · Chemotypes · Withanolides · Withaferin A · Indian ginseng

Abbreviations

WS	*Withania somnifera*
NMR	Nuclear magnetic resonance spectroscopy
RP-HPLC	Reversed phase high performance liquid chromatography
LDH	Lactate dehydrogenase
CPK	Creatine phosphokinase
LPO	Lipid peroxidation
C-H-R	Cold, hypoxia and restraint
CS	Chronic stress
GABA	Gamma-Aminobutyric acid
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PCNA	Proliferating cell nuclear antigen
TNF	Tumor necrosis factor
IL-1β, IL-6	Interleukin
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
AP-1	Activator protein 1
HUVECs	Human umbilical vein endothelial cell
Sp1	Specificity protein 1
VEGF	Vascular endothelial cell growth factor
NCI-H460	Lung tumor cell lines
HCT-116	Colon tumor cell lines
SF-268	Central Nervous System tumor cell lines
MCF-7	Breast tumor cell lines
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
TBHQ	Tert-butylhydroquinone
SOD	Superoxide dismutase
CAT	Catalase

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GPX	Glutathione peroxidase
CNS	Central nervous system
WBC	White blood cells
PFC	Plaque forming cell
SRBS	Sheep red blood cells
NADPH-d	Nicotinamide adenine dinucleotide phosphate diaphorase
SH-SY5Y	Human neuroblastoma tumor cell lines
nNOS	Neuronal nitric oxide synthase
WSG	*W. somnifera* glycowithanolides
EPM	Elevated plus maze
ECS	Electroconvulsive shock
IA	Ibotenic acid
MnPCEs	Micronucleated polychromatic erythrocytes
DMBA	Dimethylbenz (a) anthracene
$FeSO_4$	Ferrous sulfate
TBARS	Thiobarbituric acid and reactive substances
HP	Hydroperoxides
AST	Aspartate transaminase
ALT	Alanine transaminase
ALP	Alkaline phosphatase
PD	Parkinson's disease
IR injury	Ischemia and reperfusion
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
PTZ	Pentylenetetrazol
T	Testosterone
LH	Luteinizing hormone
FSH	Follicle-stimulating hormone
PRL	Prolactin

Introduction

Withania somnifera (Linn.) Dun. is commonly known as "Ashwagandha", Winter Cherry, Indian ginseng. It belongs to the family Solanaceae. 23 species of genus *Withania* are reported among which, *W. somnifera* (L.) has high medicinal value. It is one of the most valued medicinal plants in Ayurveda and other traditional systems of medicine and has been used for over 3000 years. *W. somnifera* is regarded as one of the most useful herbs having 'Vata' pacifying properties (Singh and Kumar 1998). It is widely used in traditional Indian medicine system for curing variety of diseases. It possesses adaptogenic, tonic analgesic, antipyretic, anti-inflammatory and abortifacient properties and is one of the most extensively used plants in various systems of medicine (Chopra et al. 1958). Clinical trials and animal research support the use of *W. somnifera* for hepatotoxicity (Bhattacharya et al. 2000a), anxiety (Bhattacharya et al. 2000b), cognitive (Bhattacharya et al. 1995), neurological disorders (Kuboyama et al. 2005), inflammation (Al-Hindawi et al. 1992), hyperlipidemia (Visavadiya and Narasimhacharya 2007) and Parkinson's disease (Ahmad et al. 2005). The fruits of this plant are rich in saponins and can be used as a substitute for soaps. The leaves are also known to act as an insect repellent (Schmelze et al. 2008).

Both leaves and roots of the plant are used as drug. Steroidal lactones known as withanolides (a group of biologically active oxygenated ergostane type steroidal lactones) occur in both parts of the plant (Kaushik et al. Communicated). The production of withanolides in the plant could be monitored through seasonal changes or growth periods.

Several studies concerning the chemistry, biological properties and genetics of withanolides have been carried out. These compounds have been intensely investigated because of their pronounced anti-tumor properties and novel steroidal structure. Alkaloids constitute another major group of components which have been isolated from *W. somnifera*. A number of alkaloids have also been isolated from the roots of *W. somnifera,* among them withanine is the main alkaloid comprising of 38% of the total alkaloid material (Atal et al. 1975). The chief withanolides of *W. somnifera* species found in India are withaferin A and withanolide D. Both withaferin A and withanolide D show antitumor and cytotoxic activities (Yoshida et al. 1979).

Present review summarizes the phytochemical diversity and pharmacological advances reported in literature.

Description of the plant

A small or middle-sized under shrub, erect, grayish, branched, 30–150 cm high, with greenish or lurid yellow flowers. Fruits are a berry enclosed in the green persistent calyx, green when unripe, turns to orange red when mature. The fruit contains numerous small

capsicums like seeds. Flowering occurs nearly throughout the year.

The shoots specially stem, veins and the calyx are covered with minute star-shaped hairs. Leaves are simple, ovate, petiolate, entire, exastipulate, acute, glabrous and up to 10 cm long and petioles are around 1.25 cm long. On vegetative shoots, the leaves are alternatively arranged and large while on floral branches, they are oppositely arranged in pairs of one large and one small leaf and arranged somewhat parallel, having a cymose cluster of 5–25 inconspicuous pale green flowers in their axil.

The roots are fairly long and tuberous (~20–30 cms and 6–12 mm in diameter) with a short stem and with few (2–3) lateral roots of slightly smaller size, straight, unbranched. They have buff to grayish-yellow outer surface with longitudinal wrinkles and soft, solid mass with scattered pores in the center. The roots taste bitter and acrid (John 2014).

Geographical distribution of the plant

Withania somnifera (Linn.) Dunal has a fairly wide geographic distribution. Besides the Indian subcontinent, it is widely distributed in dry subtropical regions along the shores of the Mediterranean Sea, South Africa, Israel, Italy, Pakistan, Afghanistan, Palestine, Egypt, Jordan, Morocco, Spain, Canary Island, Eastern Africa Congo, Madagascar and South Africa, representing extensive variations of soil, rainfall, temperature and altitude (Atal et al. 1975).

In India, it is cosmopolitan and grows throughout the drier parts and sub-tropical regions (Hooker 1885). The plant is widely distributed in Northwestern, Bombay, Gujarat, Rajasthan, Madhya Pradesh, and Uttar Pradesh, Punjab plains, which extends up to the mountain region of Punjab, Himachal Pradesh and Jammu.

Various morphotypes

Extensive study by Atal in India revealed various morphotypes. He reported, extreme degree of variability in *W. somnifera* regarding the morphological characteristics and growth habits of plants found in different parts of India and in plants from other countries (Atal et al. 1975).

Five different morphotypes have been identified in India by Atal, in 1975, the details of which are described as follows:

Morphotype I Plant are usually not more than 30 cm high; stems growing from crown vary from one to many in number. It grows as an annual crop. Cultivated exclusively in Madhya Pradesh (Center India) and yields roots of commercial value.
Morphotype II Plants are 0.6–1.5 m long, stem is single erect, grows from crown giving off branches above the ground level. This form grows in the sandy desert soil of Marwar, Pilani and some other parts of Rajasthan.
Morphotype III Plants are 0.6–1 m high, branching starts from 15 to 30 cm above ground level. It India, it grows in Chandigarh and some other mountainous areas of Punjab and Uttar Pradesh.
Morphotype IV Plants are 0.6–0.75 m tall, profusely branches near the ground. The plant was found growing near Delhi.
Morphotype V Plants are exceptionally tall; 1.2–2.1 m. Wild growth of this form was seen near Delhi and Ahmedabad. The plant prefers shady habitats and is found in or along hedges, sometimes extending into open.

Phytochemical investigations

Many compounds have been isolated so far from different chemotypes of *W. somnifera*. These include alcoholic, alkaloids and withanolides compounds.

Alcoholic compounds

The earliest report available on the phytochemistry of the plant is by Power and Salway (1911) that studied the chemical principles of *W. somnifera* and reported the presence of a number of compounds from the roots and leaves of the plant. They reported two new monohydric alcohols, withaniol, $C_{25}H_{33}O_4OH$ and somnirol, $C_{32}H_{43}O_6OH$, a new dihydric alcohol, somnitol, $C_{33}H_{44}O_5(OH)_2$, an acidic hydrolytic product, withanic acid, $C_{29}H_{45}O_6$ COOH, a nitrogen containing component, $C_{12}H_{16}N_2$, phytosterol, $C_{27}H_{46}O$ and ipuranol, $C_{25}H_{38}O_2$ $(OH)_2$. In addition a mixture of fatty acids, consisting of stearic, cerotic,

palmitic, oleic and linoleic acids; an essential oil and sugar were obtained.

Alkaloidal compounds

Later on Majumdar (1952, 1955), examined the roots of Indian variety from Bengal and South African varieties and identified several nitrogenous bases and partially characterized seven amorphous bases namely withanine, withananine, withananinine, pseudo-withanine, somniferine, somniferinine, somnine along with nicotine as eight component. The first six compounds were found to be alkaloids and the seventh one is a disintegrated product of withanine. Among these, withanine was found to be the main alkaloid, with 38% of the total alkaloid content. Schwarting et al. (1963) made the major breakthrough by isolating and characterizing eight bases present in the extract namely, tropine, pseudotropine, 3α-tigloyloxytropane, choline, cuscohygrine, *dl*-isopelletierine, anaferine and anahygrine the latter two being the new ones (Schwarting et al. 1963). Further, Schröter et al. (1966) isolated a pyrazole alkaloid withasomnine from the root of *W. somnifera*.

Jayaprakasam et al. (2004) purified novel withanamides A-I from the methanolic extract of *W. somnifera* fruits. The structure of these compounds was determined by using serotonin, glucose and long-chain hydroxyl fatty acid moieties (Jayaprakasam et al. 2004).

Withanolides

After reports of alcoholic compounds by Power and Salway (1911) and alkaloids by Majumdar (1952, 1955), Lavie and co-workers in series of papers reported a new group of steroidal lactones characterized by C_{28} basic skeleton with 9 C atoms side chain and a 6 membered lactone ring from *W. somnifera* which they termed as "Withanolides" (Lavie et al. 1965, 1966, 1968). The withanolides possess a highly oxygenated cholestane type side chain bearing an extra methyl group at C-24. viz. withaferin A (Lavie et al. 1965; Kirson et al. 1970). Table 1 describes various withanolides identified in different chemotypes of *W. somnifera* with their molecular formula, IUPAC name, physiochemical analysis and geographical location whereas Fig. 1 provides structures of major withanolides.

Withaferin A is a polyfunctional steroid-lactone. The structure of withaferin A and its 2,3-dihydro derivative has been elucidated by chemical studies and X-ray crystallography by Lavie et al. (1965, 1966) and Kirson et al. (1970). Basic skeleton of withaferin A was confirmed after several selenium dehydrogenations which lead to isolation of a derivative of cyclopentenophenantharene and of a trimethylnaphthalene. It is mainly known for its anti-cancerous property. It possesses three likely positions which might be involved in in vivo alkylation reaction with the biological nucleophiles thus result in activity. These includes position 3 in ring A, the epoxide i.e. position 5 (or 6) and position 24 in unsaturated lactone ring E. Anti-tumor activity of the analogues with cholesterol side chain was weaker than that of withanolides thus proved the presence of unsaturated lactone in the side chain is necessary for the activity (Yoshida et al. 1979). There are number of withanolides which has structure similar to withaferin A i.e. unsaturation at position 2 and 24 and epoxy group at position 5 and 6 (Fig. 1). Withanolide D which has been isolated in year 1968 by Lavie et al. has the same structure similar to withaferin A but presence of hydroxyl group at position 20 was found instead of position 27 (Lavie et al. 1968).

Withanolide A which was previously isolated from *Withania coagulans* has been isolated from roots of *W. somnifera* in 1971 (Menssen and Stapel 1973).

A chlorinated withanolides i.e. withanolide C was isolated from chemotype III of *W. somnifera* (Besselle and Lavie 1992). Structure of withanolide C revealed the opening of 5β,6β epoxide ring and possesses chloro group at position 5.

Glotter et al. (1973) isolated nine withanolides (withanolide E–M) and found five withanolides viz. G, H, I, J, K possess an unusual Δ8(14) double bond. Very few reports are available on the presence of Δ8(14) double bond in natural steroids. Withanolide F, possesses a double bond instead of 5β,6β epoxy group present in withanolide E (Glotter et al. 1973). X-ray analysis of withanolide E and F has disclosed that the side-chain possesses the unusual 17α-orientation (Lavie et al. 1972). Withanolide S was obtained during a study of biogenesis of withanolides in *W. somnifera* involving combination of various types through cross pollination. In NMR, it has revealed a close similarity to withanolide E, the only difference was lack of epoxide ring signal at δ 3.20 and the

Table 1 Bioactivity and other parameters of withanolides isolated from *W. somnifera* from different geographical locations

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
Withaferin A	Chemotype I	5β,6β-Epoxy-4β, 27-dihydroxy-1-oxo-22 R-witha-2, 24-dienolide	1. Antiinflammatotry 2. Anti-tumour: IC_{50} at 0.24 ± 0.01–11.6 ± 1.9 μg/mL 3. Anti-arthritic: LD_{50} at 120 mg/kg 4. Antibacterial and Anti fungal 5. Anti-angiogenic activity at IC_{50} = 12 nM	Israel/Indian/South Africa	1. Melting point: 252°–253° 2. Optical rotation: $[\alpha]_D^{28}$ +125 (c, 1.30 in $CHCl_3$) 3. Partition coefficient (calculated): Log *P* 2.22 (uncertain value) (calc)	Kirson et al. (1970) Abraham et al. (1975) Jayaprakasam, et al. (2003) Zhao et al. (2002) Kaushik et al. (communicated) Bhattacharya and Muruganandam (2003) Kaileh et al. (2007) Mohan et al. (2004) Mikolai et al. (2009) Kirson et al. (1977)
Withanolide A	Chemotype I	(20R)6α,7α-Epoxy-5α,20β-dihydroxy-1-oxowitha-2,24-dienolide	1. Neuritic regeneration and Synaptic reconstruction at dose of 10 mmol/kg/day 2. Cholinesterase inhibiting activity 3. Neurite outgrowth activity at 1 mM 4. Immunomodulator	Indian	1. Melting point: 282°–284° 2. Optical rotation: $[\alpha]_D$ +88 (c, 0.02 in $CHCl_3$)	Menssen and Stapel (1973) Malik et al. (2007) Choudhary et al. (1996) Choudhary et al. (2004)
Withanolide C	Chemotype III	5α-Chloro-6β,14α,17β,20α-tetrahydroxy-1-oxo-22R-witha-2,24-dienolide	NR	Israel	1. Melting point: 180°–182°	Bessalle and Lavie (1992)
Withanolide D	Chemotype I, Chemotype II	5β,6β-Epoxy-4β-20α-dihydroxy-1-oxo-witha-2,24-dienolide	1. Immunodepressive 2. Anti-metastatic activity	Israel/Indian/South Africa	1. Melting point: 253°–255° 2. Optical rotation: $[\alpha]_D$ +80 ($CHCl_3$)	Lavie et al. (1968) Kirson et al. (1970) Abraham et al. (1975) Eastwood et al. (1980) Abraham et al. (1968) Ray and Jha (2001) Leyon and Kuttan (2004) Shohat et al. (1978) Sethi and Subramanian (1976)
Withanolide E	Chemotype III	5β, 6β-Epoxy-14α,17β,20α-trihydroxy-1-oxo-17S, 20S, 22R-witha-2, 24-dienolide	1. Immunosuppresive activity	Sicilian and Sardinian/Israel	1. Melting point: 167°–168° 2. Optical rotation: $[\alpha]_D$ +103.5 (c, 0.7 in CH+I36Cl_3) 3. Partition coefficient (calculated): Log *P* −0.03 (uncertain value) (calc)	Lavie et al. (1972)

Table 1 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
Withanolide F	Chemotype III	14α,17β,20α-Trihydroxy-1-oxo-17S, 20S, 22R-witha-2, 5,24-trienolide	Anticancer	Israel	1. Melting point: 192°–193°	Glotter et al. (1977) Seth et al. (2016)
Withanolide G	Chemotype II and Chemotype III	20α-Hydroxy-1-oxo-20R, 22R-witha-2, 5,8(14), 24-tetraenolide	NR	Sardinian/ Israel	1. Melting point: 194°–195° 2. Optical rotation: $[\alpha]^D$ +52.5 (c, 0.5 in $CHCl_3$)	Glotter et al. (1973) Kirson and Glotter (1980)
Withanolide G2	NR	(20R)-20-Hydroxy-1-oxowitha-2,5,14,24-tetraenolide	NR	NR	NR	Kirson and Glotter (1980)
Withanolide H	Chemotype III	14,20,27-Trihydroxy-1-oxo-20R,22R-witha-2,5,24-trienolide	NR	Israel	1. Melting point 141°–142° (27-°Ac) 2. Optical rotation: $[\alpha]^D$ +35.5 (c, 0.5 in $CHCl_3$) (27-Ac)	Glotter et al. (1973) Kirson et al. (1980)
Withanolide I	Chemotype III	14,20-Dihydroxy-1-oxo-20R,22R-witha-3,5,24-trienolide	NR	Israel	1. Melting point:184° 2. Optical rotation: $[\alpha]^D$ +118 (c, 0.3 in $CHCl_3$)	Glotter et al. (1973) Kirson et al. (1980)
Withanolide J	Chemotype III	14α,17α,20α-Trihydroxy-1-oxo-20S,22R-witha-2,5,24-trienolide	NR	Sicilian and Sardinian/ Israel	1. Melting point: 215°–216° 2. Optical rotation: $[\alpha]_D$ +32.7 (c, 0.65 in $CHCl_3$)	Glotter et al. (1973)
Withanolide K	Chemotype III	14α,17α,20α-Trihydroxy-1-oxo-20S,22R-witha-3,5,24-trienolide	NR	Israel	1. Melting point: 218°–219° 2. Optical rotation: $[\alpha]_D$ +92 (c, 0.3 in $CHCl_3$)	Glotter et al. (1973) Kirson et al. (1980)
Withanolide L	Chemotype III	20S,17α-Dihydroxy-1-oxo-22R-witha-2,5,14,24-tetraenolide	NR	Israel	1. Melting point: 213° 2. Optical rotation: $[\alpha]_D$ +9.6 (EtOH)	Glotter et al. (1973) Kirson et al. (1980)
Withanolide M	Chemotype III	14α,15α-Epoxy-17α,20S-dihydroxy-1-oxo-22R-witha-2,5,24-trienolide	NR	Israel	1. Melting point: 240° 2. Optical rotation: $[\alpha]_D$ +44.6 ($CHCl_3$)	Glotter et al. (1973)
Withanolide N	Chemotype I	17α,27-Dihydroxy-1-oxo-20R,22R-witha-2,5,14,24-tetraenolide	NR	Israel	NR	Abraham et al. (1975)
Withanolide O	Chemotype I	4β,17α-Dihydroxy-1-oxo-20R,22R-witha-2,5,8(14),24-tetraenolide	NR	Israel	1. Melting point: 201°–202° 2. Optical rotation: $[\alpha]_D$ +112.5 (c, 0.1 in $CHCl_3$) 3. ν_{max} = 1690 $cm^{-1,}$ 4. λ_{max} 214 nm (ε 18,000) (MeOH)	Abraham et al. (1975)
Withanolide P	Chemotype I	14α,17β-Dihydroxy-1-oxo-20R,22R-witha-2,5,24-trienolide	NR	Israel	1. Melting point: 216°–217° 2. Optical rotation: $[\alpha]_D$ +51 (c, 0.2 in $CHCl_3$)	Glotter et al. (1977)
Withanolide Q	NK	(22S, 23S) 17,23,27-Trihydroxy-1-oxowitha-2,5,24-trienolide	NR	Indian	1. Melting point: 200°–202° 2. Optical rotation: $[\alpha]_D$ −6.6 (c, 1.2 in $CHCl_3$)	Kirson et al. (1975)
Withanolide R	Chemotype I	6α,7α-Epoxy-5α,23S-dihydroxy-1-oxo-20S,22S-witha-2,24-dienolide	NR	NR	NR	Kirson et al. (1975)

Table 1 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
Withanolide S	Chemotype III	5α,6α,14α,17β,20α-Pentahydroxy-1-oxo-22R-witha-2,24-dienolide	NR	Sicilian and Sardinian/ Israel	1. Melting point: 272° dec 2. Optical rotation: $[\alpha]_D$ +95.5 (c, 0.2 in MeOH)	Glotter et al. (1977) Nittala and Lavie (1981)
Withanolide T	Chemotype II	6α,7α-Epoxy-5α,17α,20α-trihydroxy-1oxo-22R-witha-2,24-dienolide	NR	NR	1. Optical rotation: $[\alpha]_D$ +60.5 (c, 0.15 in $CHCl_3$)	Glotter et al. (1977)
Withanolide U	Chemotype III	4β,20α-Dihydroxy-1-oxo-20R,22R-witha-2,5,8(14),24-tetraenolide	NR	NR	NR	Glotter et al. (1977) Kirson et al. (1977)
Withanolide Y	NR	5α,6α-Epoxy-7α,17α,20R-trihydroxy-1-oxo-22R-witha-2,24-dienolide	NR	NR	1. Melting point: 270°–273°	Besselle and Lavie (1987)
Withanone	Chemotype I	6α,7α-Epoxy-5α,17α-dihydroxy-1oxo-22R-witha-2,24-dienolide	Anti-inflammatory & anti Anti-arthritic LD_{50} at > 400 mg/kg.	Indian	1. Melting point: 275°–276° 2. Optical rotation: $[\alpha]_D$ +81 (c, 0.5 in $CHCl_3$)	Kirson et al. (1971)
Tubocapsenolide F	NR	(20R,22R)-5β,6β-epoxy-4β,17α-dihydroxy-1-oxowitha-2,24-dienolide	Treatment of cancer cells with Skp2 overexpression (Lung cancer).	NR	1. Melting point: 200°–202° 2. Optical rotation: $[\alpha]_D^{25}$ +75.7 (c, 0.07 in MeOH) 3. UV: [neutral] λ_{max} 214 (MeOH)	Kirson et al. (1971) Chang et al. (2007)
Dunawithagenin	NR	(20R, 22R) 1α,3α,20-trihydroxywitha-5,24-dienolide	NR	NR	1. Melting point: 273° 2. Optical rotation: $[\alpha]_D$ +19.8 (c, 0.11 in $CHCl_3$)	Velde and Lavie (1981)
D16-withanolide	Chemotype III	(20R,22R)-14α,20α-dihydroxy-1-oxowitha-2,5,16,24-tetraenolide	NR	Israel	1. Melting point: 273° 2. Optical rotation: $[\alpha]_D$ +19.8 (c, 0.11 in $CHCl_3$)	Velde and Lavie (1982)
Withasomniferin A	NR	6α,7α-Epoxy-17-hydroxy-1-oxowitha-4,24-dienolide	NR	Indian	1. UV λ_{max} 225 (MeOH)	Rahman et al. (1991)
Sominolide	NR	14α,15α-Epoxy-4β,27-dihydroxy-1-oxowitha-2,24-dienolide	NR	Pakistan variety	NR	Rahman et al. (1992)
Pubesenolide or Sominone	NR	1α,3β,27-Trihydroxywitha-5,24-dienolide	Enhances neurite Outgrowth activity	NR	1. Melting point: 145°–146° 2. Optical rotation: $[\alpha]_D$ +28.5 (c, 1.8 in $CHCl_3$) 3. UV: [neutral] λ_{max} 225 (ε 7400) (EtOH)	Rahman et al. (1992) Tohda and Joyashiki (2009)
Withasomidienone	NR	(22R)-27-Hydroxy-3-oxowitha-1,4,24-trienolide	NR	NR	1. Optical rotation: $[\alpha]_D^{25}$ +13.2 (c, 0.075 in MeOH)	Rahman et al. (1993) Misra et al. (2005)
Withaoxylactone	NR	5,6:14,15-Diepoxy-3,4,27-trihydroxy-1-oxowith-24-enolide	NR	NR	1. Optical rotation: $[\alpha]_D^{20}$ −26 (c, 0.6 in $CHCl_3$)	Choudhary et al. (1996)
Somnifericin	NR	4β,5β,6α,27-Tetrahydroxy-1-oxo-20S,22R-witha-24-dienolide	NR	NR	1. Optical rotation: $[\alpha]_D^{20}$ +162 (c, 0.024 in $CHCl_3$)	Choudhary et al. (1996)

Table 1 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
Withasomnilide	Chemotype I	6β,7β-Epoxy-5,8-dihydroxy-1-oxowitha-2,24-dienolide	NR	Indian	1. Melting point: 251°–252° 2. UV: [neutral] λ_{max} 229 (log ε 6.1) (MeOH)	Ali et al. (1997)
Withasomniferanolide	Chemotype I	(20R,22R)-1-oxo-8β,11β,16β-trihydroxywitha-2,5,24-trienolide	NR	Indian	NR	Ali et al. (1997)
Somniferanolide	Chemotype I	(20R,22R)-16α,17α-epoxy-1-oxo-8β,11β-dihydroxywitha-2,5,24-trienolide	NR	Indian	1. Melting point: 230°–231° 2. UV: [neutral] λ_{max} 225 (log ε 7.6) (MeOH)	Ali et al. (1997)
Somniferawithanolide	Chemotype I	(20R,22R)-1-oxo-8β,18,20β-trihydroxywitha-2,5,24-trienolide	NR	Indian	1. Melting point: 127°–128° 2. UV: [neutral] λ_{max} 228 (log ε 7.1) (MeOH)	Ali et al. (1997)
Somniwithanolide	Chemotype I	(20R,22R)-1-oxo-7β,18,20β,27-tetrahydroxywitha-2,4,24-trienolide	NR	Indian	1. Melting point: 144°–146° 2. UV: [neutral] λ_{max} 230 (log ε 7.5); 286 (log ε 3.4); 317 (log ε 13.3) (MeOH)	Ali et al. (1997)
Withasomniferol A	NR	6α,7α-Epoxy-5,20R,27-trihydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 271°–273° 2. Optical rotation: $[\alpha]_D$ +74.9 (c, 0.4 in MeOH) 3. UV: [neutral] λ_{max} 225 log ε 4.19) (EtOH)	Anjaneyulu and Rao (1997)
Withasomniferol B	NR	6α,7α-Epoxy-5,20R,27-trihydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 281°–283° 2. Optical rotation: $[\alpha]_D$ −124 (c, 0.5 in MeOH) 3. UV: [neutral] λ_{max} 228 log ε 4.19) (EtOH)	Anjaneyulu and Rao (1997)
Withasomniferol C	NR	5,14,20-trihydroxy-1-Oxowitha-2,7,24-trienolide	NR	NR	1. Melting point: 294°–295° 2. Optical rotation: $[\alpha]_D$ +67.8 (c, 0.09 in $CHCl_3$) 3. UV: [neutral] λ_{max} 229 (log ε 4.2) (EtOH)	Anjaneyulu and Rao (1997)
4-Deoxywithaperuvin	NR	(20S,22R) 5β,6α,14α,17β,20-Pentahydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 278°–280° 2. UV: [neutral] λ_{max} 225 (ε 18,000) (MeOH)	Abou-Douh et al. (2002)
Viscosalactone B	NR	5β,6β-epoxy-3,4,27-Trihydroxy-1-oxowith-24-enolide	1. Anti-tumour: IC_{50} ranging from 0.32 ± 0.05 to 0.47 ± 0.15 μg/mL	NR	1. Melting point: 184°–186° 2. Optical rotation: $[\alpha]_D$ −19.4 (c, 0.57 in MeOH)	Jayaprakasam et al. (2003)

NR not reported

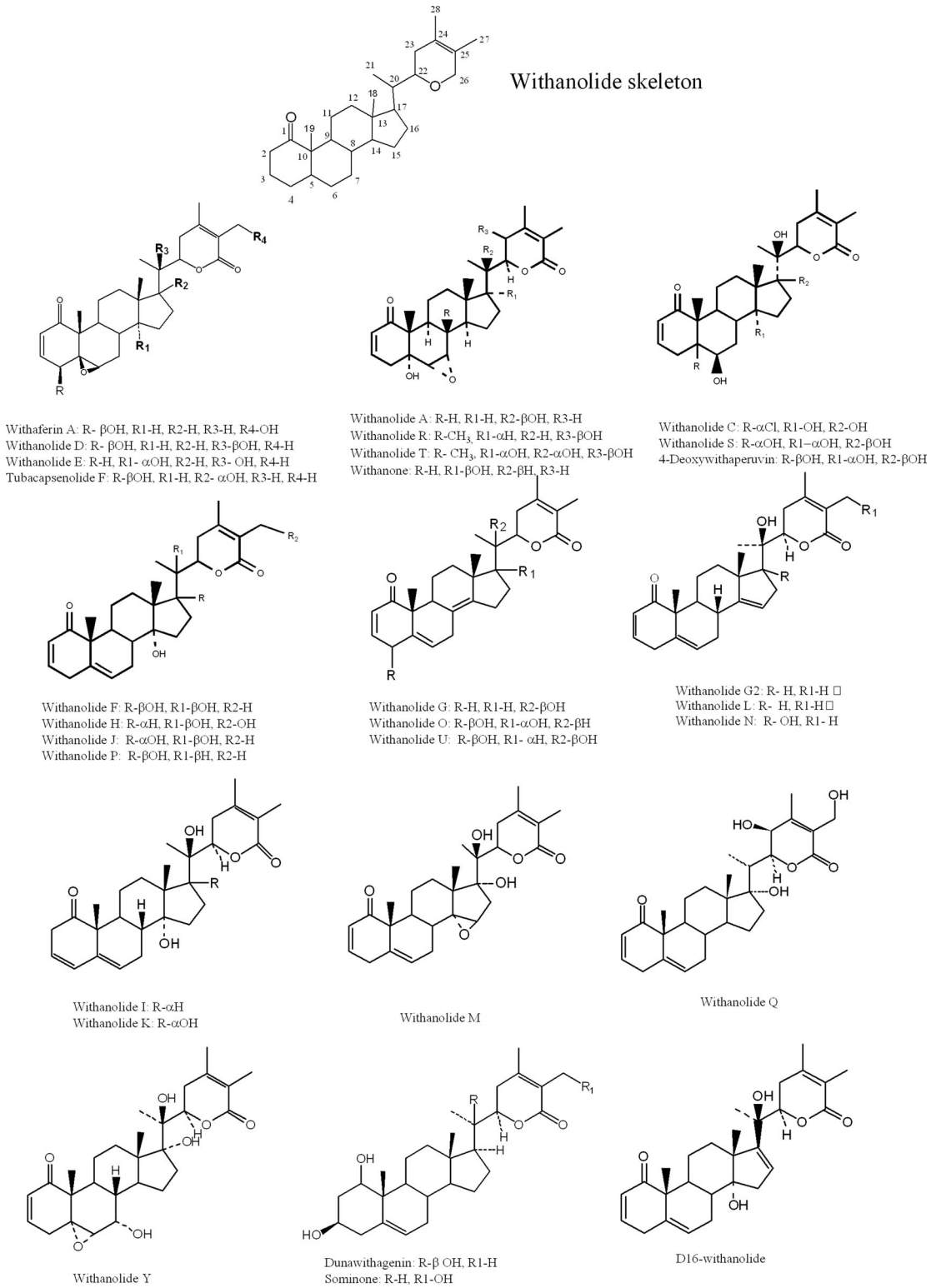
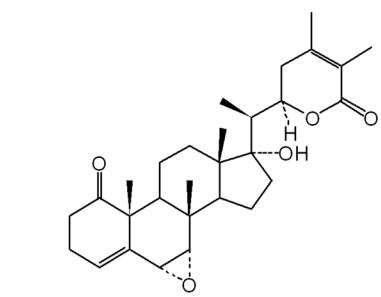
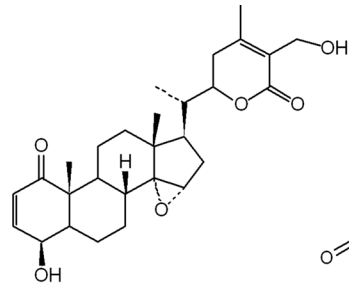


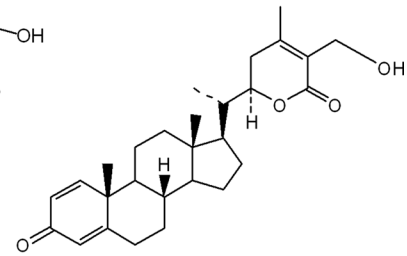
Fig. 1 Major withanolides isolated from *Withania somnifera*



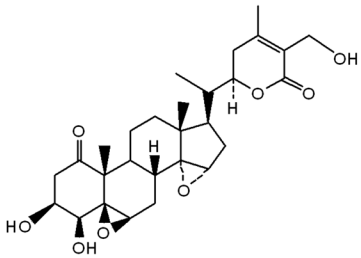
Withasomniferin A



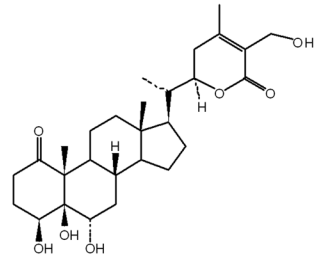
Sominolide



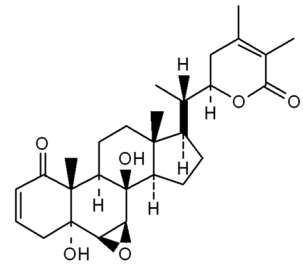
Withasomidienone



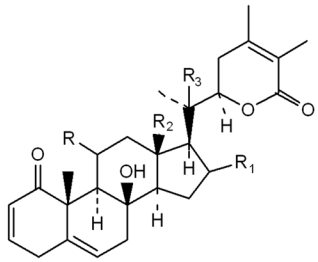
Withaoxylactone



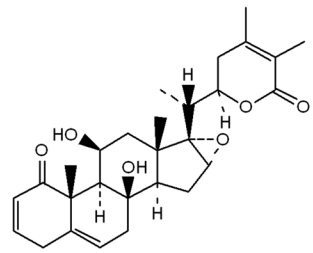
Somnifericin



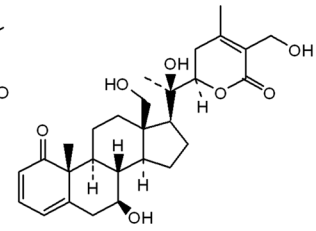
Withasomnilide



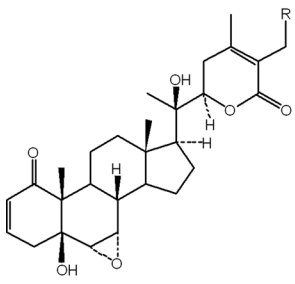
Withasomniferanolide: R-βOH, R1-βOH, R2-CH_3, R3-H
Somniferawithanolide: R-H, R1-H, R2-CH_2OH, R3-βOH



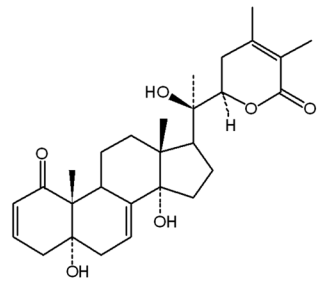
Somniferanolide



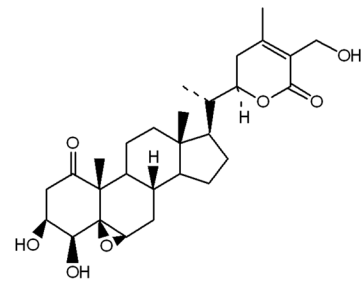
Somniwithanolide



Withasomniferol A: R -OH
Withasomniferol B: R -H



Withasomniferol C



Viscosalactone B

Fig. 1 continued

presence of secondry axial hydroxyl group at δ 4.10 (Glotter et al. 1977).

In the AB ring system, 2,5-dien-1-one, present *inter alia* in withanolide F, G, G2, H, J, L, M, N, O, P, Q, U. Withanolide Q has the same NMR signal in the low-field region for three vinylic proton as found in withanolide G thus allowing assignment of 2,5-dien-1-one structure to the AB ring system. Withanolide Q and R both have a hydroxyl group at position 23 whereas comparison of NMR signals confirms that the only difference between the side chain is the absence of hydroxyl group at position 27 in the latter.

Structure of withanolide Y has been elucidated as 5α, 6α-epoxy-7α, 17α, 20R-trihydroxy-1-oxo-22R-witha-2, 24-dienolide by X-ray single structure analysis. Among all the withanolides it is the only example of having hydroxy group at 7th position (Abraham et al. 1975).

Kirson et al. (1971) investigated *W. somnifera* growing in North-Western India and have reported the isolation of eight new steroidal lactones. e.g. withanone and tubacapsenolide F along with six derivatives. Upfield chemical shift of H-2 and H-3 at δ 6.07 and δ 6.56 respectively, suggest the presence of 2-en-1-one withasteroid moiety having epoxy group at 6, 7 position which can be further verify by presence selectively of 2-mercaptoethanol with the 5β,6β-epoxy steroids substituting the epoxide by a six-membered oxyethylene-2′-thio ring whereas it failed to show such reactivity on 6α,7α-epoxy withasteroids as in withanone (Misra et al. 2008). Tubacapsenolide F has structure similarity with withaferin A, the only difference is the presence of hydroxyl group at position 17 instead of position 27.

Velde et al. isolated a naturally occurring steroidal lactone of withanolide G i.e. dunawithagenin (Velde and Lavie 1981) and Δ16-withanolide (Velde and Lavie 1982) has been isolated from the *W. somnifera* Chemotype III. Dunawithagenin was found to be naturally occurring steroidal lactone of withanolide G and has hydroxyl group at position 1, 3 and 20 whereas Δ16-withanolide is considered to be an intermediate in the biosynthesis of withanolide E. It was the first withanolide having unusual α-oriented side chain in its structure.

Rahman et al. in 1991 isolated withasomniferin-A and in 1992 isolated sominolide and sominone (Rahman et al. 1992) from *W. somnifera.* Withasomniferin-A has epoxy group at position 6, 7 as in withanone but the major difference is the presence unsaturation in ring A at position 4. Also, it has only one hydroxyl group at position 17 but interestingly sominolide has 14,15-epoxy group, inspite of 4,5 or 6,7 epoxy group as present in withaferin A and withanone respectively. Sominone has been characterized by the absence of epoxy group but it has three hydroxyl group at position 1, 3 and 27.

Withasomidienone has also been isolated by Rahman and coworkers and spectroscopic studies showed the presence of three double bond at position 1, 4 and 24 instead of position 2 and 24 as in most of withanolides (Rahman et al. 1993; Misra et al. 2005).

Choudhary et al. (1996) isolated withaoxylactone and somnifericin from *W. somnifera*. Withaoxylactone is the only withanolide which has been characterized by the presence of two epoxy moiety, one is at 5, 6 position and another is at 14, 15 position whereas somnifericin is differentiated by the presence of four hydroxy group at position 4, 5, 6 and 27.

Ali et al. (1997) isolated five new withanolides from the stem bark of *W. somnifera,* collected from the southern region of Delhi, namely withasomnilide, withasomniferanolide, somniferanolide, somniferawithanolide and somniwithanolide. Withasomnilide is structurally similar to withanone but differ only in the position of hydroxyl group as in withasomnilide hydroxyl group present at position 5, 8 instead of 5 and 17 as in withanone whereas somniferanolide has been elucidated as epoxy group at position 16, 17, two hydroxyl group at position 8 and 11 and three double bond at position 2, 5, and 24. Furthermore, withasomniferanolide, somniferawithanolide and somniwithanolide has been characterized by the absence of epoxy group in basic moiety.

Anjaneyulu and Rao (1997) isolated three new withanolides, withasomniferol A, withasomniferol B and withasomniferol C from the non-basic fraction of the benzene and ethyl acetate extracts of the roots of *W. somnifera*. Withasomniferol A has the same basic moiety as present in withanone, the only difference is the presence of hydroxyl groups. Structural elucidation of withasomniferol A showed the presence of hydroxyl group at position 5, 20 and 27 instead of position 5 and 17 as in withanone whereas withasomniferol B has hydroxyl group at position 5 and 20 and also having only one unsaturated position i.e. 2 instead of 2 and 24 in withanone.

Abou-Douth (2002) isolated 4-deoxywithaperuvin. This has been characterized by the presence of five

hydroxyl group at position 5, 6, 14, 17 and 20. Jayaprakasam and Nair (2003) isolated viscosalactone B from *W. Somnifera.* Viscosalactone B is structurally analog to withaferin A as presence of 6,7 epoxy group but it has only one double bond at position 24 instead of 2 and 24 as in withaferin A and also has three hydroxyl group at position 3, 4 and 27 (Jayaprakasam et al. 2003).

A number of withanolide having glycocidal linkage and withanolide derivatives has also been isolated from *W. somnifera* summarized in Tables 2 and 3.

Miscellaneous compounds

Misra et al. 2012 has isolated two compounds, 2,5-dioxo-3tetratriacont-3'-enyl-1,4-dioxane and 1,4-dioxo-3,25,26 trihydroxyergosta-24(28)-ene from *W. somnifera*.

Chemotypes

Extensive studies carried out by Israeli group led by Lavie et al. revealed presence of various chemotypes in *W. somnifera*. Their study revealed that *Withania* has fairly wide geographic distribution and several chemotypes have been identified based on the type and content of various substituted steroidal lactones of withanolide series present in the species. In Israel several chemotypes of *W. somnifera* have been identified differing in their total leaf content of withanolides with various substitution patterns (Lavie et al. 1972; Glotter et al. 1973; Kirson and Glotter 1980). These substitutions are characteristic of each chemotype and seem to be genetic in character. Three chemotypes (I, II, III) of *W. somnifera* L. (Dun.) were found to occur in Israel (Abraham et al. 1968). Figure 2 describes the three chemotypes of *W. somnifera* with their major withanolides as reported by Ganzera et al. (2003), Kirson et al. (1977), Leyon and Kuttan (2004), Ray and Jha (2001), Sethi and Subramanian (2006) and Shohat et al. (1978).

The main withanolides of chemotype I and chemotype II is withaferin A and withanolide D respectively (Kirson and Glotter 1980). In chemotype III, two groups of compounds have been characterized, one with compounds possessing a normal stereochemistry at C_{17} (i.e. β-oriented side chain) *e.g.* Withanolide G and J, and other with α-oriented side chain e.g. withanolide E and F (Lavie et al. 1972). Distinct ecotypes of *W. somnifera* L. Chemotype III have been found to grow in Israel, which are characterized by possessing the same major components in the plant but differing in the relative concentrations. The existence of such ecotypes was confirmed by semi-quantitative reversed phase high performance liquid chromatography (RP-HPLC).

Chemotype I Chemotype I grow in southern and central parts of Israel. The main character in the chemistry of this plant is its ability to introduce OH groups at various sites of the carbon skeleton. The predominant features of this plant are substituents found in A/B rings, the 4β-OH, 5,6-β-epoxy system and absence of OH group at C-20 of the side chain (Kirson and Glotter 1980). Withaferin A was the major product obtained from the leaves of chemotypes-I (ca. 0.2% of the dried leaves). Chemotype I has also been reported from India (Kaushik et al. communicated).

Chemotype II This chemotype of *W. somnifera* occurs mainly in the northern parts of Israel. Withanolide D was found to be present in major quantities (0.53 g/kg dry leaves) others being found in quantities of mg. The compounds present in chemotype II are mainly characterized by presence of OH group at C-20, 4β-OH and 5,6β-epoxy system. Withanolide G being an exception to this was supposed to be as unreacted precursor of this group (Choudhary et al. 2004).

Chemotype III Located in southern coastal plains of Israel. This type of *W. somnifera* contains two groups of compounds all characterized by presence of OH group at C-20 position. Some of them possess three separate double bonds in A, B, and C rings. One type of compound contains a α-oriented side chain e.g. withanolide E and F and other containing a normal β-oriented side chain e.g. withanolide G and J. Withanolide E and J also contain C_{17}–OH group (Choudhary et al. 2004).

Israeli chemotype

Three chemotypes of *W. somnifera* (L.) Dun., *Solanaceae*, each containing different steroidal lactones of the withanolide type, have been found to occur in Israel; they have been called types I, II and III (Abraham et al. 1975; Gupta et al. 2011). Morphological differences could not be detected between the three types, although each of them has a definite and separate area of distribution. No qualitative

Table 2 Bioactivity and other parameters of steroidal glycosides isolated from *Withania somnifera*

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
Withanoside I	NR	6,7-Epoxy-1,3,5-trihydroxy-1-oxowitha-24-enolide 3-*O*-β-D-glucopyranoside	NR	NR	1. Optical rotation: $[\alpha]_D^{28}$ +48.6 (c, 0.1 in MeOH) 2. UV: [neutral] λ_{max} 227 (log ε 3.7) (MeOH)	Matsuda et al. (2001)
Withanoside II	Chemotype I	6,7-Epoxy-1α,3β,5α-trihydroxywitha-24-enolide 3-*O*-β-D-glucopyranoside	NR	Indian	1. Optical rotation: $[\alpha]_D^{28}$ −9.6 (c, 0.6 in MeOH) 2. UV: [neutral] λ_{max} 227 (log ε 3.8) (MeOH)	Matsuda et al. (2001)
Withanoside III	Chemotype I	6,7-Epoxy-1α,3β,5α,27-tetrahydroxywitha-24-enolide 3-*O*-β-D-glucopyranoside	NR	Indian	1. Optical rotation: $[\alpha]_D^{28}$ −24 (c, 0.1 in MeOH) 2. UV: [neutral] λ_{max} 228 (log ε 3.7) (MeOH)	Zhao et al. (2002)
Withanoside IV	Chemotype I	1α,3β,27-Trihydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside	1. Neurite outgrowth activity at 1 mM 2. Inhibit lipid peroxidation by 25% at 100 mg/mL	Indian	1. Optical rotation: $[\alpha]^{28}$ + 5.2 (c, 0.2 in MeOH) 2. UV: [neutral] λ_{max} 228 (log ε 3.7) (MeOH)	Jayaprakasam et al. (2004) Zhao et al. (2002)
Withanoside V	Chemotype I	1α,3β-Dihydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside	Inhibit lipid peroxidation by 82.5% at 10 ppm	Indian	1. Optical rotation: $[\alpha]_D^{29}$ +7.8 (c, 0.3 in MeOH) 2. UV: [neutral] lmax 227 (log ε 3.7) (MeOH)	Jayaprakasam et al. (2004) Zhao et al. (2002)
Withanoside VI	Chemotype I	1α,3β,20-Trihydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside	1. Inhibitory activity for tachyphylaxis (10 and 30 mM) 2. Neurite outgrowth activity at 1 mM 3. Inhibit lipid peroxidation by 86% at 50 ppm	Indian	1. Optical rotation: $[\alpha]_D^{27}$ −11.6 (c, 0.5 in MeOH) 2. UV: [neutral] λ_{max} 228 (log ε 3.8) (MeOH)	Jayaprakasam et al. (2004) Matsuda et al. (2001) Zhao et al. (2002)
Withanoside VII	NR	1,3,7-Trihydroxy-1-oxowitha-5,24-enolide 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside	NR	NR	1. Optical rotation: $[\alpha]_D^{29}$ +5 (c, 0.1 in MeOH) 2. UV: [neutral] λ_{max} 228 (log ε 3.7) (MeOH)	Matsuda et al. (2001)
Withanoside VIII	Chemotype I	27-*O*-β-D-Glucopyranosylpubesenolide 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside	NR	Indian	1. Optical rotation: $[\alpha]_D^{23}$ +10.4 (c, 0.264 in MeOH)	Zhao et al. (2002)
Withanoside IX	Chemotype I	27-*O*-β-D-Glucopyranosyl (1 → 6)-β-D-glucopyranosylpubesenolide 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside	NR	Indian	1. Optical rotation: $[\alpha]_D^{23}$ +16.7 (c, 0.096 in MeOH)	Zhao et al. (2002)

Table 2 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
Withanoside X	Chemotype I	27-*O*-β-D-Glucopyranosylpubesenolide 3-*O*-β-D-glucopyranoside	NR	Indian	1. Optical rotation: $[\alpha]_D^{23}$ +21.1 (c, 0.11 in MeOH)	Zhao et al. (2002)
Withanoside XI or Coagulin Q	Chemotype I	(20R,22R)-1α,3β,20,27-Tetrahydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranoside	Neurite outgrowth activity at 1 mM	Indian	1. Optical rotation: $[\alpha]_D$ +30 (c, 0.31 in MeOH) 2. UV: [neutral] λ_{max} 223 (log ε 4.06) (MeOH)	Zhao et al. (2002) Rahman et al. (1999)
24,25-Dihydrowithanoside VI	NR	24,25 Dihydro-1α,3β,20-trihydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside	NR	NR	NR	Jayaprakasam et al. (2004)
Sitoindoside VII	NR	3β-Hydroxyergost-5,24-diene-3-*O*-[6-*O*-palmitoyl-β-D-glucopyranoside]	Antistress activity	Indian	1. Optical rotation: $[\alpha]_D^{28}$ −16.8 (c, 0.55 in $CHCl_3$)	Bhattacharya et al. (1987)
Sitoindoside VIII	NR	24,25-Epoxy-3β-hydroxyergost-5-ene-3-*O*-[6-*O*-palmitoyl-β-D-glucopyranoside]	Antistress activity	Indian	1. Optical rotation: $[\alpha]_D^{28}$ −11.2 (c, 0.32 in $CHCl_3$)	Bhattacharya et al. (1987)
Sitoindoside IX	NR	5,6-Epoxy-4,27-dihydroxy-1-oxowitha-2,24-dienolide 27-*O*-β-D-glucopyranoside	1. Immunomodulatory at 100–400 g/mouse 2. Anti-stress at 50–200 mg/kg 3. In Alzheimer's disease 4. In Iron induced hepatoxicity	NR	1. Optical rotation: $[\alpha]_D^{28}$ −7.5 (c, 1 in H_2O)	Ghosal et al. (1989)
Sitoindoside X	NR	5,6-Epoxy-4,27-dihydroxy-1-oxowitha-2,24-dienolide 27-*O*-(6-*O*-hexadecanoyl-β-D-glucopyranoside).	1. Immunomodulatory at 100–400 g/mouse 2. Anti-stress at 50–200 mg/kg 3. In Alzheimer's disease 4. In Iron induced hepatoxicity	NR	NR	Ghosal et al. (1989)
Glucosomniferanolide	NR	20-Hydroxy-1-oxowitha-2,5,24-trienolide 20-*O*-β-D-glucopyranoside	NR	NR	1. Melting point: 214°–215° 2. Optical rotation: $[\alpha]_D^{22}$ −30.6 (c, 0.1 in $CHCl_3$) 3. UV: [neutral] λ_{max} 207 (log ε 3.1); 267 (log e 0.9) (MeOH)	Kumar et al. (2004)
Physagulin D	NR	(20S,22R)-1α,27-Dihydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranoside	Anti-tumour: weak or no activity at conc. 30 µg/mL	NR	1. Optical rotation: $[\alpha]_D$ +21 (c, 0.8 in MeOH)	Jayaprakasam et al. (2003)
Physagulin D (1-6)-β-D-glucopyranosyl-(1-4)-β-D-glucopyranoside	NR	(20S,22R)-1α,27-Dihydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside	NR	NR	NR	Jayaprakasam and Nair (2003)
27-*O*-β-D-glucopyranosylphysagulin D	NR	(20S,22R)-1α-Hydroxywitha-5,24-dienolide 27-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranoside	Anticancer	NR	NR	Jayaprakasam and Nair (2003)

Table 2 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
4,16-Dihydroxy-5β,6β-epoxyphysagulin D	NR	5β,6β-Epoxy-1α,4,16,27-tetrahydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranoside	NR	NR	NR	Jayaprakasam and Nair (2003)
27-*O*-β-D-glucopyranosylviscosalactone B	NR	5β,6β-Epoxy-3,4-dihydroxy-1-oxowith-24-enolide 27-*O*-β-D-glucopyranoside	1. Anti-tumour: IC_{50} ranging from 7.9 ± 2.9 and 17.3 ± 3.9 μg/mL	NR	NR	Jayaprakasam and Nair (2003)
Ashwagandhanolide	NR	Unknown	1. Anti-tumour: IC_{50} ranging from 0.43 to 1.48 μg/mL 2. Inhibit lipid peroxidation and activity of enzyme COX-2	NR	NR	Subbaraju et al. (2006)

NR not reported

ontogenetic changes in the withanolide content could be observed.

Italian chemotypes

In Italy, *W. somnifera* is only present in Sicily and Sardinia. Absence of substitution at C-4, configurations 20S and 14α-OH, and predominance of 17α-OH compounds in Sicilian species is evident of the fact that Sicilian plants belong to the Israelian chemotype III. The only difference is in the presence of withanolide J as chief constituent as compared to the withanolide E in Israelian species. Similar research studies on Sardinian plants led to the isolation of identical components as of Sicilian, thus confirming the Italian chemical race (Nittala and Lavie 1981).

Indian chemotypes

The chemotype I growing in India is characterized by presence of two main constituents i.e. withaferin A and withanone (Besselle and Lavie 1987). A number of samples of leaves from North Western India were found to show the predominant characteristics of chemotypes I. Beside this, intermediate of chemotype I and chemotype II were also discovered in the study. This chemotype is characterized by the presence of withaferin A and withanolide D as its chief constituents (TERI 2006).

Hybridisation of various chemotypes

Many hybrids of various chemotypes of *W. somnifera* have been attempted, which lead to production of new withanolides. The withanolides presents in hybrid of various chemotypes are shown in Fig. 3 and details of such new recombination are discussed below.

Hybrids of chemotype II (Israel) and Indian I (Delhi)

Hybrid plants of *W. somnifera* from cross-pollinations of chemotypes II (Israel) and Indian I (Delhi) have been examined. The chief constituent isolated was 14β-hydroxywithanone (6α, 7α-Epoxy-5α, 14β, 17α-trihydroxy-1-oxo-22*R*-witha-2, 24-dienolide) This compound is the first example of a 14β-substitution among withanolides (Nittala and Lavie 1981).

Table 3 Bioactivity and other parameters of withanolides derivatives isolated from *Withania somnifera*

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
Quresimine A or 2,3-dihydro-3-methoxywithaferin A	NR	5,6-Epoxy-4,27-dihydroxy-3-methoxy-1-oxowith-24-enolide	NR	NR	1. Melting point: 245°–247°	Lavie et al. (1965)
					2. Optical rotation: $[\alpha]_D$ +11.5 (c, 1.6 in $CHCl_3$)	Anjaneyulu and Rao (1997)
					3. UV: [neutral] λ_{max} 212 (EtOH)	
Quresimine B or 2,3dihydro-3-methoxy-27-deoxywithaferin A	NR	5,6-Epoxy-4-hydroxy-3-methoxy-1-oxowith-24-enolide	NR	NR	1. Melting point: Mp 256°–257°	Lavie et al. (1965)
					2. Optical rotation: $[\alpha]_D$ −40.5 (c, 0.4 in $CHCl_3$)	Anjaneyulu and Rao (1997)
					3. UV: [neutral] l_{max} 224(EtOH)	
27-Deoxy-14-hydroxy withaferin A	Chemotype I	5β, 6β-Epoxy-4β, 14-dihydroxy-1-oxo-22 R-witha-2, 24-dienolide	NR	Israel/ India	1. Melting point: 265°–267° dec.	Glotter et al. (1966)
					2. Optical rotation: $[\alpha]_D$ +67 (c, 0.46 in $CHCl_3$)	
27-Deoxywithaferin A	Chemotype I	5β, 6β-Epoxy-4β-hydroxy-1-oxo-22 R-witha-2, 24-dienolide	NR	Israel/ India	1. Melting point: 168°–169°	Kirson et al. (1970)
					2. Optical rotation: $[\alpha]_D$ +101.5 (c, 0.5 in $CHCl_3$)	
5β,6β-Epoxy-4β-hydroxy-1oxowitha-2-enolide	NR	5β,6β-Epoxy-4β-hydroxy-1oxowitha-2-enolide	NR	South Africa	NR	Kirson et al. (1970)
5β,6β-Epoxy-4β,20α(R)-dihydroxy-1-oxowitha-2-enolide	NR	5β,6β-Epoxy-4β,20α(R)-dihydroxy-1-oxowitha-2-enolide	NR	South Africa	1. Melting point: 275°	Kirson et al. (1970)
					2. Optical rotation: $[\alpha]_D$ +14 (c, 0.3 in $CHCl_3$)	
27-Deoxy-2,14,24-trienolide withaferin A	Chemotype I	5β, 6β-Epoxy-4β-hydroxy-1-oxo-22 R-witha-2,14,24-trienolide	Cholinesterase inhibiting activity	Indian	NR	Kirson et al. (1971)

Table 3 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
17α,27-Dihydroxy-1-oxo-22R-witha-2,5,24-trienolide	Chemotype I	27-hydroxy-17α-hydroxy-1-oxo-22R-witha-2,5,24-trienolide	Cholinesterase inhibiting activity	Indian	NR	Kirson et al. (1971) Choudhary et al. (2004)
5α,17α-Dihydroxy-1oxo-22R-witha-2,6,24-trienolide	Chemotype I	5α,17α-Dihydroxy-1oxo-22R-witha-2,6,24-trienolide	NR	Indian	NR	Kirson et al. (1971)
6α,7α-Epoxy-1,3,5-trihydroxy-with-24-enolide	NR	6α,7α-Epoxy-1,3,5-trihydroxy-with-24-enolide	NR	NR	1. Melting point: 217°–219° 2. Optical rotation: $[\alpha]_D^{30}$ +86 (c, 0.25 in $CHCl_3$)	Kirson et al. (1971)
6α,7α-Epoxy-5α,27-dihydroxy-1-oxo-22R-witha-2,24-dienolide	Chemotype I	6α,7α-Epoxy-5α,27-dihydroxy-1-oxo-22R-witha-2,24-dienolide	NR	Indian	NR	Kirson et al. (1971)
7α,27-Dihydroxy-1-oxo-22R-witha-2,5,24-trienolide	Chemotype I	7α,27-Dihydroxy-1-oxo-22R-witha-2,5,24-trienolide	NR	Indian	NR	Kirson et al. (1971)
Dihydrowithaferin A	Chemotype I	2,3-Dihydro-5β, 6β-epoxy-4β, 27-dihydroxy-1-oxo-22 R-witha-24-enolide	Cholinesterase inhibiting activity	NR	1. Melting point: 229°–230° 2. Optical rotation: $[\alpha]_D$ +8 (c, 0.95 in $CHCl_3$)	Choudhary et al. (2004) Lavie et al. (1975)
27-Hydroxy-withanolide D	Chemotype II	5β,6β-Epoxy-4β,20α,27-trihydroxy-1-oxo-20*R*,22*R*-witha-2,24-dienolide	NR	Israel	NR	Abraham et al. (1975)
14α-Hydroxy-withanolide D	Chemotype II	5β,6β-Epoxy-4,14,20-trihydroxy-1-oxowitha-2,24-dienolide	NR	Israel	NR	Abraham et al. (1975)
17α-Hydroxy-Withanolide D	Chemotype II	5,6-Epoxy-4,17,20-trihydroxy-1-oxowitha-2,24-dienolide	NR	Israel	NR	Abraham et al. (1975)

Table 3 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
4β-Hydroxywithanolide E	NR	4β,14α,17β,20α-tetrahydroxy-5β, 6β-Epoxy-1-oxo-17S, 20S, 22R-witha-2, 24-dienolide	Anticancer	NR	1. Melting point: 205°–214° (197°–198°) 2. Optical rotation: $[\alpha]_D$ +95.8 (c, 0.5 in $CHCl_3$) 3. Solubility: Sol. MeOH, C_6H_6; poorly sol. H_2O 4. UV: [neutral] λ_{max} 219 (e 14,700) (EtOH) (Berdy)	Kirson et al. (1976) Yoshida et al. (1979)
27-Hydroxywithanolide B	NR	6,7-Epoxy-5,27-dihydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 230°–232° (27-°Ac) 2. Optical rotation: $[\alpha]_D$ +73 (c, 0.11 in $CHCl_3$) (27-Ac)	Sethi and Subramanian (2006)
5,6-Epoxy-20-hydroxy-1,4-dioxowith-2-enolide or 24,25-dihydro-4-dehydrowithanolide D	Hybrids South Africa chemotype X chemotype II originating in Israel	5,6-Epoxy-20-hydroxy-1,4-dioxowith-2-enolide	NR	NR	1. Melting point: 260°–263° 2. Optical rotation: $[\alpha]_D$ +49.2 (c, 0.25 in $CHCl_3$)	Eastwood et al. (1980)
5β,6β-Epoxy-4,20-dihydroxy-1-oxowith-2-enolide or 24,25-dihydrowithanolide D	Hybrids South Africa chemotype X chemotype II originating in Israel	5β,6β-Epoxy-4,20-dihydroxy-1-oxowith-2-enolide	NR	NR	Melting point: 275° Optical rotation: $[\alpha]_D$ +14 (c, 0.3 in $CHCl_3$)	Eastwood et al. (1980)
5,6-Epoxy-20-hydroxy-1,4-dioxowitha-2,24-dienolide or 4-dehydrowithanolide D	Hybrids South Africa chemotype X chemotype II originating in Israel	5,6-Epoxy-20-hydroxy-1,4-dioxowitha-2,24-dienolide	NR	NR	1. Melting point: 273°–274° 2. Optical rotation: $[\alpha]_D$ +113.5 (c, 0.22 in $CHCl_3$)	Eastwood et al. (1980)

Table 3 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
5β,6β-Epoxy-4β,20R-dihydroxy-1-oxo-with-24-enolide or 2,3-dihydrowithanolide D	Hybrids South Africa chemotype X chemotype II originating in Israel	5β,6β-Epoxy-4β,20R-dihydroxy-1-oxo-with-24-enolide	NR	NR	1. Melting point: 277°–278° 2. Optical rotation: $[\alpha]_D$ −28 (c, 0.2 in $CHCl_3$)	Eastwood et al. (1980)
Withanolide D chlorohydrin	Hybrid chemotypes II or III (Israel) X Indian I (Delhi)	6-Chloro-4,5,20-trihydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 245°–247° 2. Optical rotation: $[\alpha]_D$ +19.8 (c, 0.3 in $CHCl_3$)	Nittala and Lavie (1981)
6-Cl-withanolide S OR 4-deoxyphysalolactone	Chemotype III	6-Chloro-5,14,17,20-tetrahydroxy-1-oxowitha-2,24-enolide	NR	Israel	1. Melting point: 207°–208° 2. Optical rotation: $[\alpha]_D$ +103 (c, 0.1 in $CHCl_3$) 3. UV: [neutral] λ_{max} 220 (e 17,900) (EtOH) (Derep)	Besselle and Lavie (1987) Nittala et al. (1981)
6α-Chloro-5β-hydroxywithaferin A	NR	6α-Chloro-5β-hydroxywithaferin A	NR	NR	NR	Nittala et al. (1981)
14α-Hydroxywithanone	Hybrid chemotypes II or III (Israel) X Indian I (Delhi)	6,7-Epoxy-5,14α,17-trihydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 250° 2. Optical rotation: $[\alpha]_D$ +29.75 ($CHCl_3$/MeOH)	Nittala and Lavie (1981)
14β-Hydroxywithanone	Hybrid chemotypes II or III (Israel) X Indian I (Delhi)	6,7-Epoxy-5α,14β,17α-trihydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 280° 2. Optical rotation: $[\alpha]_D$ +36.7 ($CHCl_3$/MeOH)	Nittala and Lavie (1981)
6β,7β-Epoxywithanone	NR Hybrid chemotypes II or III (Israel) X Indian I (Delhi)	6α,7α-Epoxy-5,14,17-trihydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 250° 2. Optical rotation: $[\alpha]_D$ +29.8 (c, 0.3 in $CHCl_3$/MeOH, 4:1)	Nittala and Lavie (1981)

Table 3 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
14,20-Dihydroxy-1-oxowitha-2,4,6,24-tetraenolide	Hybrid chemotypes II or III (Israel) X Indian I (Delhi)	14,20-Dihydroxy-1-oxowitha-2,4,6,24-tetraenolide	NR	NR	1. Melting point: 220°–222° 2. Optical rotation: $[\alpha]_D$ +12.2 (c, 0.3 in $CHCl_3$)	Nittala and Lavie (1981)
(22R)-5β-formyl-6β,27-dihydroxyl-1-oxo-4-norwith-24-enolide	NR	(22R)-5β-Formyl-6β,27-dihydroxyl-1-oxo-4-norwith-24-enolide	NR	NR	1. Optical rotation: $[\alpha]_D$ +33.8 (c, 0.21 in $CHCl_3$)	Nittala and Lavie (1982)
2,3-Dihydrowithaferin A-3β-*O*-sulfate	NR	2,3-Dihydrowithaferin A-3β-*O*-sulfate	Anti-cancer activity due to conversion into withaferin-A in cell culture condition		1. Optical rotation: $[\alpha]_D^{25}$ +14.5 (c, 0.21 in MeOH) 2. Melting point >167 °C 3. UV (MeOH) λmax 214 nm	Xu et al. (2009)
27-Hydroxywithanolide I	NR	14,20,27-Trihydroxy-1-oxowitha-3,5,24-trienolide	NR	Israel	1. Melting point: 184° 2. Optical rotation: $[\alpha]_D$ +98.7 (c, 0.12 in $CHCl_3$)	Velde and Lavie (1981)
5α-Ethoxy-6β,14α,17β,20α-tetrahydroxy-1-oxo-20S,22R-witha-2,24-dienolide	Chemotype III	5α-Ethoxy-6β,14α,17β,20α-tetrahydroxy-1-oxo-20S,22R-witha-2,24-dienolide	NR	Israel	1. Melting point: 167° 2. Optical rotation: $[\alpha]_D$ +62.7 (c, 0.11 in $CHCl_3$)	Velde and Lavie (1981)
3β,20α-Dihydroxy-1-oxo-20R,22R-witha-5,24-dienolide	Chemotype III	3β,20α-Dihydroxy-1-oxo-20R,22R-witha-5,24-dienolide	NR	Israel	NR	Velde and Lavie (1981)
14,20-Dihydroxy-1-oxowitha-2,5,16,22-tetraenolide	NR	14,20-Dihydroxy-1-oxowitha-2,5,16,22-tetraenolide	NR	NR	1. Melting point: 212°–213° 2. Optical rotation: $[\alpha]_D$ +44 (c, 0.34 in $CHCl_3$)	Velde and Lavie (1981)
5-Dehydroxywithanolide R	NR	6,7-Epoxy-23-hydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. UV λ_{max} 224 (MeOH)	Rahman et al. (1991)

Table 3 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
17-Isowithanolide E	Chemotype III	5,6-Epoxy-14,17,20-trihydroxy-1-oxowitha-2,24-dienolide	NR	Sicilian/ Sardinian	1. Melting point: 262° 2. Optical rotation: $[\alpha]_D$ +21.3 (c, 0.2 in MeCN)	Vitali et al. (1996)
5-Ethyl-withanolide S	Chemotype III	5α-etoxy-6α,14α,17β,20α-pentahydroxy-1-oxo-22R-witha-2,24-dienolide	NR	Israel	NR	Vitali et al. (1996)
4β,5β,6α,27-Tetrahydroxy-1-oxo-with-2,24-enolide or 2,3-didehydrosomnifericin	Chemotype I	4β,5β,6α,27-Tetrahydroxy-1-oxo-with-2,24-enolide	NR	Indian	NR	Kuroyanagi et al. (1999)
5β,6β-Epoxy-4,20-dihydroxy-3-methoxy-1-oxowithanolide	NR	5β,6β-Epoxy-4,20-dihydroxy-3-methoxy-1-oxowithanolide	NR	Indian	NR	Kuroyanagi et al. (1999)
5β,6β-Epoxy-3,4,20-trihydroxy-1-oxowithanolide	NR	5β,6β-Epoxy-3,4,20-trihydroxy-1-oxowithanolide	NR	Indian	NR	Kuroyanagi et al. (1999)
14,17-Dihydroxywithanolide R	NR	6,7-Epoxy-5,14,17,23-tetrahydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 260°–262° 2. UV: [neutral] λ_{max} 221 (ε 17,400) (MeOH)	Abou-Douh (2002)
3α,6α-Epoxy-4β,5β,27-trihydroxy-1-oxo-20S,22R-witha-24-enolide	Chemotype I	3α,6α-Epoxy-4β,5β,27-trihydroxy-1-oxo-20S,22R-witha-24-enolide	1. Neurite outgrowth activity at 1 mM	Indian	Optical rotation: $[\alpha]_D^{23}$ −17.4 (c, 0.109 in MeOH)	Zhao et al. (2002)
24, 25-Dihydro-27-desoxywithaferin A	NR	5β, 6β-Epoxy-4β, 14-dihydroxy-1-oxo-22 R-witha-2-enolide	1. Cyclooxygenase-2 enzyme inhibitory	NR	NR	Jayaprakasam and Nair (2003)
5β,6β-Epoxy-1α,3β,4β,16β,27-pentahydroxywith-24-enolide	NR	5β,6β-epoxy-1α,3β,4β,16β,27-pentahydroxywith-24-enolide	NR	NR	NR	Kirson et al. (1975)

Table 3 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
4-Dimethyloxocyclopropyl-2,3-dihydrowithaferin A	Chemotype I	4-Dimethyl oxocyclopropyl-2,3-dihydro-5β, 6β-epoxy-4β, 27-dihydroxy-1-oxo-22 R-witha-24-enolide	Anticancer	NR	NR	Jayaprakasam et al. (2003)
5β, 6β-Epoxy-1-oxo-witha-2-ene-27-ethoxy-olide	NR	5β, 6β-Epoxy-1-oxo-witha-2-ene-27-ethoxy-olide	Adaptogenic activity at dose 2.5 mg/kg body weight	NR	NR	Kaur et al. (2003)
5β,6β-Epoxy-4,17,27-trihydroxy-1-oxowitha-2,24-dienolide	NR	5β,6β-Epoxy-4,17,27-trihydroxy-1-oxowitha-2,24-dienolide	Cholinesterase inhibiting activity	NR	1. Optical rotation: $[\alpha]_D^{25}$ +12 (c, 0.11 in CH_2Cl_2) 2. UV: [neutral] λ_{max} 221 (log ε 2.68) ($CDCl_3$)	Choudhary et al. (2004)
6α,7α-Epoxy-3,5,20-trihydroxy-1-oxowith-24-enolide	NR	6α,7α-Epoxy-3,5,20-trihydroxy-1-oxowith-24-enolide	Cholinesterase inhibiting activity	NR	1. Optical rotation: $[\alpha]_D^{25}$ −196 (c, 0.006 in MeOH) 2. UV: [neutral] λ_{max} 200 (log ε 3.46) (MeOH)	Choudhary et al. (2004)
5β,6β-Epoxy-1oxowitha-2-enolide	NR	5β,6β-Epoxy-1oxowitha-2-enolide	Pretreatment in dose: 20 mg/kg bwt prevent skin carcinoma	NR	NR	Mathur et al. (2004)
5β,6β-Epoxy-4β-hydroxy-1-oxowitha-2,16,24-trienolide	NR	5β,6β-Epoxy-4β-hydroxy-1-oxowitha-2,16,24-trienolide	NR	NR	1. Melting point: 268° 2. Optical rotation: $[\alpha]_D^{30}$ +92.6 (c, 0.25 in $CHCl_3$)	Misra et al. (2005)
6α,7α-Epoxy-3,5,17-trihydroxy-1-oxowith-24-enolide	NR	6α,7α-Epoxy-3,5,17-trihydroxy-1-oxowith-24-enolide	NR	NR	1. Melting point: 258° 2. Optical rotation: $[\alpha]_D^{30}$ +66 (c, 0.25 in MeOH)	Misra et al. (2005)

Table 3 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
6α,7α-Epoxy-5α,17α-dihydroxy-1-oxo-3β-*O*-sulfate-witha-24-enolide	NR	6α,7α-Epoxy-5α,17α-dihydroxy-1-oxo-3β-*O*-sulfate-with a-24-enolide	NR	NR	1. Melting point: 158° 2. Optical rotation: $[\alpha]_D^{30}$ +59.40 (c, 0.25 in MeOH)	Misra et al. (2005)
Isowithanone	NR	6,7-Epoxy-5,17-dihydroxy-1-oxowitha-2,24-dienolide	NR	NR	UV: [neutral] lmax 228 (no solvent reported)	Bandhoria et al. (2006) Lal et al. (2006)
6α,7α-Epoxy-16β-acetoxy-5α-hydroxy-1-oxo-witha-2,17(20,)24-trienolide	NR	6α,7α-Epoxy-16β-acetoxy-5α-hydroxy-1-oxo-witha-2,17(20,)24-trienolide	NR	NR	1. Melting point: 238° 2. Optical rotation: $[\alpha]_D^{30}$ +0.97 (c, 0.24)	Misra et al. (2008)
5α,7α-Epoxy-6α,20α-dihydroxy-1-oxowitha-2,24-dienolide	NR	5α,7α-Epoxy-6α,20α-dihydroxy-1-oxowitha-2,24-dienolide	NR	NR	1. Melting point: 242° 2. Optical rotation: $[\alpha]_D^{30}$ +12.73 (c, 0.14)	Misra et al. (2008)
27-Acetoxy-4-β,6α-dihydroxy-5β-chloro-1-oxowith-2,24-dienolide	NR	27-Acetoxy-4-β,6α-dihydroxy-5β-chloro-1-oxowith-2,24-dienolide	Anticancer against human lung cancer cell lines	Karachi, Pakistan	1. UV ($CHCl_3$) λ_{max} (log ε) 249 (1.5) nm	Choudhary et al. (2010)
5β,6β,14α,15α-Diepoxy-4β,27-dihydroxy-1-oxowitha-2,24-dienolide	NR	5β,6β,14α,15α-Diepoxy-4β,27-dihydroxy-1-oxowitha-2,24-dienolide	Anticancer against human lung cancer cell lines	Karachi, Pakistan	1. UV ($CHCl_3$) λmax (log ε) 249 (1.1) nm	Choudhary et al. (2010)
6α-Chloro-5β,17α-dihydroxywithaferin A	NR	6α-Chloro-5β,17α-dihydroxywithaferin A	NR	NR	1. Melting point: 238–240 °C 2. Optical rotation: $[\alpha]_{D\,546}^{25}$ + 53.1 (c, 0.001 in MeOH) 3. UV (MeOH) λ_{max} (log ε) 220 (3.93) nm	Tong et al. (2011)

Table 3 continued

Withanolides	Chemotype	IUPAC nomenclature	Bioactivity	Reported from	Physiochemical analysis	References
3α-(Uracil-1-yl)-2,3-dihydrowithaferin A	NR	3α-(Uracil-1-yl)-2,3-dihydrowithaferin A	NR	NR	1. Optical rotation: $[\alpha]_D^{20}$ +39.5 (c, 0.07 in $CHCl_3$) 2. UV (MeOH) λ_{max} (log ε) 210 (4.18), 264 (3.95) nm	Xu et al. (2011)
3β-(Adenin-9-yl)-2,3-dihydrowithaferin A	NR	3β-(Adenin-9-yl)-2,3-dihydrowithaferin A	NR	NR	1. Optical rotation: $[\alpha]_D^{20}$ +14.4 (c, 0.03, 1:1 MeOH–$CHCl_3$) 2. UV (MeOH) λ_{max} (log ε) 208 (4.38), 261 (4.13) nm	Xu et al. (2011)
3β-*O*-Butyl-2,3-dihydrowithaferin A	NR	3β-*O*-Butyl-2,3-dihydrowithaferin A	NR	NR	1. Optical rotation: $[\alpha]_D^{20}$ −16.4 (c, 0.14, MeOH) 2. UV (MeOH) λ_{max} (log ε) 210 (4.02)	Xu et al. (2011)
6α,7α-Epoxy-5α,17α, 27-trihydroxy-1-oxo-22R-witha-2, 24-dienolide	NR	6α,7α-Epoxy-5α,17α, 27-trihydroxy-1-oxo-22R-witha-2, 24-dienolide	NR	India	NR	Gupta et al. (2011)
5,6-De-epoxy-5-en-7-one-17-hydroxy withaferin A	NR	5,6-De-epoxy-5-en-7-one-17-hydroxy withaferin A	Cytotoxic	India	1. Melting point: 206°–208° 2. Optical rotation: $[\alpha]_D^{20}$ +55 (c, 0.02 in MeOH	Siddique et al. (2014)

NR not reported

Hybrids of chemotype III (Israel) and Indian I (Delhi)

Hybrid plants of *W. somnifera* obtained from cross-pollinations of chemotypes III (Israel) and Indian I (Delhi) have also been found to contain 14β-hydroxywithanone as major compound similar to hybrids of chemotype II (Israel) and Indian I (Delhi). Beside this, three new compounds viz.14α-hydroxywithanone(5α, 14α,17α-trihydroxy-6α,7α-epoxy-1-oxo-22-*R*-witha-2,24 dienolide), 6β,7β-epoxywithanone (5α,14α,17α-trihydroxy-6β,7β-epoxy-1-oxo-22 *R*-witha-2,24-dienolide) and 2,4,6-trien-1-one (14α, 20α-dihydroxy-1-oxo-22 R-witha-2,4,6,24-tetraenolide) have been reported (Nittala and Lavie 1981).

Hybrids of chemotype II (Israel) and South African Chemotype

Crossbreeding of *W. somnifera* chemotype II (Israel) by South African chemotype led to the isolation of six compounds, three of them being new ones. The principle withanolide was found to be withanolide D followed by 24,25-dihydro-4-dehydrowithanolide D, 24,25-dihydrowithanolide D, 4-dehydrowithanolide D, 2,3-dihydrowithanolide D and withaferin A (Sethi and Subramanian 1976).

Pharmacological utility

The biological activity of Ashwagandha is mainly attributed to steroidal components, withanolides present in them, making plant useful in a wide variety of pathological states. Various activity reported by various workers is discussed in this section of review:

Adaptogenic activity

Withanolide 5β, 6β-epoxy-1-oxo-witha-2-ene-27-ethoxy-olide isolated from the roots of *W. somnifera* when evaluated for the stress-related parameters, namely serum lactate dehydrogenase (LDH) activity, serum creatine phosphokinase (CPK) activity, serum corticosterone levels, and serum lipid peroxidation (LPO) level showed significant decrease in a serum CPK, LDH, and LPO levels was observed in animal pretreated with 5β, 6β-epoxy-1-oxo-witha-2-ene-27-ethoxy-olide at the dose of 2.5 mg/kg body weight) in comparison to control when subjected to C-H-R stress. Thus concluded, the withanolide, 5β, 6β-epoxy-1-oxo-witha-2-ene-27-ethoxy-olide could prove to be an effective agent to counteract C-H-R stress (Misra et al. 2008).

In another study, the adaptogenic activity of a standardized extract of WS roots was also investigated

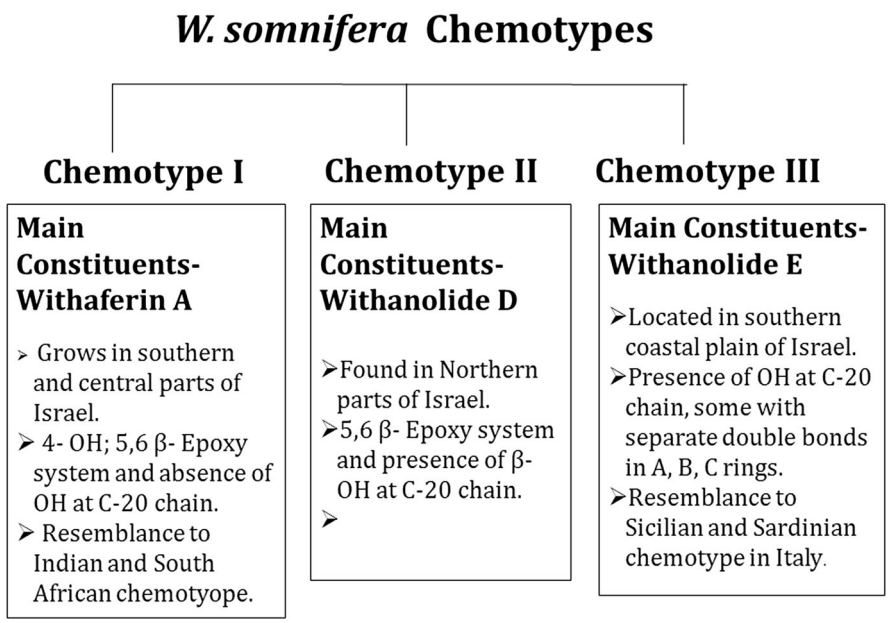


Fig. 2 Major chemotypes reported from *Withania somnifera*

against a rat model of chronic stress (CS) and significant hyperglycaemia, glucose intolerance, increase in plasma corticosterone levels, gastric ulcerations, male sexual dysfunction, cognitive deficits, immunosuppression and mental depression were induced and these perturbations were attenuated by *W. somnifera* at a dose of 25 and 50 mg/kg (*p. o*) in comparison to 100 mg/kg (*p. o*), of dose of prostaglandin administered 1 h before footshock for 21 days (Bhattacharya and Muruganandam 2003). Results indicate that *W. somnifera*, like Prostaglandin, has significant antistress adaptogenic activity confirming the clinical use of the plant in Ayurveda. Similar result was further confirmed by another investigation in which ethanolic extract of roots of *W. somnifera* 23 mg/kg (*p.o*), on acute stress induced biochemical and immunological perturbations in mice improved the swim duration in mice and significantly restored back the stress induced alterations in plasma cortisol, blood glucose and triglyceride levels (Anju 2011). Very recently, Candelario and his group proved that differential activation of GABA receptor subtypes explains a potential mechanism for its reported adaptogenic properties (Candelario et al. 2015).

Anti-inflammatory activity

The transcription factor NFκB and the signaling pathways that regulate its activation play a critical role in normal and pathophysiological immune responses thus leaf extract of *W. somnifera* and its major constituent withaferin A, was tested for its effect on NFκB. It was found it potentially inhibits NF κB activation by preventing the tumor necrosis factor-induced activation of IκB kinase β whereas other *W. somnifera* derived steroidal lactones, such as withanolide A is far less effective (Kaileh et al. 2007). Additionally, withaferin A hampers NFκ-β activation by targeting cysteine 179 located in catalytic site of IKK-β (Heyninck et al. 2014). Thus concluded that pure withaferin A or withaferin A-enriched *W. somnifera* extract have a considerable NFκB inhibitors activity, which hold promise as novel anti-inflammatory agents for treatment of various inflammatory disorders and/or cancer.

Methanolic fractions of the plant extract possess anti-inflammatory activity comparable to that of a 5 mg/kg dose of hydrocortisone sodium succinate and were attributed to the high content of biologically active steroids (withanolide) in the plant of which withaferin A is the major component (Al-Hindawi et al. 1992). Similarly, hydro-alcoholic extract of *W. somnifera* also possessed marked anti-inflammatory effect against denaturation of protein in vitro. The effect was plausibly due to the alkaloid and withanolide contents of *W. somnifera* (Chandra et al. 2012). The anti-inflammatory activity of the plant was further supported by a study conducted by Khan et al. (2011) in assessment of cholinesterase and lipoxygenase inhibitors activity of the plant. In a very recent study leaf water extract and one of its active chloroform fraction was found to suppress the proliferation of activated microglia by causing cell cycle arrest at Go/G1 and G2/M phase along with decrease in cell cycle regulatory protein expression such as PCNA and Cyclin D1. Both the extracts attenuated the TNF-α, IL-1β, IL-6, RNS, and ROS production via downregulating the expression of inflammatory proteins like NFkB and AP1 and also restricted the migration of activated microglia by downregulating metalloproteinase expression and may prove to be a potential therapeutic candidate for the suppression of neuroinflammation in the treatment of neurodegenerative diseases (Gupta and Kaur 2016).

Anti-tumour

As anti-inflammatory, cardioactive and central nervous system effects of *W. somnifera* involve angiogenic processes thus it was hypothesized that the *W. somnifera* extracts might contain angiogenesis inhibitors. It was found that withaferin A inhibited cell proliferation in human umbilical vein endothelial cell (HUVECs) with IC_{50} value of 12 nM through a process associated with inhibition of cyclin D1 expression (Mohan et al. 2004). Withaferin A was found to have anti-angiogenic activity in vivo at doses that are 500-fold lower than those previously reported to exert anti-tumor activity in vivo thus hold a promise for potent anti-tumor drug. Studies at molecular level revealed that withaferin A inhibits binding of Sp1 transcription factor to VEGF (vascular endothelial cell growth factor) gene promoter, in order to exert its antiangiogenic activity (Prasanna et al. 2009). These results clearly indicate the antiangiogenic potential of withaferin A in modulating antitumor activity. Withanolides from *W. somnifera* inhibited growth of central nervous system, lung, human breast and colon

Chemotype III (Israel) X Indian I	Chemotype II (Israel) X Indian I	Chemotype II (Israel) X South African
Withaferin A Withanolide D 27-hydroxy-Withanolide D 14α–hydroxy-Withanolide D Withanolide D chlorohydrin Withanolide E, G,H,I,J,S,T,U,Y Withanone 14α-Hydroxywithanone 14β-Hydroxywithanone 6β,7β-Epoxywithanone 14,20-Dihydroxy-1-oxowitha-2,4,6,24-tetraenolide	Withanolide D 27-hydroxy-Withanolide D Withanolide G Withanolide T Withanone 14β-Hydroxywithanone	Withaferin A Withanolide D

Fig. 3 Major withanolides presents in hybrid of various chemotypes of *W. somnifera*

cancer cell lines comparable to doxorubicin. Withaferin A inhibited growth of breast and colon cancer cell lines more effectively than doxorubicin. These results suggest *W. somnifera* extracts may prevent or inhibit tumor growth in cancer patients and suggest a potential for development of new chemotherapeutic agents. Withaferin A, sitoindoside IX, 4-(1-hydroxy-2, 2-dimethylcyclpropanone)-2, 3-dihydrowithaferin A, 2, 3-dihydrowithaferin A, 24,25-dihydro-27-desoxywithaferin A, physagulin D (1 → 6)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside, 27-*O*-β-D-glucopyranosylphysagulin D, physagulin D, withanoside IV, 27-*O*-β-D-glucopyranosylviscosalactone B, 4, 16-dihydroxy-5β, 6β-epoxyphysagulin D, viscosalactone B from the leaves of this species and diacetylwithaferin A were tested for their antiproliferative activity on NCI-H460 (Lung), HCT-116 (Colon), SF-268 (Central Nervous System; CNS and MCF-7 (Breast) human tumor cell lines. Withaferin A and its derivatives exhibited IC_{50} ranging from 0.24 ± 0.01 to 11.6 ± 1.9 μg/mL. Viscosalactone B showed IC_{50} ranging from 0.32 ± 0.05 to 0.47 ± 0.15 μg/mL whereas its 27-*O*-glucoside derivative exhibited IC_{50} between 7.9 ± 2.9 and 17.3 ± 3.9 μg/mL (Jayaprakasham et al. 2003b).

Withanolides inhibit cyclooxygenase enzymes, lipid peroxidation, and proliferation of tumor cells because several genes that regulate cellular proliferation, carcinogenesis, metastasis and inflammation are regulated by activation of nuclear factor-κB (NF-κB) (Ichikawa et al. 2006). Thus withanolides was supposed to suppress NF-κB activation. Suppression was not cell type specific, as both inducible and constitutive NF-κB activation was blocked by withanolides. Overall, it is suggested that withanolides inhibit activation of NF-κB and thus NF-κB regulated gene expression, which may explain the ability of withanolides to enhance apoptosis and inhibit invasion and osteoclastogenesis. Beside this, withanolide D, an important bioactive withanolide, purified from the leaves of an Indian chemotype (NMITLI 135) exhibited antileukemic activity, targeting multiple pathways along with ceramide accumulation through N-SMase 2 activation, ultimately inducing apoptosis in neoplastic cells (Mondal et al. 2012). In an another investigation worked on the structure activity relationship analysis of withanolides with respect to its anti-proliferative activity against an array of cell lines, human head and neck squamous cell carcinomas cell lines, breast cancer cell line and non-malignant human cell line confirmed the importance of the presence of a Δ 2-1-oxo-functionality in ring A, 5β,6β-epoxy or 5α-chloro-6β-hydroxy grouping in ring B, and nine-carbon side chain with a lactone moiety for cytotoxic activity whereas the presence of –OH or –OR groups at C-4, 7, 11, 12, 14, 15, 16, 17, 18, 19, 20, 23, 24, 27, and 28 were not contributors to the observed antiproliferative activity (Zhang et al. 2012).

Anti-oxidant activity

Clinical effectiveness of antioxidants generally showing that reactive oxygen species (ROS) and oxidative damage are important factors in the processes involved. Withanamides A, B, C, D, E, F, G, H and I (alkaloids from *W. somnifera*) and three withanolides from the methanolic extract of *W. somnifera* fruits were tested for their ability to inhibit lipid peroxidation in a model system using large unilamellar vesicles. All the nine withanamides inhibited lipid peroxidation at 1 and at 0.5 μg/mL and one withanolide inhibited the lipid peroxidation by 82% at 10 μg/mL. Commercial antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) were also tested in this assay at 1 μg/mL and showed 80, 81 and 85% of inhibition, respectively (Jayaprakasam et al. 2004). Thus results suggest that hydroxylated long-chain acyl group may be responsible for the potent antioxidant activity exhibited by novel withanamides. Other compounds viz. Sitoindosides VII-X and withaferin

A were found to increase the free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain frontal cortex and striatum (Bhattacharya et al. 1997). An increase in these enzymes would represent increased antioxidant activity and a protective effect on neuronal tissue.

Immunomodulatory activity and hematopoiesis

The role of *W. somnifera* as immunomodulator has been extensively studied. Sitoindoside IX and sitoindoside X, isolated from *W. somnifera* Dun, have immunomodulatory and CNS effects (anti-stress, memory and learning) in doses of 100–400 g/mouse and produced statistically significant mobilization and activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal enzymes secreted by the activated macrophages. Thus *W. somnifera* attenuate cerebral function deficits in the geriatric population and to provide non-specific host defence (Rahman et al. 1999). Root extract of *W. somnifera* has immunomodulatory effects in three myelosuppression models in mice: cyclophosphamide, azathioprin, or prednisolone and significant increases in hemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight were observed in *W. somnifera* treated mice compared to untreated control mice (Ziauddin et al. 1996). The authors also reported significant increases in hemolytic antibody responses toward human erythrocytes, which indicated immunostimulatory activity.

Treatment with *W. somnifera* root extract at the dose of 20 mg/dose/animal; *i.p.* was found to enhance the total WBC count (17,125 cells/mm^3) on 10th day, bone marrow cellularity (27×10^6 cells/femur) as well as α-esterase positive cell number (1800/4000 cells) that was found to increase significantly ($P < 0.001$) after the administration of *Withania* extract (Davis and Kuttan 2000). Antibody titre and the number of plaque forming cells (PFC) in the spleen were also found to increase with *Withania* extract treatment along with the antigen (SRBC). Maximum number of PFC (985 PFC/10^6 spleen cells) was obtained on the fourth day. Delayed type hypersentivity reaction in mice (Mantoux test) was also inhibited. Administration of *Withania* extract also showed an enhancement in phagocytic activity of peritoneal macrophages (76.5 pigmented cells/200) when compared to control (31.5/200 cells) in mice. These results confirm the immunomodulatory activity of *W. somnifera* extract, which is a known immunomodulator in indigenous medicine. The immunologic effects of *W. somnifera* on four types of immune cells in a human sample were investigated. After 96 h, significant increases in the expression of CD4 on CD3+ T cells and CD56+ NK cells were observed (Mikolai et al. 2009). Chandran and Patwardhan (2017) revealed the immunomodulation mechanism of *W. somnifera* involved five bioactive viz. 2,3-dihydrowithaferin A-3-β-*O*-sulfate, β-sitosterol, daucosterol, withaferin-A, and withasomniferol-A which are capable of regulating 15 immune system pathways through a network of six bioactive-targets interactions and ten other protein-proteins interactions (Chandran and Patwardhan 2017).

Neuritic regeneration activity

Neurodegenerative diseases are characterized by progressive dysfunction and death of cells that frequently affect specific neural systems, implying some form of selective vulnerability. In order to reconstruct neuronal networks, neuritic regeneration and synaptic reconstruction must take place in the damaged brain thus the compounds that would facilitate the regeneration of neurites and the reconstruction of synapses, even in severely damaged neurons, provides new insights for drug development to prevent, treat, and cure these diseases. Withanolide A (10 mmol kg/1 day/1, for 13 days, *p.o.*) could regenerate neurites and reconstruct synapses in severely damaged neurons and also recovered Ab (25–35)-induced memory deficit in mice (Kuboyama et al. 2005). Some withanolides from methanolic extract of the roots of *W. somnifera* showed significant neurite outgrowth activity at a concentration of 1 mM on a human neuroblastoma SH-SY5Y cell line (Jayaprakasam et al. 2003). Possible mechanism of neuroprotective action of the root extract of *W. somnifera* Dunal (WS) is inhibition of nitric oxide production, which is known to mediate neurodegeneration during stress. Treatment of adult mice with *W. somnifera* extract for 30 days during stress significantly reversed the stress induced NADPH-d activation via suppressing corticosterone release and activating cholineacetyltransferase, which in turn increase serotonin level in hippocampus to

inhibit NADPH-d (Bhatnagar et al. 2009). Thus down regulation of nNOS and neurochemical alterations of specific neurotransmitter systems attributed to neuroprotective action of *W. somnifera*.

Anxiety and depression

W. somnifera has been used to stabilize mood in patients with behavioral disturbances. Bioactive glycowithanolides (WSG), isolated from *W. somnifera* roots, have been assessed at the dose of 20 and 50 mg/kg, orally once daily for 5 days for anxiolytic and antidepressant actions in rats. It was found that WSG gives results compared to standard bendodiazepine lorazepam in the dose of 0.5 mg/kg, ip for anxiolytic studies and standard tricyclic anti-depressant, imipramine in the dose of 10 mg/kg, ip for the antidepressant investigations (Bhattacharya et al. 2000b). In an another study, *W. somnifera* in the dose of 100, 200 or 500 mg/kg, oral dependently increased the time spent and entries into the open arms on EPM test and showed the anxiolytic activity. It also helped to potentiate the anxiolytic action of diazepam (0.5, 1 or 2 mg/kg, ip) at subeffective dose *i.e.* 50 mg/kg, oral (Gupta and Rana 2007). Similar result were reported by Kaurav et al. 2012, revealed aqueous and methanolic extracts *W. somnifera* (50 mg/kg) successively decreased the marble burying behavior activity without affecting motor activity which is comparable to the activity of standard fluoxetine, ritanserin and parachlorophenylalanine (Kaurav et al. 2012).

Nootropic effect

W. somnifera is a traditional Ayurvedic medicine, used for centuries as a memory-enhancing agent. The plant, plant extract and isolated withanolides (the major active principles) have been extensively investigated in several laboratories for their neuropharmacological effects and a number of reports are available confirming their nootropic action. *W. somnifera* root extract in the dose of 50, 100 and 200 mg/kg; orally improved retention in a step-down paradigm in mice also in the dose of 50, 100 and 200 mg/kg; orally reversed the scopolamine (0.3 mg/kg)-induced disruption of acquisition and retention and attenuated the amnesia produced by acute treatment with electroconvulsive shock (ECS), immediately after training (Dhuley 2001). Alzheimer's disease is a syndrome induced by ibotenic acid (IA) lesioning of the nucleus basalis magnocellularis and thus caused a marked cognitive deficit. Equimolar amounts of sitoindosides VII-X and withaferin A in the dose of 20–50 mg/kg significantly reversed both IA-induced cognitive deficit and the reduction in cholinergic markers after 2 weeks of treatment (Bhattacharya et al. 1995).

Antigenotoxic effect

Pretreatment with Withaferin A in Syrian golden hamsters significantly reduced the frequency of micronucleated polychromatic erythrocytes (MnPCEs) and chromosomal aberrations such as chromosomal break, gap, minute, and fragment caused by 7, 12-dimethylbenz (a) anthracene (DMBA). Results thus demonstrated the antigenotoxic effect of withaferin-A in DMBA-induced genotoxicity in the bone marrow of golden Syrian hamsters (Panjamurthy et al. 2008).

Antihepatotoxic

Glycowithanolides, consisting of equimolar concentrations of sitoindosides VII-X and withaferin A, isolated from the roots of *W. somnifera* on 10 days of oral administration in graded dose of 10, 20 and 50 mg/kg attenuate iron overload ($FeSo_4$, 30 mg/kg, *i.p.*) induced hepatotoxicity in rats in comparison to Silymarin in the dose of 20 mg/kg, *p.o.* and that may be due the antioxidant action of glycowithanolides WSG (Bhattacharya et al. 2000a).

Influence of *W. somnifera* root powder on the levels of circulatory ammonia, urea, lipid peroxidation products such as TBARS (thiobarbituric acid and reactive substances), HP (hydroperoxides) and liver marker enzymes such as AST (aspartate transaminase), ALT (alanine transaminase) and ALP (alkaline phosphatase), for its hepatoprotective effect in ammonium chloride induced hyperammonemia were investigated. *W. somnifera* offers hepatoprotection by influencing the levels of lipid peroxidation products and liver markers in experimental hyperammonemia and this could be due to (1) the presence of alkaloids, withanolids and flavonoids, (2) normalizing the levels of urea and urea related compounds, (3) its free radical scavenging property and (4) its antioxidant property (Harikrishnan et al. 2008). Sabina et al. (2013) investigated the protective effect of *W. somnifera*

against paracetamol-induced hepatotoxicity and found that treatment with *W. somnifera* significantly reversed elevated levels of liver marker enzymes and bilirubin. It also helped to improve the total protein content, histological observations and antioxidant status which was affected by paracetamol treatment (Sabina et al. 2013).

Antimicrobial

Monomeric glycoprotein with a molecular mass of 28 kDa isolated from the *W. somnifera* root tubers demonstrated potent antimicrobial activity against the phytopathogenic fungi and bacteria tested and exerts a fungistastic effect against *Aspergillus flavus, Fusarium oxysporum, Fusarium verticilloides* and antibacterial activity against *Clvibacter michiganensis subsp. michiganensis* by inhibiting spore germination and hyphal growth in the tested fungi (Girish et al. 2006). In an another study conducted using both disc diffusion and serial dilution method different extracts of leaf, flower, root and stem part of *W. somnifera* inhibited six bacteria (two Gram +ve and four Gram −ve bacteria) i.e. *Staphylococcus aureus* (Gram +ve), *Bacillus Subtilis* (Gram +ve), *Escherichia coli* (Gram −ve), *Raoultella planticola* (Gram −ve), *Pseudomonas aeruginosa* (Gram −ve), *Enterobactor aerogens* (Gram −ve), and two fungi *Candida albicans* and *Aspergillus flavus* to varying degrees whereas water extract of leaves of *W. somnifera* showed highest activity against *R. planticola* (Singariya et al. 2011, 2012a, b, Datta et al. 2011). Beside antibacterial and antifungal activity, hydro-alcoholic extract of *W. somnifera* roots also showed the inhibition of bursal disease virus at maximum 99.9% in its highest nontoxic concentration, 25 μg/mL in cytopathic effect reduction assay (Pant et al. 2012).

Antidote activity

A large family of peptides includes proteins of different origin, neurotoxins, myotoxins, cardiotoxins/cytotoxins and enzymatic toxins are the peptides present in snake venom (Girish et al. 2004). A glycoprotein WSG isolated from *W. somnifera* is an inhibitor of hyaluronidase of *Naja naja* and *Daboia russelii* venom and IC_{50} value was found to be 52 and 36 μg for *N. naja* and *D. russelii* venoms, respectively. It also inhibits phospholipase A2 of the toxic cobra venom, thus it may help in preventing the rapid diffusion of toxins (Machiah et al. 2006). Recently Kumar et al. (2015a) also reported the antidote activity of *W. somnifera* against arsenic induced toxicity.

Anti-Parkinson's activity

Parkinson's disease (PD) is a neurodegenerative disorder caused by the loss of dopaminergic neurons in the substantia nigra pars compacta. To assess the efficacy of *W. somnifera* extract 100 mg/kg was feed to the mouse. The midbrain and corpus striatum of parkinson affected mouse showed increased levels of catalase, superoxide dismutase and malondialdehyde; and reduced levels of glutathione and glutathione peroxidase compared to the normal mouse while treatment with *Withania* extract 100 mg/kg for 7 days significantly improved all these enzyme levels compared to *Withania* untreated Parkinson affected mouse brain thus suggest that *Withania* is a potential drug in treating Parkinson affected oxidative damage (RajaSankar et al. 2009).

Cardio protective effect

Wistar rats were used to evaluate the cardio protective mechanisms of *W. somnifera*, in the setting of ischemia and reperfusion (IR) injury (Mohanty et al. 2008). Post-ischemic reperfusion injury resulted in significant cardiac necrosis, apoptosis, and decline in antioxidant status and elevation in lipid peroxidation in the IR control group as compared to sham. *W. somnifera* prior-treatment favorably restored the myocardial oxidant-antioxidant balance, exerted marked anti-apoptotic effects [upregulated Bcl-2 ($p < 0.001$) protein and attenuated TUNEL positivity ($p < 0.01$)], and reduced myocardial damage as evidenced by histopathologic evaluation.

Anti-convulsant

Kulkarni et al. 2008, analyzed the effects of *W. somnifera* extract (100 or 200 mg/kg, po) against pentylenetetrazol (PTZ) seizure threshold in mice. The drug was tested alone and in combination with exogenous gamma-amino butyric acid (GABA), a GABA receptor agonist or with diazepam. *W. somnifera* increased the PTZ seizure threshold for the onset of tonic extension phase. Co-administration of a

sub-effective dose of *W. somnifera* (50 mg/kg, po) with a sub-protective dose of either GABA (25 mg/kg, ip) or diazepam (0.5 mg/kg, ip) increased the seizure threshold.

Male infertility

Stress has been reported to be a causative factor for male infertility. *W. somnifera* has been documented in Ayurveda and Unani medicine system for its stress-combating properties. Treatment of infertile male with *W. somnifera* extract is found to inhibit lipid peroxidation and protein carbonyl content and improved sperm count and motility and also men recovered the seminal plasma levels of antioxidant enzymes and vitamins A, C, and E and corrected fructose (Ahmad et al. 2010). The treatment effectively reduced oxidative stress, as assessed by decreased levels of various oxidants and improved level of diverse antioxidants. Moreover, the levels of T, LH, FSH and PRL, good indicators of semen quality, were also reversed in infertile subjects. Mahdi et al. 2011 also defined the role of stress in male infertility after measuring various biochemical and stress parameters before and after treatment. Study reported that root powder at the dose of 5 g/day for 3 months treat stress-related infertility improved the level of anti-oxidants and improved overall semen quality in a significant number of individuals (Mahdi et al. 2011; Shukla et al. 2011). Kumar et al. (2015b) revealed that *W. somnifera* also reversed the effect of sodium arsenite administration on sperm counts and sperm motility and also maintains the cellular integrity of testicular cells leading to normal functioning of it.

Activity in agriculture

Herbicidal activity

Aqueous, methanol and n-hexane shoot and root extracts of 5, 10, 15 and 20% w/v (fresh weight basis) concentrations of *W. somnifera* were tested against the germination and seedling growth of *Parthenium* in which aqueous and methanol extracts markedly suppressed the germination, root and shoot growth of *Parthenium* (Javaid et al. 2011). The activity was checked by two bioassay method, first is foliar spray bioassay, the aqueous and methanol shoot extracts of 10% w/v (on a dry weight basis) concentration were sprayed on 1-week and 2-week-old pot-grown parthenium seedlings. Two subsequent sprays were carried out 5 and 10 days after the first spray. The aqueous and methanol extracts significantly reduced the length and biomass of parthenium shoots.

In another soil amendment bioassay, the crushed shoots of *W. somnifera* were incorporated in the soil at 1–5% w/w. *Parthenium* seeds were sown one week after the residue incorporation and plants were harvested 40 days after sowing. All the soil amendment treatments significantly reduced seed germination by 43–89%.

Pesticidal activity

Spodoptera litura is a Noctuid moth which is considered as an agricultural pest. Treatment of sixth instar larvae and pupae of the polyphagous pest *Spodoptera litura* with an acetone extract of leaves of *W. somnifera*, caused toxicity, molt disturbances, formation of larval-pupal, pupal-adult intermediates and adultoids. Thus *W. somnifera* acts as an insect growth regulator causing disruption of the endocrine mechanism regulating molting and metamorphosis (Gaur and Kumar 2010).

Conclusion

The literature survey revealed that *W. somnifera* is an important source for the group of compounds, withanolides, which are having important pharmacological activity. Beside this, preliminary studies have also found that group of constituents of *Withania* exhibit a variety of therapeutic activity. It is concluded from the review that there is significant variation in the biological activity of the withanolide studied, which may be due to stereochemical specificity in their steroidal structure. The extensive survey of literature revealed that *W. somnifera* is an important source of many other pharmacologically and medicinally important chemicals, sitoindosides and various useful alkaloids.

The plant has also been widely studied for their various pharmacological activities like immunomodulatory activity and hematopoiesis, adaptogen, antivenom, anti-inflammatory, antitumor properties. Various other effects like immunomodulation,

antioxidant, anxiolytic, hypolipidemic, antibacterial have also been studied.

Studies of total withanolide content in the hybrid plants and their respective parents also revealed that a hybrid plant contains more total content of withanolides as compare to parent plant. Also chemotype variation of *W. somnifera* has not been studied much in India, so this review throws a fresh perspective of chemotypic study of this plant in India, production of superior hybrids with potential for the commercial exploitation and development of improved varieties with distinct chemoprofiles targeting specific bioactive molecules for different pharmacological applications.

Also, information on the different chemotypes is not available for *Withania* plants found in different parts of world. Such studies would be interesting to develop genetic linkages.

Although the results from this review promises new prospects for the use of *Withania somnifera* as a multipurpose medicinal agent, several limitations currently exist in the recent literature.

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