REVIEW ARTICLE

Withanolides: Phytoconstituents with significant pharmacological activities

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Withanolides are a group of naturally occurring oxygenated ergostane type steroids, having lactone in side chain and 2-en-1-one system in the ring. Withanolides are present in medicinal plants of Solanaceae family. Formulations based on these medicinal plants are widely used in Ayurveda and traditional Chinese medicine. Withanolides have shown a wide range of pharmacological activities including hypno¬sedative, immunomodulatory, anti-inflammatory, antiarthritic, angiogenesis inhibitor, anticholinesterase, antioxidant, antibacterial and above all, antitumour. Withaferin A is the best studied withanolide as far as pharmacological investigations are concerned. The present review summarises the investigative work carried out on bioactive withanolides.

Key words: Bioactivity, withaferin A, *withania somnifera,* withanolide, withanone

INTRODUCTION

Some 50 new withanolides have been found in plants, mostly in roots and leaves, during the period under review [Table 1]. Lavie, Glotter and Shro in 1965 studied the basic structure of withanolides. Chemically, they are a group of naturally occurring oxygenated ergostane type steroids [Figure 1], having lactone in side chain and 2en1one system in ring A.[1]

Several withanolides are saponins containing an additional acyl group; the remainders have glucose at carbon 27.[1] This class of steroid derivative is largely restricted in distribution to the genera *Acnistus*, *Datura*, *Discopodium*, *Dunalia*, *Jaborosa*, *Lycium*, *Nicandra*, *Physalis*, *Solanum*, and *Withania*, all belonging to the plant family Solanaceae.[2]

Previous Reported Work

Previously, 1α,3β,20-trihydroxywitha-5,24-dienolide, 7α,27-hydroxy-1-oxo-witha-2,5,24-trienolide, and 7α,27-dihydroxy-1-oxo-witha-2,5,24-trienolide had been isolated from *Withania somnifera* chemotype III.[3] Two minor constituents, 7α,27-hydroxy-1-oxo-witha-2,5,24-trienolide and 7α,27-dihydroxy-1-oxo-witha-2,5,24-trienolide, have been found in Indian chemotype of *W. somnifera*.[4] Three withanolides, G [Figure 2], H, and J, along with 20-hydroxy-1-oxo-witha-2,5,16,24-trienolide have been isolated from *W. somnifera* chemotype III.[5-7] Three withanolides, I, J, and K, have been isolated from *W. somnifera* chemotype III.[5] Withanolide U was isolated from *W. somnifera* chemotype III.[8]

Withaferin A [Figure 3] was the first compound isolated as a major compound from *W. somnifera* chemotype I.[1] 27-deoxy-withaferin [Figure 4] was also reported to have been isolated with withaferin A.[9] Withaferin A is thought to be the primary pharmacological agent present in the roots and leaves of *W. somnifera*.[10,11]

Withanolide D [Figure 5] was isolated from *W. somnifera* chemotype II.[12] Several withanolides like chlorohyrdin II, 27-O-glucosides (sitoindoside IX and X), and withasomidienone [Figure 6] have been characterised from the roots of *W. somnifera*.[13,14] In the Indian chemotype of *W. somnifera*, jaborosalactone A [Figure 7] and withanolide Y have been isolated.[3,15] Two withanolides, Q and R, have been isolated from the offspring of Indian chemotypes I and III of *W. somnifera*.[16]

Withanolides F, E, and 4β-hydroxy-withanolide E were isolated from *W. somnifera* chemotype III.[17,18] Further, withanolides S and T have been isolated from *W. somnifera* chemotype III.[19] A variety of withanolides including sominolide, soinone, withasomnilide, withasomniferabolide, somniferanolide, and somnwithanolide have been reported to be present in the stem bark of *W. somnifera*.[10,20-23]

Recent Advances in Biological Activities

Previous studies have reported anti-inflammatory, antiarthritic, antibiotic, antitumour immunomodulatory and central nervous system effects of withanolides [Table 1].[24-36]

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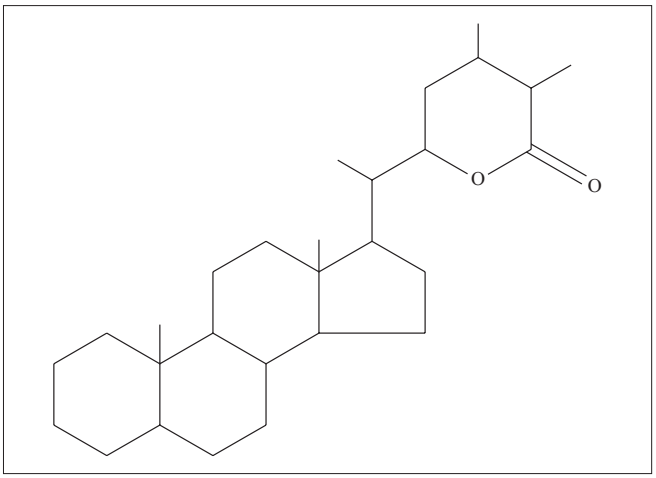

Figure 1: Basic withanolide skeleton

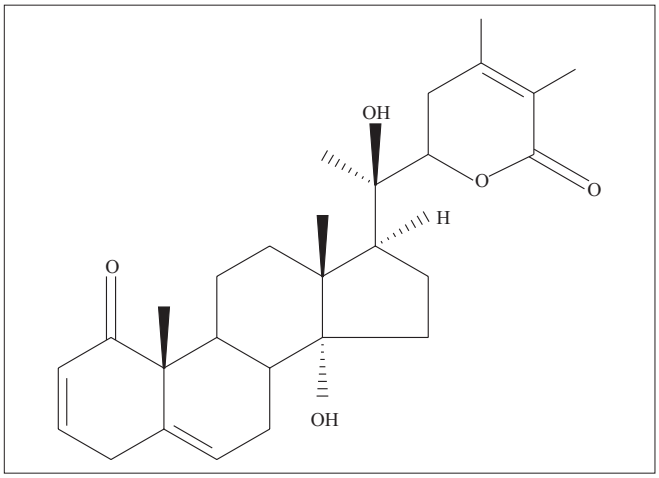

Figure 2: Structure of withanolide G

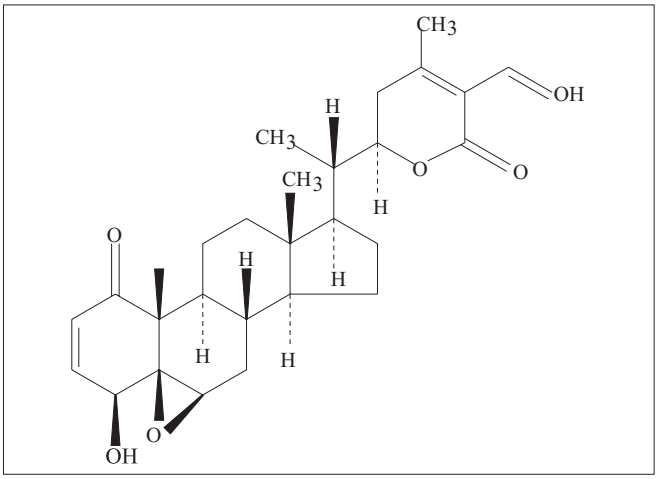

Figure 3: Structure of withaferin A

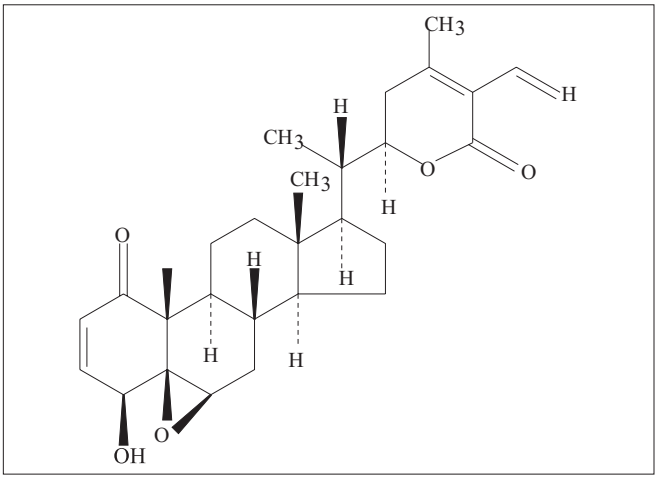

Figure 4: Structure of 27-deoxywithaferin A

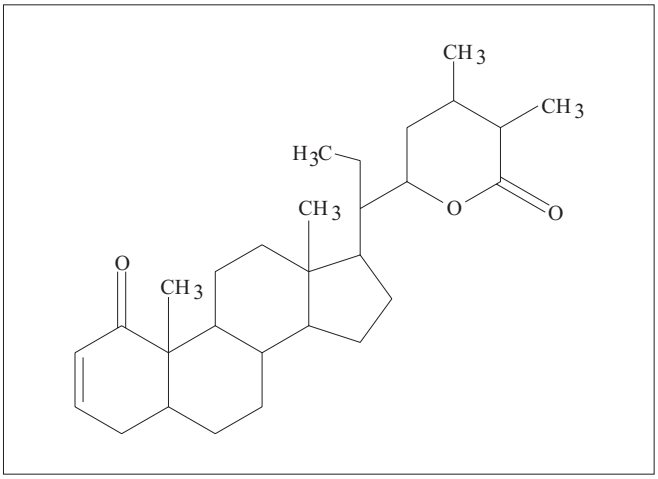

Figure 5: Structure of withanolide D

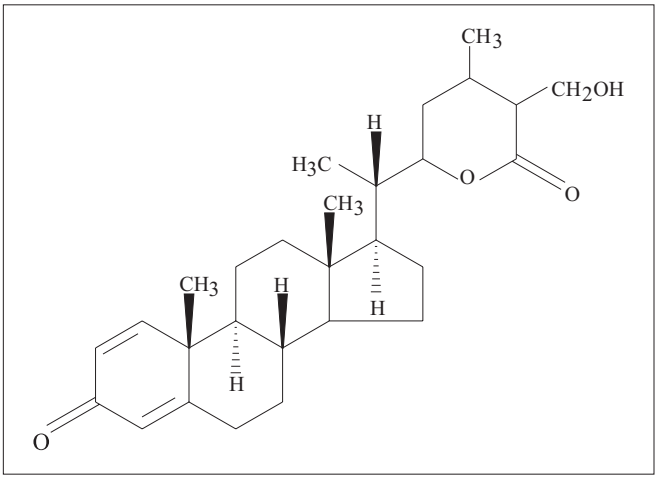

Figure 6: Structure of withasomidienone

Anti-tumour

Withaferin A, isolated from the roots of *W. somnifera*, reduced the survival of V79 cells in a dose-dependent manner. LD50 for survival was 16 μM. Onehour treatment with a nontoxic dose of 2.1 μM before irradiation significantly enhanced cell killing, giving a sensitiser enhancement ratio of 1.5 for 37% survival and 1.4 for 10% survival. Withaferin A induced a G2/M block, with a maximum accumulation of cells in G2-M phase at 4 hours after treatment with 10.5 μM withaferin-A in 1 hour.[37]

Table 1: Reported pharmacological activities of withanolides

Withanolide	Biological activity	Reference
Withaferinil	Antitumour	Palyi *et al*., 1969
Withaferin-A	Antitumour	Palyi *et al*., 1969; Chakrabotri *et al*., 1974; Ascher *et al*., 1981; Budhiraja *et al*., 1987
4β, 20-dihydroxy-i-oxo-5β,6β,-epoxy-witha-2,24-dienolide	Antitumour	Chakrabotri *et al*., 1974
Compound WS-1	Hypno-sedative	Kundu *et al*., 1976
Withanolide-E	Antifeedent	Ascher *et al*., 1981
Withanolide-5, 20α,(*R*)-dihydroxy-6α,7α-epoxy-1-oxo-5α-witha-2,24-dienolide	Immunomodulatory	Bahr *et al*., 1982
Withanolide-D	Antitumour	Das *et al*., 1985
3-β-hydroxy-2,3-dihydro-withanolide	Antibacterial, antitumour, immunomodulatory and anti-inflammatory	Budhiraja *et al*., 1987
Sitoindoside IX, X	Immunomodulatory and CNS effects	Bhattacharya *et al*., 1987; Ghosal *et al*., 1989
Compound WS-2	Antibacterial	Khan *et al*., 1993
Jaborosalactones R, S and T	–	Bonetto *et al*., 1995

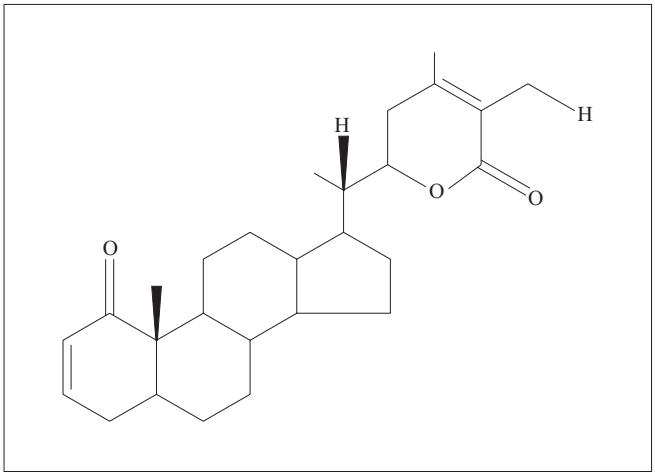

Figure 7: Structure of jaborosalactone A

In another animal study; the alcoholic extract of the dried roots of *W. somnifera,* as well as withaferinA, showed significant antitumour and radiosensitising effects in experimental tumours *in vivo*, without any noticeable systemic toxicity. WithaferinA gave a sensitiser enhancement ratio of 1.5 for *in vitro* cell killing of V79 Chinese hamster cells at a nontoxic concentration of approximately 2 μM.[38]

WithaferinA showed marked tumour inhibitory activity when tested *in vitro* against cells derived from human carcinoma of nasopharynx. It also acted as a mitotic poison arresting the division of cultured human larynx carcinoma cells at metaphase and in HeLa cultures similar to star-metaphase. Withaferin-A produced significant retardation of the growth of Ehrlich ascites carcinoma, Sarcoma 180, Sarcoma Black, and E 0771 mammary adenocarcinoma in mice in doses of 10, 12, 15 mg/kg b.wt.[38]

In this study, it was found that 6 of the 18 compounds isolated from the methanol extract isolated from *Physalis philadelphica* enhanced neurite outgrowth in human neuroblastoma SHSY5Y cells. In withanolideA treated cells, the length of NFHpositive processes was significantly increased compared with vehicle treated cells, whereas the length of MAP2positive processes was increased by withanosides IV and VI. The results suggest that axons are predominantly extended by withanolide A, and dendrites by withanosides IV and VI.[39]

Five new withanolide derivatives have been isolated from the roots of *W. somnifera*, together with 14 known compounds. Withanoside VIII, withanoside IX, withanoside XI, withanolide A, withanoside IV, withanoside VI, and coagulin showed significant neurite outgrowth at a concentration of 1 μM on a human neuroblastoma SHSY5Y cell line [Table 2].[40]

Fifteen new withanolides and two known withanolides, withanolide D and 17α-hydroxywithanolide D, were isolated from the stems, roots, and leaves of *T. anomalum* using bioassay-directed fractionation. Majority of the withanolides 1, 4–6, 8–10, and 13 showed significant cytotoxic activity against Hep G2, Hep 3B, A-549, MDA-MB-231, MCF-7, and MRC-5 cell lines.[41]

Three new withanolide glycosides, namely daturametelins H–J [Figure 8] together with two known ones, daturataturin A and 7,27-dihydroxy-1-oxowitha-2,5,24-trienolide, isolated from the MeOH extract of the aerial parts of *D. metel* were tested for their antiproliferative activity towards the human colorectal carcinoma (HCT-116) cell line. 7,27-dihydroxy-1-oxowitha-2,5,24-trienolide exhibited the highest activity of all the tested withanolides, with an IC50 value of 3.2±0.2 μM.[42]

Extracts of *Withania adpressa* were tested for their cytotoxicity towards a panel of cancer cell lines (Hep2, HT29, RD, Vero, and MDCK), using 3-(4,5-dimethylthiazol-2-yl)-

Table 2: Withanolides reported during 1996–2009

Withanolide	Source	Reference
Withaferin-A	*W. somnifera* (Solanaceae)	Devi 1996; Uma *et al*., 1996; Gupta *et al*., 1996; Ali *et al*., 1997; Mohan *et al*., 2004; Jung *et al*., 2008; Oh *et al*., 2008
(20*R*, 22*R*) 5α, 6β, 14α, 20,27pentahydroxy1oxo with 24 enolide, (20*S*,22*R*)-5β, 6β-epoxy-4β, 14β, 15α-trihydroxy-1-oxowith-2,24-dienolide, withaphysanolide, and viscosalactone B	*Physalis peruviana* (Solanaceae)	Ahmad *et al*., 1999
Ajugin C (=(20*R*,22*R*)-4β, 14α, 20, 27-tetrahydroxy-1-oxoergosta-2,5,24-trieno-26, 22-lactone and ajugin D (=(20*R*,22*R*)-8β, 14α, 17β, 20, 27-pentahydroxy-1-oxoergosta-5, 24-dieno-26, 22-lactone	*Ajuga parviflora* (Lamiaceae)	Khan *et al*., 1999
Withanoside VIII, withanoside IX, withanoside XI, withanolide A, withanoside IV, withanoside VI and coagulin	*W. somnifera* (Solanaceae)	Zhao *et al*., 2002
Withanolide A, withanosides IV and VI	*W. somnifera* (Solanaceae)	Kuboyama *et al*., 2002
Physagulin D (1→6)-β-D-glucopyranosyl-(1-4)-β-D-glucopyranoside, 27-O-β-D-glucopyranosyl physagulin D, 27-O-β-D-glucopyranosyl viscosalactone B, 4, 16-dihydroxy-5β, 6β-epoxyphysagulin D, 4-(1-hydroxy-2,2-dimethylcyclo-propanone)-2, 3-dihydrowithaferin A, withaferin A, 2, 3-dihydrowithaferin A, viscosalactone B, 27-desoxy-24, 25-dihydrowithaferin A, sitoindoside IX, physagulin D, and withanoside IV	*W. somnifera* (Solanaceae)	Jayaprakasam and Nair, 2003
Ixocarpalactone A, 2, 3-dihydro-3β-methoxyixocarpalactone A, 2, 3-dihydro-3β-methoxyixocarpalactone B, 2, 3-dihydroixocarpalactone B, and 4β, 7β, 20*R*-trihydroxy-1-oxowitha-2, 5-dien-22, 26-olide	*Physalis philadelphica* (Solanaceae)	Gu *et al*., 2003
Subtrifloralactones A-E, F-L, new C-18 oxygenated withanolide, 13β-hydroxymethylsubtrifloralactone E and philadelphicalactone A	*Deprea subtriflora* (Solanaceae)	Kinghorn *et al*., 2003; Su *et al*., 2003
20β-hydroxy-1-oxo-(22*R*) -witha-2, 5, 24-trienolide, withacoagulin and a known withanolide, 17β-hydroxy-14α, 20α-epoxy-1-oxo-(22*R*)-witha-3, 5, 24-trienolide	*Withania coagulans* (Solanaceae)	Rahman *et al*., 2003
Bracteosin A (= (22*R*) -5β, 6α: 22, 26-diepoxy-4β, 28-dihydroxy-3β-methoxyergost-24-ene-1,26-dione), bracteosin B (= (22*R*)-5β,6β: 22,26-diepoxy-4β,28-dihydroxy-3β α-methoxy-1, 26-dioxoergost-24-en-19-oic acid), and bracteosin C (= (22*R*)-22, 26-epoxy-4β, 6β, 27-trihydroxy-3β-methoxyergost-24-ene-1, 26-dione)	*Ajuga bracteosa* (Lamiaceae)	Naheed *et al*., 2004
Withanolide A, withanoside IV, and withanoside VI	*W. somnifera* (Solanaceae)	Tohda *et al*., 2005
Withanolides 1 and 2	*Jaborosa caulescens* (var. *caulescens* and var. *bipinnatifida*)	Nictota *et al*., 2005
Daturametelins C, D, E, F and G	*Datura metel* (Solanaceae)	Shingu *et al*., 2005
Witharifeen (11α, 12β-dihydroxy (20*R*, 22*R*) -21,24-epoxy-1-oxowitha-2,5,25(27)-trien-22,26-olide) and daturalicin (20*R*,22*R*)-5β, 6β-14α, 15α.-21, 24-triepoxy-1-oxowitha-2, 25(27)-dien-22, 26-olide)	*Datura innoxia* (Solanaceae)	Siddique *et al*., 2005
Withanone, 27-hydroxy withanolide A, two new withanolides, iso-withanone and 6α,7β-epoxy-1β,3β, 5α-trihydroxy-witha-24-enolide	*W. somnifera* (Solanaceae)	Lal *et al*., 2006
Cilistepoxide and cilistadiol	*Solanum sisymbifolium* (Solanaceae)	Niero *et al.,* 2006
Ashwagandhanolide	*W. somnifera* (Solanaceae)	Subbaraju, 2006
Daturametelins H–J, daturataturin A and 7, 27-dihydroxy-1-oxowitha-2,5,24-trienolide	*Da. metel* (Solanaceae)	Ma *et al*., 2006
Fifteen new withanolides, withanolide D and 17α-hydroxywithanolide D	*Tubocapsicum anomalum*	Hsieh *et al*., 2007
Withanolide Z	*W. somnifera* (Solanaceae)	Pramanick *et al*., 2008
Withangulatin A (1) and withangulatin I	*Physalis angulata* (Solanaceae)	Lee *et al*., 2008
Physacoztolides A–E	*Physalis coztomatl* (Solanaceae)	Perez-Castorena *et al*., 2006
Withanolide Z	*W. somnifera* (Solanaceae)	Pramanick *et al*., 2008
Coagulanolide and withanolides 1–3 and 5	12β-acetoxy-4-deoxy-5,6-deoxy-Δ^5–withanolide D and withanolide D	Maurya *et al*., 2008

Table 2: ***(contd...)***

Withanolide	Source	Reference
Withaferin A and witharistatin	*Withania aristata* (Solanaceae)	Benjumea *et al*., 2009
Withanolide sulfoxide	*W. somnifera* (Solanaceae)	Vanisree *et al*., 2009
	Acnistus arborescens	Codero *et al*., 2009
Withacoagulins A–F	*W. coagulans* (Solanaceae)	Huang *et al*., 2009
Withanoside IV, withanoside VI, physagulin D and withastraronolide	*W. somnifera* (Solanaceae)	Ahuja *et al*., 2009

2,5-diphenyltetrazolium bromide. The bioassay-guided fractionation of the plant extracts resulted in isolation of a novel withanolide, 14α,15α,17β,20β-tetrahydroxy-1-oxo-(22*R*)-witha-2,5,24-trienolide, and the already identified withanolides, F and J. Extract, semi-purified fractions and pure compounds exhibit potent cytotoxicity against human cancer cell lines tested, in a dose-dependent manner.[43]

Four new withanolides, physagulins L–O, with seven known withanolides were isolated from the MeOH extract of the aerial parts of *P. angulata*. All the 11 compounds were tested for their antiproliferative activities towards HCT-116 and human non-small-cell lung cancer (NCI-H460) cells. Fifth withanolide compound exhibited the highest anticancer activity against the HCT-116 cell line, with an IC50 value of 1.64±0.06 μM. Ninth withanolide compound exhibited the highest cytotoxicity towards the NCI-H460 cell line, with an IC50 value of 0.43 ± 0.02 μM.[44]

Withangulatin A and withangulatin I, from the whole plant of *P. angulata*, were tested for their cytotoxic activities against two human cancer cell lines, colorectal carcinoma COLO 205 and gastric carcinoma AGS, *in vitro*. Compounds 1 and 2 exhibited inhibitory activities against these two human cancer cells with IC50 values of 16.6 and 1.8, and 53.6 and 65.4 μM, respectively.[45]

The present study demonstrated that a major component of i-Extract and withanone [Figure 9] (i-Factor) protected the normal human fibroblasts against the toxicity caused by withaferin A. It increased the *in vitro* division potential of normal human cells that appeared to be mediated by decreased accumulation of molecular damage, downregulation of the senescence-specific β-galactosidase activity and the senescence marker protein, $p21^{WAF-1}$, protection against oxidative damage, and induction of proteasomal activity. From the findings it was concluded that i-Extract and withanone have both anticancer and antiaging activities. It further points to the molecular link between aging and cancer.[46]

A new withanolide, 12β-acetoxy-4-deoxy-5,6-deoxy-Δ^5–withanolide D, and withanolide D were isolated from

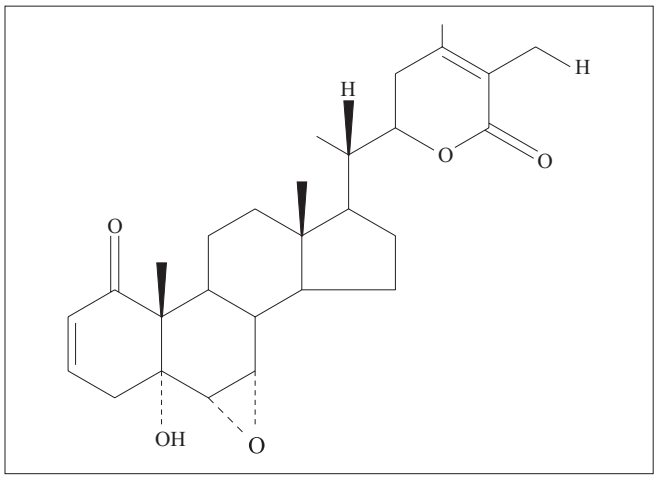

Figure 8: Structure of daturametelin

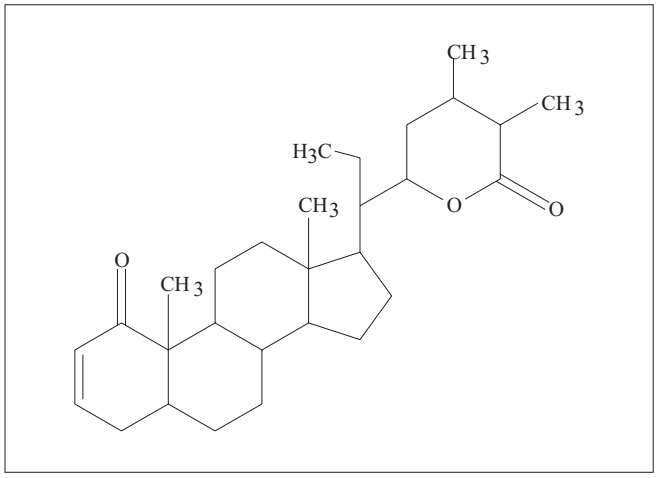

Figure 9: Structure of withanone

the leaves of *A. arborescens*. Cytotoxic activity of these two compounds against human tumour cell lines HT-29, MCF-7, MKN-45, HEp-2, HeLa, U-937 and two human normal fibroblast cultures, Fib04 and Fib05, were studied. Withanolide D presented *in vitro* cytotoxic activity against tumour cell lines at the low micromolar range (LC_{50}: 1.01.69 μM) and showed a slightly lower activity against Fib04, suggesting moderate selectivity among tumoural and normal cells. No cytotoxic effect was observed for 12β-acetoxy-4-deoxy-5,6-deoxy-Δ^5–withanolide D.[47]

The chemokine receptor, CCR7, is important for lymphatic invasion of cancer cells and is overexpressed in metastatic breast cancer cells. A bioactive withanolide, tubocapsanolide, suppressed NF-κB-mediated CCR7 expression in breast cancer cells and attenuated their migration towards lymphatic endothelial cells.[48]

Anti-inflammatory and Antioxidant

In raw 264.7 cells stimulated with lipopolysaccharide (LPS) to mimic inflammation, withaferin A inhibited LPS-induced expression of both iNOS protein and mRNA in a dose-dependent manner. To investigate the mechanism by which withaferin A inhibits iNOS gene expression, the researchers examined activation of mitogen-activated protein kinases (MAPKs) and Akt in Raw 264.7 cells. Withaferin A prevented IκB phosphorylation, blocking the subsequent nuclear translocation of nuclear factor-KB (NF-KB) and inhibiting its DNA binding activity. Moreover, LPS-induced NO production and NF-KB activation were inhibited by SH-6, a specific inhibitor of Akt. The results suggest that withaferin A inhibited inflammation through inhibition of NO production and iNOS expression, by blocking Akt and subsequently downregulating NF-KB activity.[49]

Four novel withanolide glycosides and a withanolide [physagulin D (1→6)-β-D-glucopyranosyl-(1-4)-β-D-glucopyranoside, 27-O-β-D-glucopyranosyl physagulin D, 27-O-β-D-glucopyranosyl viscosalactone B, 4,16-dihydroxy-5β,6β-epoxyphysagulin D, and 4-(1-hydroxy-2,2-dimethylcyclo-propanone)-2,3-dihydrowithaferin A] were isolated from the leaves of *W. somnifera*. In addition, seven known withanolides (withaferin A, 2,3-dihydrowithaferin A, viscosalactone B, 27-desoxy-24,25-dihydrowithaferin A, sitoindoside IX, physagulin D, and withanoside IV) were isolated. These withanolides were assayed to determine their ability to inhibit cycloxygenase-1 and cyclooxygenase-2 (COX-2) enzymes and lipid peroxidation. The withanolides tested, except 27-desoxy-24,25-dihydrowithaferin A, showed selective COX-2 enzyme inhibition ranging from 9 to 40% at 100 μg/ml. 4,16-dihydroxy-5β,6β-epoxyphysagulin D, sitoindoside IX, and physagulin D also inhibited lipid peroxidation by 40, 44 and 55%, respectively.[50]

Investigation of the methanol extract of *W. somnifera* roots for bioactive constituents yielded a novel withanolide sulfoxide compound along with a known withanolide dimmer, ashwagandhanolide. Withanolide sulfoxide was highly selective in inhibiting COX-2 enzyme by 60% at 100 μM with no activity against COX-1 enzyme. The IC50 values of compound 1 against human gastric (AGS), breast (MCF-7), central nervous system (SF-268) and colon (HCT-116) cancer cell lines were in the range 0.74–3.63 μM.[51]

Anticholinesterase

Three new withanolides, bracteosin A (= (22*R*)-5β,6β: 22,26-diepoxy-4β,28-dihydroxy-3β-methoxyergost-24-ene-1,26-dione;), bracteosin B (= (22*R*)-5β,6β: 22,26-diepoxy-4β,28dihydroxy3βmethoxy1,26dioxoergost24en19oic acid, and bracteosin C (= (22*R*)-22,26-epoxy-4β,6β,27-trihydroxy-3β-methoxyergost-24-ene-1,26-dione), and dihydroclerodin-1, clerodinin A, lupulin A, and dihydroajugapitin were isolated from the whole plants of *Aj. bracteosa*. Bracteosins A–C, exhibited evident inhibitory potential against cholinesterase enzymes in a concentration-dependent fashion.[52]

Neuroprotective

In a study, the researchers screened the neurite outgrowth activity of herbal drugs and identified several active constituents. In each compound, neurite outgrowth activity was investigated under amyloid-beta-induced neuritic atrophy. Most of the compounds with neurite regenerative activity also demonstrated memory improvement activity in Alzheimer's disease model mice. Withanolide derivatives (withanolide A, withanoside IV, and withanoside VI), isolated from *W. somnifera*, also showed neurite extension in normal and damaged cortical neurons.[53]

In another animal study, it was investigated whether withanolide A, isolated from the root of *W. somnifera*, could regenerate neurites and reconstruct synapses in severely damaged neurons. Further, the effect of withanolide A on memory-deficient mice showing neuronal atrophy and synaptic loss in the brain was also investigated. Subsequent treatment with withanolide A induced significant regeneration of both axons and dendrites, in addition to the reconstruction of pre- and post-synapses in the neurons. Withanolide A recovered A β (25–35)-induced memory deficit in mice. Also, the decline of axons, dendrites, and synapses in the cerebral cortex and hippocampus was almost recovered.[54]

Angiogenesis Inhibitor

In an endothelial cell-sprouting assay, it was demonstrated that withaferin A inhibits human umbilical vein endothelial cell (HUVEC) sprouting in three-dimensional collagen-I matrix at doses which are relevant to NF-κ inhibitory activity. Withaferin A inhibits cell proliferation in HUVECs (IC50 =12 nM) at doses that are significantly lower than those required for tumour cell lines through a process associated with inhibition of cyclin D1 expression. It was proposed that the inhibition of NF-κB by withaferin A in HUVECs occurs by interference with the ubiquitin-mediated proteasome pathway, as suggested by the increased levels of poly-ubiquitinated proteins. Withaferin A was shown to exert potent anti-angiogenic activity *in vivo* at doses that are 500-fold lower than those previously reported to exert anti-tumour activity *in vivo*.[55]

Diuretic

Four *W. aristata* extracts at 100 mg/kg were orally administered to laboratory animals to evaluate their diuretic activity. From the most active fraction, two withanolides were isolated. Both of them, individually and as a mixture at 5 and 10 mg/kg, were analysed for their diuretic activity. Water excretion rate and content of Na(+) and K(+) electrolytes were measured in the urine of saline-loaded animals. *W. aristata* water fraction, the two withanolides and the mixture of these compounds displayed high diuretic activity, with a significant excretion of sodium and potassium ions in laboratory animals. The activity was ascribed to withaferin A and witharistatin.[55]

Hypoglycaemic

A new withanolide, coagulanolide, along with four known withanolides, 1–3 and 5, have been isolated from *W. coagulans* fruits and their structures were elucidated by spectroscopic techniques. All the compounds showed significant inhibition of postprandial rise in hyperglycaemia post-sucrose load in normoglycaemic rats as well as in streptozotocin-induced diabetic rats. Withanolide 5 showed significant fall of fasting blood glucose profile and improved the glucose tolerance of *db*/*db* mice. Further, Withanolide 5 showed antidyslipidemic activity in *db*/*db* mice.[56]

Immunosuppression

Six new withanolides, withacoagulins A–F (1–6), together with 10 known withanolides, 7–16, were isolated from the aerial parts of *W. coagulans*. These compounds, including the crude extracts of this herb, exhibited strong inhibitory activities on the T- and B-cell proliferation.[57]

Miscellaneous

Two new withanolides, (20*R*, 22*R*)-5α,6β,14α,20,27-pentahydroxy-1-oxowith-24-enolide and (20*S*, 22*R*)-5β,6β-epoxy-4β,14β,15α-trihydroxy-1-oxowitha-2,24-dienolide, in addition to the known withanolides, withaphysanolide and viscosalactone B, were isolated from the whole plant material of *P. peruviana*.[58] The phytochemical study of two species of *J. caulescens* (var. *caulescens* and var. *bipinnatifida*) yielded four new withanolides, 1–4.[59]

The whole plant extract of *D. subtriflora* yielded subtrifloralactones A–E and F–L, a new C-18 oxygenated withanolide, 13β-hydroxymethylsubtrifloralactone E [Figure 10], a new α-ionone derivative, (+)-7α,8α-epoxyblumenol B, and philadelphicalactone A [Figure 11].[60-61]

Two new withanolides, ajugin C (=(20*R*,22*R*)-4β,14α,20,27-tetrahydroxy-1-oxoergosta-2,5,24-trieno-26,22-lactone and ajugin D (=(20*R*,22*R*)-8β,14α,17β,20,27-pentahydroxy-1-oxoergosta-5,24-dieno-26,22-lactone, were isolated from

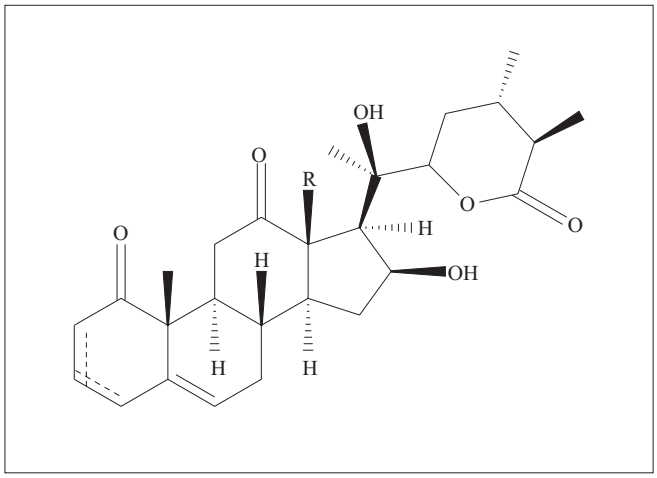

Figure 10: Structure of 13β-hydroxymethylsubtrifloralactone E

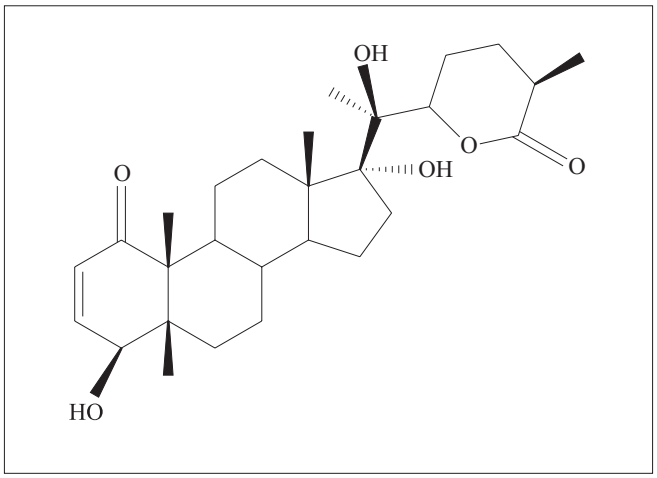

Figure 11: Structure of philadelphicalactone A

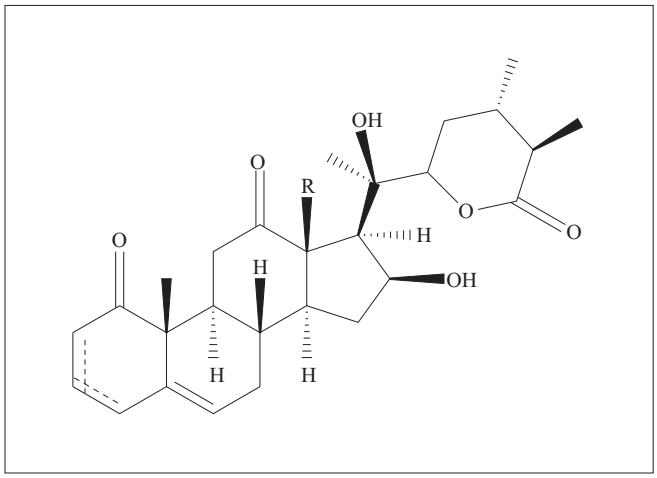

Figure 12: Structure of ixocarpalactone A

the whole plant of *A. parviflora*.[62]

Two withanolides, 20β-hydroxy-1-oxo-(22*R*)-witha-2,5,24-trienolide and withacoagulin, along with a known withanolide, 17β-hydroxy-14α,20α-epoxy-1-oxo-(22*R*)-

witha-3,5,24-trienolide were isolated from *W. coagulans*.[63] Minor new withanolides, daturametelins C, D, E, F, and G-Ac, were isolated from the methanolic extract of the fresh aerial parts of *D. metel*.[64] Cilistepoxide and cilistadiol, two new withanolides have been isolated from *S. sisymblifolium*.[65]

Aerial parts of *P. coztomatl* afforded a new labdane diterpene (physacoztomatin) and five new withanolides, physacoztolides A–E (5–9).[66] Bioactivity-guided search for novel, plant-derived cancer chemopreventive agents yielded ixocarpalactone A [Figure 12] and minor new withanolides; 2,3-dihydro-3β-methoxyixocarpalactone A, 2,3-dihydro-3β-methoxyixocarpalactone B, 2,3-dihydroixocarpalactone B, and 4β,7β,20*R*-trihydroxy-1-oxowitha-2,5-dien-22,26-olide were isolated from the leaves and stems of *P. philadelphica*.[67]

The methanolic extract of the aerial parts of *D. innoxia* yielded two new withanolides namely witharifeen (11α,12β-dihydroxy (20*R*,22*R*)-21,24-epoxy-1-oxowitha-2,5,25(27)-trien-22,26-olide) and daturalicin ((20*R*,22*R*)-5β,6β-14α,15α.-21,24-triepoxy-1-oxowitha-2,25(27)-dien-22,26-olide).[68] The chloroform extract of the fresh berries of *W. somnifera* has been found to yield stigmasterol, its glucoside, withanone, 27-hydroxy withanolide A along with two new withanolides, namely, iso-withanone and 6β,7β-epoxy-1β,3β,5β-trihydroxy-witha-24-enolide.[69]

A new dimeric withanolide, ashwagandhanolide, was isolated from the roots of *W. somnifera*. It displayed growth against human gastric (AGS), breast (MCF-7), central nervous system (SF-268), colon (HCT-116) and lung (Ncl h460) cancer cell lines, with IC50 values in the range 0.43–1.48 μg.[70]

n-Butanol fraction of the methanolic extract of leaves of *W. somnifera* leaves afforded a novel chlorinated withanolide, withanolide Z, along with known withanolides, withanolide B, withanolide A, 27-hydroxywithanolide B, and withaferin A.[71] Recently, four glycowithanolides, namely, withanoside IV, withanoside VI, physagulin D, and withastraronolide, were characterised from multiple shoot cultures of selected accessions AGB002 and AGB025 of *W. somnifera*.[72]

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