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Review

Chemistry and pharmacology of *Withania coagulans*: an Ayurvedic remedy

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Abstract

Objectives The use of *Withania coagulans*, a member of the solanaceae family, has been highlighted in Ayurveda. We have reviewed the chemical constituents and pharmacological properties of *W. coagulans,* as well as its morphology. This has included therapeutic effects of the whole plant and its extracts, fractions and isolated withanolides. The hepatoprotective, anti-inflammatory, antihyperglycaemic, hypolipidaemic, free radical scavenging, antimicrobial, cardiovascular, central nervous system depressant, immunomodulating, antitumour and cytotoxic activities of *W. coagulans* have been described.
Key findings Research carried out using different biological testing in-vitro and in-vivo techniques supported the claims.
Summary This review has covered the morphology, chemistry and pharmacology of the plant. It has described 37 compounds containing 46 references.
Keywords Solanaceae; steroidal lactones; *Withania coagulans*; withanolides

Introduction

Plants play a dominant role in the discovery of new therapeutics. They have always been a rich source of lead compounds e.g. morphine, cocaine, digitalis, quinine etc. Pharmacological screening of natural products has led to the discovery of a number of drugs. Different civilizations have developed their own indigenous system of medicines. *Withania coagulans* Dunal (synonym: *Puneeria coagulans* Stocks), commonly known as Indian rennet, Indian cheese maker, vegetable rennet (English), Panir ke phool, Panir band, Paneer bandh, Punir dodi (Hindi), Ning gu shui qie (Chinese), is distributed in the drier parts of India.[1] The plant is native of the Asia-temperate (Western Asia: Afghanistan) and Asia-tropical (Indian Subcontinent: India, Nepal) regions. A survey of the literature has shown that in various traditional systems of medicine the plant has been recommended for the treatment of various disorders.

It is an erect greyish under-shrub, 60–120 cm high. The leaves are lanceolate, entire, clothed with a persistent greyish tomentum on both sides. The flowers are dioecious, in axillary clusters. The calyx is 6 mm long, clothed with grey tomentum and the corolla is 8 mm long, with lobes that are ovate-oblong, subacute. The male flowers have stamens approximately level with the top of the corolla-tube, the filaments are 2 mm long and glabrous, and the anther 3–4 mm long. The ovary is ovoid, without style or stigma. The female flowers have stamens reaching halfway up the corolla-tube, the filaments are approximately 0.85 mm long, the anthers smaller than in the male flowers and sterile. The ovary is ovoid, glabrous, the style glabrous, the stigma mushroom-shaped, 2-lamellate. The berry is 6–8 mm in diameter, globose and smooth. The seeds are 2.5–3 mm in diameter, somewhat ear-shaped and glabrous.[2] In India two species of the genus *Withania*, *Withania somnifera* and *Withania coagulans*, are found.[3] *W. somnifera* is well known by the name 'Ashwagandha' (in Hindi) and 'Indian ginseng' and 'Winter cherry' (in English). The morphology of both species is similar.

Taxonomical Classification

Kingdom: Plantae, Plants; subkingdom: Tracheobionta, Vascular plants; super division: Spermatophyta, Seeds plants; division: Angiosperma; class: Dicotyledons; order: Tubiflorae; family: Solanaceae; genus: *Withania*; species: *coagulans.*[3]

Phytochemistry

The defatted meal from the seeds of *W. coagulans* Dunal contains 17.8% free sugars, consisting of D-galactose and D-arabinose in the ratio 1 : 1, with maltose in traces. Enzymatic studies showed the absence of a β-galactosidic linkage in the polysaccharide.[4] The seeds of *W. coagulans* are reported to contain 12–14% fatty oil. A hydrocarbon triacontaine and sterol dihydrostigmasterol are obtained from the unsaponifiable portion of the fruits. The oil was found to contain a high percentage of linoleic acid and β-sitosterol i.e. the factors which in combination are reported to be responsible for the hypocholesterolaemic effect of corn oil.[5]

W. coagulans is rich in steroidal lactones, which are known as withanolides (Figure 1). Withanolides are naturally occurring polyhydroxy C_{28} steroidal lactones. In the basic structure of all withanolides a six- or five-membered lactone or lactol ring is attached to an intact or rearranged ergostane skeleton. They give a positive Dragendorff's test even though they are not N-containing. On spraying the TLC with H_2SO_4–MeOH they give a characteristic blue colour spot. This class of compounds does not occur in all members of the Solanaceae family. However, the occurrence of withanolides is not restricted to Solanaceae. They have also been reported from marine organisms (soft corals) and from members of plant families Taccaceae and Leguminoseae.

Isolated compounds

One of the characteristic features of the plants that produce withanolides is their extraordinary ability to introduce oxygen functions at almost every position of the carbocyclic skeleton and side chain. Modifications either of the carbocyclic skeleton or of the side chains result in many novel structural variants of withanolides. Previous phytochemical examination of the whole plant resulted in the isolation of 25 compounds, including 24 withanolides and one dimeric lignan, bispicropodophyllin glucoside (**25**). In total nine compounds have been isolated from the fruits of *W. coagulans*, including ergosta-5,25-diene-3β,24ξ-diol and sitosterol-β-D-glucoside along with withanolides. Five withanolides have been isolated from the root of this plant (Table 1, Figure 2).[6–32] Withanolides having regular 17β-oriented as well as unusual 17α-oriented side chains have been identified from *W. coagulans*. Withaferin A (**32**) is a very common withanolide present in many plants (*Withania*, *Acnistus* etc.). Hairy roots, induced by the inoculation of leaf sections of *W. coagulans* with *Agrobacterium tumefaciens* strain C58C1 (pRiA4), have the capacity to produce withanolide A and withaferin A. Time course studies of withanolide production showed that withanolide A accumulated during the first part of the culture, whereas the maximum accumulation of withaferin A occurred at the end of the culture period.[25]

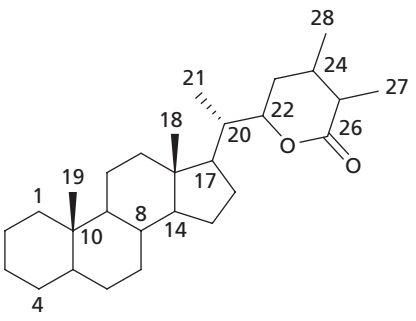


Figure 1 Basic skeleton of *Withania coagulans* withanolides

Pharmacological Properties

The berries of the plant are used for milk coagulation.[3] It has always had a prominent place in Ayurvedic, Unani, and ancient Indian systems of medicine. A number of reports have revealed that the withanolides isolated from *W. coagulans* possess interesting biological activities. The fruits of the plant are sweet and have been reported to have sedative, emetic, alterative and diuretic effects. They are useful in chronic complaints of the liver. In some places, they have been used as a blood purifier. They are also used in dyspepsia, flatulent colic and other intestinal infections. These are used for the treatment of asthma, biliousness and stranguary.[2]

Hepatoprotective activity

The aqueous extract of fruits of this plant has been shown to exert hepatoprotective activity. Since the steroidal compounds (glucocorticoids) having anti-inflammatory properties are used in some hepatic disorders, 3-β-hydroxy-2,3 dihydrowithanolide F has been screened for its hepatoprotective effect. It has shown hepatoprotective activity against CCl_4-induced hepatotoxicity in adult albino rats of either sex (150–200 g) at 10 mg/kg (i.p.). The protective effect was assessed by observing pentobarbitone (30 mg/kg; i.p.)-induced hypnosis, the determination of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels, and histopathological examination of hepatic tissues after staining with haematoxylin and eosin solutions. Concomitant treatment of the rats with 10 mg/kg withanolide protected the liver significantly ($P < 0.05$).[19]

Anti-inflammatory activity

The alcoholic extract and total alkaloids showed a significant anti-inflammatory effect in acute inflammation induced with egg albumin, subacute inflammation induced with formalin and granulation tissue formation by the cotton pellet method.[33]

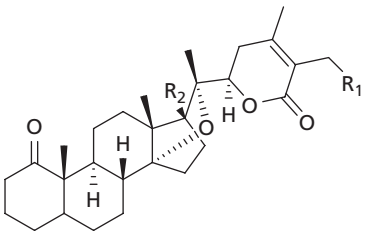
3-β-Hydroxy-2,3-dihydrowithanolide F exhibited a significant anti-inflammatory activity at 10 mg/kg in subacute models of inflammation such as granuloma formation and formalin-induced arthritis in rats. The effect was comparable with that obtained with 50 mg/kg phenylbutazone and 10 mg/kg hydrocortisone. However, it did not show any significant activity in acute models of inflammation.[20,21]

Antihyperglycaemic activity

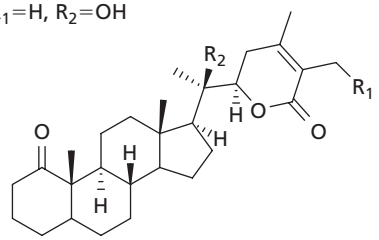
Administration of an aqueous extract of fruits of *W. coagulans* (1 g/kg; p.o.) significantly lowered the blood sugar, serum cholesterol, serum lipid peroxide (LPO) and hepatic LPO levels in streptozocin-induced diabetic rats after

Table 1 Isolated compounds from *Withania coagulans* and the reported biological activity

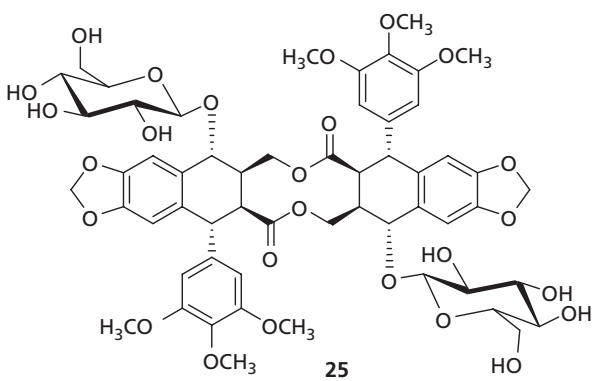
Structure no.	Name of compound	Plant part	Activity
1	Coagulin (17*β*,27-dihydroxy-14,20-epoxide-1-oxo-(22*R*)-witha-3,5,24-trienolide)[6]	Whole plant	
2	14,15*β*-Epoxywithanolide I ((20*S*,22*R*) 17*β*,20*β*-dihydroxy-14*β*,15*β*-epoxy-1-oxowitha-3,5,24-trienolide)[7,8]	Whole plant	
3	17*β*-Hydroxywithanolide K ((20*S*,22*R*) 14*α*,7*β*,20*β*-trihydroxy-1-oxowitha-2,5,24-trienolide)[7–9]	Whole plant & fruits	Antihyperglycaemic, antimicrobial
4	Coagulin B[10]	Whole plant	
5	Coagulin C[9,10]	Whole plant & fruits	Antihyperglycaemic
6	Coagulin D[10]	Whole plant	
7	Coagulin E[10]	Whole plant	
8	Coagulin F (27-hydroxy-14,20-epoxy-1-oxo-(22*R*)-witha-3,5,24-trienolide)[11]	Whole plant	
9	Coagulin G (17*β*,27-dihydroxy-14,20-epoxy-1-oxo-(22*R*)-witha-2,5,24-trienolide)[11]	Whole plant	
10	Coagulin H ((17*S*,20*S*,22*R*)-5*α*,6*β*,14*α*,15*α*,17,20-hexahydroxy-1-oxowitha-2,24-dienolide)[12,13]	Whole plant	Immunosuppressive
11	Coagulin I ((14*R*,17*S*,20*S*,22*R*)-5*α*,6*β*,17-trihydroxy-14,20-epoxy-1-oxowitha-2,24-dienolide)[12]	Whole plant	
12	Coagulin J ((14*R*,17*R*,20*R*,22*R*)-3*β*,27-dihydroxy-14,20-epoxy-1-oxowitha-5,24-dienolide)[12]	Whole plant	
13	Coagulin K ((14*R*,17*R*,20*R*,22*R*)-14,20-epoxy-3*β*-(*O*-*β*-D-glucopyranosyl)-1-oxowitha-5,24-dienolide)[12]	Whole plant	
14	Coagulin L ((14*R*,17*S*,20*S*,22*R*)-14,17,20-trihydroxy-3*β*-(*O*-*β*-D-glucopyranosyl)-1-oxowitha-5,24-dienolide)[9,12]	Whole plant & fruits	Antihyperglycaemic
15	Coagulin M ((14*R*,17*R*,20*R*,22*R*)-5*α*,6*β*,27-trihydroxy-14,20-epoxy-1-oxowitha-24-enolide)[14]	Whole plant	
16	Coagulin N ((14R,17S,20*R*,22R)-15*α*,17-dihydroxy-14,20-epoxy-3*β*-(*O*-*β*-D-glucopyranosyl)-1-oxowitha-5,24-dienolide)[14]	Whole plant	
17	Coagulin O ((14*R*,20*S*,22*R*)-14,20-dihydroxy-3*β*-(*O*-*β*-D-glucopyranosyl)-1-oxowitha-5,24-dienolide)[14]	Whole plant	
18	Coagulin P (20,27-dihydroxy-3*β*-(*O*-*β*-D-glucopyranosyl)-1-oxo-(20*S*,22*R*)-witha-5,14,24-trienolide)[15]	Whole plant	
19	Coagulin Q (1*α*,20-dihydroxy-3*β*-(*O*-*β*-D-glucopyranosyl)-(20*S*,22*R*)-witha-5,24-dienolide)[15]	Whole plant	
20	Coagulin R (3*β*,17*β*-dihydroxy-14,20-epoxy 1-oxo-(22*R*)-witha-5,24-dienolide)[15]	Whole plant	
21	20*β*-Hydroxy-1-oxo-(22*R*)-witha-2,5,24-trienolide[16]	Whole plant	
22	Withacoagulin[16]	Whole plant	
23	17*β*-Hydroxy-14*α*, 20*α*-epoxy-1-oxo-(22*R*)-witha-3,5,24-trienolide[16]	Whole plant	
24	Coagulin S[17]	Whole plant	
25	Bispicropodophyllin glucoside[17]	Whole plant	
26	3*β*,14*α*,17*β*,20α_F-Tetrahydroxy-1-oxo-20S,22*R*-witha-5,24-dienolide (or 3*β*-hydroxy-2,3-dihydrowithanolide F)[18–22]	Fruits	Hepatoprotective, anti-inflammatory, blood pressure lowering, central nervous system depressant
27	Ergosta-5,25-diene-3*β*,24*ξ*-diol[18]	Fruits	
28	3*β*,14*α*,20α_F,27-Tetrahydroxy-1-oxo-20*R*,22R-witha-5,24-dienolide (or 3*β*-hydroxy-2,3-dihydrowithanolide H)[23]	Fruits	
29	Sitosterol-*β*-D-glucoside[23]	Fruits	
30	Coagulanolide ((17*S*,20*S*,22*R*)-14*α*,15*α*,17*β*,20*β*-tetrahydroxy-1-oxowitha-2,5,24-trienolide)[9]	Fruits	Antihyperglycaemic
31	Withanolide F[9]	Fruits	Antihyperglycaemic
32	Withaferin A[24–29]	Root	Antimicrobial, immunomodulating, antitumour, cytotoxic
33	5,27-Dihydroxy-6*α*,7*α*-epoxy-1-oxo-(5*α*)-witha-2,24-dienolide[30]	Root	
34	Withacoagin ((20*R*,22*R*)-5*α*,20-dihydroxy-1-oxowitha-2,6,24-trienolide)[31]	Root	
35	(20*R*,22*R*)-6*α*,7*α*-Epoxy-5*α*-20-hydroxy-1-oxowitha-2,24-dienolide[31]	Root	
36	(20*S*,22*R*)-6*α*,7*α*-Epoxy-5*α*-dihydroxy-1-oxowitha-2,24-dienolide[31,32]	Root	Immunosuppressant



1. $\Delta^{3,5}$, R_1=OH, R_2=OH
4. $\Delta^{2,5}$, R_1=OH, R_2=H
5. $\Delta^{2,5}$, R_1=H, R_2=OH
6. $\Delta^{2,5}$, R_1=H, R_2=H
7. $\Delta^{3,5}$, R_1=H, R_2=H
8. $\Delta^{3,5}$, R_1=OH, R_2=H
9. $\Delta^{2,5}$, R_1=OH, R_2=OH
11. Δ^{2}, 5α-OH, 6β-OH, R_1=H, R_2=OH
12. Δ^{5}, 3β−OH, R_1=OH, R_2=H
13. Δ^{5}, 3β-(O-β-D-glucopyranosyl), R_1=H, R_2=H
15. 5α-OH, 6β-OH, R_1=OH, R_2=H
16. Δ^{5}, 3β-(O-β-D-glucopyranosyl), R_1=H, R_2=OH
20. Δ^{5}, 3β-OH, R_1=H, R_2=OH
23. $\Delta^{3,5}$, R_1=H, R_2=OH



18. $\Delta^{5,14}$, 3β-(O-β-D-glucopyranosyl), R_1=OH, R_2=OH
21. $\Delta^{2,5}$, R_1=H, R_2=OH
22. $\Delta^{2,5,14}$, R_1=OH, R_2=OH
32. Δ^{2}, 4β-OH, 5β, 6β-epoxy, R_1=OH, R_2=H
33. Δ^{2}, 5α-OH, 6α, 7α-epoxy, R_1=OH, R_2=H
34. $\Delta^{2,6}$, 5α-OH, R_1=H, R_2=OH



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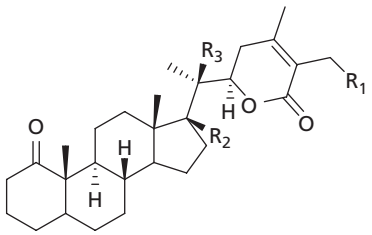
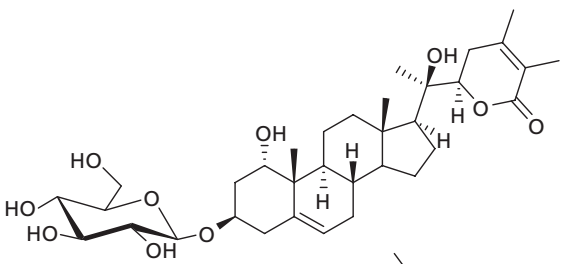


2. $\Delta^{3,5}$, 14β, 15β-epoxy, R_1=H, R_2=OH, R_3=OH
3. $\Delta^{3,5}$, 14α-OH, R_1=H, R_2=OH, R_3=OH
10. Δ^{2}, 5α-OH, 6β-OH, 14α-OH, 15α-OH, R_1=H, R_2=OH, R_3=OH
14. Δ^{5}, 3β-(O-β-D-glucopyranosyl), 14α-OH, R_1=H, R_2=OH, R_3=OH
17. Δ^{5}, 3β-(O-β-D-glucopyranosyl), 14α-OH, R_1=H, R_2=H, R_3=OH
24. 5α-OH, 6β-OH, 14α-OH, 15α-OH, R_1=OH, R_2=OH, R_3=OH
26. Δ^{5}, 3β-OH, 14α-OH, R_1=H, R_2=OH, R_3=OH
28. Δ^{5}, 3β-OH, 14α-OH, R_1=OH, R_2=H, R_3=OH
30. $\Delta^{2,5}$, 14α-OH, 15α-OH, R_1=H, R_2=OH, R_3=OH
31. $\Delta^{2,5}$, 14α-OH, R_1=H, R_2=OH, R_3=OH
35. Δ^{2}, 14α-OH, 6α, 7α-epoxy, R_1=H, R_2=H, R_3=OH
36. Δ^{2}, 5α-OH, 6α, 7α-epoxy, R_1=H, R_2=H, R_3=H



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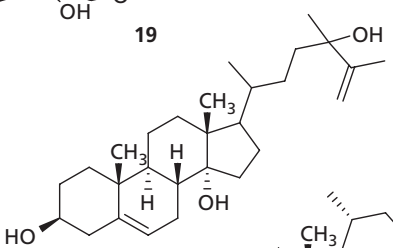

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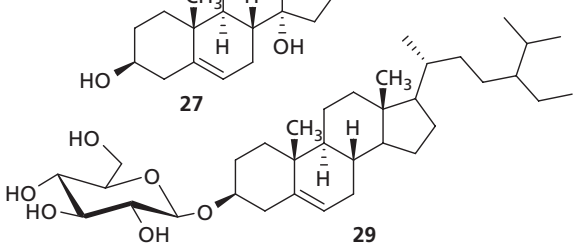

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Figure 2 Compounds isolated from *Withania coagulans*

seven days of treatment ($P < 0.001$). Such lipid lowering activity in streptozocin-induced diabetic rats may have helped in preventing associated atherogenesis and other secondary complications of diabetes mellitus. Its serum LPO and liver LPO reducing activity suggested that it may prevent lipid peroxidation and may protect tissues from free radicals. It also significantly ($P < 0.01$) decreased blood glucose level in normal rats (at 1 g/kg; p.o.).[34] Coagulin C (**5**),

17β-hydroxywithanolide K (**3**), withanolide F (**31**), coagulanolide ((17*S*,20*S*,22*R*)-14α,15α,17β,20β-tetrahydroxy-1-oxowitha-2,5,24-trienolide) (**30**) and coagulin L (**14**) were evaluated for their antihyperglycaemic activity in the normoglycaemic rat model (SLM) and in the streptozocin-induced diabetic rat model (STZ). Compound **14** was also evaluated in C57BL/KsJ-db/db mice. Compound **14** improved glucose tolerance up to 29.8% in SLM and 23.3% in STZ-induced diabetic rats at a dose of 100 mg/kg body weight. Compounds **5**, **3**, **31** and **30** exhibited significant antihyperglycaemic activity, 22.8, 20.4, 24.9 and 28.1% in SLM and 16.9, 15.8, 18.2 and 19.3% in STZ, models, respectively. Compound **5** (at 50 mg/kg body weight) in db/db mice for 10 consecutive days significantly lowered the postprandial blood glucose level by 22.7% ($P < 0.01$), whereas metformin decreased the postprandial blood glucose by 18.6% ($P < 0.05$). For compound **14** in the db/db mice group, the area under the curve (AUC) of blood glucose decreased by approximately 40.5% compared with the vehicle-treated group. Compound **5** (at 50 mg/kg body weight) showed significant improvement in plasma lipid profiles of dyslipidaemic db/db mice after 10 days of consecutive treatment. Increasing oral doses of compound **14** declined the postprandial glucose level, with a calculated ED50 (effective dose 50%) of approximately 25 mg/kg of body weight following oral administration.[9]

Hypolipidaemic activity

The aqueous extract of fruits of *W. coagulans* (1 g/kg; p.o.) showed 15% reduction in serum cholesterol level in Triton-induced hyperlipidaemic rats in comparison with untreated animals. In rats with a high fat diet-induced hyperlipidaemia, the aqueous extract at the same dose administered for seven weeks showed a significantly reduced body weight, elevated serum cholesterol, triglycerides and lipoprotein levels. The animals treated with aqueous extract of fruits of *W. coagulans* and the reference drug Navaka guggulu showed less degenerative changes along with microvesicular fatty changes.[35]

Free radical scavenging activity

The aqueous extract (2 mg/ml) exhibited free radical scavenging activity in an in-vitro system using 1,1-diphenyl-2-picrylhydrazyl (DPPH). This method, used to determine free radical scavenging activity, was based on the reduction of a methanolic solution of the coloured free radical DPPH. The decrease in absorption of DPPH at its absorption maximum of 517 nm is proportional to the concentration of free radical scavenger added to the DPPH reagent solution. The activity was expressed as the effective concentration at 50%. The presence of free radical scavenging potential might help in protecting against oxidative damage to pancreatic beta cells.[34]

Antimicrobial activity

Antifungal and antibacterial properties have been demonstrated in the withanolides isolated from the ethanolic extract of the whole plant and leaves, respectively.[7,36] Withaferin A exhibited a significant antibacterial activity against Gram-positive microorganisms at the concentrations 6–100 μg/ml, whereas it was inactive against Gram-negative bacteria or nonfilamentous fungi. The following minimal inhibitory concentrations (mg/ml) have been found for withaferin A: 6.25 for *Staphylococcus pyogenes*, 12.5 for *Sarcinia lutea*, 100 for *Streptococcus pyogenes*, 200 for *Streptococcus viridans*, 25 for *Bacillus subtilis*, 50 for *Corynebacterium diphtheriae*, 6.26 for *Bacillus anthracis*, 200 for *Escherichia coli*, and 200 for *Pseudomonas aeruginosa*.[27,28] Antifungal activity against *Aspergillus niger, Candida albicans* and *Taenia rubrum* at doses of 12.5–50 μg/ml, 100, 150 and 200 μg/ml has been shown. It was found to be more inhibitory to the filamentous fungi than to the yeast group of fungi.[29] The volatile oil obtained from fruits of *W. coagulans* had antibacterial activity against *Staphylococcus aureus* and *Vibrio cholerae* and was also found to have anthelmintic activity.[37] 17β-Hydroxywithanolide K (**3**) exhibited antifungal activity against human pathogens *Nigrospora oryzae*, *Aspergillus niger*, *Curvularia lunata*, *Stachybotrys atra*, *Allescheria boydii*, *Drechslera rostrata*, *Microsporum canis* and *Epidermophyton floccosum* and plant pathogen *Pleurotus ostreatus* (minimal inhibitory concentration 300 μg/ml).[7]

Cardiovascular effects

An alcoholic solution of 3β-hydroxy-2,3-dihydrowithanolide F (**26**) 5 mg/kg exhibited a moderate fall of blood pressure (34 ± 2.1 mmHg) in mongrel dogs (weight 12–15 kg). The hypotensive response was blocked by atropine (2 mg/kg) but not by mepyramine (2 mg/kg) and propranolol (1 mg/kg). At the same dose, the hypotensive response was less with a suspension of the withanolides. On administration of a 10 mg/kg bolus dose in alcohol, a depression of the S-T segment was caused in ECG studies of dog. A 2 mg dose in suspension produced a positive inotropic and chronotropic effect in perfused frog heart. The heart rate increased from 61.2 ± 1.39 to 77 ± 1.94 beats/min ($P < 0.01$). In rabbit Langendorff preparations 2 mg withanolide produced negative inotropic and chronotropic effects. The heart rate decreased from 71 ± 2.4 to 19 ± 0.28 beats/min. In rat limb preparation, 1 mg withanolide caused insignificant ($P > 0.05$) vasoconstriction. On administration of 5 mg/kg it increased the rate and depth of respiration. The rate of respiration increased from 18 ± 1.4 to 65 ± 5.3 breaths/min in dogs, which was insignificant ($P < 0.01$).[22]

Central nervous system depressant activity and acute toxicity

The total extract of *W. coagulans* fruit has been reported to have central nervous system (CNS) depressant activity in mice, rabbits and dogs. The extract was hypotensive in animals and had respiratory stimulant and smooth muscle relaxant activity. Alcoholic extract, total alkaloids and aqueous extract at doses of 1 g/kg, 200–400 mg/kg and 5 mg/100 g exhibited CNS depression in albino rats characterized by sedation, reduced exploratory, spontaneous activity and hypothermia. At the same doses but administered 30 min before a hypnotic, they potentiated pentobarbitone sleeping time in rats. They did not show any analgesic and diuretic activity in albino rats. Alcoholic extract (1 g/kg) and total alkaloids (200–400 mg/kg) did not protect against

convulsions induced by pentylenetetrazol (70 mg/kg). They increased the lethal effect of amphetamine in aggregated mice.[33] 3β-Hydroxy-2,3-dihydrowithanolide F (**26**) was tested for its CNS depressant activity. It was found nonlethal to mice up to a dose of 625 mg/kg (i.p.). It did not show analgesic, hypothermic or local anaesthetic activity.[21]

Immunomodulating activity

Withaferin A (**32**) has been reported in various studies to possess both immunoactivating and immunosuppressive properties, even at a low dose of 10 mg/kg for six consecutive days. Withaferin A was found also to impart immunoactivation by specifically inducing proliferation of peritoneal macrophages in mice but not in splenocytes, resulting in regression of tumour cells in a mouse carcinoma model, which was persistent even after passive transfer of the serum or macrophages of the treated mice into another model.[38] Withaferin A had specific immunosuppressive effects on human B and T lymphocytes as well as on mice thymocytes. It inhibited E rosettes and EAC rosette formation by normal human T and B lymphocytes at very low concentrations. It was demonstrated to affect the functional activity of normal human T lymphocytes as assessed by a local xenogeneic graft versus host reaction. It had specific action on antigen recognition as well as proliferative capacity of T lymphocytes and B lymphocytes.[39]

5,20α(*R*)-dihydroxy-6α,7α-epoxy-1-oxo-(5α)-witha-2,24-dienolide is known to show immunosuppressant activity in spleen cell culture. On administration of doses above 1 μg/ml, it inhibited proliferation of murine spleen cell cultures. A solution of 5,20α(*R*)-dihydroxy-6α,7α-epoxy-1-oxo-(5α)-witha-2,24-dienolide in dimethyl sulfoxide was mixed with RPMI 1640 medium to achieve a fine suspension (0.37% dimethyl sulfoxide).[32]

Coagulin H (**10**) exhibited effects on the immune response, including an inhibitory effect on lymphocyte proliferation, and expression of interleukin-2 (IL-2) cytokine. A complete suppression of phytohaemagglutinin-activated T-cells was observed at $\geq$2.5 μg/ml coagulin H and this suppression activity was similar to that of prednisolone, a commonly used immune modulating drug. Coagulin H also significantly inhibited IL-2 production by 80%. Docking studies predicted that coagulin H bound to the receptor binding site of IL-2 more effectively than prednisolone. Based on the computational and the experimental results, coagulin H was identified as a potential immunosuppressive candidate.[13]

Antitumour activity

Withaferin A showed marked tumour-inhibitory activity when tested in-vitro against cells derived from human carcinoma of the nasopharynx (KB).[40] Withaferin A inhibited RNA synthesis of Sarcoma-180 ascites tumour cells. At 40 μg/ml, within 30 min of incubation it showed inhibition of RNA synthesis of more than 50%. It inhibited protein synthesis of Sarcoma-180 cells. In this way, withaferin A inhibited transcription and translation processes of these cells.[41] At concentrations of 0.01–0.5% it showed inhibition on the growth of roots of *Allium cepa* by arresting the cell division at metaphase after 2-h treatment.[42] Withaferin A acted as a mitotic poison via arresting the division of cultured human larynx carcinoma cells at metaphase. It showed a similar, but less marked effect on HeLa and embryonal chicken fibroblast cells.[43,44] Withaferin A inhibited human umbilical vein endothelial cell (HUVEC) sprouting in three-dimensional collagen-I matrix at doses which were relevant to nuclear factor-kappa B-inhibitory activity. Withaferin A inhibited cell proliferation in HUVECs at doses that were significantly lower than those required for tumour cell lines through a process associated with the inhibition of cyclin D1 expression.[45]

Cytotoxic activity

In-vitro effects of Withaferin A on P388 cells have been studied. The cytotoxicity was calculated from the utilization of precursors in protein and nucleic acid synthesis and from capacity to suppress cell proliferation. Withaferin A stopped cell proliferation and, at the same time, killed the cells. Cytotoxicity was found to be due to a double bond at position C2–3; on dissociating this bond the cytotoxicity markedly decreased. A dissociation of the double bond at C24–25 or a removal of OH group from C27 did not cause any significant changes in the biological effects. An addition of a carbonyl group at C4 increased the effect. As withaferin A promptly reacted with L-cysteine, it was presumed that one of the possible target sites in the cell might be the SH groups of enzymes, which react with the lactone and epoxide groups of the agent.[46]

Conclusions

The use of herbal drugs is increasing worldwide as they has fewer or no side effects as compared with synthetic drugs. Ayurveda claims therapeutic potentials of various plants. In India, two species of the genus *Withania* are found, *W. somnifera* and *W. coagulans*. *W. somnifera* is known by the name 'Ashwagandhain' in Hindi and 'Indian ginseng' in English. Both species closely resemble each other. Though withanolides are the principal compounds found in both species, there are some withanolides specific to each of them. Withaferin A is a major compound found in *W. somnifera,* whereas, coagulin L has been found in major amounts in *W. coagulans*. Where antihyperglycaemic leads from *W. coagulans* have been identified, it is still to be determined in *W. somnifera*.[9] A unique thio-dimer of withanolide named Ashwagandhanolide has been found in *W. somnifera.*[47,48] Withanolides containing a 14,20-epoxide bridge are specific to *W. coagulans*. *W. somnifera* has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent and astringent and more recently as an antibacterial, antihyperglycaemic, hypolipidaemic and antitumoral, as well as to treat ulcers and senile dementia.[48] Hepatoprotective, anti-inflammatory, antihyperglycaemic, hypolipidaemic, free radical scavenging, antimicrobial, cardiovascular, central nervous system depressant, immunomodulating, antitumour and cytotoxic activities have been studied in *W. coagulans*. *W. coagulans* had the greater therapeutic value overall. The variety of activities reported for the extracts, fractions and withanolides isolated from *W. coagulans* provide promising evidence for future research.

Withanolides could achieve an important place in the world of modern drugs. Isolation on a large scale, chemical transformations and synthesis of the active compounds will definitely enhance their pharmacological value. The pharmacophores of various pharmacologically active withanolides have not yet been identified. Clinical trials using the active compounds for a variety of conditions need conducting. All these advantages prove the significance of *W. coagulans* in natural product research.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Kirtikar KR, Basu BD. *Indian Medicinal Plants.* Dehradune, India: International Book Distributors, 1995.
2. Kirtikar KR, Basu BD. *Indian Medicinal Plants*, vol. 3. Allahabad, India: L M Basu, 1933.
3. Chadha YR. *The Wealth of India, Raw Materials*, vol. 10. New Delhi: CSIR, 1976.
4. Salam A, Wahid MA. Free sugars and a galactoaraban from *Withania coagulans* seeds. *Pakistan J Biochem* 1969; 2: 18–21.
5. Atal CK, Sethi PD. A preliminary chemical examination of *Withania coagulans*. *Indian J Pharm* 1963; 25: 163–164.
6. Rahman Atta-Ur *et al*. New withanolides from *Withania* Spp. *J Nat Prod* 1993; 56: 1000–1006.
7. Choudhary MI *et al*. Antifungal steroidal lactones from *Withania coagulance*. *Phytochemistry* 1995; 40: 1243–1246.
8. Rahman Atta-Ur, Choudhary MI. New natural products from medicinal plants of Pakistan. *Pure Appl Chem* 1998; 70: 385–389.
9. Maurya R *et al*. Coagulanolide, a withanolide from *Withania coagulans* fruits and antihyperglycemic activity. *Bioorg Med Chem Lett* 2008; 18: 6534–6537.
10. Rahman Atta-Ur *et al*. New steroidal lactones from *Withania coagulance*. *Heterocycles* 1998; 47: 1005–1011.
11. Rahman Atta-Ur *et al*. Two new ergostane-type steroidal lactones from *Withania coagulans*. *J Nat Prod* 1998; 61: 812–814.
12. Rahman Atta-Ur *et al*. Five new withanolides from *Withania coagulans*. *Heterocycles* 1998; 48: 1801–1811.
13. Mesaik MA *et al*. Biological and molecular docking studies on coagulin-H: human IL-2 novel natural inhibitor. *Mol Immunol* 2006; 43: 1855–1863.
14. Rahman Atta-Ur *et al*. New withanolides from *Withania coagulans*. *Chem Pharm Bull* 1998; 46: 1853–1856.
15. Rahman Atta-Ur *et al*. Three withanolides from *Withania coagulans*. *Phytochemistry* 1999; 52: 1361–1364.
16. Rahman Atta-Ur *et al*. Withanolides from *Withania coagulans*. *Phytochemistry* 2003; 63: 387–390.
17. Alam Nur-e *et al*. A novel dimeric podophyllotoxin-type lignan and a new withanolide from *Withania coagulans*. *Helv Chim Acta* 2003; 86: 607–614.
18. Velde VV *et al*. Potential biogenetic precursors of withanolides from *Withania coagulans*. *Phytochemistry* 1983; 22: 2253–2257.
19. Budhiraja RD *et al.* Protective effect of 3-β-hydroxy-2,3 dihydrowithanolide F against CCl_4 induced hepatotoxicity. *Planta Med* 1986; 28–29.
20. Budhiraja RD *et al*. Anti-inflammatory activity of 3-β-hydroxy-2,3-dihydro-withanolide F. *Planta Med* 1984; 50: 134–136.
21. Budhiraja RD, Sudhir S. Review of biological activity of withanolides. *J Sci Ind Res* 1987; 46: 488–491.
22. Budhiraja RD *et al*. Cardiovascular effects of a withanolide from *Withania coagulans* Dunal fruits. *Indian J Physiol Pharmacol* 1983; 27: 129–134.
23. Ramaiah PA *et al*. Spectroscopic studies on a withanolide from *Withania coagulans*. *Phytochemistry* 1984; 23: 143–149.
24. Subramanian SS, Sethi PD. Withaferin A from the roots of *Withania coagulans*. *Curr Sci* 1969; 38: 267–268.
25. Mirjalili HM *et al*. Morphology and withanolide production of Withania coagulans hairy root culture. *Eng Life Sci* 2009; 9: 197–204.
26. Subramanian SS *et al*. 5,20α(R)-Dihydroxy-6α,7α-epoxy-1-oxo(5α) with-2,24-dienolide, a new steroidal lactone from *Withania coagulans*. *Phytochemistry* 1971; 10: 685–688.
27. Sethi PD *et al*. Antibacterial activity of some C_{28} steroidal lactones. *Indian J Pharm* 1974; 36: 122–123.
28. Chatterjee S, Chakraborti SK. Antimicrobial activities of some antineoplastic and other withanolides. *Antonie van Leeuwenhoek* 1980; 46: 59–63.
29. Dasgupta LR *et al*. Antifungal activity of withaferin A. *Indian J Pharm* 1970; 32: 70.
30. Sethi PD, Subramanian SS. Steroidal constituents of *Withania coagulans* roots. *Indian J Pharm* 1976; 38: 22–23.
31. Neogi P *et al*. Withacoagin, a new withanolide from *Withania coagulans* roots. *Bull Chem Soc Jpn* 1988; 61: 4479–4481.
32. Bahr V, Hansel R. Immunomodulating properties of 5,20α(R)-dihydroxy-6α,7α-epoxy-1-oxo(5α)-witha-2,24-dienolide and solasodine. *Planta Med* 1982; 44: 32–33.
33. Budhiraja RD *et al*. Pharmacological investigations on fruits of *Withania coagulans* Dunal. *Planta Med* 1977; 32: 154–157.
34. Hemalatha S *et al*. Hypoglycemic activity of *Withania coagulans* Dunal in streptozotocin induced diabetic rats. *J Ethnopharmacol* 2004; 93: 261–264.
35. Hemalatha S *et al*. Hypolipidemic activity of aqueous extract of *Withania coagulans* Dunal in Albino rats. *Phytother Res* 2006; 20: 614–617.
36. Khan MTJ *et al*. Antibacterial activity of *W. coagulans*. *Fitoterapia* 1993; 64: 367–378.
37. Gaind KN, Budhiraja RD. Antibacterial and anthelmintic activity of *Withania coagulans* Dunal. *Indian J Pharm* 1967; 29: 185–186.
38. Shohat B, Joshua H. Effect of withaferin A on ehrlich ascites tumor cells. II. Target tumor cell destruction in vivo by immune activation. *Int J Cancer* 1971; 8: 487–496.
39. Shohat B *et al*. Immunosuppressive activity of two plant steroidal lactones withaferin A and withanolide E. *Biomedicine* 1978; 28: 18–24.
40. Kupchan SM *et al*. The isolation and structural elucidation of a novel steroidal tumor inhibitor from *Acnistus arborescens*. *J Am Chem Soc* 1965; 87: 5805–5806.
41. Chowdhury K, Neogy RK. Mode of action of Withaferin A and Withanolide D. *Biochem Pharmacol* 1975; 24: 919–920.
42. Shohat B, Joshua H. The effect of withaferin A on plant cells. *Eur J Cancer* 1971; 7: 561–562.
43. Palyi I *et al*. Cytological effects of compounds isolated from *Withania somnifera*. *Herba Hungarica* 1969; 8: 73–77.
44. Shohat B. Antimitotic properties of Withaferin A in tissue culture. *Harefuah* 1972; 8312: 582–583.

45. Mohan R *et al*. Withaferin A is a potent inhibitor of angiogenesis. *Angiogenesis* 2004; 72: 115–122.
46. Fuskova A *et al*. Novel cytotoxic and antitumor agents. IV. Withaferin A: relation of its structure to the in vitro cytotoxic effects on P388 cells. *Neoplasm* 1984; 312: 31–36.
47. Subaraju GV *et al*. Ashwagandhanolide, a bioactive dimeric thiowithanolide isolated from the roots of *Withania somnifera*. *J Nat Prod* 2006; 69: 1790–1792.
48. Mirjalili MH *et al*. Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. *Molecules* 2009; 14: 2373–2393.