

PHYTOPHARMACOLOGICAL PROPERTIES OF *WITHANIA COAGULANS*: A REVIEW

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Summary

In the natural system of medicine many plants have possess a variety of biological activities. There are two species of *Withania*, small genres of shrubs distributed in east of the Mediterranean region extending to South Asia are found in India. *Withania coagulans* Dunal (family: Solanaceae) commonly known as Indian cheese maker, reported to have different phytochemicals like withanolides, withacoagin, coagulan, withaferin and withasomidienone. The different pharmacological activities reported with this shrub are sedative, CNS depressant, antibilious, emetic, antiasthmatic, diuretic, anti-inflammatory; carminative, depurative, used for dyspepsia, flatulence, hepatoprotective activity, antifungal antibacterial activity, hypolipidemic activity and calcium channel blocking activity. So the present paper enumerates an overview of phytochemical and pharmacological properties of *withania coagulans*.

Keywords: *withania coagulans*, Phytochemicals, pharmacological profile

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Introduction

In the natural system of medicine many plants have possess a variety of biological activities. *Withania Coagulans* belongs to the genus *Withania* and family Solanaceae. *Withania coagulans* Dunal commonly known as Indian cheese maker, reported to have different phytochemicals and pharmacological properties. The actions reported in literature are Ripe fruits-sedative, CNS depressant, antibilious, emetic, antiasthmatic, diuretic, anti-inflammatory; used in chronic liver troubles and strangury. Dried fruits - carminative, depurative; used for dyspepsia, flatulence and strangury. Leaf-alterative, febrifuge. Seeds- anti-inflammatory, emetic, diuretic activity.

The berries contain a milk-coagulating enzyme, esterases, free amino acids, fatty oil, an essential oil and alkaloids. The amino acid composition fairly agrees with that of papain. The essential oil was active against *Micrococcus pyogenes* var. *aureus* and *Vibro cholerae*; also showed anthelmintic activity. The withanolides, withacoagin, coagulan and withasomidienone have been isolated from the plant, along with other withanolides and withaferin. 3- beta-hydroxy-2,3-dihydrowithanolide E, isolated from the fruit showed significant hepatoprotective activity and anti-inflammatory activity equal to hydrocortisone. The ethanolic extract of the fruit showed antifungal and that of the leaves and stem antibacterial activity [1].

Botanical Name *Withania Coagulans* Dunal

Family *Solanaceae*.

Habitat Dried parts of Punjab, Gujarat, Shimla and Kumaon.

Synonymes

Though known as Desi Asgandh, the root is not used in Indian medicine. Ashwagandhaa (Bengali) and Ashwagandhi (Kannada) are confusing synonyms of *W. coagulans*. In the market no distinction is made between the berries of *W. coagulans* and *W. somnifera*.

English- Vegetable Rennet, Indian Cheese-maker.

Hindi- Paneer, Paneer-band. Akri(fruit).

Unani -Desi Asgandh, Kaaknaj-e-

Siddha/Tamil -Ammukkura.

Botany

Withania Coagulans belongs to the genus *Withania* and family *Solanaceae*. Two species, viz, *W. coagulans* Dunal and *W. somnifera* Dunal are found in India. *W. coagulans* is a rigid grey under shrub of 60-120cm high.

Distribution

Withania Coagulans is believed to have oriental origin. It is found wild in the forests of Mandsaur and Bastar in Mandhya Pradesh, the foot hills of Punjab, Himachal Pradesh, Uttar Pradesh and western Himalayas in India. It is also found wild in the Mediterranean region in North America. In India it is cultivated in Madhya Pradesh, Rajasthan and other drier parts of the country [2].

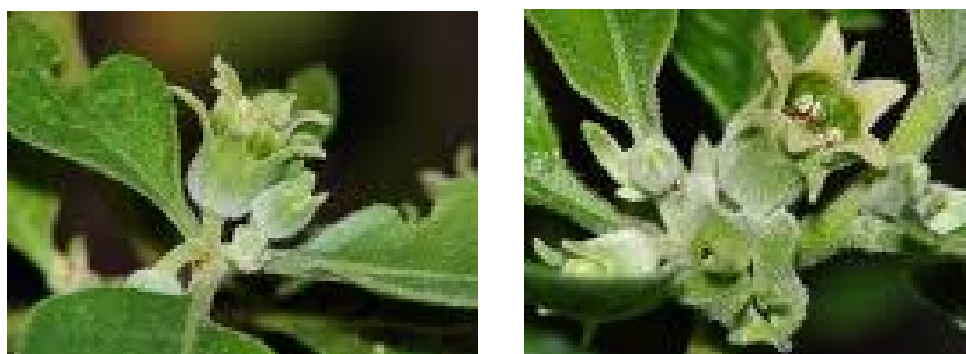


Fig.1 Leaves and flowers of *Withania Coagulans*

Fig. 2 Dunals of *Withania Coagulans*

Phytochemicals

One new withanolide, (17S,20S,22R)-14a,15a,17b,20b-tetrahydroxy-1-oxowitha-2,5,24-trienolide) named coagulanolide (4) along with four known withanolides 1–3 and 5 have been isolated from *Withania coagulans* fruits and their structures were elucidated by spectroscopic techniques. The compounds 1–5 showed significant inhibition on postprandial rise in hyperglycemia post-sucrose load in normoglycemic rats as well as streptozotocin-induced diabetic rats. The compound 5 also showed significant fall on fasting blood glucose profile and improved the glucose tolerance of db/db mice. Further compound 5 showed antidyslipidemic activity in db/db mice. The median effective dose of the compound 5 was determined to be around 25 mg/kg in streptozotocin-induced diabetic rats, which is comparable to the standard antidiabetic drug metformin. Our results provide further support to explain the traditional use of *W. coagulans* as antihyperglycemic cum antidyslipidemic agent by the traditional medical practitioners [3].

Three new withanolides, coagulins P, Q and R, were isolated from the whole plant of *Withania coagulans*. Their structures were determined as 20,27-dihydroxy-3b-(O-b-D-glucopyranosyl)-1-oxo-(20S,22R)-witha-5,14,24-trienolide, 1a,20-dihydroxy-3b-(O-b-D-glucopyranosyl)-20S,22R)-witha-5,24-dienolide and 3b,17b-dihydroxy-14,20-epoxy-1-oxo-(22R)-witha-5,24-dienolide by a combination of 1D- and 2D-NMR and mass spectroscopic studies[4].

Two new withanolides (steroidal lactones) named coagulin F [27-hydroxy-14,20-epoxy-1-oxo-(22R)-witha-3,5,24-trienolide] (1) and coagulin G [17 β ,27-dihydroxy-14,20-epoxy-1-oxo-(22R)-witha-2,5,24-trienolide] (2) were isolated from the whole plant of *Withania coagulans*, and their structures were deduced by spectral analysis[5].

Squalene synthase (SS) is able to dimerize two molecules of farnesyl diphosphate to synthesize squalene, a shared precursor in steroid and triterpenoid biosynthesis in plants. The *SSI* gene encoding SS from *Arabidopsis thaliana* was introduced in *Withania coagulans* under the control of the CaMV35S promoter together with the TDNA of *Agrobacterium rhizogenes* A4. The engineered hairy roots were studied for withanolide production and phytosterol accumulation and the results were compared with those obtained from control roots harbouring only the TDNA from pRiA4. Both kinds of roots were able to biosynthesize the most important bioactive withanolides, withanolide A and withaferin A, the latter only in very low concentrations. The engineered root line SS135 exhibited the highest withanolide content (4.93 g/g DW), more than doubling the most productive control (C26), and thereby showing the effectiveness of the transgene in increasing withanolide production.

This root type also exhibited a greater capacity than the control (nearly threefold) for accumulating squalene-derived primary metabolites such as phytosterols[6].

Root cultures of *Withania coagulans* (Solanaceae) were obtained from *in vitro* germinated sterile plantlets. The roots grew axenically in MS medium containing 0.25 mgL⁻¹ indole-3-butyric acid and 30 gL⁻¹ sucrose. A RP-HPLC method for the determination of withanolides in plant tissues was developed using a binary gradient solvent system comprising acetonitrile and water containing 0.1% (v/v) acetic acid. The method was validated according to ICH guidelines. The linearity was found to be in the range of 1.2-720 µM⁻¹. The method described is simple, sensitive, accurate and reproducible. The developed method was applied for quantification of withanolides level in cultured roots of *W. coagulans*. The root cultures synthesized withanolides of which withaferin A was the major compound. The productivity of withaferin A in the threeweek- old cultured roots was 11.65 µgg⁻¹. Withaferin A production showed consistent increase with root growth during the three weeks of culture period [7].

The structure of two new compounds, a withanolide and an ergostene derivative, are described and identified as 3β,14α,17β,20α_F-tetrahydroxy-1-oxo-20S,22R-witha-5,24-dienolide ergosta-5,25-diene-3β,24ξ-diol. These are considered interesting intermediates in the biogenetic sequence leading to the formation of withanolides, the ergostene diol being a very early precursor [8].

The structure of a new withanolide was elucidated as 3β,14α,20α_F,27-tetrahydroxy-1-oxo-20R,22R-witha-5,24-dienolide using chemical and spectroscopic methods. The structure was corroborated by comparative studies with known closely related withanolides. Sitosterol-β-D-glucoside was identified through chemical and spectroscopic means [9].

Inoculation of leaf sections of *Withania coagulans* with *Agrobacterium tumefaciens* strain C58C1 (pRiA4) induced transformed roots with the capacity to produce the most important bioactive compounds of *Withania* species, withanolide A and withaferin A. The hairy roots obtained showed two morphologies: callus-like roots (CR) with a high capacity to produce withanolides and typical hairy roots (HR) with faster growth capacity and lower withanolide accumulation. The *aux1* gene of pRiA4 was detected by PCR analyses in all roots showing callus-like morphology. However, this gene was only detected in 12.5% of the roots showing typical hairy root morphology. This fact suggests a significant role of *aux* genes in the morphology of transformed roots. 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. Some transformed root lines, such as HR112 and CR26, showed considerable potential to produce withanolides in a scaled up bioreactor system, especially the important pharmaceutical compound withanolide A. [10].

Pharmacological Properties

Pharmacological investigations with alcoholic extract, total alkaloids and aqueous extract of the fruits of *Withania coagulans* showed the presence of central nervous system depressant effect characterised by decreased spontaneous activity and potentiation of pentobarbitone hypnosis. However, the extracts showed no analgesic and anticonvulsant activity. In addition, alcoholic extract and total alkaloids showed a significant antiinflammatory effect in acute inflammation induced with egg albumin, subacute inflammation induced with formalin and granulation tissue formation by cotton pellet method [11].

Administration of an aqueous extract of fruits of *Withania coagulans* (1 g/kg; p.o.) to high fat diet induced hyperlipidemic rats for 7 weeks, significantly reduced elevated serum cholesterol, triglycerides and lipoprotein levels. This drug also showed hypolipidemic activity in triton induced hypercholesterolemia. The histopathological examination of liver tissues of treated hyperlipidemic rats showed comparatively lesser degenerative changes compared with hyperlipidemic controls. The hypolipidemic effect of *W. coagulans* fruits was found to be comparable to that of an Ayurvedic product containing *Commiphora mukul* [12].

A new withanolide, with a unique chemical structure similar to the aglycones of the cardiac glycosides, with mol. wt. 488.6, m. p. 260-261 degrees, isolated from the fruits of *Withania coagulans*, was screened for cardiovascular effects. At doses of 5 mg/kg body weight, the withanolide produced a moderate fall of blood pressure in dogs (34 +/- 2.1, mm Hg) which was blocked by atropine and not by mepyramine or propranolol. In rabbit Langendorff preparation and ECG studies, it produced myocardial depressant effects but in perfused frog heart it produced mild positive inotropic and chronotropic effects [13].

The effects of crude methanolic extract of *Withania coagulans* (Wc.cr) were studied for possible calcium channel blocking activities in rabbit's jejunum preparations at different doses. Potassium chloride (KCl) induced contractions were also inhibited by the Wc.cr extract suggesting calcium channel blocking activity. Calcium channel blocking activity was confirmed by the right shift in the dose response curves of the tissues in calcium free tyrode's solution. The dose response curves of the Wc.cr resemble the curves produced by verapamil, a standard calcium channel blocker. The results confirm the presence of calcium channel blocker(s) in the extract and the plant species could be a potential target for activity-guided isolation of the active moieties [14].

Administration of aqueous extract of fruits of *Withania coagulans* Dunal significantly lowered the blood sugar, serum cholesterol, serum LPO, and hepatic LPO levels at the highest concentration of 1 g/kg; po in streptozotocin induced diabetic rats. In normal rats as well the blood sugar levels were significantly decreased following treatment with the above drug. *Withania coagulans* also exhibited free radical scavenging activity in an in vitro system using DPPH [15].

The systematic evaluation and the role of minerals in glycemic potential of aqueous extract of *Withania coagulans* fruits in order to develop an effective and safe alternative treatment for diabetes mellitus was evaluated. Laser Induced Breakdown Spectroscopy was used for glycemic element detection. This study was based on the results of lowering in blood glucose levels of normal, sub, mild and severely diabetic rats assessed during fasting blood glucose, glucose tolerance test and post prandial glucose studies. The dose of 1000mg/kg was identified as the most effective dose, which reduces the Fasting Blood Glucose level maximum by 33.2% at 4h in normal rats during fasting blood glucose studies. Glucose tolerance test studies of normal, sub and mild diabetic rats showed the maximum reduction of 15.7, 28.9 and 37.8% at 3h respectively. Long-term study in case of severely diabetic rats showed reduction of 52.9 and 54.1% in Fasting Blood Glucose and Post Prandial Glucose levels respectively after 30 days of treatment. This study, besides confirmed hypoglycemic and antidiabetic activities of aqueous extract of *W. coagulans*, but also helped in identifying the role of trace minerals like Mg & Ca responsible for antidiabetic potential of this potent indigenous shrub [16].

The combined effect of *withania coagulans* dunal and *Trigonella foenum-graecum* Linn was studied in the clinical trial. Both the drugs *withania coagulans* dunal and *Trigonella foenum-graecum* Linn exhibited hypoglycemic activity with significant improvement in symptoms and signs of type 2 diabetes mellitus [17].

Different parts of this plant have been reported to possess a variety of biological activities [18]. A compound isolated from aqueous extract of fruit of *Withania coagulans* has been shown to exert hepatoprotective and anti-inflammatory activity [19]. Antifungal and antibacterial properties have also been demonstrated in the ethanolic extract of the whole plant and leaves, respectively [20].

Conclusion

Numerous studies have been conducted on different parts of *Withania coagulans* with isolation of different phytochemicals and evaluation of various pharmacological activities. In view of the nature of the shrub, more research work can be done to explore the unexploited potential of this shrub so that a drug with multifarious effects will be available in the future market to serve to the mankind.

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