

WITHANIA COAGULANS



- **ICNI/Botanical Name:** Withania coagulans
- **Common Name:** Paneer dodi, Panner, doda, Panir bed, Paneer dhodi
- **Arabic Name:** Hab kaknaj
- **Family:** Solanaceae
- **Bioactive Component:** Withanolides, Alkaloids, Flavonoids, Saponin, Tannins
- **Plant Part Use:** Flower

INTRODUCTION

Ayurveda, the science of life, aims to maintain health and treat various diseases. Plants play a pivotal role in Ayurvedic medicine for the treatment and prevention of diseases, as well as the promotion of a healthy lifestyle. Since ancient times, medicinal plants have been extensively documented in Ayurvedic classics and are still employed to successfully address numerous ailments. One such plant, *Withania coagulans* Dunal, commonly known as Indian Rennet or Paneer Dodi, has been recognized for its extensive medicinal properties. This shrub is particularly valued for its milk-coagulating property, attributed to its berries, which are widely used for this purpose in North-West India and neighboring regions. The milk-coagulating activity is due to the presence of a proteolytic enzyme that can be extracted by treating the berries with water and precipitated using ammonium sulfate or acetone.

Beyond its role in milk coagulation, *Withania coagulans* exhibits various pharmacological properties, including anti-inflammatory, antioxidant, hypoglycemic, hepatoprotective, and antimicrobial activities. It has been traditionally used to manage conditions such as diabetes, liver disorders, asthma, and cardiovascular diseases. The berries, rich in bioactive compounds like withanolides, alkaloids, and flavonoids, contribute to its medicinal potential. Recent studies have also highlighted its role in reducing cholesterol levels, enhancing immunity, and alleviating stress-related disorders. Furthermore, the plant is gaining attention in modern phytomedicine for its potential in developing nutraceuticals and functional foods. The dried berries are often used in herbal teas or formulations to promote overall well-being.

MORPHOLOGICAL DESCRIPTION

- **Growth Habit:** A perennial, erect shrub reaching a height of 1–1.5 meters, exhibiting branched and woody stems, particularly at the base.
- **Stem:** Cylindrical, pubescent, and woody at maturity, with a pale brown coloration.
- **Leaves:** Simple, alternate, and ovate to lanceolate in shape; measuring 5–10 cm in length. The leaf surface is pubescent, with prominent venation.
- **Inflorescence:** Axillary clusters comprising small, actinomorphic, greenish-yellow flowers.
- **Flowers:** Bisexual, pentamerous with a tubular calyx and corolla. The calyx is persistent and pubescent, while the corolla is yellowish-green. Stamens are epipetalous, with a superior ovary.
- **Fruit:** A globose berry, 5–12 mm in diameter, turning yellowish-orange upon ripening. The berry contains bioactive compounds and is the source of the milk-coagulating enzyme.
- **Seeds:** Small, reniform (kidney-shaped), and brown with a smooth surface.
- **Root:** Taproot system, woody, and fibrous, extending deep into the soil.

PHYTOCHEMICAL CONSTITUENT

1. **Withanolides:** Withanolides are the primary active constituents, belonging to the class of steroidal lactones. They are structurally characterized by an ergostane framework substituted with a lactone ring.
 - Coagulin A, Coagulin B, and Coagulin L: Steroidal lactones with milk-coagulating activity, anti-inflammatory, antimicrobial, and hepatoprotective properties.
 - Withaferin A: Exhibits potent anti-cancer, anti-inflammatory, and immunomodulatory effects.
 - These withanolides play a role in adaptogenic activities, helping to modulate stress responses, and contribute to the plant's neuroprotective potential.
2. **Alkaloids:** Alkaloids are nitrogenous compounds known for their bioactivity. In *Withania coagulans*, these contribute to neuroprotective, anxiolytic, and mild sedative effects.
 - Alkaloids in the plant may also assist in reducing stress and enhancing cognitive functions.
3. **Flavonoids:** Flavonoids, a group of polyphenolic compounds, are abundant in *Withania coagulans*.
 - They possess antioxidant properties by neutralizing reactive oxygen species (ROS) and preventing oxidative damage to cells.
 - These compounds also exhibit anti-inflammatory effects and help in maintaining cardiovascular health.
4. **Tannins:** Tannins are polyphenolic compounds with astringent properties.
 - They contribute to antimicrobial activity by disrupting microbial cell walls.
 - Tannins in the plant are also involved in wound healing and reducing inflammation, making them valuable in skin and mucosal applications.
5. **Saponins:** Saponins are amphiphilic glycosides that exhibit multiple bioactivities.
 - They play a critical role in reducing serum cholesterol levels through bile acid binding.
 - Saponins also enhance immune responses and exhibit anti-inflammatory effects.
6. **Sterols:** Steroidal compounds such as β -sitosterol are abundant in *Withania coagulans*.
 - These sterols have been reported to possess hypolipidemic, antioxidant, and anti-inflammatory activities.
 - They contribute to the maintenance of membrane stability and hormonal balance.
7. **Phenolic Compounds:** Phenolic acids and other phenolic compounds act as potent antioxidants.
 - They scavenge free radicals and inhibit lipid peroxidation, protecting against chronic degenerative diseases.
 - These compounds are also involved in anti-aging and protective effects against cardiovascular and neurological disorders.
8. **Proteolytic Enzymes:** The berries of *Withania coagulans* contain a proteolytic enzyme responsible for its milk-coagulating activity.
 - This enzyme is extracted using aqueous methods and precipitated with ammonium sulfate or acetone, yielding a brownish-white powder with high enzymatic activity.
9. **Essential Oils:** Minor amounts of essential oils contribute to the plant's aroma and exhibit mild antimicrobial and anti-inflammatory properties.

EXPERIMENTAL STUDIES DONE ON WITHANIA COAGULANS

1. Antihyperglycaemic Activity:

The drug *Withania coagulans* exhibited hypoglycaemic activity which is an effective and safe alternative treatment for diabetes. Hypoglycemic activity of *Withania coagulans* was exhibited in streptozotocin induced rats. Significant improvements in symptoms and signs were observed and euglycemia was attained in diabetes mellitus type 2. A withanolide, named coagulanolide isolated from *Withania coagulans* fruits has antihyperglycemic activity in rats. The median effective dose of isolated coagulanolide from fruits of *Withania coagulans* was determined about 25 mg/kg in streptozotocin-induced Vandana Gupta, International journal of ayurvedic & herbal medicine 3(5) Sep-Oct. 2013(1330-1336) 1333 diabetic rats, which is comparable to the standard antidiabetic drug metformin (Maurya et al 2008)11. The 4- week treatment with *Withania coagulans* dried fruit extract significantly reversed hyperglycemia in streptozotocin-induced diabetic rats that was comparable to glipizide.

2. Antihyperlipidemic activity:

The aqueous extract of *Withania coagulans* fruits in high fat diet induced hyperlipidemic rats, significantly reduced elevated serum cholesterol, triglycerides, lipoprotein and the LPO levels. The hypolipidemic effect of *Withania coagulans* fruits were found to be comparable with ayurvedic product containing *Commiphora mukul*. The coagulanolide isolated from fruits of *Withania coagulans* has antidyslipidemic effect. The hydroalcoholic extract of *Withania coagulans* dried fruits was effective and comparable to atorvastatin in controlling the high cholesterol diet-induced hyperlipidemia in rats.

3. Anti-inflammatory activity:

The alcoholic extract of *Withania coagulans* showed significant anti-inflammatory effect in acute inflammation induced with egg albumin. A withanolide from *Withania coagulans* showed significant anti-inflammatory effects in acute inflammation. The hydro alcoholic extract of *Withania coagulans* fruits showed significant anti-inflammatory activity in carragenin induced rat paw oedema model.

4. Antifungal and Antibacterial Effects:

The essential oil obtained by steam distillation of the petroleum ether extract of the fruits was active against *Micrococcus pyogens* var. *aureus* and *Vibrio cholerae*. The volatile oil from the fruits of *Withania coagulans* showed antibacterial activity against *Staphylococcus aureus* and *Vibrio cholerae*. The antifungal activity of the crude extract, 17 β -hydroxy withanolides k and withanolide F were tested against nine highly pathogenic fungi. These compounds also showed activity against gram positive bacteria.

5. Cardiovascular Effects:

A steroidal lactone, Withanolide isolated from the aqueous extract of fruits of *Withania coagulans*, has cardiovascular effect. A new withanolide, with a unique chemical structure similar to the aglycones of the cardiac glycoside, isolated from the fruits of *Withania coagulans*. This withanolide produced a moderate fall of blood pressure in dogs which has blocked by atropine and not mepyramine or propranolol. In rabbits Langendorff preparation and ECG studies, produced myocardial depressant effects but in perfused frogs hearts it caused mild positive inotropic and chronotropic effects.

6. Immunosuppressive Effects:

Withaferin A and withanolide E were reported to have specific immunosuppressive effects on human B and T lymphocytes as well as on mice thymocytes. A known withanolide, coagulin-H, was evaluated for its effect on various cellular functions related to immune responses including lymphocyte proliferation, interleukin-2 (IL-2) cytokine expression. These results were compared with prednisolone. Coagulin-H was found to have a powerful inhibitory effect on lymphocyte proliferation and the Th-1 cytokine production. The inhibition of the phytohaemagglutinin (PHA) activated T-cell proliferation by coagulin-H.

ANALYTICAL METHOD USE FOR WITHANIA COAGULANS:

High-Performance Liquid Chromatography (HPLC):

- Application: Separation, identification, and quantification of withanolides, flavonoids, and alkaloids in plant extracts.

Gas Chromatography-Mass Spectrometry (GC-MS):

- Application: Identification and quantification of essential oils and volatile compounds in Withania coagulans.

Thin-Layer Chromatography (TLC):

- Application: Preliminary qualitative screening and fingerprinting of withanolides, flavonoids, and other phytochemicals in plant extracts.

Ultra-High-Performance Liquid Chromatography (UHPLC):

- Application: High-resolution quantification of complex phytochemicals, including withanolides, saponins, and alkaloids.

Fourier Transform Infrared Spectroscopy (FTIR):

- Application: Structural characterization and identification of functional groups, such as flavonoids and tannins, in the plant extract.

Nuclear Magnetic Resonance (NMR) Spectroscopy:

- Application: Structural elucidation of complex compounds, especially withaferin A and other withanolides in the extract.

High-Performance Thin-Layer Chromatography (HPTLC):

- Application: Quantitative and qualitative analysis of withanolides and other bioactive constituents in plant extracts.

UV-Visible Spectroscopy:

- Application: Quantitative estimation of flavonoids, phenolic compounds, and withanolides in plant extracts.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

- Application: Elemental analysis of trace minerals such as calcium, magnesium, and iron in Withania coagulans.

Fluorescence Spectroscopy

- Application: Detection of naturally fluorescent compounds like withanolides in the plant extract.

HEAVY METALS TESTING METHODS

- ICP-MS (Inductively Coupled Plasma Mass Spectrometry) – Sensitive for detecting trace metals like lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg).
- AAS (Atomic Absorption Spectroscopy) – Detects metals such as lead, cadmium, and zinc.
- GFAAS (Graphite Furnace Atomic Absorption Spectroscopy) – More sensitive than AAS, used for low-concentration metals like lead and cadmium.
- XRF (X-ray Fluorescence) – Non-destructive, rapid screening for heavy metals in plant materials.

PESTICIDE TESTING METHODS

- GC-MS (Gas Chromatography-Mass Spectrometry) – Detects and quantifies volatile pesticides.
- LC-MS (Liquid Chromatography-Mass Spectrometry) – Sensitive for non-volatile, polar pesticides.
- QuEChERS – Quick, simple extraction method for pesticide residues.
- HPLC (High-Performance Liquid Chromatography) – Analyzes pesticide residues, particularly non-volatile ones.
- ELISA (Enzyme-Linked Immunosorbent Assay) – Rapid screening for specific pesticide residues.

APPLICATION

- **Pharmaceutical Industry:** Used in the development of natural medicines and supplements, especially for its anti-inflammatory and immune-boosting properties.
- **Nutraceutical Industry:** Incorporated into dietary supplements and functional foods for enhancing vitality, reducing stress, and promoting overall health.
- **Cosmetics Industry:** Added to skincare products for its anti-aging, anti-inflammatory, and skin-soothing effects.
- **Food and Beverage:** Used as a functional ingredient in health drinks, energy bars, and snacks to improve energy levels and manage stress.
- **Agricultural Industry:** Explored for its potential as a natural pesticide and plant growth enhancer in organic farming.

FORMULATION

- **Powder:** Often used in capsule or tablet form for easy oral consumption.
- **Capsules/Tablets:** Standardized dosages of the active compounds like withanolides for targeted therapeutic effects.
- **Tinctures/Extracts:** Liquid forms that allow for rapid absorption of the active compounds.
- **Ointments/Creams:** Topical formulations used for local application, especially for its anti-inflammatory and skin-healing properties.
- **Syrups:** Used as a liquid form for children or individuals who have difficulty swallowing tablets or capsules.
- **Teas:** Herbal infusions made from the dried plant parts, often consumed for their adaptogenic and calming effects.

SAFETY AND QUALITY:

- **Heavy Metal Testing:**
 - Ensures that levels of harmful metals (lead, arsenic, cadmium, mercury) are within permissible limits.
 - Methods used: ICP-MS, AAS, GFAAS, XRF.
- **Pesticide Residue Testing:**
 - Detects and quantifies harmful pesticide residues in plant extracts.
 - Methods used: GC-MS, LC-MS, QuEChERS, HPLC, ELISA.
- **Regulatory Compliance:**
 - Adheres to safety standards set by organizations like WHO, FDA, and EMA.
 - Ensures products meet maximum residue limits (MRLs) for both heavy metals and pesticides.
- **Consumer Health Protection:**
 - Ensures that Withania coagulans extracts are safe for use in nutraceuticals, functional foods, and pharmaceuticals.
- **Quality Assurance:**
 - Prevents contamination by harmful substances, maintaining the purity and potency of the extract.

