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A COMPREHENSIVE REVIEW ON THE PHYTOCHEMISTRY AND THERAPEUTIC APPLICATIONS OF WITHANIA COAGULANS (PANEER DODA)

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ABSTRACT
Withania coagulans, commonly known as Paneer Doda or Indian Rennet, is a medicinal plant of the Solanaceae family widely used in traditional medicine for its therapeutic properties. This plant is rich in phytochemicals such as withanolides, flavonoids, saponins, and alkaloids, which contribute to its pharmacological activities, including antidiabetic, anti-inflammatory, antioxidant, hepatoprotective, and immunosuppressive effects. It has applications in treating diabetes, hyperlipidemia, cancer, wound healing, and inflammatory conditions. This review highlights the botanical characteristics, phytochemical constituents, and pharmacological activities of Withania coagulans, emphasizing its potential in modern medicine and pharmaceutical formulations.

INTRODUCTION

The science of life is known as Ayurveda. In Ayurveda, plants are the primary source of medicine for treating and preventing diseases and maintaining a healthy lifestyle [1] . Many plants in the natural system of medicine must have a wide range of biological activities. Withania is a minor genus in the Solanaceae family of shrubs (which has about 2000–3000 species divided around 90 families) [2]. Withania species, from the East Mediterranean to South Asia, may be found worldwide. Withania coagulans and Withania somnifera are two species found in Pakistan [2] . Withania is a flowering plant of the Solanaceae family with roughly 23 species endemic to North Africa, the Middle East and the Canary Islands [3] . In various sections of Afghanistan, Pakistan, India, and Nepal, W.coagulans is prevalent. It may be found in Punjab, Rajasthan, Simla, Kumaun and Garhwal in India [4].



Fig. 1: Freshplantof W. Coagulans

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Fig. 2: Dry Fruits of W. Coagulans

The seeds are used to cure liver problems, piles, ophthalmia and as an emmenagogue and diuretic. They are used as blood purifier and used in nervous exhaustion, insomnia, impotence, dyspepsia, flatulent and intestinal infections[5]. Milcoagulation can be achieved by the berries of the shrub. Milk coagulation is only caused by aspartic protease, as determined by mass spectrometry 2 analysis of purified protease and enzyme assays carried out in the presence of protease inhibitors . [6,7] Chewing on twigs can help with tooth cleaning and inhaling the plant's smoke can ease toothache pain. W. coagulans flowers are used to cure diabetes; locally, leaves and roots are utilised to cure a range of diseases.[8] Anti-mutagenic, antioxidant, antidiabetic, anti-microbial, anti-fungal, anti-bacterial, anti-hyperglycemic and anti-cancer effects have been found for W. coagulans fruit extracts.[9,10] The main active chemical constituents of plant are alkaloids, carbohydrates, phenolic compounds, steroids, tannins, amino acids, organic acids, withacoagin, withaferin, essential oils, flavonoids, vitamin, triterpenes etc.[11,12] Withania coagulans as a coagulant for tofu production by assessing its impact on quality and sensory properties.[10]

Taxonomical Classification: [13]

- Kingdom: Plantae
- Division: Magnoliophyta
- Order: Solanales
- Family: Solanaceae
- Genus: Withania

Botanical description of Withania Coagulans

- Botanical Name : Withania coagulans Dunal
- Family : Solanaceae
- Subfamily : Solanoideae
- Tribe : Physaleae
- Subtribe : Withaninae
- Sanskrita Name : Rishyagandha1&2
- Hindi Name : Punir, Punir bandh, Akri, Binputakah, Paneer doda
- English Name : Indian Cheese maker, Indian Rennet, Vegetable Rennet
- Trade Name : Paneer dodi, Panner, doda, Panir bed, Paneer dhodi.

Vernacular Name of Withania coagulans : [15]

- Bengal - Asvagandha
- Bombay – Kaknaj
- Gwalior - Asgandha
- Panjab - Khamjaria, Khamjira,
- Panir Sindhi - Punirjafota, Punirband
- Persian - Kaknajehindi,
- Punirbad Arabic - Javzulmizaja,
- Kaknajehindi Canares - Asvagand
- Telgu - Panneru-gadda

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- Urdu - Hab kaknaj

Geographical Description[36]:] It is found in the Eastern Mediterranean region and extends northern Africa to Southwest Asia. Across India, it grows in drier regions such as Punjab, Gujarat, Rajasthan, Shimla, Kumaon, and Garhwal.

Morphological Discription[16]

A rigid grey- tomentose undershrub 0.3-0.9 m. high, branches terete, clothed with dense grey or yellowish white tomentum. Leaves : 2.5-5.7 by 1-2.2 cm., lanceolate-oblong, obtuse, entire, clothed with a persistent not easily detachable greyish tomentum, of a uniform colour on both sides, thick, more or less rugose, base acute, running down into an often obscure petiole; petiole 6 mm. long but often indistinct. Flowers : Dioecious, in axillary clusters; pedicles 0-6mm. long, deflexed, slender. Calyx 6 mm. long, campanulate, clothed with fine stellate grey tomentum; teeth triangular, 2.5 mm. long. Corolla 8 mm. long, stellately mealy outside, devided about 1/3 the way down; lobes ovate-oblong, subacute. Male flowers: Stamens about level with the top of the corolla- tube; fillaments 2 mm. long, glabrous; antherrs 3-4 mm. long. Ovary ovoid, without style or stigma. Female flowers: Stamens scarcely reaching ½ way up the corolla-tube; filaments about 0.85 mm. long; anthers smaller than in the male flowers, sterile. Ovary ovoid, glabrous; style glabrous; stigma mushroom- shaped, 2-lamellate. Fruits : Berry 6-8 mm. diam., globose,smooth, closely girt by the enlarged membranous calyx which is scurfypubescent outside. Seeds 2.5-3 mm. diam., somewhat ear-shaped, glabrous. Seeds : 2.5-3mm diam., dark brown, ear-shaped, glabrous; Flowering period: from January to April and berries ripen during January to May. The natural regeneration is from seed.

Microscopic Characters[17]

The pedicel's outer layer, the epidermis, consists of rectangular to slightly elongated cells with a smooth cuticle. Below the epidermis lies the cortex, composed of parenchymatous cells. These cells are thin-walled and store various nutrients. The vascular bundles are arranged in a ring and include both xylem and phloem. The xylem vessels are thick-walled and lignified, while the phloem consists of thin-walled sieve elements and companion cells. At the center of the pedicel is the pith, made up of large parenchymatous cells shown in Figure 3 (i). The calyx's outermost layer consists of polygonal cells with a thick cuticle. Trichomes (hair-like structures) are often present and can be unicellular or multicellular. The internal tissue of the calyx, the mesophyll, is divided into palisade and spongy parenchyma shown in Figure 3 (iii). The palisade cells are elongated and arranged tightly, while the spongy parenchyma cells are more loosely packed. The vascular bundles are scattered throughout the mesophyll and contain xylem and phloem tissues. The xylem vessels are thick-walled, and the phloem elements are thin-walled. The outer layer of the seed is the testa, which consists of several layers of cells. The outermost layer is composed of thick-walled sclerenchymatous cells that provide protection. The endosperm is a nutritive tissue inside the seed, composed of thin-walled parenchymatous cells. It is rich in starch and oils. The embryo is the young plant inside the seed, consisting of embryonic root (radicle), shoot (plumule), and cotyledons (seed leaves). The cells of the embryo are meristematic, meaning they have the capacity to divide and differentiate shown in Figure 3 (iv). The outermost layer of the pericarp is the epicarp, made up of polygonal cells with a thick cuticle. It may also have trichomes. The middle layer of the pericarp, the mesocarp, consists of parenchymatous cell sthat are thin-walled and storenutrients. Vascular bundles arepresent within this layer. The innermost layer of the pericarp, theendocarp, is composed of thick-walled sclerenchymatous cells shown in Figure 3 (ii) that provide protection to the seed shown in Table 1.

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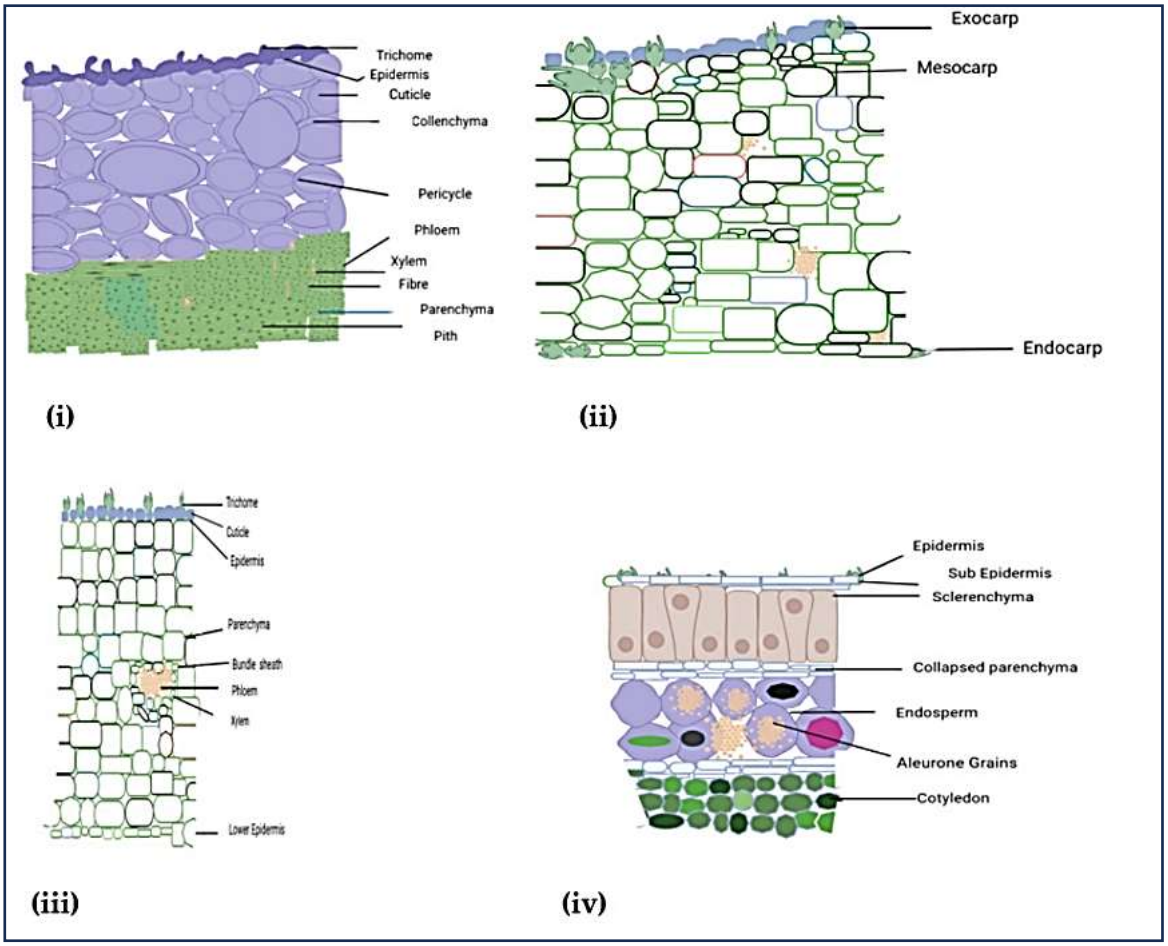


Figure 3: (i) Transverse section of Pedicle. (ii) Transverse section of Pericarp (Fruit wall). (iii) Transverse section of calayx. (iv) Transverse section of Seed.

Part Used	Microscopic Characters	References
Pedicel	A single layer of tabular cells with many branching and unbranched trichomes covers the epidermis in the transverse section of the pedicel. This is followed by a cortex consisting of five to ten layers of collenchymatous cells. The pericycle has parenchymatous cells interspersed with pericyclic threads. The pericycle has parenchymatous cells interspersed with pericyclic threads. The xylem in the central region is bordered by a narrow band of phloem, which is enclosed by a ring of intra-xylary phloem. Parenchymatous cells encircle the hollow pith in the core and a few lignified fibres with robust walls that point in the direction of the phloem are visible	19,20
Calyx	The calyx's bottom epidermis is made up of a single layer of thin-walled cells, while the upper epidermis has a few branching and unicellular covering trichomes. The mesophyll is made up of spongy parenchyma that is crossed by several tiny veins and wrapped in bundle sheath cells made of thin-walled parenchymatous cells	19,20
Pericarp	The transverse section reveals a large zone of parenchymatous cells with significant cellulose thickening may be seen in the mesocarp of the pericarp (fruit wall). The exocarp, which is composed of a single layer of cells, is also visible. An isolated layer of cells makes up the endocarp.	19,20

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CHEMICAL CONSTITUENT

Plant Part	Chemical Constituent	Specific Chemicals	Role
Fruits	**Withanolides**	- Coagulin F (27-hydroxy-14,20-epoxy-1-oxo-(22R)-witha-3,5,24-trienolide) -Coagulin G (17beta,27-dihydroxy-14,20-epoxy-1-oxo-(22R)-witha-2,5,24-trienolide) -Withacoagulin (20(β)-hydroxy-1-oxo-(22R)-witha-2,5,24-trienolide)	These steroidal lactones exhibit anti-inflammatory, anti-cancer, and adaptogenic properties. They help regulate stress responses and enhance immunity.
	Esterases	- Various esterase enzymes	Facilitate milk coagulation, essential for cheese-making processes.
	Free Amino Acids	-Proline -Tyrosine - Glycine	Important for protein synthesis and metabolic functions.
	Fatty Acids	- Linoleic acid (C18H32O2) - Oleic acid (C18H34O2)	Essential for maintaining cell membrane integrity and providing energy; have anti-inflammatory effects.
	Alkaloids	- Withanine	Exhibits analgesic and anti-inflammatory effects; used in traditional medicine.
Leaves	**Phenolic Compounds**	- Flavonoids (e.g., quercetin)	Antioxidants that reduce oxidative stress and inflammation.
	Tannins	- Various tannins	Possess astringent properties; inhibit microbial growth and may aid in digestive health.
Seeds	**Saponins**	- Various saponins	Lower cholesterol levels and exhibit immune-boosting properties; may induce apoptosis in cancer cells.
	Proteins	- Enzymes and structural proteins	Essential for growth, repair, and maintenance of body tissues.
Whole Plant	**Carbohydrates**	- Polysaccharides (e.g., cellulose)	Provide energy; contribute to dietary fiber promoting digestive health.
	Vitamins	- Vitamin C (ascorbic acid)	Important for immune function and antioxidant protection against cellular damage.
	Minerals	- Calcium (Ca) - Iron (Fe)	Essential for various bodily functions including bone health (Calcium) and oxygen transport (Iron).

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FORMALATION TABLE [21,22]

Ingredients	F1	F2	F3	F4	F5	F6
Azadirachta Indica	60	60	60	60	60	60
Withania Coagulans	60	60	60	60	60	60
Picrorhiza Kurroa	60	60	60	60	60	60
Pterocarpus Marsupium	60	60	60	60	60	60
Lactose / Mannitol	235	225	215	205	195	185
Pregelatinised Starch	0	10	20	30	40	50
Talc	20	20	20	20	20	20
Sodium Benzoate	5	5	5	5	5	5
Total (Mg)	500	500	500	500	500	500

Table 1: Formulation of Anti-Diabetic Tablet

Ingredients	F1	F2	F3	F4	F5	F6
Plant Extract	300	300	300	300	300	300
Carbopol	20	30	40	-	-	-
Ethyl cellulose	-	-	-	20	30	40
Microcrystalline cellulose	40	40	40	40	40	40
Dibasic calcium phosphate	30	20	10	30	20	10
PEG 4000	10	10	10	10	10	10
Methyl Paraben	0.1	0.1	0.1	0.1	0.1	0.1
Weight per tablet (Mg)	400	400	400	400	400	400

Table 2: Formulation of Anti-Diabetic Tablet

PHARMACOLOGICAL USES

1. **Antihyperglycemic activities:** Diabetes can be effectively managed with Withania coagulans since it exhibits hypoglycemic properties, which is a safe and effective alternative treatment option [36]. An aqueous extract of Withania coagulans berries (1gm/kg; P.O.) significantly lowers vital signs, serum glucose, and lipid peroxide. It promotes the correct amount of insulin secretion. Withania coagulans improve glucose utilization and carbohydrate metabolism, thereby depleting blood glucose. Hyperglycaemia is reduced due to it [37]. Treatment with coagulanolide alongside four known withanolides 1-3 and 5 isolated from four fruits of W. coagulans, shows significant inhibition on the postprandial rise in hyperglycemia post sucrose load in normoglycemic rats also as streptozotocin-induced diabetic rats [38]. Hence, Withania coagulans is considered an antihyperglycemic and antidyslipidemic agent [39]. Withania coagulans are commonly used in the management of type-2 diabetes mellitus [40].

2. **Antihyperlipidemic activities:** In high-fat diet-induced hyperlipidemic rats, extracts of Withania coagulans fruits significantly reduced levels of elevated serum cholesterol, triglyceride, lipoprotein, and therefore LPO levels. Ayurvedic products containing Commiphora Mukul are analogous to the hypolipidemic effect of fruits of Withania coagulans[41].

3. **Anti-inflammatory activities**: Withania coagulans alcoholic extract has a significant anti-inflammatory effect in acute inflammation induced by egg albumin [42,43]. Withanolides from Withania coagulans are effective in reducing inflammation in acute inflammation [44]. The hydroalcoholic extract of Withania coagulans berries exhibits significant anti-inflammatory activity in a carrageenin induced rat paw edema model [45].

4. **Antifungal and antibacterial effects:** Micrococcus pyrogens var. aureus vibrio cholera is inhibited by the volatile oil produced by steam distillation of the petroleum ether extract of the fruits.Two new withanolides 14,15β epoxywithanolides I [(20S, 22R) 17ϭ,20ϭ-dihydroxy-14ϭ,15ϭ-epoxy-1-oxo-with a-3,5,24 trienolides] and 17β-hydroxy withanolides K[(20S,22R)14α,17ϭ,20ϭ-trihydroxy-1-oxo-witha-2,5,24-trien-olide],isolated from ethanolic extract of whole plant Withania coagulans found to move against a variety of potentially pathogenic fungi [45].

5. **Cardiovascular Effects:** Withanolide, a steroidal lactone derived from the aqueous extract of Paneer Dodi fruits, has a cardiovascular effect[46,47]. This withanolide replacement, isolated from the fruits of Withania coagulans, has a similar chemical structure to the aglycones of cardiac glycosides [48]. Withanolide produced a moderate fall in blood pressure in dogs (34 +/- 2.1, mm Hg), which was blocked by atropine and not by mepyramine or propranolol at doses of 5 mg/kg body weight.

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It produces a myocardial depressant effect in rabbit Langendorff or preparation of ECG studies but produces mild positive ionotropic and chronotropic effects in perfused dogs' hearts [49].

6. **Immunosuppressive Effects:** Withanolide E and Ashwagandha possess specific immunosuppressive properties on human B and T lymphocytes as well as mice thymocytes[50]. Withanolides, such as coagulin-H, acts on several cellular functions involved in immune responses, including lymphocyte proliferation and interleukin-2 (IL-2) cytokine expression [51]. It is comparable to the effects of prednisolone. The coagulin-H possesses a strong inhibitory effect on lymphocyte proliferation, and therefore, cytokine production by Th-1 cells. Coagulin-H inhibits phytohaemagglutinin (PHA)-induced T-cell motivation[52].

7. **Antimutagenic and anticarcinogenic effects:** The genotoxicity of herbal drugs is determined by their phytoconstituents. Withania coagulans contain withanolides, which have antitumor properties, as well as flavonoids that exert antimutagenic and anticancer effects [53]. The antimutagenic properties of Withania coagulans remain unknown. Withania coagulans extract cyclophosphamide-induces micronucleus formation in mice bone marrow cells. The results show that a single i.p. injection of Withania coagulans fruits extracts at doses of 500,1000,1500 mg/kg weight before 24 hours effectively reduces micronucleus development in bone marrow cells of mice in a dose department manner as compared to the cyclophosphamide group [55]. The plant is ethnobotanically reported in cancer treatment [54].

8. **Hepatoprotective Activity:** In adult albino rats, 3-hydroxy-2, 3-dihydro-withanolide F derived from a fruit of Withania coagulans has been shown to have hepatoprotective effects against CCl4-induced hepatotoxicity. A weight-based comparison revealed that it is more active than hydrocortisone and exhibits a marked protective effect [57].

9. **Antitumor properties:** Withaferin (3β-hydroxy-2, 3-dihydro-withanolide F) exhibits antitumor effects. Withania coagulans aqueous extract has anticyto toxic properties. The extract shows the remarkable inhibitory activity of DMSO-induced cytotoxicity and decreases in TNF-α production in chicken Lymphocytes [56].

10. **Wound healing activity:** A study suggests the wound healing activity of Withania coagulans in streptozotocin-induced diabetic rats. In both topical and oral forms, hydro-alcoholic fractions of the methanolic extract (standardized by withaferin A) of Withania coagulans increase the rate of wound contraction. Withaferin-A enhances collagen, protein, DNA, SOD, and CAT levels, and decreases the levels of hexosamine [59]. Hydroalcoholic fractions of Withania coagulans methanolic extract sort of 10 %w/w ointment were applied topically and orally at a dose of 500mg/kg weight to streptozotocin-induced diabetic rats [58]. In models of open and incised wounds, the aqueous-methanolic phase of the methanolic extract of Withania coagulans has shown significant wound healing activity. It accelerates collagen, mucopolysaccharides, DNA, and protein synthesis [60].

11. **Anti-arteriosclerosis Activity:** The aqueous extract of Withania coagulans also exhibits radical scavenging activity in an in vitro system using DPPH and. Aqueous extract of fruits of Withania coagulans has antioxidant potential against several diseases like aging, atherosclerosis, etc[61].

12. **Anthelmintic activity:** In the steam distillation of the petroleum ether extract of Withania coagulans fruits, essential oil appears to possess anthelmintic properties. The upper parts of Withania coagulans have anthelmintic activity in ruminants [62].

13. **Diuretic activity:** Withania coagulans fruits exhibit diuretic potential in an aqueous extract when studied in rats. When compared with other Withania species, Withania coagulans have more polar Withanolides[64]. Using furosemide as a standard, the diuretic activity of the aqueous extract of paneer Dodi roots can be studied in the Lipschitz test model. The results showed significant increases in urine volume by 71.02% and 79.12% at 500mg/kg and 75mg/kg weight dosages, respectively, when compared to regulate. Urinary electrolyte excretion where increases in both the dosage compared to regulate. The diuretic effect is due to the presence of the active principles of polar nature, of which withanolides are the chemical protagonists. Research supports the use of Withania coagulans as a diuretic agent in folk medicine [63]. Withania coagulans extract has hypotensive, respiratory stimulant, and muscle relaxing properties [38].

Toxicology: When the body is exposed to medications or poisons, nephrotoxicity is a common adverse outcome. It leads to uremia due to a failure of the kidneys to filter excess urea, nitrogenous substances, and creatinine. There is no specific treatment for acute renal failure; only supportive care is required to restore renal function. This condition can only be avoided by avoiding nephrotoxic substances and maintaining adequate hydration and perfusion.

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EVALUATION PARAMETERS

1. Pre-Formulation Studies[34,35]

Flow Properties: Evaluate the physical properties of the capsule's powder to ensure proper filling and handling during manufacturing.

Bulk Density & Tapped Density: Measure the volume occupied by the powder under standard and compressed conditions.

Carr's Index: Indicates compressibility, calculated from bulk and tapped density values.

Hausner's Ratio: Measures flow efficiency, derived from bulk and tapped density.

Angle of Repose: Determines the powder's flowability; lower angles indicate better flow properties.

2. Organoleptic Properties[33,35]

Appearance: Capsules should be visually inspected for uniformity in size, shape, and color.

Odor and Taste: Ensures that the capsule masks any unpleasant characteristics of the active pharmaceutical ingredients (APIs) to improve patient compliance.

3. Weight Uniformity[34,35]

- Capsules from a batch are weighed individually, and the average weight is calculated. Variations must fall within pharmacopoeial limits to ensure consistent dosing.

4. Disintegration Time[34]

- Measures the time taken for capsules to break apart in simulated gastrointestinal fluids. This test ensures the release of the drug within an acceptable time frame for therapeutic efficacy.
- Performed using disintegration testing equipment.

5. Dissolution Profile[33,34]

- Evaluates how quickly the active ingredients are released from the capsule into a dissolution medium (simulating body fluids).

Method: Carried out using a dissolution apparatus at 37°C with specified media and stir rates.

Sampling: Periodic sampling followed by analysis using spectrophotometry or HPLC

6. Moisture Content[33,34]

- Ensures that the powder inside the capsules is dry to prevent clumping, microbial growth, or degradation of active ingredients.

Method: Measured using techniques like Karl Fischer titration or loss on drying.

7. Phytochemical Screening[34,35]

- Confirms the presence of key active compounds such as:
- Alkaloids: Tested using Mayer's or Wagner's test.
- Flavonoids: Confirmed using HPTLC fingerprinting or colorimetric assays.
- Glycosides, Tannins, and Saponins: Identified using standard phytochemical tests.

8. Bioactivity Studies[34]

- In Vitro Antidiabetic Assays:
- **Alpha-Amylase Inhibition:** Tests the ability of the capsule contents to inhibit the enzyme alpha-amylase, simulating the reduction of glucose absorption in the gut.
- **Procedure:** Incubation of the capsule's powder with starch solution and alpha-amylase, followed by measuring the reaction using DNSA and UV spectrophotometry.
- **Alpha-Glucosidase Inhibition:** Similar test for assessing the inhibition of carbohydrate breakdown enzymes.

9. Stability Testing[34,35]

- Conducted under accelerated conditions (temperature, humidity) to check the integrity of the capsule over time.

parameters: Physical appearance, weight, dissolution, and potency are evaluated periodically.

10. Microbial Testing[33,34]

- Assesses contamination levels to ensure the product is safe for consumption.
- Total Viable Count (TVC): Measures bacterial and fungal contamination.
- Specific Pathogens: Checks for harmful bacteria like E. coli, Salmonella, etc.

11. High-Performance Thin Layer Chromatography (HPTLC)[34]

- Used to fingerprint the active compounds in the formulation (e.g., flavonoids, saponins) to ensure consistency across batches.

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12. FTIR Analysis[34]

- Identifies functional groups in the active ingredients, ensuring the presence of expected compounds (e.g., phenolic groups in antidiabetic herbal extracts).

CONCLUSION

Withania coagulans is a promising medicinal plant with diverse pharmacological applications rooted in traditional medicine. Its phytochemical diversity and therapeutic efficacy make it an essential candidate for developing herbal remedies and pharmaceutical formulations. Further research is needed to standardize its extracts, validate its clinical efficacy, and ensure its safe incorporation into modern healthcare practices. By bridging the gap between traditional knowledge and scientific validation, Withania coagulans could play a vital role in addressing contemporary health challenges.

Conflict Of Interest

The Authors declared that they do not have any conflict of interest.

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