

**PHARMACOGNOSTICAL AND PHARMACOLOGICAL EVALUATION OF WITHANIA  
COAGULANS - AN IMPORTANT ETHNOMEDICINAL PLANT**<sup>1</sup>Harish Vishnoi\*, <sup>2</sup>Ayush Kumar Garg, <sup>3</sup>Gulab Chand Meena, <sup>4</sup>Chandan Singh and <sup>5</sup>L. N. Sharma<sup>1,2,3</sup>,PG Scholar, <sup>4,5</sup>Associate Professor

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**ABSTRACT**

In ancient system of medicine, many plants have been reported to cure various health problems and diseases. *Withania coagulans* (*W. coagulans*) Dual commonly known as 'Indian cheese maker' or 'vegetable rennet' belongs to family Solanaceae and is one of the important medicinal plants. *W. coagulans*Dunal is a gray-whitish small shrub distributed in east of the Mediterranean region extending to South Asia. It is a common medicinal plant in many parts of Pakistan and India. In Ayurvedic system of medicine, *W. coagulans* is widely used in diabetic cases. *W. coagulans* is also used to treat nervous exhaustion, disability, insomnia, wasting diseases, failure to thrive in children and impotence. The fruits of the plant are reported to be sedative, emetic, alterative and diuretic. Further, they are used for liver complaints, asthma and biliousness. The active compounds, in particular, withanolides isolated from the plant are considered to have antimicrobial, anti-inflammatory, antitumor, hepatoprotective, anti-hyperglycemic, cardiovascular, immuno-suppressive, free radical scavenging and central nervous system depressant activities. This review gives a bird's eye view mainly on the biological activities of some of the *W. coagulans* compounds isolated and phytochemistry as well as pharmacognostic properties of the plant extracts.

**KEYWORDS:** Indian cheese maker, Anti-inflammatory, Withanolides, Cyclophosphamide.**INTRODUCTION**

Ayurveda is the science of life. The basic aim of Ayurveda is maintenance of health and treatment of various diseases. The plants are the key source of medicine in Ayurveda for treatment and prevention of diseases and maintenance of healthy life. The plants are used in medicine since antiquity. Much of the medicinal plants are documented in the Ancient Ayurvedic classics and these plants are still used successfully to treat different ailments. One of these plants which are used to treat various disease is *Withania coagulans* Dunal. The shrub is important for the property of coagulating milk, possessed by its berries; they are used for this purpose in North-West India and adjoining country. The milk-coagulating activity is due to the presence of an enzyme, which can easily be isolated by extracting the berries with water and precipitating the enzyme either by ammonium sulphate or by adding two volumes of acetone. The precipitate is dried at low temperature and the enzyme is obtained as a brownish white powder.

In ancient system of medicine, many plants have been reported to be useful to cure various health problems and diseases. Herbalism refers to traditional or folk medicine practice based on the use of plants and plant extracts.

Charaka Samhita and Sushruta Samhita give extensive description on various medicinal plants. Medicinal plants play an important role in the development of new herbal drugs. Presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient.<sup>[1]</sup>

*Withaniacoagulans* (*W. coagulans*) Dual belongs to family Solanaceae and is one of the important medicinal plants. There are two species of *Withania*, viz., *W. somnifera* and *W. coagulans*, which are distributed in east of the Mediterranean region extending to South Asia. It is found in many parts of Pakistan and India.<sup>[2]</sup> *W. coagulans*Dunal, is commonly known as 'Indian cheese maker' or 'vegetable rennet' because fruits and leaves of this plant are used as a coagulant. The milk coagulating property of the fruits is attributed to the pulp and husk berries which contain an enzyme called Withanin, having milk-coagulating activity. One ounce of the fruits of *W. coagulans* when mixed with 1 quart of boiling water makes a decoction, one table spoonful of which is capable to coagulate a gallon of milk in just an hour. In Pakistan, the berries of *W. coagulans* are commonly used to clot milk which is called, 'paneer'. The milk of buffalo or sheep is boiled to 100o F and treated with crushed berries of the plant, tied in a cloth.

This causes the milk to curdle within 30-40 minutes. The fruits of the plant are sweet and are reported to be sedative, emetic, alterative and diuretic. In some parts of Pak-Indian sub-continent, the berries are used as a blood purifier. The twigs are often chewed for cleaning of teeth and the smoke of the plant is inhaled for relief in toothache.<sup>[1]</sup> In addition, *W. coagulans* is used to treat nervous exhaustion, disability, insomnia, wasting diseases, failure to thrive in children, impotence. Its fruits are used for liver complaints, asthma and biliousness. Flowers of the plant are useful in the treatment of diabetes.<sup>[2]</sup> This plant has been reported to possess antimicrobial, anti-inflammatory, antitumor, hepatoprotective, anti-hyperglycemic, cardiovascular, immuno-suppressive, free radical scavenging and central nervous system depressant activities.<sup>[3]</sup>

#### Taxonomical Classification

Botanical Name : *Withania coagulans* Dunal  
 Family : Solanaceae  
 Subfamily : Solanoideae  
 Tribe : Physaleae  
 Subtribe : Withaninae  
 SanskritaName : Rishyagandha<sup>[4,5]</sup>  
 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.

#### Botanical description

This shrub is, common in, East India, Nepal and Afghanistan,. In India it occurs in Punjab, Rajasthan, Simla, Kumaun and Garhwal.

#### Morphological characteristics

*Withania* is a small or medium undershrub, 30-150cm height, erect, grayish, branching perennial, with strong disagreeable odour like horse's urine. *Withania somnifera* is a small shrub of 1m to 2m height across. Almost the complete plant is covered and surrounded with very short, small, fine, branched hairs and silver-grey in colour. The stems of *Withania* are brownish dark colour and erect, sometimes leaves are absent or less on lower part of stem. The leaves are in an alternate manner (opposite on flowering shoots), simple, possessing margins are slightly waved, narrowed into the 5–20mm long petioles, normally broadly ovate or oblong, 29–80mm long and 21–50mm broad. It is generally referred to as stellatomentose, grayish, under shrub of 30-150cm high with long woody tuberous roots. Flowers are generally small, greenish, axillary, monoceous or bisexual and solitary or in few-flowered cymes. Seeds are normally many, discoid, reniform and yellow. The number of chromosome is  $2n = 48$  (Schonbeck 1972; Hepper 1991; Mozaffarian 2003). The corolla is 5-lobed, constrictly campanulate, 5–8mm long and light yellow to yellow-green in colour. *Ashwagandha* fruit is usually a round hairless berry, 5–8mm across, orange-red to red in

ripped condition and is enveloped by the enlarged calyx. Most of the seeds are very pale brown, 2.5mm across, sometimes kidney-shaped and squeezed with a rough surface and netted surface. In *Withania* flowering time is generally from October to June, whereas the fruiting time is usually from October to July. *Withania somnifera* can be identified by the red fruit enclosed by the brownish, papery, turgid calyx. Collectors have mentioned it as a bad-smelling bush with generally strong-smelling roots and have also described that the leaves have a strong smell of green tomatoes (Mirjalili et al 2009).

#### Flowers

Flowers sessile, axillary clusters, 1cm long, it blossoms nearly throughout the year. Corolla greenish or yellow or white-yellowish, 5mm long, lobes lanceolate, acute and thin, calyx is visibly expanded around the fruit. The flowers appear in a brightly yellow or greenish and it carries small berries which are orange-red in color. Fruits are orange-red, carrying numerous seeds. Florescence appears and falls in spring season (Davis & Kuttan 2000). The stamens or filament base is appeared to the ovary and a groove between every stamen helps to allow the nectar to flow upward from the nectary at the ovary base (Kothari et al 2003). There are large numbers of trichomes present in stamen which also secrete some amount of nectar. Trichomes are also present on outer surface of calyx and corolla. Nectaries on different floral parts attract insects. There are 3200- 4000 pollen/anther and 16,250- 20,000 pollen/flower. Each ovary contains 26-32 ovules. Thus, the pollen ovule ratio is 625:1 indicating facultative xenogamy (Cruden 1977).

#### Roots

The roots of *Ashwagandha* are fleshy when dry, they are straight, cylindrical, tapering down, gradually unbranched of about 10-17.5cm long and 6-12mm diameter in thick. The main roots are brownish outer and creamy interior and bear fiber similar secondary roots having acrid taste and bitter (Anonymous 1982). Roots are stout, fleshy and whitish brown in colour. Leaves simple, petiolate, elliptic-ovate to broadly ovate, entire, exstipulate, cunate or oblique, glabrous, up to 10cm long, those in the floral region are smaller and opposite. Single layered epidermis present in young root with a 4-5 layers of cells of parenchymatous cortex while the endodermis being present as casparian stripes. Outer most layer of cortex consists of cork cambium. The endodermis is always persists even after the secondary growth has taken.



**Fig 1: Fruit Sample of Withania coagulans Collected from herbal garden of DSRRAU, Jodhpur.**



**Fig 2: Sample of Fruit powder of Withania coagulans.**

#### Phytochemical Properties

Chemical constituents: The berries contain the milk-coagulating enzyme, two esterases, free amino acids, fatty oil, an essential oil and alkaloids. The amino acids present are proline, hydroxyproline, valine, tyrosine, aspartic acid, glycine asparagin, cysteine and glutamic acid. Fourteen alkaloidal fractions have been isolated from the alcoholic extract of the fruits. The seeds on petroleum ether extraction, give a yellow fatty oil and unsaponifiable matter. Fatty acid composition are oleic, linoleic, palmitic, stearic and arachidonic acid. The unsaponifiable matter consist of triacontan, three sterols

**Table 1: Physico-chemical observations (% W/w).<sup>[2]</sup>**

Test	Observation 1	Observation 2	Observation 3
Total ash value	19.9	19.2	19.25
Acid insoluble ash	13.9	12.55	13.7
Water soluble extractive value	29.7	29.0	27.1
Alcohol soluble extractive value	6.8	6.5	6.0

**Table 2: Phyto-chemical screening of 50% extract of fruits.<sup>[3]</sup>**

Sr. No	Tests	50% ethanolic extract
1.	<b>Alkaloids</b>	
	a. Dragendorff's test	+ve
	b. Wagner's test	+ve
	c. Mayer's test	+ve
2.	<b>Carbohydrate</b>	
	a. Molisch test	+ve
	b. Fehling's Test	+ve

including dihydrostigmasterol and  $\beta$ -sitosterol. The defatted meal from the seeds contains free sugar consisting of D-galactose and D-arabinose and traces of Vandana Gupta, International journal of ayurvedic& herbal medicine 3(5) Sep-Oct. 2013(1330-1336) 1332 maltose. The leaves contain four steroidal lactones called Withanolides, viz Withaferin-A, 5, 20 $\alpha$ (R)- dihydroxy-6 $\alpha$ ,7 $\alpha$ -epoxy-1-oxo-(5 $\alpha$ )-with a-2,24-dienolide and two minor withanolides, of which one is probably 5 $\alpha$ , 17 $\alpha$ -dihydroxy-1-oxo-6 $\alpha$ , 7 $\alpha$ -epoxy-22R-witha-2,24-dienolide (the so called withanone).<sup>[7]</sup> Withaferin A is the most important of the withanolides isolated so far. It has good antibiotic and anti tumor activities. Withaferin A in concentration of 10 $\mu$ ml. inhibited the growth of various gram-positive bacteria, acid fast bacilli, aerobic bacilli and pathogenic fungi. It was active against Micrococcus pyogenes var. aureus and Bacillus subtilis glucose-6-phosphate-dehydrogenase.

Withaferin A has marked tumorinhibitory property when studied in vitro against cells derived from human carcinoma of nasopharynx (KB). It also act as mitotic poison arresting the division of cultured human larynx carcinoma cells at metaphase. The studies also showed growth inhibitory and radio sensitizing effects in vivo on mouse Ehrlich ascites carcinoma. It also caused mitotic arrest in embryonal chicken fibroblast cells. Withaferin A exhibits positively potent anti-arthritic and anti-inflammatory effect. It suppress effectively arthritic syndrome without any toxic effect. In contrast to hydrocortisone treated animals which shows weight loss, the animal treated with withaferin A showed weight gain in arthritic syndrome.<sup>[4]</sup> Withaferin A inhibits angiogenesis (Mohan et al., 2002).<sup>[8]</sup> It has been reported that some of the withanolide affects events in the cholinergic signal transduction cascade of the cortical and the basal forebrain, indicating their promising role in the treatment of Alzheimer's disease (Kuboyama et al., 2005).<sup>[9]</sup>

	<b>c. Benedict's Test</b>	<b>+ve</b>
<b>3.</b>	<b>Protein</b>	
	<b>a. Biuret Test</b>	<b>+ve</b>
	<b>b. Xanthoprotein Test</b>	<b>+ve</b>
	<b>c. Lead Acetate Test</b>	<b>+ve</b>
<b>4.</b>	<b>Amino acids</b>	
	<b>a. Ninhydrin test</b>	<b>+ve</b>
<b>5.</b>	<b>Glycoside</b>	
	<b>a. Baljet Test</b>	<b>-ve</b>
	<b>b. Keller Kiliani Test</b>	<b>-ve</b>
<b>6.</b>	<b>Saponin test</b>	
	<b>a. Foam test</b>	<b>-ve</b>
<b>7.</b>	<b>Tannins and phenol compounds</b>	<b>+ve</b>
<b>8.</b>	<b>Essential oils</b>	
	<b>a. spot test</b>	<b>+ve</b>

Table 3: Fluorescence characteristics.<sup>[4]</sup>

Treatment with reagent	Fluorescence under long UV(366nm)
Powder as such	Brown
Powder treated with nitrocellulose in amyl acetate	Dark brown
Powder treated with 1N NaOH in methanol	Reddish green
Powder treated with 1N NaOH in methanol, dried and mounted in nitrocellulose in amyl acetate	Dark green
Powder treated with 1N Hcl	Light green

#### Experimental studies done on *Withania coagulans* Antihyperglycaemic and antihyperlipidemic effects

Aqueous and chloroform extracts prepared from *W. coagulans* fruits showed pharmacological effects on blood glucose, lipid profile and body weight in type 2 diabetic rats causing significant decrease in blood levels of glucose, triglyceride, total cholesterol, LDL and VLDL and an increase in HDL when administered alone or in combination once daily p.o., at a dose of 1 g/kg body weight for 14 days in different groups of normoglycemic and hyperglycemic rats. In addition, aqueous extract of fruits of the plant at the same dose significantly lowered serum LPO and hepatic LPO levels in streptozotocin induced diabetic rats as well as db/db mice. The dose of 1g/ kg body weight was identified as the most effective dose of the plant extract to reduce Fasting Blood Glucose level maximum by 33.2 % at 4h in normal rats. On the other hand, 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 confirming hypoglycemic and antidiabetic activities of aqueous extract of *W. coagulans*. Treatment with coagulanolide 4 along with four known withanolides 1-3 and 5 isolated from *W. coagulans* fruits, 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 caused significant fall in fasting blood glucose profile and improved the glucose tolerance of db/db mice. Further, compound 5 showed antidiabetic activity in db/db mice. The median effective dose of the compound 5 was determined to be around 25 mg/kg body weight in streptozotocin-induced diabetic rats, which is comparable to the standard

antidiabetic drug metformin. This explains the traditional use of *W. coagulans* as antihyperglycemic cum antidiabetic agent by the traditional medical practitioners.<sup>[10]</sup>

#### Wound healing activity

The hydroalcoholic fraction of the methanolic extract of *W. coagulans* was administered in the form of 10% w/w ointment topically and at a dose of 500 mg/kg body weight orally to streptozotocin-induced diabetic rats. The hydroalcoholic fraction in both the forms, i.e., topical (10% w/w ointment) and oral (500 mg/kg body weight, p.o.) showed a significant increase in the rate of wound contraction compared to diabetic controls.<sup>[11]</sup>

#### Cardiovascular effects

A new withanolide, with a unique chemical structure similar to the aglycones of the cardiac glycosides (mol. wt. 488.6, m. p. 260-261 degrees), was isolated from the fruits of *W. coagulans*, and was screened for cardiovascular effects. At a dose of 5 mg/kg body weight, the withanolide produced a moderate fall of blood pressure in dogs ( $34 \pm 2.1$ , 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's heart, it produced mild positive inotropic and chronotropic effects.<sup>[12]</sup>

#### Hepatoprotective effects

Protective effect of 3β-hydroxy-2,3-dihydrowithanolide F isolated from *W. coagulans* was tested against CCl<sub>4</sub>-induced hepatotoxicity, and the compound was found to possess marked protective effect. A comparison of the

protective properties showed that it is more active than hydrocortisone on a weight basis.<sup>[13]</sup>

#### Immuno-suppressive effects

Six new withanolides, withacoagulins A-F (1-6, resp.), together with ten known withanolides, 7-16, were isolated from the aerial parts of *W. coagulans*. These compounds, including the crude extracts of this herb, exhibited strong inhibitory activities on the T- and B-cell proliferation.<sup>[14]</sup>

#### Diuretic effect

The diuretic activity of the aqueous extract of fruits of *W. coagulans* was studied by *in vivo* Lipschitz test model with slight modifications using furosemide as a standard. The results indicated significant increase in the urine volume by 79.12 % and 71.02 % at 500 mg/kg and 750 mg/kg body weight doses respectively compared to controls. Urinary electrolyte excretions were increased at both the doses compared to controls.<sup>[15]</sup>

#### Antimutagenic and anticarcinogenic effects

The genotoxic nature of any herbal drug is determined on the basis of presence of phytoconstituents. *W. coagulans* contains withanolides, which are reported for antitumor activity, and flavonoids which have been shown to possess antimutagenic and anticarcinogenic activities. The underlying mechanism behind anti-mutagenic action of *W. coagulans* is still unknown. The antimutagenic activity of *W. coagulans* fruit extracts was investigated on cyclophosphamide induced micronucleus formation in mouse bone marrow cells. The results confirmed that a single i.p administration of *W. coagulans* fruit extract at the doses of 500, 1000 and 1500 mg/kg body weight prior to 24 h significantly prevented the micronucleus formation in dose dependent manner in bone marrow cells of mice as compared to cyclophosphamide group.<sup>[16]</sup>

#### Anti-inflammatory activity

Different extracts prepared from fruits of *W. coagulans* have been shown to possess anti inflammatory properties.<sup>[17]</sup>

#### Antifungal activity

Two new withanolides, 14,15 $\beta$ -epoxywithanolide I [(20*S*,22*R*) 17 $\beta$ ,20 $\beta$ -dihydroxy-14 $\beta$ ,15 $\beta$ -epoxy-1-oxo-witha-3,5,24-trienolide] and 17 $\beta$ -hydroxywithanolide K (20*S*,22*R*) 14 $\alpha$ ,17 $\beta$ ,20 $\beta$ -trihydroxy-1-oxo-witha-2,5,24-trien-olide], isolated from ethanolic extract of the whole plant of *W. coagulans*, have been found to be active against a number of potentially pathogenic fungi.<sup>[18]</sup>

#### Antibacterial and antihelmintic activities

The volatile oil obtained from alcoholic extract of fruits of *W. coagulans* has antibacterial activity against *S. aureus* and *Vibrio cholera*, and it is also found to have antihelmintic activity.<sup>[19,20]</sup>

#### Neuro Protective Activities

WCE showed neuroprotective activity by significant decrease in MDA level and increase in the SOD, CAT and GPx activity in pretreated groups as compared to I/R groups ( $p < 0.001$ ). The number of intact neurons was increased while the number of TUNEL positive neurons in CA1 hippocampal region in pretreated groups were decreased as compared to I/R group ( $p < 0.001$ ).<sup>[21]</sup>

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#### CONCLUSION

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. *W. coagulans* has been found to contain a vast array of biologically active compounds, which are chemically diverse and have got an enormous therapeutic potential. Very little work has been done on the biological activity and plausible medicinal applications of the compounds and hence extensive investigation is needed to exploit their therapeutic utility to combat diseases. Although crude extracts from various parts of *W. coagulans* particularly fruits have medicinal applications, modern drugs can be developed only after extensive investigation of their bioactivity, mechanism of action, pharmacotherapeutics, toxicity and after proper standardization and clinical trials.

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