

Chapter

Indian Cheese Revolution: *Withania coagulans* in Dairy Industry

Mayur Ram, Bimal S. Desai and Sumankumar S. Jha

Abstract

Commonly called as Indian Cheese Maker, Paneer dodi, Paneer phool and Vegetable rennet, *Withania coagulans* belongs to family Solanaceae and widely used in Indian System of Medicines due to its anti-diabetic, anti-microbial and immune modulator properties. The specific epithet coagulans reveals its coagulating properties and hence used in Punjab and parts of Northern India in cheese and paneer industries. The plant is rich in enzyme Withanin which is responsible for the coagulating properties. Many herbal prescriptions like Liv 52, (Liver Health Support Supplement) which is an Ayurvedic poly herbal formulation consists of extracts of both *Withania somnifera* and *Withania coagulans*. Commercial cultivation of this plant is in its initial phase in parts of Punjab, Haryana and also in neighboring countries as it has wide distribution extending up to South Asia. Plant is also rich in Withanolide contents and can be a future prospect for South Gujarat region, as coagulating agent for dairy industries and rennet enzyme production. Seeds are available in local markets of Surat and Navsari, routinely used for controlling diabetes. The chapter aims at the possibilities of cultivating this plant in South Gujarat conditions in India, since the other species *Withania somnifera* is also available and has naturalized in AES Zone III of South Gujarat.

Keywords: rennet, Withanin, withanolides, poly herbal, anti-diabetic

1. Introduction

South Asia is home to many rich Traditional Systems of Medicine (TSM) including Ayurvedic, Unani, Siddha and Tibetan systems, which have been helpful in sustaining healthy life of tens of millions of people for centuries. India possesses abundant reserves of medicinal and aromatic plants (MAPs) across a vast territory characterized by diverse environmental conditions. The strategic geographical location, unique geomorphology, the existence of ancient flora from geological eras, and the harmonious interplay between biotic and non-biotic factors have collectively contributed to India being recognized as a region of remarkable plant diversity and endemism. These factors directly influence the wide range of medicinal and aromatic plant species found within the country. Himalayan sage scholars of TSM have said “*Nanaushadhi Bhootam Jagat Kinchit*” i.e., there is no plant in the world, which does not have

medicinal properties.’ These venerable scholars possessed extensive knowledge about the medicinal properties of numerous plant species, with estimates suggesting their understanding extended to hundreds of plants. It is not an overstatement to assert that the utilization of plants for enhancing human health dates back to the very origins of human existence [1].

The genus *Withania*, belonging to the Solanaceae family, holds a prominent position in the Indian Ayurvedic system of medicine due to its remarkable pharmaceutical and nutraceutical properties. Out of the 26 identified species within the genus *Withania*, only two species, namely *Withania somnifera* (L.) Dunal and *Withania coagulans* (Stocks) Dunal, have garnered significant economic importance [2]. *Withania* has been widely utilized in traditional folk medicine for treating a wide range of ailments. Additionally, one particular species, *W. coagulans*, is commonly referred to as the Indian cheese maker or Vegetable rennet. This species has been traditionally employed in various regions of India for the preparation of rennet ferment used in cheese production from vegetables. Different parts of this plant have been reported to possess a variety of biological activities [3].

Since decades ago, fruits of this plant were used widely in the production of traditional cheese from raw cow’s milk. It is believed that the milk coagulation ability corresponds to the presence of an enzyme in the berries of the plant as it is related to the pulp and husk of the berry. Cottage and Cheddar cheese can be made using fruit extracts of *W. coagulans* as a good alternative to animal rennet, though the cheese produced with the described extract has a notable bitter flavor that can be reduced by increasing the ripening time [4].

1.1 Indian cheese: a wealth of health benefits

Indian cheese, particularly varieties like paneer has been a staple in Indian cuisine for centuries. Beyond its culinary appeal, Indian cheese offers a plethora of health benefits, making it a popular choice not just for its taste, but for its nutritional value as well.

- *Rich protein source:* One of the standout features of Indian cheese is its high-quality protein content. Protein is the building block of the body, essential for repairing tissues, building muscles, and maintaining a strong immune system. For vegetarians, in particular, Indian cheese provides a crucial source of this vital nutrient.
- *Calcium and bone health:* Indian cheese is a rich source of calcium, a mineral critical for bone and teeth health. Adequate calcium intake is essential throughout life to prevent osteoporosis and ensure the structural integrity of bones. Along with calcium, it also provides phosphorus, further enhancing bone health.
- *Weight management and satiety:* Despite being calorie-dense, Indian cheese can aid in weight management. Its high protein and fat content provide a sense of fullness, curbing hunger and reducing overall calorie intake. This makes it a valuable addition to diets aimed at weight control.
- *Vitamins and minerals:* Indian cheese contains an array of vitamins and minerals, including B-vitamins such as B12 and minerals like zinc. Vitamin B12 is crucial

for nerve health and red blood cell production, while zinc supports the immune system and promotes wound healing.

- *Digestive health*: Some Indian cheeses, through their fermentation process, contain probiotics. These beneficial bacteria are excellent for gut health, aiding digestion, and promoting a balanced intestinal environment. A healthy gut is linked to improved overall well-being.
- *Versatility in diets*: For lacto-vegetarians, Indian cheese provides an excellent source of protein, ensuring that they meet their nutritional needs without relying on meat-based protein sources. Its versatility in Indian cuisine means it can be included in various dishes, making it easier to incorporate into different diets.
- *Heart and brain health*: Indian cheese contains healthy fats, including omega-3 fatty acids, which are beneficial for heart health and cognitive function. These good fats contribute to overall cardiovascular health and support brain function.

2. Distribution

W. coagulans is distributed in the East of the Mediterranean region extending to South Asia i.e., Iran, Afghanistan, Pakistan (Sindh and Baluchistan), Nepal and India, up to 1700 m. In India, it is found in (North-West India) Himachal Pradesh, Punjab, Uttarakhand and Rajasthan. In Rajasthan it is sporadically distributed in Barmer, Jaisalmer and Jodhpur districts of Western Rajasthan desert and it is not common, categorized as “vulnerable species” by Pandey *et al.* [5]. It’s important to note that *Withania coagulans* has also been introduced and cultivated in other parts of the world, including certain regions of the United States, Australia, and Europe. However, its natural distribution is primarily centered in South Asia. Overall, *Withania coagulans* is adapted to grow in arid and semi-arid environments and is commonly found in the regions mentioned above.

3. Cultivation

W. coagulans, commonly known as Indian Rennet or “Paneer Doda,” is primarily cultivated in specific regions where it is native or adapted to grow. The main cultivation areas for *Withania coagulans* include:

- *India*: *W. coagulans* is extensively cultivated in various parts of India. It is particularly grown in the arid and semi-arid regions of Rajasthan, Gujarat, Punjab, and Haryana. These regions provide the suitable climate and soil conditions for its growth.
- *Pakistan*: *W. coagulans* is also cultivated in Pakistan, primarily in areas with similar climatic conditions to those found in India. It is grown in regions like the Thar Desert and other arid parts of the country.
- *Afghanistan*: *W. coagulans* is known to be cultivated in certain regions of Afghanistan, particularly in areas with dry and arid conditions.

Outside of its native range, *W. coagulans* has also been introduced and cultivated in some other countries, such as the United States, Australia, and parts of Europe. However, the cultivation in these areas might be limited and less widespread compared to its native regions. It's worth noting that successful cultivation of *W. coagulans* requires specific growing conditions, including well-drained soil, warm temperatures, and a semi-arid climate. The plant is well-adapted to arid and dry regions, and its cultivation is typically focused in areas that provide the necessary environmental factors for its growth.

W. coagulans cultivation is primarily concentrated in India. India is one of the main countries where *Withania coagulans* is cultivated due to its suitability for the plant's growth requirements and its traditional use in Ayurvedic medicine. In India, the cultivation of *W. coagulans* is particularly prominent in regions with arid and semi-arid climates. States like Rajasthan, Gujarat, Punjab, and Haryana are known for their significant cultivation of *W. coagulans*. These areas provide the necessary conditions,



Figure 1.
Morphology of Withania coagulans.

Kingdom	Plantea, Plants
Subkingdom	Tracheobionta, vascular plants
Super division	Spermatophyte, seeds plants
Division	Angiosperma
Class	Dicotyledons
Order	Tubiflorae
Family	Solanaceae
Genus	Withania
Species	Coagulance

Deshmukh et al. [6].

Table 1.
Taxonomical classification.

Sanskrit name	Rishyagandha
Hindi name	Punir, Punir bandh, Akri, Binputakah, Paneer doda
English name	Indian cheese maker, Indian organic compound, Vegetable organic compound
Trade name	Paneer dodi, panner, doda, panir bed, Paneer dhodi

Table 2.
Synonyms.

including dry and warm climates, well-drained soil, and limited rainfall, which are favorable for the growth of *W. coagulans*.

Farmers in these regions have been cultivating *W. coagulans* for generations and have developed knowledge and expertise in its cultivation practices. The plant is typically grown as a cash crop by farmers who recognize its value in traditional medicine and Paneer Industries demand in the market. Overall, India is a major contributor to the cultivation and production of *W. coagulans*, meeting both domestic demand and supplying it to various other countries for its medicinal and therapeutic applications (**Figure 1**, **Tables 1** and **2**).

4. Botanical description

W. coagulans is a sturdy gray undershrub, reaching a height of 60–120 cm. Its flowers from November to April, while the berries ripen from January to May. The natural regeneration is from the seed. The flowers dioceous, in auxiliary clusters; pedicels 0.6 mm long, Deflexed, slender. The calyx of *W. coagulans* is campanulate, measuring 6 mm in length and covered with a delicate stellate gray tomentum. Its teeth are triangular, approximately 2.5 mm long. The corolla of *W. coagulans* measures 8 mm in length and is covered with a stellate mealy texture on the outside. It is divided approximately one-third of the way down, and its lobes are ovate-oblong with a sub-acute shape. Male flowers stamens about level with the top of the corolla-tube; filament 2 mm long, glabrous; anthers 3–4 mm long. Ovary ovoid, without style or stigma. Female flowers stamens scarcely reaching 1/2 way up the corolla-tube; filaments about 0.85 mm long; anther smaller than in the male flowers, sterile. Ovary

ovoid, style glabrous; stigma mushroom-shaped, 2-lamellate. Berry 6–8 mm globose, smooth, closely girt by the enlarged membranous calyx, which is scurfy -pubescent outside. Seeds 2.5–3.0 mm diameter, somewhat ear shaped, glabrous [7, 8].

5. Phytochemistry

Different chemical components such as alkaloids, hormones, tannins, saponins, carbohydrates, protein, amino acids and organic acid are shown by aqueous and methanolic extracts from the *W. coagulans*. Seeds have 17.8% free sugars, maltose, fatty oil, D-galactose & D-arabinose. The fruit contains a milk-coagulating enzyme, two esterases, free amino acids, essential oil and fatty oil and alkaloids, triacontaine hydrocarbon, dihydrostigmasterol sterol. Proline, hydroxyproline, valine, tyrosine, aspartic acid, glycine, asparagine, cysteine, and glutamic acid are amino acids.

Alkaloidal fractions were isolated from the fruit's alcoholic extract. Leaves contain four withanolides-called steroidal lactones, Withaferin-A, 5, 20 α (R)-dihydroxy-6 α ,7 α epoxy-1- oxo-(5 α)-with a-2,24-dienolide and two minor withanolide, of which one is probably 5 α , 17 α -dihydroxy-1- oxo-6, 7 α -epoxy-22R-witha-2,24- dienolide [9].

The term “withanolide” is a structural designation coined by combining “withan” from the genus *Withania* with “olide,” which is the chemical term for a lactone. Major bioactive phytoconstituents isolated from *W. coagulans* are lactone steroids called withanolides. A new group of steroidal lactones called withanolides has been recently isolated from different species of the solanaceae family, mainly *Withania Somnifera*. The whole plant of *W. coagulans* contains various withanolides, including coagulin F, coagulanolide, withacoagulin, and coagulin G. Additionally, the roots, leaves, and fruits of this plant have been reported to contain four, two, and two withanolides respectively, which serve as important biogenetic precursors of withanolides, withanolides shows antitumorous, anti-inflammatory, antibacterial, immunosuppressive activities [10]. A new withanolide, with a unique chemical structure similar to the aglycones of the cardiac glycosides, was isolated from the fruits of *W. coagulans*, and was screened for cardiovascular effects. The diverse therapeutic applications of withanolides found in *W. coagulans* have garnered significant interest in the scientific community. A new Withanolide isolated from *W. coagulans* have been found to be active against several potentially pathogenic fungi. Withanolides have been reported to possess both immunostimulating and immunosuppressive effects in different studies. Withanolides have been reported to have effect on haemopoietic system and bone marrow. Glycowithanolides have been found to have effects on CNS [11].

Withanolides, which constitute a major component of *W. coagulans*, can be classified chemically into the following groups [6].

- a. Withanolide glycoside
- b. Withaphysalin
- c. Physalin
- d. Nicadrenons or ring D aromatic withanolides
- e. Acnistins

f. Perculactones

g. Withajardines

6. Medicinal use of different parts of *Withania coagulans*

Different parts of *W. coagulans*, including the leaves, roots, seeds, and fruit, have been traditionally used for their medicinal properties. Here are some of the medicinal uses associated with each part [12].

- **Leaves:** The leaves of *W. coagulans* have been used in traditional medicine for their anti-inflammatory and analgesic properties. They are often used topically to alleviate pain, reduce inflammation, and promote wound healing. The leaves may also be used in poultices or as a paste for skin conditions and joint ailments.
- **Roots:** The roots of *W. coagulans* are highly valued in traditional medicine systems like Ayurveda. They are known for their adaptogenic properties, meaning they help the body cope with stress and promote overall well-being. The root extracts are used to support the immune system, improve vitality, and enhance mental clarity. *W. coagulans* root extracts are also used for their potential anti-inflammatory, anti-diabetic, and anti-cancer effects.
- **Seeds:** *W. coagulans* seeds are used for their medicinal properties, particularly in the management of diabetes. The seeds contain compounds that have blood sugar-lowering effects and may help regulate blood glucose levels. They are used in traditional medicine as a natural remedy for diabetes and to support healthy blood sugar control [13].
- **Fruit:** The fruit of *W. coagulans* is traditionally used for its digestive and laxative properties. It is believed to aid in digestion, alleviate constipation, and promote regular bowel movements. The fruit is also used as a natural coagulant or rennet substitute in cheese making, as mentioned earlier.

7. Pharmacological properties

The plant's berries are used to coagulate milk. It has always played a significant role in the Ayurvedic, Unani, and traditional Indian medical systems. Numerous studies have shown that the isolated withanolides from *W. coagulans* have interesting biological properties. The plant's sweet fruits are also said to have sedative, emetic, alterative, and diuretic properties. They are helpful for liver complaints that are persistent. They have occasionally been employed as blood purifiers. Additionally, they are used to treat other intestinal infections, flatulent colic, and dyspepsia. These are used to treat asthma, biliary conditions, and stuttering Maurya [11].

- **Anti-inflammatory:** *W. coagulans* exhibits anti-inflammatory properties, which can help reduce inflammation in the body. These effects are attributed to the presence of bioactive compounds like withanolides, flavonoids, and other phytochemicals.

- **Antioxidant:** *W. coagulans* possesses antioxidant activity, meaning it can help neutralize harmful free radicals and reduce oxidative stress. The plant contains withanolides, flavonoids, and other compounds that contribute to its antioxidant potential.
- **Immunomodulatory:** *W. coagulans* has immunomodulatory properties, meaning it can modulate or regulate the immune system. It may help enhance immune function, support immune responses, and exert immunomodulatory effects. These properties are attributed to various bioactive compounds present in the plant.
- **Anti-diabetic:** *W. coagulans* has been studied for its potential anti-diabetic properties. There is evidence suggesting that *Withania* contribute to the regulation of blood sugar levels, enhancement of insulin sensitivity, and mitigation of complications associated with diabetes. The seeds of *W. coagulans*, in particular, have shown promising effects in managing diabetes [14].
- **Anti-cancer:** *W. coagulans* exhibits potential anti-cancer properties. Research suggests that its bioactive compounds, such as withanolides, may have cytotoxic effects on cancer cells, inhibit tumor growth, and induce apoptosis (programmed cell death) in certain cancer types. However, further studies are needed to fully understand its mechanisms and potential applications in cancer treatment.
- **Adaptogenic:** *W. coagulans* is considered an adaptogen, a category of natural substances that help the body adapt to stress and promote overall well-being. It may help reduce stress, improve resilience, and support the body's ability to cope with physical and mental challenges [15].

8. Significance of *W. coagulans* in dairy industries

The primary step in cheese production is milk coagulation. For thousands of years, coagulating enzymes, which are preparations of proteins that break down other proteins, have been utilized in the process of making cheese. Interestingly, this practice of using enzymes in cheesemaking is believed to be one of the earliest known applications of enzymes. The first evidence of cheesemaking can be traced back to cave paintings dating around 5000 BC [16]. Over time, scientists have developed alternatives to animal rennet, such as rennet substitutes produced by microorganisms and genetically engineered microorganisms. However, there is a growing interest in vegetable coagulants, which are milk-clotting enzymes extracted from plants. Cheeses made with vegetable coagulant can be found mainly in Mediterranean, West African, and southern European countries. Spain and Portugal have the largest variety and production of cheeses. *Withania coagulans*, commonly known as Indian Rennet or “Paneer Doda,” has traditional uses in the dairy industry. It is used as a natural coagulant or rennet substitute in the process of cheese and paneer (Indian cottage cheese) production.

In traditional cheese making, rennet is commonly used as a coagulant to separate milk into curds and whey. However, *W. coagulans* offers an alternative source of coagulant enzymes that can achieve similar effects. The ripe fruit of *W. coagulans* contains enzymes known as milk-clotting proteases or chymosin-like proteases. These

enzymes have the ability to coagulate milk proteins, resulting in the formation of curds [17]. This property makes *W. coagulans* a natural substitute for animal-based rennet in cheese making, particularly in vegetarian or vegan cheese production. In the dairy industry, *W. coagulans* is sometimes used to produce a variety of cheeses, including traditional Indian paneer. Paneer is made by coagulating milk with an acid or coagulant, and *W. coagulans* can be used as a natural coagulant in this process. The concentrated aspartic protease can be used instead of rennet for cheese preparation especially “cheddar”. Cheese can be prepared by lyophilised extract has highest content of fat, total solids, crude protein, and ash which result in highest cheese yield as compared to pure chymosin and fungi rennet [18].

It's important to note that while *W. coagulans* can be used as a rennet substitute in cheese making, the specific techniques and processes may vary depending on the cheese recipe and desired outcome. Industrial cheese production often utilizes standardized rennet sources, but *W. coagulans* may find applications in small-scale or artisanal cheese production, especially in regions where it is traditionally used [19].

Overall, *W. coagulans* offers a natural alternative for coagulating milk in the dairy industry, particularly for those seeking vegetarian or vegan options in cheese making.

8.1 Some key points highlighting the importance of *W. Coagulans* in the dairy industry

Importance in the dairy industry due to its potential applications as a natural coagulant or rennet substitute in cheese and paneer production.

- *Vegetarian and vegan cheese production:* *W. coagulans* offers an alternative source of coagulant enzymes for cheese making, particularly for those seeking vegetarian or vegan options. Traditionally, animal-based rennet derived from the stomach lining of young calves is used as a coagulant in cheese production.: *W. coagulans* provides a natural, plant-based substitute, allowing for the production of vegetarian and vegan cheeses.
- *Cultural significance:* *W. coagulans* has historical and cultural significance in regions where it is traditionally used. In India, it is often employed in the production of paneer, a popular cheese-like dairy product widely consumed in Indian cuisine. By preserving traditional cheese-making practices and utilizing *W. coagulans*, the dairy industry can maintain cultural authenticity.
- *Market demand:* The demand for vegetarian and vegan cheese products has been growing globally, driven by consumer preferences for plant-based alternatives. *W. coagulans* enables dairy manufacturers to cater to this expanding market segment by offering cheese products that align with vegetarian and vegan dietary choices.
- *Sustainability and animal welfare:* *W. coagulans* contributes to sustainable and ethical practices in the dairy industry. By reducing reliance on animal-based rennet, the industry can minimize its impact on animal welfare and promote more sustainable production methods.
- *Traditional medicine integration:* *W. coagulans* is not only relevant to the dairy industry but also holds significance in traditional medicine systems like

Ayurveda. The integration of traditional medicinal knowledge and practices into the dairy industry allows for a holistic approach to product development, incorporating both nutritional and therapeutic aspects.

While it has potential advantages, industrial-scale cheese production often utilizes standardized rennet sources. However, *W. coagulans* finds value in small-scale or artisanal cheese production and meets the demand for vegetarian and vegan options.

9. The significance of *W. coagulans* in food industries

9.1 Nutritional profile

Both macro and micronutrients are abundant in *W. coagulans*. The *W. coagulans* mineral constitution. In addition to being an excellent source of carbohydrates, it also contains a small amount of hydration, protein, fat, and fiber. Research studies have revealed that *W. coagulans* exhibits comparatively higher levels of essential minerals like magnesium (greater than *Alhagi maurorum*, *Berberis lyceum*, and *Tecomella undulate*), calcium (greater than *Dature alba*, *A. maurorum*, *Chenopodium album*, *B. lyceum*, *T. undulate*), potassium (greater than *B. lyceum* and *T. undulate*), and iron (greater than *D. alba*, *B. lyceum*, and *T. undulate*) [20]. The composition of roots, leaves, and fruit varies in terms of their nutrient content. Roots predominantly consist of carbohydrates (75.71%), followed by fiber (5.76%), lipids (5.5%), protein (2.95%), and ash (1.92%). In comparison, leaves contain carbohydrates (65.31%) as the major component, accompanied by fiber (11.76%), lipids (5%), protein (2.95%), and ash (3.26%). As for fruit, it consists of carbohydrates (60.14%) as the primary constituent, along with protein (4.65%), lipids (5%), and ash (4.21%). Hameed and Hussain [21]. The berries of *W. coagulans* include two esterases, free amino acids, milk coagulating enzymes, and essential oil. The primary amino acids found in the plant are proline, tyrosine, valine, hydroxyproline, glycine, cysteine, asparagine, glutamic, and aspartic acids. The major fatty acids found include arachidonic acid, stearic acid, palmitic acid, linoleic acid, and oleic acid [22]. Similarly, research found 20 constituents in the essential oil of the fruit of *W. coagulans* is primarily composed of sesquiterpenes (54%) and esters (21.50%), which are the dominant compounds. Additionally, fatty acids (5.5%) such as nonanoic acid, hexanoic acid, methyl ester of hexadecanoic acid, methyl ester of nonadecanoic acid, methyl ester of 8,11-octadecadienoic acid, methyl ester of 9-octadecenoic acid, and ethyl ester of linoleic acid are present. Alkanes (9.11%) and aldehydes (0.32%) are present in smaller percentages Shahnaz *et al.* [23]. In addition, it was shown that a de-fatted meal made from *W. coagulans* seeds included free sugar (17.8%) in the form of D-galactose and D-arabinose (1:1), with trace amounts of maltose also present. Additionally discovered and reported for the hypocholesterolemic impact of corn oil combined with *W. coagulans* were higher concentrations of -sitosterol and linoleic acid. Maurya [11].

The fruit of the plant is known to contain certain constituents that may contribute to its potential health benefits. Here are some components found in *W. coagulans* fruit:

- *Withanolides*: *W. coagulans* is known to contain bioactive compounds called withanolides. These are steroidal lactones that are believed to have various pharmacological activities, including anti-inflammatory, antioxidant, and immunomodulating properties [24].

- **Flavonoids:** Flavonoids are a group of plant compounds known for their antioxidant activity. *W. coagulans* fruit may contain flavonoids, which contribute to its potential antioxidant effects.
- **Alkaloids:** *W. coagulans* may also contain alkaloids, which are natural compounds found in various plant species. Alkaloids can have diverse physiological effects and may contribute to the overall chemical composition of the fruit [4, 25].
- **Antioxidant compounds:** *W. coagulans* is known to contain bioactive compounds, such as withanolides and flavonoids, which possess antioxidant properties. These compounds help neutralize harmful free radicals in the body and protect against oxidative stress [26].

While detailed information on the specific nutrient composition of *W. coagulans* is limited, the following nutrients are commonly found in similar plants within the same family or genus:

- **Carbohydrates:** *W. coagulans* likely contains carbohydrates, which are the primary source of energy in plants. The specific types and amounts may vary.
- **Protein:** *W. coagulans* is likely to contain proteins, which are important for various physiological processes in the body.
- **Fiber:** Like many plant-based foods, *W. coagulans* may contain dietary fiber, which can contribute to digestive health and help regulate blood sugar levels.
- **Vitamins:** *W. coagulans* may contain various vitamins, although specific quantities are unknown. Common vitamins found in similar plants include vitamin C, vitamin A, and certain B vitamins.
- **Minerals:** Similar to other plants, *W. coagulans* may contain minerals such as potassium, calcium, magnesium, and iron. The exact mineral profile can vary (Table 3).

The coagulating properties of *W. coagulans* berries on milk are widely recognized. Additionally, the milk coagulating potential of *W. coagulans* fruit extract was evaluated. *W. coagulans* fruit, an enzyme called aspartic protease was isolated using fractional ammonium-sulfate precipitation and cation-exchange chromatography. Casein was used to test the protease enzyme's proteolytic activity. Using skim milk, the ability of *W. coagulans* crude fruit extract to cause milk to coagulate was evaluated [17]. Therefore, it was discovered using mass spectrometry and inhibitory experiments that aspartic protease is the sole enzyme responsible for milk coagulation. Additionally, the activity of the enzyme was steadily decreased by the rising salt concentrations (NaCl, CaCl₂). As a result, it was determined that this enzyme would be suitable to create reduced salt cheese [6].

Buffalo milk mozzarella cheese was made using the fruits of *W. coagulans*, which served as milk coagulants. Therefore, using an aqueous fraction of *W. coagulans* to make cheese might be an option. Buffalo milk cheese was made using an extract from the fruit of *W. coagulans*, and its storage capabilities were examined (5 months). Cheese produced using lyophilized berry extract showcased the highest

Macro-minerals	Minerals (mg/kg)
Calcium	9260
Magnesium	35,280
Potassium	2450
Sodium	125
Micro-Minerals	Minerals (mg/kg)
Iron	98.8
Copper	2.2
Zinc	40.2
Chromium	0.6
Cadmium	1.4
Lead	1.9
Nickel	1.8

Khan et al. [27].

Table 3.
Mineral composition of *W. coagulans*.

concentrations of ash, fat, crude protein, and total solids. To create the cheese, *W. coagulans* alcoholic and aqueous fractions containing plant proteinase were utilized at varying concentrations (0.5, 1, and 1.5%) [28].

Additionally, cottage cheese made with an aqueous plant fraction had a significantly higher moisture content and pH, whereas cheese made with calf rennet and *W. coagulans* had the same levels of ash, fat, and crude protein. a white cheese of acceptable grade can be produced using a 0.5% alcoholic plant extract. Tofu made with calcium sulphate and *W. coagulans* extract were compared in terms of how well they produced coagulation of soy milk. The two types of tofu could not be distinguished based on sensory evaluation, while the tofu produced by *W. coagulans* had a lower yield and more moisture.

10. Future prospects and potential of *W. coagulans*

W. coagulans, also known as Indian rennet or vegetable rennet, is a medicinal plant that has been traditionally used in Ayurvedic medicine. General insights and potential avenues for exploration.

- *Medicinal applications:* *W. coagulans* has been studied for its potential health benefits, including anti-inflammatory, antioxidant, anti-diabetic, and anti-cancer properties. Future research could focus on investigating the specific bioactive compounds responsible for these effects and their mechanisms of action. This could lead to the development of novel therapeutic agents or natural remedies for various ailments.
- *Functional food and nutraceuticals:* *W. coagulans* has been explored for its potential as a functional food ingredient or nutraceutical. It contains high levels of bioactive compounds like withanolides, flavonoids, and saponins, which could

contribute to its health-promoting properties. Future studies may investigate the formulation of *W. coagulans* extracts or derivatives into various food products or supplements, evaluating their efficacy and safety profiles.

- *Agricultural applications:* *W. coagulans* is a perennial shrub that is drought-tolerant and adaptable to arid environments. It may have potential for cultivation in regions where other crops struggle to thrive, thus offering economic opportunities for farmers in such areas. Additionally, research efforts could focus on improving cultivation practices, optimizing yield, and enhancing the quality of harvested plant material.
- *Phytochemical exploration:* *W. coagulans* is known to contain various bioactive compounds, such as withanolides, which have been of interest in pharmaceutical and herbal medicine research. Further exploration of the phytochemical profile of *W. coagulans*, including isolation and characterization of novel compounds, could open up new avenues for drug discovery, natural product synthesis, or plant breeding programs.

It's important to note that these prospects are speculative, and future research and developments will determine the actual potential and applications of *W. coagulans*.

Author details

Mayur Ram^{1*}, Bimal S. Desai² and Sumankumar S. Jha³

1 Department of Forest Products and Utilization, College of Forestry, Navsari Agricultural University, Navsari, Gujarat, India

2 Department of Basic Sciences and Humanities, College of Forestry, Navsari Agricultural University, Navsari, Gujarat, India

3 Department of Forest Biology and Tree Improvement, College of Forestry, Navsari Agricultural University, Navsari, Gujarat, India

*Address all correspondence to: rammayur132@gmail.com

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