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Full Length Article

Pharmacognostic evaluation of *Withania coagulans* Dunal (Solanaceae) - an important ethnomedicinal plant

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ABSTRACT

Withania coagulans Dunal, belonging to the family Solanaceae, is a small bushy shrub which is widely spread in South Asia. The plant is commonly known as 'Indian cheese maker' or 'paneer dodi' due to its milk coagulating characteristics of the fruits. In traditional system of medicine, different parts of plant especially fruits are used as magic healer of various diseases. In the present work, pharmacognostical studies of fruits and seeds are carried out for authentication of drug plant. Physico-chemical and phyto-chemical screening of drug material are done for determination of quality/purity of crude drug and for detection of plant constituents respectively. The plant is characterized by shrubby habit with dioecious and polygamous flowers; fruits (berries) enclosed in persistent leathery calyx; seeds ear-shaped, with fruity smell. Fruit pedicel with branched and unbranched trichomes, massive collenchymatous cortex, intra-xylary phloem and hollow pith; calyx with spongy parenchyma; pericarp with exocarp, mesocarp and endocarp; seeds with highly lignified sclerenchyma cells and strongly thickened endosperm. The plant is rich in alkaloids, esterase, carbohydrates, steroids, phenolic compounds, tannins, free amino acids and organic acids.

Key Words: Indian cheese maker, Pharmacognostic evaluation, *Withania coagulans*, conservation.

INTRODUCTION

In Indian systems of medicine, a large number of herbal and herbo-mineral drugs are in use for ailment of various types of diseases of human since long (Brekhman and Dardimov, 1969). The traditional medicines are increasingly solicited through the traditional practitioners and herbalists in the treatment of different diseases. Currently more than 80% of the world population depends on plant derived health care products for daily regime because of no side effect (Hassan et al., 2009; Gangadhar *et al.,* 2012). *Withania coagulans* Dunal (Solanaceae), commonly known as 'vegetable rennet' or 'Indian cheese maker', is well known in Indian medicine system due to its multifarous medicinal and ethno-pharmacological uses. It is distributed in the east of the Mediterranean region and extends to South Asia (Gupta, 2012). In India, the plant is common in drier parts of Punjab, Haryana, Gujarat and Rajasthan and known by different local names *viz.*, 'Akri' or 'Puni-ke-bij' in Hindi, 'Tukhme-kaknaje-hidi' in Persian, 'Spiubajja' in Afghan, 'Khamjira' in Punjabi, 'Punir band' or 'Punir-ja-fota' in Sindhi (Naz *et al*., 2009). In folk medicine, different parts of the plant, especially fruits are considered as magic healer (Gupta and Keshari, 2013). The fruits are reported to be sedative, emetic and diuretic. They are effective in chronic liver disorder, dyspepsia, flatulent coli and other intestinal infections, asthma, biliousness and strangury (Gupta, 2012; Gupta and Keshari, 2013). In some parts of the Indo-Pak sub-continent, the berries are used as a blood purifier.

The twigs are chewed for cleaning teeth and the smoke of the plant is inhaled for relief of toothache (Kirthikar and Basu, 1933). In North-western parts of India, traditional practitioners use dry fruits of this species for the treatment of diabetic patients though its anti-hyperglycemic activity has not been evaluated systematically. Being a highly valued ethnomedicinal plant of drier parts of the country, skill-less and unscientific uprooting of the whole plant is being practiced with upcoming threats. Thus, *ex-situ* and *in-situ* conservation is needed for protection of this plant in near future (Nautiyal, 2011; Rathore *et al.,* 2012).

MATERIALS AND METHODS

a) Macroscopical and Microscopical studies:

The fruits of *Withania coagulans* (with persistent calyx and pedicel), commonly known as 'paneer phul' or 'paneer dodi', were purchased from the local drug market of Kolkata and identified by Pharmacognosy Department, NRIADD, Kolkata. After proper identification with the help of standard flora, the voucher specimen (WCDP00011) has been deposited in the above mentioned department, Kolkata. The fruits have been examined macroscopically with reference to its colour, shape, size, odour and taste. Hand sections of the pedicel, calyx, pericarp and seeds of the water soaked fruits have been done, stained and mounted following standard method (Johansen, 1940). For phyto-chemical studies fruits have been dried in shade and were grinded to coarse powder (Johansen, 1940; Kay, 1938; Trease and Evans, 1983).

b) Physico-chemical studies:

Physico-chemical parameters of the powdered drug such as total ash, acid insoluble ash, water soluble extractive value and alcohol soluble extractive value have been determined according to the procedures mentioned in Pharmacopoeia of India (1996).

c) Phyto-chemical studies:

Phyto-chemical studies have been carried out by preparing 50% ethanolic extract of fruit and has been subjected to different chemical tests for identification of various bioactive compounds (Kokate, 2001).

d) Fluorescence characteristics:

For study of fluorescence characteristics, air dried coarse powder of fruits has been examined under ultraviolet light according to the method described by Chase and Pratt (1949) and Kokoski *et al*. (1958).

RESULTS AND DISCUSSION

Botanical description:

Stout, greyish-white, small shrub, 30-90 cm tall. Leaves 2.5-7.5 cm x 1.0-1.5 cm, lanceolate oblong, sometimes ovate, obtuse, entire, narrowed at the base, densely covered with minute, gray, stellated tomentum; shortly petiolate. Inflorescence axillary cymose. Flowers yellow, 7-12 mm across, dioecious and polygamous with leathery calyx. Berries globose, red or brown, smooth, covered enclosed in leathery calyx. Seeds dark brown, ear shaped, glabrous, pulp brown, having sharp fruity smell.

Fig. 1: *Withania coagulans* Dunal: **A.** Fruits; **B.** Powder of fruits (Churna)

Table 1: Physico-chemical observations (% w/w)

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

Sr. No.	Tests	50% ethanolic extract
1.	Alkaloids a. Dragendorff's test b. Wagner's test c. Mayer's test d. Hager's test	 +ve +ve +ve +ve
2.	Carbohydrate a. Molisch test b. Fehling's Test	 +ve +ve
	c. Benedict's Test	+ve
3.	Protein a. Biuret Test b. Xanthoprotein Test c. Lead Acetate Test	 +ve +ve +ve
4.	Amino acids a. Ninhydrin test	 +ve
5.	Glycoside a. Baljet Test b. Keller Kiliani Test	 -ve -ve
6.	Saponin test a. Foam test	 -ve
7.	Tannins and phenol compounds	+ve
8.	Essential oils a. spot test	 +ve

Table: 3- Fluorescence characteristics

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

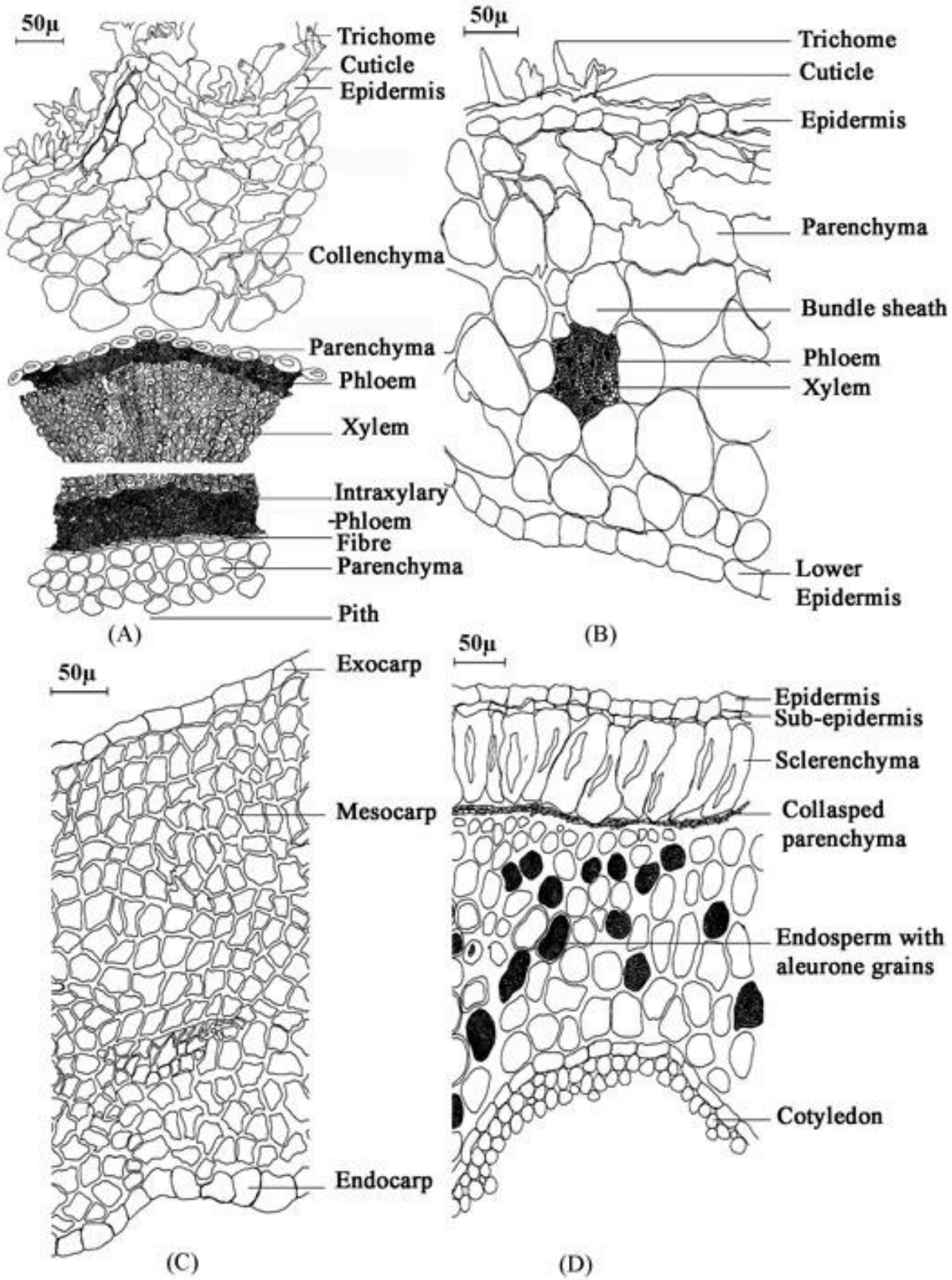

Fig. 2. *Withania coagulans* Dunal: A. Transverse section of pedicel; B. Transverse section of calyx; C. Transverse section of pericarp; D. Transverse section of seed.

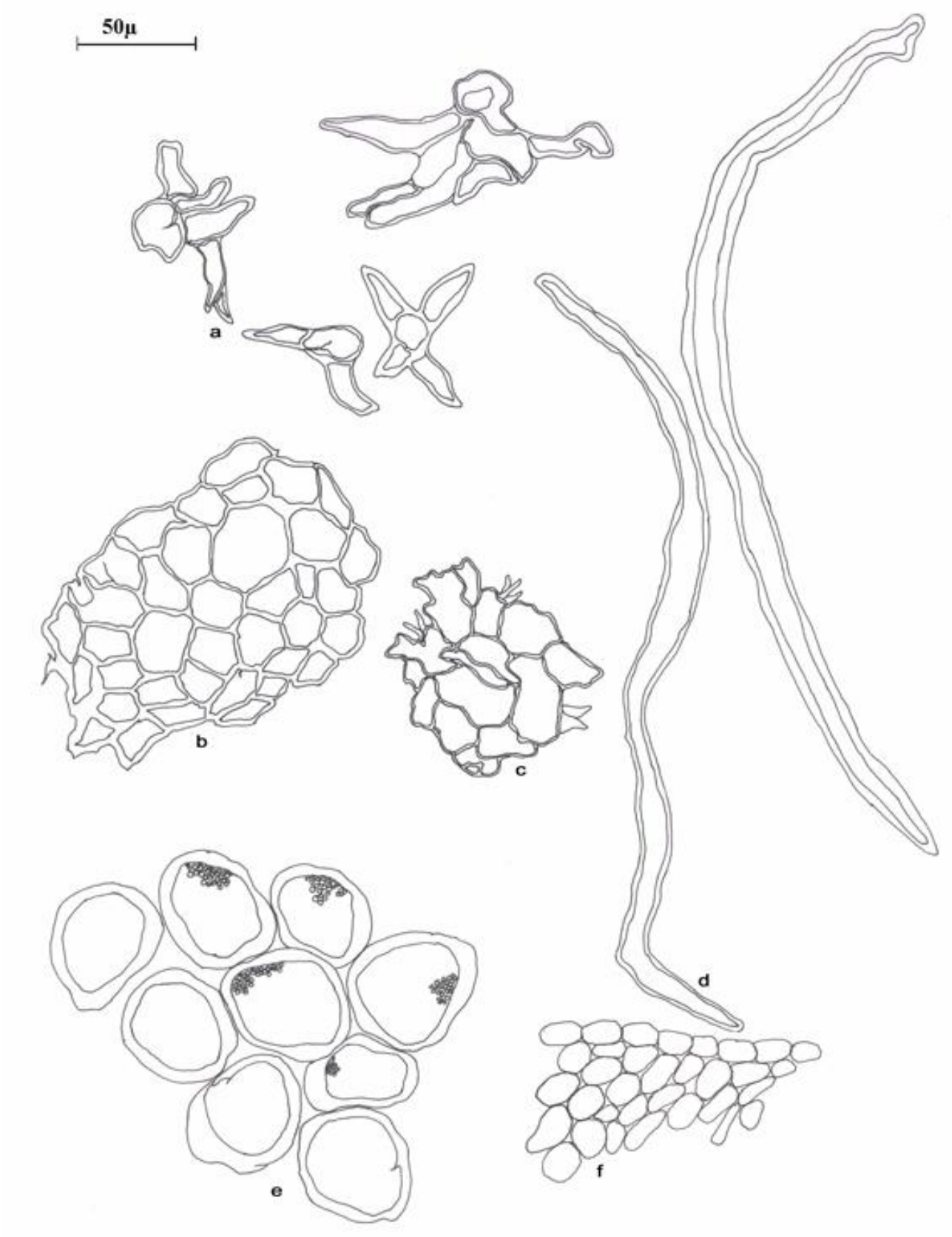

Fig. 3. *Withania coagulans* Dunal: Powder characteristics of fruits. A. Trichomes; B. Pericarp in surface view; C. Upper epidermis of calyx; D. Fibre; E. Endosperm cells with aleurone grains; F. Portion of cotyledons.

Macroscopical description:

The fruits are superior, reddish-yellow to brown in colour, indehiscent, many-seeded berry, pedicellate, round to globose, 6-9 mm in diam., enclosed within leathery persistent calyx, scurfy-puberulous outside. The pedicel persistent, 3-7 mm long. Pericarp smooth. Seeds 2.5-3 mm diameter, ear-shaped, glabrous. The fruits have an indistinct odour with a slight bitter taste [Fig. 1].

Microscopial description:

The transverse section of the pedicel shows a single layer of epidermis composing of tabular cells, covered with a large number of branched and unbranched trichomes, followed by cortex composing of 5-10 layers of collenchymatous cells. The pericycle shows the presence of pericyclic fibres with intervening parenchymatous cells. The central region consisting of a narrow band of phloem encircling the xylem beneath which is a ring of intra-xylary phloem. The centre most region is consisting of hollow pith surrounded by parenchymatous cells with a few thick-walled lignified fibres towards phloem [Fig.2(A)].

The transverse section of calyx exhibit a single layer of thin-walled cells in both upper and lower epidermis with a few branched and unicellular covering trichomes present in upper epidermis. The mesophyll is represented by spongy parenchyma traversed by a number of small veins covered with bundle sheath cells composed of thin-walled parenchymatous cells [Fig. 2(B)].

The transverse section of pericarp (fruit wall) shows the presence of exocarp which consists of a single layer of cells while mesocarp shows a wide zone of parenchymatous cells with strong cellulosic thickening. The endocarp is consists of single layer of cells [Fig.2(C)].

The transverse section of seed shows a single layer of epidermis followed by a layered of flattened thin-walled sub-epidermal cells. Beneath the sub-epidermis there is a layer of highly lignified sclerenchyma cells with narrow lumen. The inner epidermis of the seed coat comprising of 1-3 layer of thin-walled parenchymatous cells which at places are collapsed. The endosperm is represented by cells showing strong cellulosic thickening filled with aleurone grains. The cotyledon shows thin-walled radially elongated cells enclosing a wide zone of round to oval to polyhedral parenchymatous cells [Fig. 2(D)].

The powder characteristics of the fruits of *Withania coagulans* has been described in [Fig. 3] which shows (a) a large number of branched and unbranched trichomes, (b) fragments of pericarp in surface view, (c) epidermal cells of calyx with unicellular covering trichomes, (d) asepted fibres, (e) endosperm cells with aleurone grains and (f) a large number of cotyledonary cells.

Physico-chemical observations:

Ash value help in determining the quality and purity of crude drugs while extractive values help in evaluating the constituens of crude drugs. Various Physico-chemical Standards such as total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value have been recorded (in triplicate) [Table 1].

Phyto-chemical screening:

The plant extract has been subjected to preliminary phytochemical screening for the detection of various plant constituents present in the raw drug. The test results have been recorded**.** [Table 2]**.**

Fluorescence characteristics:

Fluorescence characters helps in fulfilling the inadequacy of physical and chemical methods for identification of plant drugs. Fluorescent analysis of powdered drug on treatment with different reagents has been studied under ultraviolet light and the observations are presented [Table 3].

CONCLUSION

The macroscopic and microscopic evaluations of any plant drug are considered to be the preliminary steps for establishing their quality control profile. According to WHO, botanical standards should be proposed as a protocol for the diagnosis of the herbal drugs.

Physico-chemical standards, such as total ash value help us in sophistication of drugs i.e. of determining both physiological ash of plant tissues and non-physiological ash of extraneous matters like sand and soil; where as acid insoluble ash detects presence of the heavy metals in the earthy matter in the drugs. Extractive values (both water soluble and alcohol soluble) help us in determining the amount of active constituents and the above mentioned tests are done on plant materials for which still now no suitable chemical or biological assay exists.

In folk medicine, different parts of the plant especially fruit (berry) are used as magic healer of various diseases. Fruits of the plant have a milk coagulating characteristics that is attributed as an enzymatic charisma of the plant (Naz, 2002). The milk coagulating property of the berries is due to the presence of an enzyme, esterase. Most of the activities of *Withania coagulans* is due to presence of active constituent, 'withanolides' which are a group of steroidal lactones with an ergostane skeleton (Glotter, 1991). Withanolides which are named after the name of the source plant *Withania* species, are generally defined as C-28 steroidal lactones. Modifications either of the carboxylic skeleton or of the side chains result in many novel structural variants of withanolides or ergostane-type steroids (Cardenas *et al*., 1994). As withanolides have been reported to posses anti-tumorous, anti-angiogenic, chemopreventive and inflammatory activities thus may represent useful leads for development of potential anti-cancer drug. In addition, withacoagin, coagulan, withasomidienone, withaferin, 3-β-hydroxy-2,3-dihydro-withanolide E, free amino acids, essential oil etc. have been isolated from the berries as well as aerial parts of the plant.

The natural habitat of *W. coagulans* is destructed day by day by increasing rate of agricultural practices, mining, industrialization, reduction of forest cover and population explosion throughout the country. In addition, global warming, climate change and political partition of India and Pakistan also had great impact on habitat loss of the plant in India.However, some *ex-situ* and effective micropropagation techniques have already been implemented by Arid Zone Regional Centre, Botanical Survey of India, some research institutes, Universities and NGOs.

The major aim of present study is to protect adulteration of crude drugs of plant origin and give emphasis on conservation of *W. coagulans*.The results have been obtained from the present study may play an important role in setting particular standards for the plant specimen. The pharmacognostical, botanical, chemical and economic characterizations also prove beneficial in proper identification of plant drugs. Thus with the help of these parameters the effect of adulteration of fruits of this species can be minimized. Thus the future research works with authenticated and properly identified specimens will be of great use (Prasad et al, 2010; Gupta, 2012; Salwaan *et al.,* 2012).

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