Cytotoxic Activity of *Withania coagulans*

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Abstract:

The *withania coagulans* belongs to family Solonaceae and is chiefly distributed in East of the Mediterranean region, extending to South Asia. This plant is rich source for withanolide. The Withania coagulans possess variety of medicinally necessary activities like antifungal activity, anthelmintic, antimicrobial, hypolipidemic, inhibitor, anti-cytotoxic, anti-fungal activity, hypoglycaemic activity etc. The current review explores biological description, different constituents, synthesis and structure of withanolide and cytotoxic activity of *withania coagulans.*

Key Words: Withania coagulans, Withanolides, Withaferin A, Anticancer.

Introduction:

Plants are very important source of natural products providing the raw material for diverse pharmaceutical and therapeutic applications due to the presence of phytochemicals commonly known as secondary metabolites. A large number of metabolites of plants are utilized against number of diseases including cancer and other cellular disorders.[1,2] The medicinal plants and their bioactive constituents have been extensively used as therapeutics against various cancer types, and Solanaceae family contains vast number of medicinal plants which shows anticancer activity.[3,4] Plants are used because of their cost-effectiveness, availability, and low toxicities.[5]

In the natural system of drugs several plants have been claimed to be helpful for the treatment of cancer. There are two species of Withania, a small genus of shrubs distributed in east of the Mediterranean region extending to South Asia are found in India.[6] Withania coagulans Dunal (family: Solanaceae) commonly known as Indian cheese maker, it is a rigid, grey beneath woody plant, 60-120cm high occurring in drier area of India. Dry fruits of W. coagulans used by northern Indians for the treatment of diabetic patients through its antihyperglycemic activity have not been evaluated systematically. It is well known for its wide applications. The fruits contain withanin enzyme which shows milk-coagulating properties. Withanolide, a steroidal lactone isolated from the aqueous extract of fruits of W. coagulans, has cardiovascular effect. Alcoholic extract of*W. coagulans* has shown antibacterial and antihelmintic activities.[7] Withaferin A and some other withanolides have been found to possess antimitotic and anticancer activity.[8]

Withania coagulans Dunal (Solanaceae) is native to warm, temperate (Western Asia: Afghanistan) and tropical regions (Indian Subcontinent: India, Nepal, Pakistan,). It is widely distributed in the relatively drier parts of India and Pakistan and is commonly known as Indian rennet, cheese maker, or vegetable rennet (English). It is also recommended for the treatment of various disorders in traditional medicine.[9]

The *Withania coagulans* contain several phytochemical constituents present in aqueous and methanol extracts. These phytochemical

Figure No. 1: *Withania coagulans* Fruit

Introduction to *Withania coagulans*-

Botanical Name	:	*Withania coagulans* Dunal
Kingdom	:	Plantae
Division	:	Magnoliaphyta
Class	:	Magnolipsida
Order	:	Solanales
Family	:	Solanaceae
Subfamily	:	Solanoideae
Genus	:	Withania
Species	:	*W. coagulans*
Sanskrita Name	:	Rishyagandha

Commonly known as: Punirbandh, Indian cheese maker, Indian Rennet, Vegetable rennet, Paneer ke Phool, Paneer doda[11] . Trade Name: Paneer dodi, Paneer bed, Paneer dhodi

Botanical Description

Withania coagulans Dunal is a rigid, gray-whitish small shrub about 60-120 cm tall.

Habit - Herb

Leaves - About 2.5-7.5cm long and 1.5cm broad Usually lance late oblong sometimes ovate, narrow and obtuse at the base and very short stalked

Flowers - About 7-12mmacross, Yellowish and are polygamous and dioeciously in nature, found in axiliary cymose in clusters.

constituents are responsible for antioxidant activity of plant.[10]

Figure No. 2: *Withania coagulans* Plant

Fruit (berry) - 1.5-1cm long, 0.7-1cm width, sepals covers the fruit and ended into crown like structure

Seeds - Oval to round in shape, yellowish brown, 41-59 in number, 0.1-0.3 cm long, 0.2 -

0.3 cm wide, dotted

Flowering - November-March

Cancer is hyper proliferative disorder that involves transformation, dysregulation of apoptosis, invasion, proliferation, angiogenesis and metastasis. Millions of people die every year with different types of cancer such as lung cancer and mesothelioma from inhaling asbestos fibers and tobacco smoke, or leukemia from exposure to benzene at their workplaces, breast cancer, skin cancer etc. In the last century, great advances were made in modern medical system to cure and prevent this disease. However, success rates are very low. *Withania coagulans* has been used as traditional medicine for decades for the treatment of various ailments.

Several anticancer agents derived from plants like taxol[12], vinblastine, vincristine and the camptothecin[13] derivatives are actively used clinically worldwide. But still many of the potential plants have been left unexplored.

Two species, Withania somnifera and W. coagulans, are found in India and Pakistan.

W. somnifera is known as "Indian ginseng" and "winter cherry" in English and "Ashwagandha" in Hindi. Withanolides are the important compounds found in both species. Antihyperglycemic leads have been isolated from W. coagulans, which have not yet been observed in *W. somnifera.* Furthermore, a distinctive thio-dimer of withanolide named ashwagandhnolide has been reported in W. somnifera. Withanolides which contain a 14, 20-epoxide bridge are specific to W. coagulans.[14]

Kirthikar and Basu in 1933 had shown that *Withania coagulans* has ethnopharmacological activities.[15] The W. coagulans is common in East India, Iran, Pakistan and Afganistan. According to Atal and Sethi(1963),fruits of the plant have a milk coagulating characteristics.[16]

The fruits are sweet and are reported to be emetic, sedative and diuretic. The plant contain constituent named Withanolides which are known to be as a group of steroidal lactones found amongst members of solanaceae. Withanolides are reported to have antitumor, anti-inflammatory, antibacterial, immuno- suppressive, antiulcer, cytotoxicity activities.[17]

Synthetic chemotherapeutic agents not only cause several side effects but are ineffective to kill cancer cells when these cells develop resistance against them. Ayurveda has blessed us with the numerous applications of plant derived therapeutic agents to heal several diseases. Several plants derived anticancer agents like vinblastine, vincristine, camptothecine (Hsiang et. al 1985), taxol (sparano et. al., 2008) derivatives are actively used worldwide.[18,19]

Table No. 1: Constituents of *Withania coagulans*

Sr. No.	Name of Compound	Part of Plant	Activity
1.	17β-Hydroxywithanolide K ((20S,22R) 14α, 7β,20β-trihydroxy-1-oxowitha-2,5,24- trienolide)[20-22]	Whole plant & fruits	Antihyperglycaemic, antimicrobial
2.	Coagulin C[22,23]	Whole plant & fruits	Antihyperglycaemic
3.	Coagulin H ((17S,20S,22R)-5α, 6β, 14α, 15α, 17,20 -hexahydroxy-1-oxowitha-2,24- dienolide)[24,25]	Whole plant	Immunosuppressive

Cont...

Table No. 1: Constituents of *Withania coagulans*

4.	Coagulin L ((14R,17S,20S,22R)-14,17,20-trihydroxy-3β-(O-β-D-glucopyranosyl)-1- oxowitha-5,24-dienolide)[22,24]	Whole plant & fruits	Antihyperglycaemic
5.	3β,14α,17β,20αF-Tetrahydroxy-1-oxo- 20S,22R-witha-5,24-dienolide (or 3β-hydroxy-2,3-dihydrowithanolide F)[26-29]	Fruits	Hepatoprotective, anti-inflammatory, blood pressure lower ing, central nervous system depressant
6.	Coagulanolide ((17S,20S,22R)-14α, 15α, 17β, 20β-tetrahydroxy-1-oxowitha-2,5,24- trienolide)[22]	Fruits	Antihyperglycaemic
7.	Withanolide F[22]	Fruits	Antihyperglycaemic
8.	Withaferin A[29]	Root	Antimicrobial, immunomodulating, antitumour, cytotoxic

Table No. 2: Other constituents of *Withania coagulans*

Sr. No.	Name of Compound	Part of Plant
1	Coagulin(17β,27-dihydroxy-14,20-epoxide-1-oxo-(22R)-witha-3,5,24-trienolide)[30]	Whole plant
2	14,15β-Epoxywithanolide I ((20S,22R) 17β,20β-dihydroxy-14β,15β-epoxy-1-oxowitha-3,5,24-trienolide)[20,21]	Whole plant
3	Coagulin B[23]	Whole plant
4	Coagulin D[23]	Whole plant
5	Coagulin E[23]	Whole plant
6	Coagulin F (27-hydroxy-14,20-epoxy-1-oxo-(22R)-witha-3,5,24-trienolide)[31]	Whole plant
7	Coagulin G (17β,27-dihydroxy-14,20-epoxy-1-oxo-(22R)-witha-2,5,24-trienolide)[31]	Whole plant
8	Coagulin I ((14R,17S,20S,22R)-5α, 6β,17-trihydroxy-14,20-epoxy-1-oxowitha-2,24-dienolide)[24]	Whole plant
9	Coagulin J ((14R,17R,20R,22R)-3β,27-dihydroxy-14,20-epoxy-1-oxowitha-5,24-dienolide)[24]	Whole plant
10	Coagulin K ((14R,17R,20R,22R)-14,20-epoxy-3β-(O-β-D-glucopyranosyl)-1-oxowitha-5, 24-dienolide)[24]	Whole plant
11	Coagulin M ((14R,17R,20R,22R)-5α,6β,27-trihydroxy-14,20-epoxy-1-oxowitha-24-enolide)[32]	Whole plant
12	Coagulin N ((14R,17S,20R,22R)-15α,17-dihydroxy-14,20-epoxy-3β-(O-β-D-glucopyranosyl)-1-oxowitha-5,24-dienolide)[32]	Whole plant
13	Coagulin O ((14R,20S,22R)-14,20-dihydroxy-3β-(O-β-D-glucopyranosyl)-1-oxowitha-5, 24-dienolide)[32]	Whole plant
14	Coagulin P (20,27-dihydroxy-3β-(O-β-D-glucopyranosyl)-1-oxo-(20S,22R)-witha-5,14, 24-trienolide)[33]	Whole plant
15	Coagulin Q (1α,20-dihydroxy-3β-(O-β-D-glucopyranosyl)-(20S,22R)-witha-5,24-dienolide)[33]	Whole plant
16	Coagulin R (3β,17β-dihydroxy-14,20-epoxy 1-oxo-(22R)-witha-5,24-dienolide)[33]	Whole plant
17	20β-Hydroxy-1-oxo-(22R)-witha-2,5,24-trienolide[34]	Whole plant
18	Withacoagulin[34]	Whole plant
19	17β-Hydroxy-14α, 20α-epoxy-1-oxo-(22R)-witha-3,5,24-trienolide[34]	Whole plant
20	Coagulin S[35]	Whole plant
21	Bispicropodophyllin glucoside[35]	Whole plant
22	Ergosta-5,25-diene-3β,24ξ-diol[26]	Fruits
23	3β,14α,20αF,27-Tetrahydroxy-1-oxo-20R,22R-witha-5,24-dienolide (or 3β-hydroxy-2,3-dihydrowithanolide H)[28]	Fruits

Cont...

Table No. 2: Other constituents of *Withania coagulans*

24	Sitosterol-β-D-glucoside[28]	Fruits
25	5,27-Dihydroxy-6α7α-epoxy-1-oxo-(5α)-witha-2,24-dienolide[11]	Root
26	Withacoagin ((20R,22R)-5α,20-dihydroxy-1-oxowitha-2,6,24-trienolide)[36]	Root
27	(20R,22R)-6α,7α-Epoxy-5α-20-hydroxy-1-oxowitha-2,24-dienolide[36]	Root

The defatted meal from the seeds of W. coagulans Dunal contains 17.8% free sugars, consisting of D-arabinose and D-galactose in the ratio 1:1, with maltose in traces. Enzymatic studies showed the absence of a β-galactosidic linkage in the polysaccharide.[37] The seeds of W. coagulans are reported to contain 12–14% fatty oil. A hydrocarbon triacontaine and sterol dihydrostigmasterol are obtained from the unsaponifiable portion of the fruits. The oil was found to contain a high percentage of b-sitosterol and linoleic acid i.e. the factors which in combination are reported to be responsible for the hypocholesterolaemic effect of corn oil.[16]

Withanolides are a gaggle of steroidal lactones found among members of Solanaceae. It was named after the name of the source plant Withania species generally defined as C-28 steroidal lactones. The presence of a lactone ring with C-22 and C-26 oxygen functions to form a five or six member lactone ring on an ergostane skeleton, rearranged ergostane or intact, it constitutes basic structure of all withanolides. The withanolide skeleton may be described as 22-hydroxy ergostane-26-oic acid-26, 22-olide. Carboxylic skeleton or side chain modifications results in many novel structural variants of withanolides which are known as modified withanolides or ergostane type steroids related to withanolides. Withanolides possess anti-t umour, chemopreventive and anti-inflammatory activities .Therefore withanolides may be described as useful leads for the development of potential anti-cancer drugs. Withanolides act by suppressing the transcription factor nuclear factor-EB (NF-EB). NF-EB is activated by various tumour promoters, carcinogens, and conditions in the tumour micro-environment (hypoxia and acidic pH). Most inflammatory agents activate NF-EB. Chemo preventive agents have been shown to suppress NF-EB activity on withanolides which are potent suppressors of NF-EB activation induced by various agents and that this suppression is mediated through IKK. Berries contain milk coagulating enzyme esterase, free amino acids, essential oil, fatty oil, and alkaloids. The essential oil shows effect against Micrococcus pyogenes var. aureus and also shows anthelmintic activity.[31,38,39]

Figure No. 3: General overview of WTDs biosynthesis pathway. Abbreviation: G3P, Glyceraldehyde 3 phosphate; DOXP, 1-Deoxy-D-xylulose 5-phosphate ; MEP, 2-Cmethylerythritol 4-phosphate; DMPP, Dimethylallyl pyrophosphate; IPP, Isopentenyl- 5- pyrophosphate; FPP- Farnesyl pyrophosphate; SQS - Squalene synthase.[40]

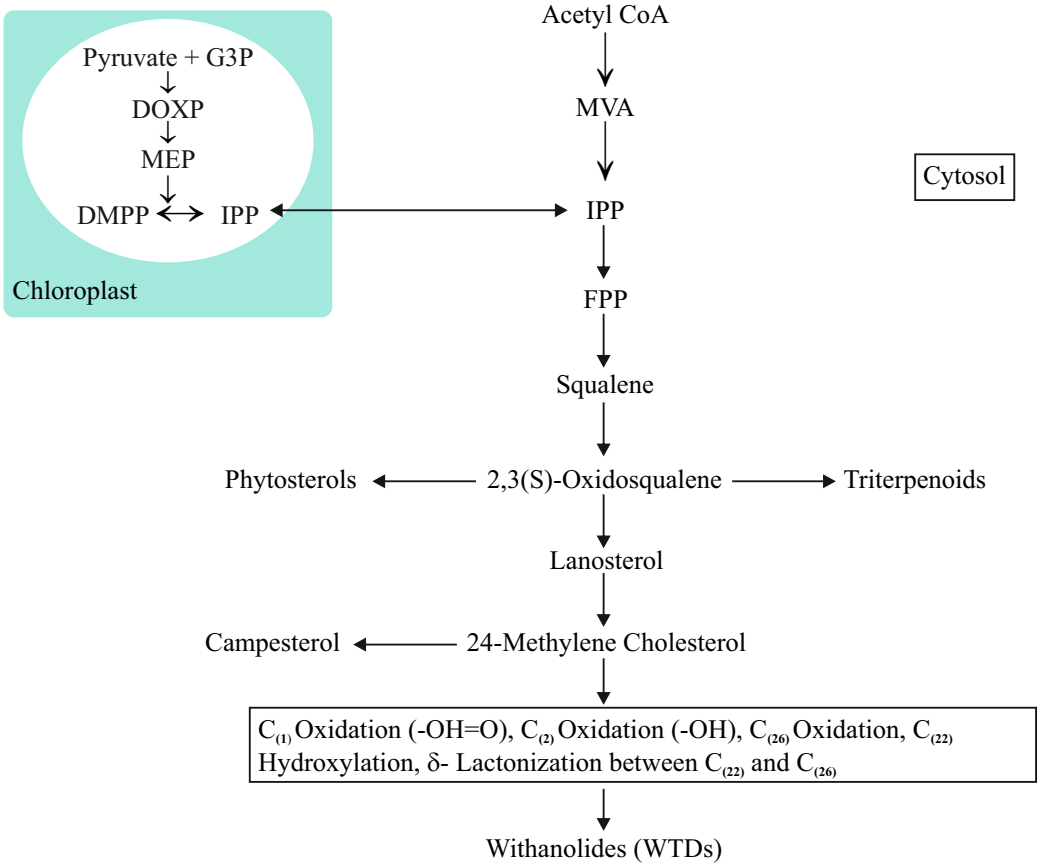
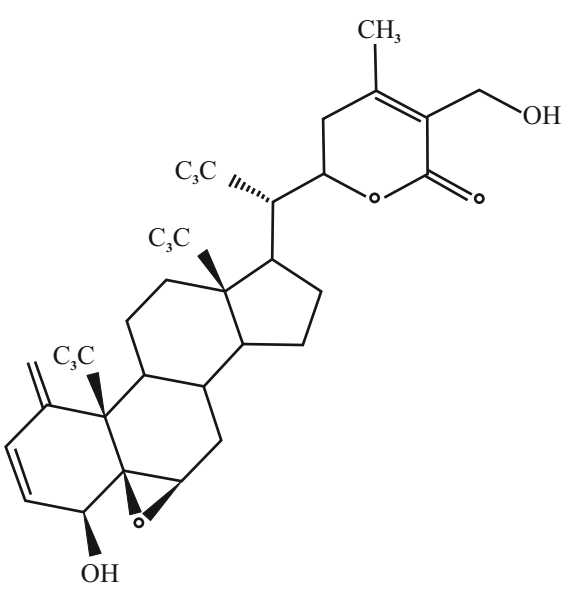
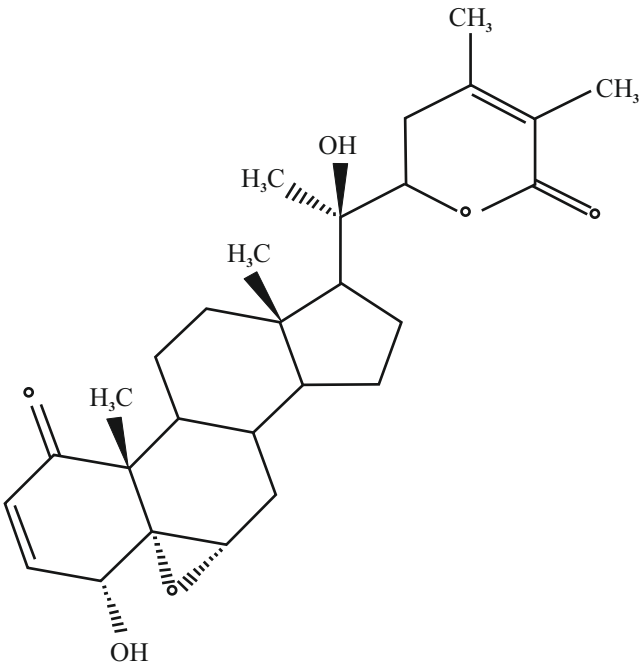


Figure No. 4: Structures of important Withanolides: (a) Withaferin A and (b) Withanolide



(a)



(b)

Withaferin A

Withaferin A is the most vital of the withanolides isolated so far. It has good antibiotic activities and also it has marked tumor inhibitory property when studied in vitro against cells derived from human carcinoma of nasopharynx (KB). It acts 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-inflammatory and antiarthritic effect. It effectively suppresses arthritic syndrome without any toxic effect. On the contrary hydrocortisone treated animals which show weight loss, the animal treated with withaferin A showed weight gain in arthritic syndrome. Withaferin A in conc. of 10µml inhibited the growth of various gram-positive bacteria, aerobic bacilli, acid fast bacilli, and pathogenic fungi.[41]

Synthesis of Withanolide

Withanolides generally contain a polyoxygenated ergostan skeleton. Kreis and Muller-Uri in 2010 synthesized Withanoloids via the mevalonate pathway of terpenoids formation arise from the initial cyclization of 3S -squalene-2, 3-epoxide.[43]

Structure

The basic structures of all withanolides contain a six- or five-membered lactone or lactol ring attached to an intact or rearranged ergostane skeleton. They give positive Dragendorff"s test even though they are not N-containing. The TLC gives a characteristic blue colour spot when spread with H_2SO_4–MeOH. This class of compounds does not occur in all members of the Solanaceae family. However, the withanolides are not restricted to Solanaceae, they have also found in members of plant families Leguminoseae and Taccaceae and also reported in marine organisms (soft corals).[43]

Withanolides of ergostane steroids are four-ring triterpenes. The steroids of plants are obtained from sterols and comprise steroid saponins, steroid alkaloids, pregnanes, estranges, androstanes, ecdysteroids, withanolides and cardiac glycosides.[42] "Withanolide" are often shown because the term for C28-skeleton 22- hydroxyergostan-26-oic acid -22, 26-olide; this γ-lactones residue containing the structure may be a theoretical.[44] The essential skeleton of withanolides is shown in Figure 1.

Withanolides contain two major groups as follows:

A- Withanolides with an unmodified skeleton

a. With a regular β-oriented side chain

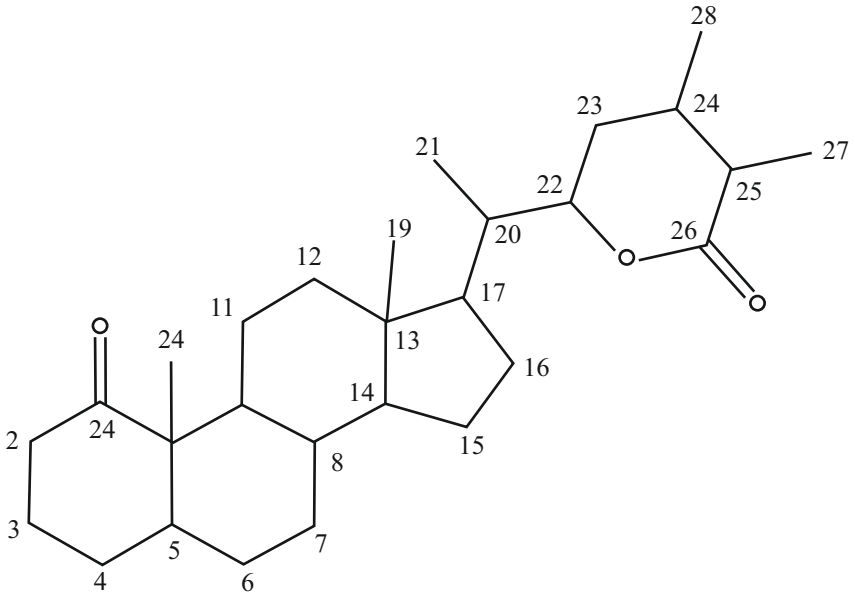
b. With an unusual α-oriented side chain.

B- Withanolides with modified carbocyclic skeletons or side chains. These withanolides are initially classified on the basis of the chemo- types of the Withania species depending on the region of the plant collection. Chemically, these compounds may be categorized as ergostane derivatives from their structural pattern; these can be broadly divided into seven groups.

1. 5β, 6 β –epoxides
2. 6 α, 7 α –epoxides
3. 5-enes
4. Intermediate compounds
5. 5 α, 6 α -epoxides
6. 6 β, 7 β -epoxides
7. Phenolic withanolides

From these, the 5β, 6β-epoxides are most common. Most of the compounds possess a 4β -hydroxyl group.

Figure No. 5: Basic Skeleton of Withanolides



Withanolides also found in certain Tacca spp. of the Taccaceae (taccalonolides)[45] and Ajuga spp., e.g., A. parviflora Benth. Lamiaceae (ajugins)[46], as well as in some marine organisms.

Structure Activity Relationship

Cytotoxicity of withanolides is associated to their structures. The presence of unsaturated lactone within the chain to which an allylic primary alcohol is attached at $C_{(26)}$ and the other end of the molecule may be specific chemical system possessing carcinostatic properties.[47] Unsaturated lactone in side chain is essential for antitumor activity of Withaferin A; 1keto-2-ene and the epoxide function being necessary for antitumor activity. The covalent bond at position $C_{(2)}$-$C_{(3)}$ is very important and dissociation of this bond at $C_{(24)}$-$C_{(25)}$ did not inhibit the activity. Removal of 27-OH acid does not cause any important modification in biological effects. Biological activity increases with presence of carbonyl group at C4.[48]

Plant Extraction

The fruits were shade dried and crushed with the help of grinder to form powder. The powder was then allowed to extraction with 100 % methanol in separating funnel by refluxing for 36 h at 50-60º C. Pellets of the drug were obtained and the double distilled water is used to dissolve the pellets to prepare required dose at different concentration.[49]

Gopinath S. et al has extracted W. Coagulans by drying berries of W. Coagulans for 5 days and milled into fine powder. For ethanol extraction, 10 g of powdered material was dissolved in 50 ml of ethanol and kept on hot water bath at 50º C for 4 hours. The extract was filtered through Whatman No.1 filter paper and the filtrate was kept in water bath at 80ºC for few hours until it get into semisolid. The stock solution of extract was prepared in DMSO at 320 mg/ml concentration and was kept on hot water bath at 60 ºC for 1 hour for proper dissolution of the pellet.[50]

Anticancer and chemoprotective activities:

Cancer, known medically as a malignant neoplasm, is a broad group of diseases involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invading nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Not all tumors are cancerous; benign tumors do not invade neighboring tissues and do not spread throughout the body. There are over 200 different known cancers that affect humans. The anticancer effect of Withania has been studied extensively and it was found that it was effective because of its ability to reduce the tumour size. *Withania coagulans* Dunal contains withanolides, which are reported for antitumor activity, and flavonoids which have been shown to possess antimutagenic and anticarcinogenic activities. Treatment of root extract of *Withania coagulans* Dunal on induced skin cancer in mice exhibited significant decrease in the incidence and average

number of skin lesions compared to control group. Withaferin A showed tumor inhibitory activity against cells derived from human carcinoma of the nasopharynx while, Shohat et al. reported antitumor and immune stimulating effects in mice suffering from tumor.[47]

The anticancer effect of *Withania* has been studied extensively and it had been found that it is the effective agent in preventing cancer through its ability to scale back tumour size. Treatment of root extract on induced carcinoma in mice exhibited significant decrease within the incidence and average number of skin lesions compared to regulate group. Withaferin A showed tumour inhibitory activity against cells of human carcinoma of the nasopharynx and it also inhibited the growth of roots of *Allium cepa* by arresting the cell division at metaphase in another study, it was evaluated for its antitumor effect in urethane-induced lung adenomas in adult male albino mice. Simultaneous administration of extract (200 mg kg-1 weight daily orally for seven months) and urethane (125 mg kg-1 biweekly for seven months) reduced tumor incidence significantly. Additionally, during a different study the aqueous extract of *W. coagulans* was used for anti-cytotoxic effect in chicken lymphocytes and remarkable inhibitory activity of dimethyl sulfoxide (DMSO)-induced cytotoxicity with a decrease in TNF-G production was reported.[49]

The presence of phytoconstituents decides the genotoxic nature of any herbal compound. W. coagulans contains withanolides, which are responsible for antitumor activity and flavonoids which shows antimutagenic and anticarcinogenic activities. The mechanism involved in anti-mutagenic action of W. coagulans is still unknown. The antimutagenic activity of W. coagulans fruit extract was investigated on cyclophosphamide induced micronucleus formation in mouse bone marrow cells. The results confirmed that a single intraperitoneal administration of W. coagulans fruit extract at the doses of 500, 1000 and 1500 mg/kg body weight prior to 24 h significantly block the micronucleus formation in dose dependent manner in bone marrow cells of mice as compared to cyclophosphamide group.[49]

Withaferin A, Withanone and Withanolide A are major withanolides present in Withania genus.[5], [52] Out of which Withaferin A is the most significant in terms of its concentration and spectrum of activity. It possesses significant antibiotic activity and its anti-cancer effect has been studied against malignant nasopharyngeal (KB) cells in vitro.It interferes in cell division by arresting mitosis at metaphase. In vivo studies have also demonstrated the growth- inhibitory and *Withania coagulans* radiosensitizing effects of Withaferin A in mouse Ehrlich ascites carcinoma.[53,54] It has also been found to arrest cell division in embryonical chicken fibroblast cells. Anticancer and apoptotic activities of *Withania coagulans* methanolic and aqueous fruit extracts have also been studied on DMBA induced skin papilomagnesis in vivo[55] as well as in vitro.

The apoptotic activity of has been tested against human breast cancer as well as normal epithelial cell lines. The results revealed that the methanolic extract of fruit possesses significant activity against breast cancer cell lines but no appreciable activity against the normal cell line. The genus Withania can be used as complementary medicine in patients undergoing aggressive treatment in clinical settings against a number of chronic diseases like cancer.[56]

Prasad S. et al in 2010 investigated the antimutagenic activity of W. Coagulans fruit extract on cyclophosphamide induced micronucleus formation in mouse bone marro cells. From the results it is confirmed that a single intraperitoneal administration of *Withania coagulans* fruit extract at the doses of 500, 1000 and 1500 mg/kg body weight prior to 24 h considerably prevented the micronucleus formation in dose dependent manner in bone marrow cells of mice as compared to cyclophosphamide group.[57]

Withacoagulin A, withacoagulin C, withacoagulin D, withacoagulin E, withanolide L, Δ3 iso withanolide F, withanolide F, withacoagulin and (22R)- 14α15 α17β- 20 β, tetrahydroxy-1oxowitha 2,5,24 trien, 26, 22- olide compounds had relatively better activities with IC50<20 mm on the inhibition of both Con A-induced T cell and LPS-induced B-cell proliferation that, among these compounds, withanolide F shows strongest activity (IC50¼1.66 mm) and the best SI value (25.5). withacoagulin C also exhibited a satisfactory SI value. Withanolides induces apoptosis in HL-60 leukemia cells via mitochondria then the cytochrome C is released and caspase activation.[58] 3F-hydroxy-2, 3dihydro-withanolide F was reported to possess anti-tumor activity.[28]

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Conflict of Interest: Nil

References:

1. Scheck AC, Perry K, Hank NC, Clark WD. Anticancer activity of extracts derived from the mature roots of Scutellariabaicalensis on human malignant brain tumor cells. BMC Complement Altern Med 2006;6:27.
2. Ramasamy TS, Yu JS, Selden C, Hodgso H, Cui W. Application of three-dimensional culture conditions to human embryonic stem cell-derived definitive endoderm cells en- hances hepatocyte differentiation and func- tionality. Tissue Eng Part A 2013;19:360–7.

3. Patel B, Das S, Prakash R, Yasir M. Natural bioactive compound with anticancer poten- tial. Int J Adv Pharma Sci2010;1:32–41.
4. Shukla S, Braz MA. Anticancer potential of medicinal plants and their phytochemicals: a review. J Bot 2015;38:199.
5. Greenwell M, Rahman PKSM. Medicinal plants: their use inanticancer treatment. Int J *Withania coagulans* Pharmaceut Sci Res 2015;6:4103–12.
6. Chadha YR. The Wealth of India, Publica- tion and Information Directorate CSIR: New Delhi, India, vol. X, 1976; pp. 580-85.
7. Gaind KN, Budhiraja, RD. Antibacterial and antihelmintic activity of *Withania coagu- lans*, Dunal. Ind J Pharmacol 1967; 29(6): 185-6.
8. Budhiraja, RD, Pawan Krishan, Sudhir S. Biological activity of Withanolides, Journal of scientific and industrial research, Vol. 59 , Nov 2000 pp 904-911.
9. Ihsan-ul-Haq, Ui Joung Youn, Xingyun Chai, Eun-Jung Park,Tamara P. Kondratyuk, Charles J. Simmons,§ Robert P. Borris, Bushra Mirza, John M. Pezzuto, and Leng Chee Chang, "Biologically Active Withano- lides from", Journal of natural products.
10. Deepika Mathur, R.C. Agrawal, Vinoy Shrivastava, "Phytochemical screening and determination of antioxidant potential of fruits extracts of *Withania coagulans*", Re- cent Research in Science and Technol- ogy2011,3(11):26-29.
11. Nidhi Agarwal, Pramod Kumar Raghav, Richa Singh, Renu Singh," Paneer Doda (*Withania coagulans* Dunal): A promising Therapeutic Agent",International Journal of Green and Herbal Chemistry, May 2014; Vol.3,No.2,701-711.
12. Sparano JA, Wang M, Martino S, Jones V, Perez EA, Saphner T, Wolff AC, Sledge GW Jr, Wood WC, Davidson NE. (2008). Weekly paclitaxel in the adjuvant treatment of breast cancer. *The New England Journal of Medicine, 358*(16): 1663-71.
13. Hsiang YH, Hertzberg R, Hecht S, Liu LF. (1985). Camptothecin induces protein- linked DNA breaks via mammalian DNA topoisomerase I. *The Journal of Biological Chemistry, 260*(27): 14873-8.
14. Samiya Rehman , Ken Keefover-Ring, Ih- san ul Haq, Erum Dilshad, Mohammad Im- ran Khan, Nosheen Akhtar and Bushra Mirza, " Drier climatic conditions increase withanolide content of *withania coagulans* enhancing its inhibitory potential against human prostate cancer cells", Applied Bio- chemistry and Biotechnology, Springer.
15. Kirthikar KR, Basu BD (1933) Indian Medical Plants. 2d ed. Bishen Singh Mahen- dra Pal Singh, Delhi
16. Atal CK, Sethi PDA (1963) Preliminary chemical examination of Withania coagu- lans. Indian J. Pharm 25: 163-164.
17. Maryam Khodaei, Mehrana Jafari, Mitra Noori. Remedial Use of Withanolides from Withania Coagolans (Stocks) Dunal, Ad- vances in Life Sciences 2012, 2(1): 6-19
18. Hsiang YH, Hertzberg R, Hecht S, Liu LF. (1985). Camptothecin induces protein- linked DNA breaks via mammalian DNA topoisomerase I. *The Journal of Biological Chemistry, 260*(27): 14873-8.
19. Sparano JA, Wang M, Martino S, Jones V, Perez EA, Saphner T, Wolff AC, Sledge GW Jr, Wood WC, Davidson NE. (2008). Weekly paclitaxel in the adjuvant treatment of breast cancer. *The New England Journal of Medicine, 358*(16): 1663-71.
20. M. Choudhary et. al. "Antifungal steroidal lactones from Withania coagulance" Phyto- chemistry 1995; 40: 1243–1246.
21. Rahman Atta-Ur, Choudhary MI. New natural products from medicinal plants of Pakistan. Pure Appl Chem 1998; 70:385–389.
22. Maurya R et al. Coagulanolide, a withano- lide from *Withania coagulans* fruits and an- tihyperglycemic activity. Bioorg Med Chem Lett2008:18:6534-6537.
23. Atta-ur-Rahman et. al.,"Two new ergostane type steroidal lactones from *Withania co- agulans*", Journal of natural products1998, 61, 812-814.
24. Rahman Atta-Ur et al. Five new withano- lides from *Withania coagulans*. Heterocy- cles 1998; 48: 1801–1811.
25. Mesaik MA et al. Biological and molecular docking studies on coagulin-H: human IL-2 novel natural inhibitor. Mol Immunol 2006; 43: 1855–1863.
26. Budhiraja RD et al. Protective effect of 3-b- hydroxy-2,3dihydrowithanolide F against CCl4 induced hepatotoxicity. Planta Med 1986; 28–29.
27. Budhiraja RD et al. Anti-inflammatory ac- tivity of 3-b-hydroxy- 2,3-dihydro- withanolide F. Planta Med 1984; 50: 134–136.
28. Budhiraja RD, Sudhir S. Review of biologi- cal activity of withanolides. J Sci Ind Res 1987; 46: 488–491.
29. Ramaiah PA et al. Spectroscopic studies on a withanolide from *Withania coagulans*. Phytochemistry

1984; 23: 143–149.

30. Rahman Attar-Ur et. al. "New Withanolides from withania spp.", Journal of Natural Products, 1993;56; 1000-1006.
31. Rahman Atta-Ur et.al.,"Two new ergostane type steroidal lactones from *Withania co- agulans*",Journal of Natural Product 1998; 61: 812-814.
32. Rahman Atta-Ur et al. New withanolides from *Withania coagulans*. Chem Pharm Bull 1998; 46: 1853–1856.
33. Rahman Atta-Ur et al. Withanolides from *Withania coagulans*. Phytochemistry 2003; 63: 387–390.
34. Alam Nur-e et al. A novel dimeric podo- phyllotoxin-type lignand and a new witha- nolide from *Withania coagulans*. Helv Chim Acta 2003; 86: 607–614.
35. Velde VV et al. Potential biogenetic precur- sors of withanolides from *Withania coagu- lans*. Phytochemistry 1983; 22: 2253–2257.
36. Neogi P. et al. Withacoagin, a new withano- lide from *Withania coagulans* roots. Bull Chem Soc Jpn 1988; 61: 4479–4481.
37. Salam A, Wahid MA. Free sugars and a ga- lactoaraban from *Withania coagulans* seeds. Pakistan J Biochem 1969; 2: 18–21.
38. Roshni Barad et. al., "*Withania coagulans* and Psidium guajama- An overview", IR- JPAS-2013:3(3);42-47.
39. Salwan C., Singh A., Mittal A., Singh P.,"Investigation of the Pharmacognistical, Phytochemical and Antioxidant Studies of Plant *Withania coagulans* Dunal", Journal of Pharmacognosy and Phytochemistry, 2012,1,36-44.
40. Zeynab Yousefian et.al. , "Production of the Anticancer Compound Withaferin A from Genetically Transformed Hairy Root Cul- tures of *Withania Somnifera*", Natural prod- uct communications 2018 vol. 13 No. 08,943-948.
41. Vandana Gupta, Bipin Bihari Keshari, "*Withania coagulans* Dunal (Paneer Doda): A review", International Journal of Ay- urvedic and Herbal Medicine 3:5 (2013) 1330–1336.
42. Kreis W, Muller-Uri F (2010) Biochemistry of sterols, cardiac glycosides, brassinoster- oids, phytoecdysteroids and steroidsaponins. In: Michael W (ed) Annual Plant Reviews, vol 40. Wiley-Blackwell, Singapore, pp304– 363.
43. Rakesh Maurya, Akanksha and Jayendra, "Chemistry and pharmacology of *Withania coagulans*: an Ayurvedic remedy", Journal of Pharmacy and Pharmacology: 2010, 62: 153–160.
44. Lavie D, Glotter E, Shvo Y (1965 a) Con- stituents of Withania somnifera. III. The side chain of withaferin A. J ORG CHEM 30:1774–1778.
45. Huang Y, Liu JK, Mühlbauer A, Henkel T (2002) three novel taccalonolides from the tropical plant Tacca subflaellata. HELV MED ACTA 85: 2553–2558 .
46. Khan PM, Malik A, Ahmad S, Nawaz HR (1999) Withanolides from Ajuga parviflora. J NAT PROD 62:1290–1292.
47. Shohat B., Gitter S., Abraham A., Lavie D., "Antitumor activity of Withaferin A", Can- cer chemotherapy report,51(1967) 271.
48. Fuska J., Fuskova A., Rosazza J., Nicholas A. W., Neoplasma 31(1984)31.
49. Mathur D and Agrawal R C. Evaluation of in vivo antimutagenic potential of fruits ex- tracts of *Withania coagulans*. Der Pharma Chemica, 3 (4): 2011, 373-376.
50. Gopinath S. M., Ismail Shareef M., Sonia Gupta and Ayushi Gupta, In-vitro anticancer activity evaluation of *withania coagulans* on breast cancer cell lines, World journal of pharmacy and pharmaceutical sciences Volume 6, Issue 9, 933-940.
51. Jain R, Kachhwaha S., Kothari SL. "Phytochemistry, Pharmacology and Bio- technology of *Withania somnifera* and *Withania coagulans*: a review", J. Med. Plants Res.2012;6;5388-99.
52. Uddin Q., Samiulla L., Singh VK, Jamil SS, "Phytochemical and Pharmacological profile of *Withania somnifera* Dunal; a review, J. Appl. Pharm Sci. 2012;2;170-5.
53. Devi PU, Sharada AC, Soloman FE. "In vivo growth inhibitory and radio sensitizing effects of withaferin A on mouse Ehrlich ascites carcinoma", Cancer Lett 1995;95:189-93.
54. Devi PU, Sharada AC, Soloman FE, Udupa N, Srinivasan KK, " Antitumour and radio sensitizing effects of Withaferin A on mouse Ehrlich ascites carcinoma in vivo, Acta On- col 1996;35:95-100.
55. Mathur D,Agrawal RC, " Anticarcinogenic potential of *withania coagulans* fruit against skin papilomagnesis in Swiss albino mice", Rec Res Sci Tech 2013;5:1-4.
56. Ahmad R, Fatima A, Srivastava AN, Moh- sin Ali Khan, "Evaluation of apoptotic activ- ity of *Withania coagulans* methanolic ex- tract against human breast cancer and vero cell lines", Journal of Ayurveda and Integra- tive Medicine.
57. Prasad SK, Kumar R, Patel DK, Hemalatha S (2010) Wound healing activity of *Witha- nia coagulans* in streptozotocin-induced dia- betic rats. Pharm Biol 48 (12):1397-1404.
58. Senthil V, Ramadevi S, Venkatakrishnan V, Giridharan P. Lakshmi BS, Vishwakarma RA, Balakrishnan A (2007) Withanolide induces apoptosis in HL-60 leukemia cells via mi-tochondria mediated cytochrome c release and caspase activation. CHEM- BIOL INTERACT 167:19–30.