



## EFFECT OF *WITHANIA COAGULANS* AND *PSIDIUM GUAJAVA* ON DIABETES MELLITUS

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### ABSTRACT

*Withania coagulans* and *Psidium guajava* are used for treatment of Diabetes mellitus. Both are compared for its actions on Diabetes mellitus. *Withania coagulans* and *Psidium guajava* are collected and authenticated. Physico-chemical Parameters of both herbs have been presented. Successive solvent extraction of *Withania coagulans* and *Psidium guajava* were performed. Preliminary Phytochemical Examination shows the presence of Steroids, Saponin, Alkaloids, Flavonoids, glucoside and Tannins in various extracts. Pharmacological evaluations by In vivo oral glucose tolerance test indicate that aqueous extract of *Withania coagulans* reduced blood sugar level significantly as compared to aqueous extract of *Psidium guajava*. In vivo anti diabetic activity was performed using Streptozotocin induced diabetic rats. 10 mg of glipizide produced 70% reduction in blood sugar level, 1106 mg of aqueous extract of *Withania coagulans* and 1193 mg of aqueous extract of *Psidium guajava* also produced 70% reduction in blood sugar level. Thus, 10 mg of glipizide is equivalent to 1106 mg of aqueous extract of *Withania coagulans* and also equivalent to 1193 mg of aqueous extract of *Psidium guajava*. *Withania coagulans* is more potent than *Psidium guajava* for diabetic patients in controlling their blood glucose level.

**Key words:** *Withania coagulans*, *Psidium guajava*, Anti diabetic Activity, OGTT test.

### INTRODUCTION

#### Herbal Medicine

In olden times, vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. But the scenario has changed now; herbal medicines are being manufactured on the large scale in Pharmaceutical units, where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of single drugs and formulation, quality control parameters. The use of herbal medicine due to toxicity and side effects of allopathic medicines, has led to sudden increase in the number of herbal drug manufactures [1].

Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today

because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health. World Health Organization (WHO) has defined herbal medicines as finished labeled medicinal product that contain active ingredients, aerial or underground parts of the plant or other plant material or combinations [2].

#### Diabetes

Diabetes mellitus is a chronic endocrine disorder in which there is an absolute or relative deficiency of insulin or deranged secretion and/or function of islet cells of pancreas. Clinically it is a complex syndrome characterized by polydipsia, polyuria, exhaustion, rapid weight loss, increasing short sightedness, nausea, vomiting, abdominal pain etc. Diabetes is associated with (1) hyperglycemia (secondary to deranged secretion and/or action of insulin); (2) specific micro angiopathy

with thickening of capillary basement membranes leading to retinopathy and nephropathy; (3) macro angiopathy producing accelerated atherosclerosis and other long term complications like autonomic neuropathy, cardio myopathy, increased tendency to infections etc [3].

### Diabetes Mellitus

- Type 1: Insulin-dependent diabetes mellitus (IDDM) (formerly known as juvenile-onset diabetes)
- Type 2: Non-insulin-dependent diabetes mellitus (NIDDM) (formerly known as Maturity onset diabetes of the young (MODY) characterized as Non-obese and obese
- Other types: Malnutrition related diabetes mellitus (MRDM) and other types associated with iatrogenic conditions[4]

### Secondary Diabetes

- Pancreatic disease (Chronic pancreatitis, hem chromatists, pancreatomy etc.)
- Hormonal (Cushing's syndrome, acromegaly, pheochromocytoma)
- Drug or chemical induced (chlorthiazide, used in the treatment of Kala-Azar etc.)
- Insulin receptor abnormalities (acanthosis, nigricans, lip dystrophy etc.)
- Genetic syndromes (ataxia telangiectasia, progeria, Laurence-Moon-Biedl syndrome.

**Fig 1. Symptoms of Diabetes**

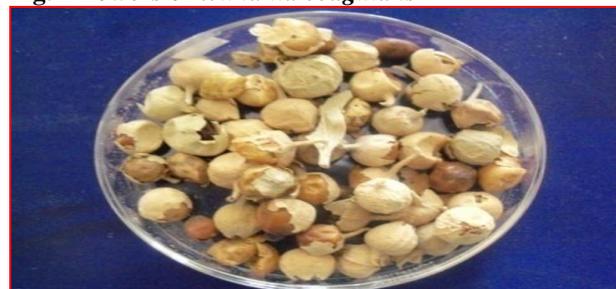


### *Withania coagulans*

Biological source: It consist of flower of *Withania coagulans*

Family: Solanaceae [5,6]

**Fig. 2 flowers of *Withania coagulans***



**Distribution:** Iran, Afghanistan, Pakistan and India. Fairly, common in dry hot and stony places up to 1700 m. The fruit is emetic and diuretic and also has coagulating properties.

### Vernacular names

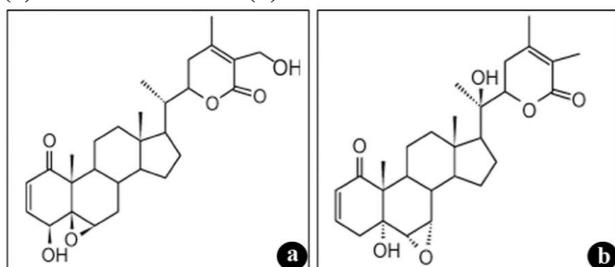
- Hindi - Akri, Punir.
- Bengali - Ashwagandha
- English - Indian cheese maker
- Punjabi — Spin bajja, panir

### Chemical Constituents

Active compounds: Withanolides are a group of steroidal lactones found among members of Solanaceae. Withanolides are named after the name of the source plant *Withania* species. They are generally defined as C-28 steroidal lactones. The presence of a lactones ring with C-22 and C-26 oxygen functions to form a six or five member lactones ring on an Ergostane skeleton, intact ergostane or rearranged, constitutes the basic structure of all Withanolides. The Withanolides skeleton may be defined as 22-hydroxy ergostane-26-oic acid-26, 22-olide. Modifications of either the carboxylic skeleton or of the side chains result in many novel structural variants of Withanolides which are described as modified Withanolides or ergostane-type steroids related to Withanolides .It was reported that Withanolides posses anti-tumour, anti-antigenic, chemo preventive and anti-inflammatory activities. Therefore, Withanolides may represent useful leads for the development of potential anti-cancer drugs. Withanolides are reported to have antitumor activity. Component Withanolides, Withaferin A, inhibits angiogenesis. Withanolides have also been reported to inhibit metastatic and quinine reductase activity. Some of them have been show to preferentially affect events in the cholinergic signal transduction cascade of the cortical and the basal forebrain, indicating their promise for the treatment of Alzheimer's diseases. Withanolides mediate their effects through suppression of the transcription factor nuclear factor-EB (NF-EB). The evidence is multifaceted. NF-EB is activated by various carcinogens, tumour promoters, and conditions in the tumour microenvironment (hypoxia and acidic pH), most inflammatory agents activate NF-EB. Chemo preventive agents have been shown to suppress NF- EB activity on Withanolides is potent suppressors of NF-EB activation induced by various agents and that this suppression is mediated through inhibition of IKK. This mechanism accounts for the ability of Withanolides to suppress the expression of gene products that regulate apoptosis, proliferation, angiogenesis and invasion. Anti proliferative, Preapoptotic, anti-invasive, anti-osteoclastogenic, anti antigenic, anti-metastatic, radio sensitizing, anti arthritic and cardio protective effects assigned to Withanolides may be mediated in part through

the suppression of NF-EB and NF-EB regulated gene products. Diverse pharmacological activities reported that Withanolides, Withaferin-A includes anti-inflammatory, anti-tumor and anti-oxidant properties. Some studies have demonstrated that Withaferin-A has potent anti-inflammatory, anti-oxidant and antitumor properties [7].

**Fig 3. Structures of important Withanolides:**  
(a) Withaferin A and (b) Withanolide



### Pharmacology

The chemical constituents of *Withania coagulans* have always been of great interest to the scientific community. The biologically active chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine, etc), steroidal compounds including ergostane-type steroidal lactones, Withaferin a, Withanolides A-Y, withasomniferin A, withasomidienone, withasomniferols A-C, withanone, etc. Withaferin A (4 $\beta$ , 27-dihydroxy-5 $\beta$ , 6 $\beta$ -epoxy-1-oxowitha-2, 24-dienolide), and withanolide A (5 $\alpha$ , 20 $\alpha$ -dihydroxy- 6 $\alpha$ , 7 $\alpha$  -epoxy-1-oxowitha-2, 24-dienolide) are the main withanolide active principles isolated from the plant. These are chemically similar but differed in their chemical constituents [8].

### Anti-inflammatory activities

The anti-inflammatory potential of *W. coagulans* has been studied in details by several workers. It showed that the aqueous extract of fruits of *W. coagulans* has significant anti-inflammatory activity at 10 mg kg<sup>-1</sup> in sub-acute models of inflammation, such as granuloma formation and formalin-induced arthritis in rats. It reported that *it* possesses efficient anti-inflammatory activity as compared with hydrocortisone, a common anti-inflammatory drug. The effect *an* on glycosaminoglycan sync-thesis in the granulation tissue of carrageen induced air pouch granuloma was studied. Oral administration of 1000 mg kg<sup>-1</sup> root powder decreased the glycosaminoglycan content by 92%, which was much higher than that of the hydrocortisone and phenylbutazone. It studied the granuloma-tissue formation inhibiting activity of various fractions of an extract of the aerial parts of drugusing subcutaneous cotton-pellet implantation in rats. The methanol fractions of the extract showed high anti-inflammatory activity as compared to

that of a 5 mg kg<sup>-1</sup> dose of hydrocortisone sodium succinct. The activity in both the species was attributed to the high content of biologically active steroids in the plant, of which Withaferin A is known to be a major component.

### Anticancer and chemo protective activities

The anticancer effect of *Withania* has been studied extensively and it was found that it is the most effective agent in preventing cancer through its ability to reduce the tumour size. Treatment of root extract 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 tumour-inhibitory activity against cells derived from 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<sup>-1</sup> body weight daily orally for seven months) and urethane (125 mg kg<sup>-1</sup> biweekly for seven months) reduced tumor incidence significantly. Additionally, in a different study the aqueous extract of *W. coagulans* was used for anti-cytotoxic effect in chicken lymphocytes and remarkable inhibitory activity of diethyl sulfoxide (DMSO)-induced cytotoxicity with a decrease in TNF-G production was reported.

### Immunomodulatory activity

Withaferin A has been reported in various studies to possess both immune-activating and immunosuppressive properties. Withaferin A has specific immunosuppressive effects on human B and T lymphocytes viz. antigen recognition and proliferative capacity of B and T lymphocytes in mice, the Ashwagandha extract was able to suppress the cyclophosphamide-induced potentiating of delayed type hypersensitivity (DTH) reaction. A protective effect in cycloid phosphamide-induced myeloid suppression was observed in animals treated with this extract. In another study, the aqueous suspension of root powder inhibited the mutagen induced lymphocyte proliferation and DTH reaction in rats. The root extract also enhanced total white blood cell count, inhibited delayed-type hypersensitivity reactions and enhanced phagocyte activity of macrophages. Significant increases in haemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight were observed in-treated mice compared to untreated control mice. It isolated novel Withanolides, withacoagulins from the areal parts of *W. coagulans* and reported the inhibitory activity of the extract on T and B-lymphocyte proliferation in murine spleen cells.

### *Psidium guajava*

Biological source: It consists of fruits of *Psidium guajava*

Family: Myrtaceae [9].

**Fig. 4. Herb of *Psidium guajava***



**Distribution:** It is a low evergreen tree or shrub 6 to 25 feet high, with wide-spreading branches and square, downy twigs, is a native of tropical America. It is a common vegetation cover by roads and in waste places in Hawaii. Guava is a tropical and semitropical plant. It is well known in the islands for its edible fruit. It is common in the backyards. The branches are crooked, bringing opposite leaves. The flowers are white, incurved petals, 2 or 3 in the leaf axils; they are fragrant, with four to six petals and yellow anthers. The fruit is small, 3 to 6 cm long, pear-shaped, reddish-yellow when ripe.

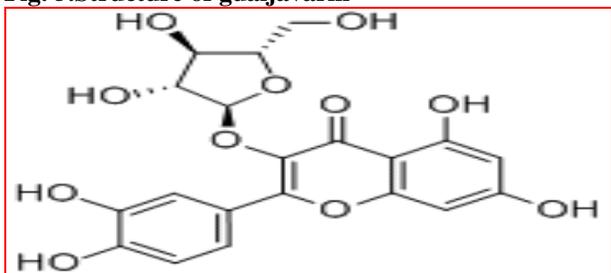
#### Vernacular names

- Common guava
- yellow guava
- apple guava
- Bayabas
- Sans: Perala; Amratafalām; Amruta-phalam.

#### Chemical Constituents

The fruits also contain vitamin C, vitamin A, iron, calcium and phosphorus. Guavas are up to 5 times richer in vitamin C than oranges. Manganese is also present in the plant in combination with phosphoric, oxalic and malice acids. The fruit contains saponin combined with oleanolic acid. Morin-3-O- $\alpha$ -L-lyxopyranoside and morin-3-O- $\alpha$ -L-arabopyranoside and flavonoids, guaijavarin and quercetin.

**Fig. 5. Structure of guaijavarin**



In the headspace, the major constituents are: hexanal (65.9%),  $\gamma$ -b(7.6%), (E)-2-hexenal (7.4%), (E,E)-2,4-hexadienal (2.2%), (Z)-3-hexenal (2%), (Z)-2-hexenal (1%), (Z)-3-hexenyl acetate (1.3%) and phenol (1.6%), while  $\beta$ -caryophyllene (24.1%), nerolidol (17.3%) 3-phenylpropyl acetate (5.3%) and caryophyllene oxide (5.1%) are the major volatile constituents present in the hydro distilled essential oil. The leaves contain essential oil with the main components being  $\alpha$ -pinene,  $\beta$ -pinene. Pinene leaves contains fixed oil 6%, and volatile oil 0.365% contains 'glycogen' 4.14% Avicularin. Bark contains 12-30% of tannin and one source says it contains tannin 27.4%, or roots also rich in tannin. The plant also contains leukocyanidins. The seeds contain 14% oil on dry weight, with 15% proteins and 13% starch. Ten phenolic and flavonoids compounds including one new acylated flavones glycoside were isolated. The structures of the new compound quercetin-3-O- $\beta$ -D-(2"-O-galloylglucoside)-4'-O-vinylpropionate and of the known compounds are elucidated. Another biologically interesting compound in the plant is guaijavarin, a glycoside (arabinopyroside) of quercetin. The leaves also contain essential oils and trite pen seeds are very small but abundant in the fruit and have been reported. Twigs contain calcium (0.30-1.00%), magnesium (0.06-0.30%), and phosphorous (0.10-m) [10].

#### Pharmacological Activity

##### Anti-bacterial activity

The bark was also shown to exhibit antibacterial effects. Four antibacterial compounds were isolated from leaves of guava (*P. guajava* new flavonoids glycosides, morin-3-O- $\alpha$ -L-lyxopyranoside and morin-3-O- $\alpha$ -L-arabopyranoside, and two known flavonoids, guaijavarin and quercetin *Psidium* sedermatophytes viz. *Trichophyton tonsurans*, *T. rubrum*, *Trichosporon beigellii*, *Microsporium fulvum*, *M. gypseum* and *Candida albicans*. Bark tincture has higher efficacy in controlling the mycelia growth of dermatophytes than the leaf tincture. The tincture showed fungicidal property in different concentrations but exhibited only fungi static property in case of *C. albicans*. Another good effect with the methanol extract. Three antibacterial substances have been detected in the leaves which are derivatives of quercetin. As in the barpolyphenols and many other substances are present [11].

##### Anti diarrhoeal activity

Leaf infusion is used for constipation, and in Adamawa with "red" potash for dysentery; a decoction is taken in Senegal to combat diarrhoea and dysentery; the shoots and roots may also be hile in neighbouring The Gambia the leaves are chewed for queasy tummy, a treatment that is said to work very well. A leaf infusion is drunk in Hawaii and Trinidad and in Indonesia for

medical purposes. The ripe fruit is mild laxative. The unripe fruit is astringent, anti-diarrhoeic, and has medicinal use the ripe fruit is a good aperient, and should be eaten with the skin, for without it, costiveness results. The unripe fruit is said to be indigestible, causing vomiting and feverishness, but it is sometimes employed for diarrhoea.

#### Anti-inflammatory effect

The anti-inflammatory and analgesic activities of 70% ethanol extract of *Psidium guajava* in rats using carrageen induced hind paw oedema model. Extracts which exhibited anti-inflammatory activity were screened for analgesic activity using the Randall-Selitto method in rats. The extracts were administered at a dose of 300 mg/kg; p.o. Aspirin (300 mg/kg, p.o.) was employed as the reference drug. *Psidium guajava* leaves, showed significant anti-inflammatory activity with percentage inhibitions of 58.27%. The anti-inflammatory and analgesic activities of 70% ethanol extract of *Psidium guajava* leaves was investigated in rats using the carrageen induced hind paw oedema model. Extracts which exhibited anti-inflammatory activity were screened for analgesic activity using the Randall-Selitto method in rats. The essential oil has also been proven to have anti-inflammatory effect. The essential oil, steam-distilled from leaves of *P. guajava* leaves, was given orally rats to study its effects on the oxidative and proliferative phases of the inflammatory reaction (Carrageen an-induced paw oedema and cotton pellet induced granuloma models). The essential oil (0.8 mg/kg) significantly reduced oedema formation induced by Carrageen an. The essential oil (0.4 and 0.8 mg/kg) significantly reduced granuloma formation induced by cotton pellets. Another paper confirmed the anti-inflammatory and also showed significant antipyretic activity and anti-arthritic activity in rats. In Peru it is said to be good for oedema and was found to inhibit paw oedema induced by Carrageen an in rats.

**Conjunctivitis:** Flowers are also used as a poultice for conjunctivitis and are also applied to painful eye conditions such as sun strain, conjunctivitis or eye injuries.

**Coughs:** Boiled with lemon grass to make a decoction that is drunk for coughs. A decoction is also taken in Senegal for trachea bronchitis. The leaves are also used for cough in Peru.

**Diabetes:** The leaves are also used for several other ailments including diabetes. The leaf infusions are used in the Cape for diabetes Water in which the fruit is soaked is good for thirst in diabetes. Malaria the leaves are used as an ingredient in the preparation of fever "teas". They are

also used as part of the pot herb used in steam treatment for malaria. Indeed, the main ethno therapeutic use in Africa is said to be for malaria. *Psidium guajava* stem bark extract contained anthraquinones, flavonoids, seccoirridoids and terpenoids and was found to be effective for the treatment and/or prophylaxis of malaria in KwaZulu Natal province of South Africa. The in vitro antiplasmodial assay was carried out using a chloroquine-sensitive strain of malaria parasite.

## MATERIALS AND METHODS

### Collection and authentication of herb

*Withania coagulans* is Pakistan herb. It is not cultivated and grown in India. It is cultivated in Pakistan .We have studied anti-diabetic activity on flower of *Withania coagulans*. We have collected it from G.Y.Hakim, Raopura, Vadodara. The small herb of *Psidium guajava* was collected from Surendra Nursery, Vadodara. It was grown in herbal garden in our college; Sigma Institute of Pharmacy. Authentication was done by Dr. Padamnabhi Nagar Assistant Professor, Department Of Botany, The M.S University, Vadodara.

### Physicochemical parameters

#### Ash value

**Total ash:** Weighed 2 gm of the powdered drug was taken in a tarred silica dish and it was incinerated at a temperature not exceeding 450°C until free from carbon. The sample was cooled and weighed. If a carbon free ash cannot be obtained in this way, the charred mass was exhausted with hot water. The residue was collected on ash less filter paper were incinerated the filtrate was evaporated to dryness, and ignited at a temperature not exceeding 450°C. The percentage of ash was calculated with reference to the air dried drug [12].

Total Ash value in percentage =  $(z - x) \times 100/y$

Where;

$z$  = weight of the dish + ash (after complete incineration)

$x$  = weight of the empty dish ,  $y$  = weight of the drug taken.

#### Extractive Value

The determination of Extractive values helps to determine the amount of soluble constituents in a given amount of medicinal plant material, when extracted with solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phyto constituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of drug and solvent used. The use of single solvent can also be used by means of providing preliminary information of quality of a particular drug sample.

**A. Petroleum Ether Soluble Extractive:** Proceed as

directed for the determination of alcohol-soluble extractive, using Petroleum Ether instead of alcohol.

**B. Benzene Soluble Extractive:** Proceed as directed for the determination of alcohol-soluble extractive, using benzene instead of alcohol.

**C. Chloroform Soluble Extractive:** Proceed as directed for the determination of alcohol-soluble extractive, using chloroform instead of alcohol.

**D. Acetone Soluble Extractive:** Proceed as directed for the determination of alcohol-soluble extractive, using acetone instead of alcohol.

**E. Alcohol Soluble Extractive:** Macerate 5 g of the air-dried drug coarsely powdered, with 100ml of alcohol of the specified strength in a closed flask for twenty-four hours shaking frequently during six hours and allowed to stand for eighteen hours. Filter rapidly taking precaution against loss of alcohol; evaporate 25ml of the filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 105°C, and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

**F. Water Soluble Extractive:** Proceed as directed for the determination of alcohol-soluble extractive, using chloroform water instead of alcohol.

#### Moisture Content (Loss on Drying)

Accurately weighed 10 gm of drug was placed in a tarred evaporating dish. After placing the drug in to the tarred evaporating dish it was dried at 105 °C for 5hours. Then sample was weighed. The drying and weighing was continued at one hour interval until constant weight was reached. Percentage moisture contents calculated on the basis of sample taken.

Loss on drying (%) =  $\frac{\text{Loss in weight}}{\text{Weight of the drug in gms}} \times 100$  [13].

#### Phytochemical evaluation

##### Successive solvent extraction

One kilogram of shade-dried leaves of Herbs were powdered and successively extracted with petroleum ether, benzene, chloroform, acetone and ethanol (95%) by utilising soxhlet apparatus and finally macerated with water (cold maceration). The nature and yield of the extracts obtained by successive extraction of the herb were recorded [14].

#### Qualitative Chemical Investigation of Extracts

Qualitative chemical tests were conducted for all the extracts of leaves of herb to identify the various phyto constituents like alkaloids, glycosides, carbohydrates, phenolic and tannins, phyto sterols, fixed oils and fats, proteins and amino acids, flavonoids, saponins, etc.

#### Pharmacological evaluation

##### In vivo oral glucose tolerance test

Groups: Group 1: Control (6 Rat) Treated group (Drug –

1000 mg / kg suspended in Distilled Water) Group contains 6 rats (fasted overnight before experiment).

Group 2: Treated group *Withania coagulans* (Drug - 1000 mg / kg suspended in Distilled Water) Group contains 6 rats (fasted overnight before experiment)

Group 3: Treated group *Psidium guajava* (Drug - 1000 mg / kg suspended in Distilled Water) Group contains 6 rats (fasted overnight before experiment)

Procedure: The animals are fasted overnight, Initial Fasting Blood Glucose (FBG) will be estimated then the Drug will be given 1000mg / kg through oral route (Suspended in distilled water). After half an hour of the Drug administration 2g/kg of glucose will be administered (Dissolved in Water) to all animals including negative control then glucose tolerance will be studied up to 3h [15].

#### Observation: For each animal.

1. Initial Blood Glucose Level
2. Blood Glucose Level after Half an hour of Glucose administration
3. Blood Glucose Level after 1 hour of Glucose administration
4. Blood Glucose Level after 2 hour of Glucose administration
5. Blood Glucose Level after 3 hour of Glucose administration [16]

#### IN VIVO ANTI DIABETIC ACTIVITY

##### Preparation Aqueous Extract

Three Kg of the shade dried powder of flower of *Withania coagulans* and *Psidium guajava* are mixed with 24 L of water. The mixture is heated at 70°-80° C until the quantity of water was reduced to approximately half (12 L). The whole mass is filtered. The filtrate is concentrated by heating at 80° C until 6 L of extract remained. This is filtrate I. The marc is mixed with fresh 12 L of water and heated at 60°-70° C till it gets reduced to half (6 L). The whole mass is filtered. The filtrate is concentrated by heating at 80° C until approximately 3 L of the extract remained. This is filtrate II. The residue is then discarded. Both the filtrate I and filtrate II are mixed and concentrated at 90° C till it get reduced to 300 ml. Sodium benzoate (2%) is added as preservative to the extract which is stored at 15°-20° C in well closed glass container. The concentration of dried powder is 10 gm/ml in this final extract [17].

#### Induction of Streptozotocin Induced Diabetes

Healthy female albino Wister rats weighing between 180-250 gm are used in the experiment. All the animals are housed in a group of 3 rats per cage at 27<sup>0</sup>-30<sup>0</sup> C with 12 hours alternating light and dark cycles. Animals have free access to standard feed and water ad libitum. Diabetes is induced by a single tail vein injection

45mg/kg of Streptozotocin, dissolved in 0.1 M citrate buffer of pH 4.5. Diabetes is confirmed by measuring blood sugar by glucometer. The animals are divided into five different groups as follows. The animals are given the aqueous extract of *Withania coagulans* and *Psidium guajava* (1000 mg/kg) P.O. daily. The treatment is continued for six weeks. Blood glucose level is measured by glucometer [18].

## RESULT AND DISCUSSION

### Collection and Authentication of Herbs

*Withania coagulans* is not cultivated and grown in India. It is cultivated in Pakistan. We have studied anti-diabetic activity on flower of *Withania coagulans*. We

have collected it from G.Y.Hakim, Raopura, Vadodara. The small herb of *Psidium guajava* was collected from Surendra Nursery, Vadodara. It was grown in our herbal garden in our college; sigma institute of pharmacy. Authentication was done by Dr. Padamnabhi Nagar, Assistant Professor, Department of Botany, The M.S University, Vadodara.

### Physicochemical Parameters

Physicochemical parameters like ash value, extractive value and moisture Content performed of both herbs. Preliminary study of *Withania coagulans* and *Psidium guajava* powder had shown presence of total ash content  $6.77 \pm 0.12$  and  $5.75 \pm 0.16$  %w/w respectively.

**Table 1. Study Plan of in vivo anti-diabetic activity**

Group	Treatment	Treatment Substance Dose (mg/kg/day) and Route of Administration
1	Negative Control	Normal Saline (25ml/Kg) P.O.
2	Positive Control	Streptozotocin(45 mg/kg) I.V.
3	Evaluating Drug Control	Streptozotocin(45mg/kg) I.V. + <i>Withania Coagulans</i> (1000 mg/kg) P.O.
4	Evaluating Drug Control	Streptozotocin (45 mg/kg) I.V. + <i>Psidium guajava</i> (1000/kg) P.O.
5	Standard Drug	Streptozotocin (45mg/kg) I.V. + Glipizide (10 mg /kg) P.O.

**Table 2. Physicochemical parameters of *Withania coagulans***

Physicochemical Parameters		Result
<b>Ash Value</b>		
1	Total Ash % W/W	$6.77 \pm 0.12$
<b>Extractive Value</b>		
1	Pet Ether Soluble Extractive	5% W/W
2	Benzene Soluble Extractive	4% W/W
3	Chloroform Soluble Extractive	5% W/W
4	Acetone Soluble Extractive	3% W/W
5	Alcohol Soluble Extractive	8% W/W
6	Water Extractive	11% W/W
Moisture Content (Loss On Drying) % W/W		$4.6 \pm 0.19$

**Table 3. Physicochemical parameters of *Psidium guajava***

Physicochemical Parameters		Result
<b>Ash Value</b>		
1	Total Ash % W/W	$5.75 \pm 0.16$
<b>Extractive Value</b>		
1	Pet Ether Soluble Extractive	3% W/W
2	Benzene Soluble Extractive	4.75% W/W
3	Chloroform Soluble Extractive	6.5% W/W
4	Acetone Soluble Extractive	7.25% W/W
5	Alcohol Soluble Extractive	11.5% W/W
6	Water Extractive	13% W/W
Moisture Content (Loss On Drying) % W/W		$14 \pm 0.09$

**Table 4. % yield of extracts obtained by successive solvent extraction of *Lithuania coagulans***

Phytochemical Parameters		Result
% yield of extracts obtained by successive solvent extraction		
1	Pet Ether	4.7 % w/w
2	Benzene	8.64 % w/w
3	Chloroform	5.38 % w/w
4	Acetone	4.06 % w/w
5	Ethanol	10.16 % w/w
6	Water	35 % w/w

**Table 5. % yield of extracts obtained by successive solvent extraction of *Psidium guajava***

Phytochemical Parameters		Result
% yield of extracts obtained by successive solvent extraction		
1	Pet Ether	2.04 % w/w
2	Benzene	1.04 % w/w
3	Chloroform	9.38 % w/w
4	Acetone	7.16 % w/w
5	Ethanol	22.46 % w/w
6	Water	31.51 % w/w

**Qualitative Phytochemical evaluation**

**Table 6. Qualitative chemical examinations of various extracts of *Withania coagulans***

General Chemical Tests of Phytoconstituents							
S. No.	Solvent Used In Extraction	Alkaloid	Glycoside	Steroid	Flavonoids	Saponin	Tannin
1	Pet. Ether	+	—	—	—	—	—
2	Benzene	+	—	—	—	—	—
3	Chloroform	+	—	++	++	—	—
4	Acetone	+	—	+	++	—	—
5	Alcohol	++	+	+++	+++	++	++
6	Water	+++	+	+++	+++	++	++

- Petroleum ether extract contain alkaloids,
- Benzene extract contain alkaloids
- Chloroform extract contain alkaloid, steroid, flavonoids.
- Acetone extract contain alkaloid, steroid, flavonoid.
- Ethanol extract contain alkaloid, glycoside, Saponin, tannins, flavonoids, steroid
- Water extract contains alkaloid, glycoside, Saponin, tannins, flavonoids, and steroid.

**Table 7. Qualitative chemical examinations of various extracts of *Psidium guajava***

General Chemical Tests of Phytoconstituents							
S. No.	Solvent used in extraction	Alkaloid	Glycoside	steroid	flavonoid	Saponin	Tannin
1	Pet. Ether	—	—	—	++	—	—
2	Benzene	+	—	—	++	—	—
3	Chloroform	—	—	—	++	—	+
4	Acetone	—	—	—	++	—	—
5	Alcohol	++	+	+	+++	++	++
6	Water	—	+	++	+++	+++	++

- Petroleum ether extract contain flavonoids
- Benzene extract contain alkaloids, flavonoids
- Chloroform extract contain flavonoids
- Acetone extract contain flavonoids
- Ethanol extract contain alkaloid, glycoside, Saponin, tannins, flavonoids, steroid
- Water extract contains glycoside, Saponin, tannins, flavonoids, steroid

## Pharmacological Activity

**Table 8. Result of *In Vivo* Oral Glucose Tolerance test**

Time (min)	Blood Sugar Level (mg/dl)		
	Control (mean± SEM )	<i>Psidium guajava</i> (mean± SEM )	<i>Withania coagulants</i> (mean± SEM )
0	85±3.44	89±1.727	93±1.770
30	95±2.088	100±1.256	99±2.155
60	146±4.169	140±3.827	120±1.880*
120	140±3.911	138±4.938	110±1.498*
180	100±3.930	93±3.631	85±2.513*

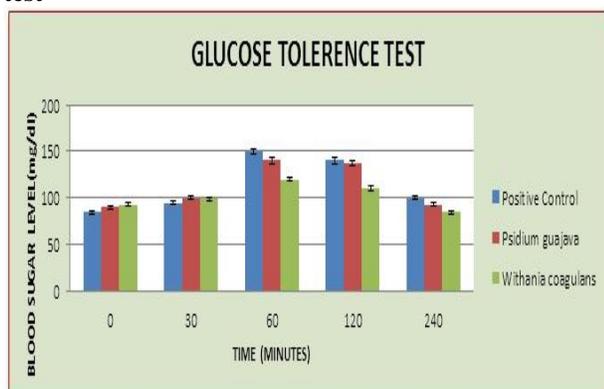
\* P< 0.05; statistically difference as compared to control

**Table 9. Result of *in Vivo* anti diabetic activity**

Days	Blood Sugar Level (Mg/dl)			
	Control(mean±SEM)	<i>Withania Coagulants</i> (mean± SEM)	<i>Psidium guajava</i> (mean± SEM)	Standard Control (mean± SEM)
0	92±2.89	448±23.15	484±13.69	382±32
42	85±3.1	284±7.2*	284±18.11*	268±17*

\* P< 0.05; statistically difference as compared to control

**Fig 6. Graphical presentation of oral glucose tolerance test**



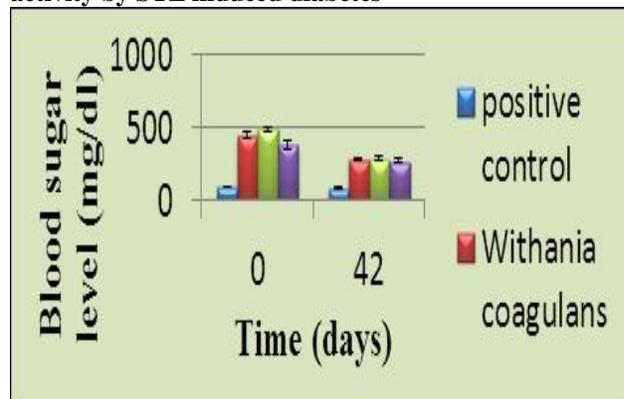
The result indicates that herb contained inorganic matter, which are insoluble in acid. Preliminary study for extractive value in different herb powder had shown. Petroleum ether, benzene, Chloroform, acetone, alcohol and water soluble extractives were found to be 5 % w/w, 4% w/w, 5% w/w, 3% w/w, 8% w/w, 11% w/w of *Withania coagulants* and 3% w/w, 4.75% w/w 6.5% w/w, 7.25% w/w, 11.5% w/w, 13% w/w of *Psidium guajava*. Water soluble extractive was higher for both herbs. Moisture content of both herbs powder was found to be 4.6 ±0.19 % w/w and 14±0.09% w/w and indicating that it is safer to prevent microbial growth.

## PHYTOCHEMICAL EVALUATION

### Successive solvent extraction

The presences of different chemical constituents in the crude drug can be detected by subjecting them to successive extraction using solvents in the order

**Fig 7. Graphical presentation of *in vivo* anti diabetic activity by STZ induced diabetes**



increasing polarity. The extracts were dried completely and kept in vacuum desiccators.

### Statistical analysis

The results were expressed as Mean±SEM, and the statistical evaluation carried out by t-test, and the probability of 0.05 was chosen as level of significance. It indicates that *Withania coagulants* is more potent as compared to *Psidium guajava*. It is statistically proved. The dose of *Withania coagulants* of aqueous extract was 1000mg/kg P.O. and it reduces blood sugar level by 63.3%. For producing 70% reduction in blood sugar level, the required aqueous extract of *Withania coagulants* is 1106 mg, thus, 1106 mg of dried aqueous extract of *Withania coagulants* gives equivalent reduction in blood sugar level. The anti-diabetic effect produced by 1106 mg of aqueous extract of *Withania coagulants* is equivalent to the anti-diabetic effect produced by 10 mg of glipizide

.The dose of *Psidium guajava* of aqueous extract was 1000mg/kg P.O. and it reduces blood sugar level by 58.7%. For producing 70% reduction in blood sugar level, the required aqueous extract of *Psidium guajava* is 1193 mg, thus, 1193 mg of dried aqueous extract of *Psidium guajava* gives equivalent reduction in blood sugar level. The anti-diabetic effect produced by 1193 mg of aqueous extract of *Psidium guajava* is equivalent to the anti-diabetic effect produced by 10 mg of glipizide. From the data obtained Streptozotocin induced diabetic rats showed a significant reduction in blood glucose levels (\* $p < 0.05$ ) with aqueous extract of *Withania coagulans* and *Psidium guajava*, Streptozotocin produced rapid induction of diabetes; the standard glipizide 10mg/kg produced a significant reduction of blood glucose levels. Aqueous extract of *Withania coagulans* and *Psidium guajava* produced a very significant reduction in blood glucose levels similar to glipizide.

## CONCLUSION

*Withania coagulans* and *Psidium guajava* are used for treatment of diabetes. Both are compared for its actions on diabetes. *Withania coagulans* and *Psidium guajava* are collected and authenticated. Physico-chemical Parameters of both herbs have been presented. Results indicate that all parameters are found within limits. Phytochemical evaluation has been presented. Successive solvent extraction is performed. It indicates that *Withania coagulans* and *Psidium guajava* are more soluble in water. It gives more yield than other solvent. The Preliminary Chemical Examination shows the presence of Steroids, Saponin, Alkaloids Flavonoids, glucoside and Tannins in various extracts. Pharmacological evaluation of by In vivo oral glucose tolerance test indicate that aqueous extract of *Withania coagulans* reduced blood sugar level as compared to aqueous extract of *Psidium guajava*. In vivo anti-diabetic activity have been performed. The dose of *Withania coagulans* of aqueous extract was 1000mg/kg P.O. and it reduces blood sugar level by 63.3%. For producing 70% reduction in blood sugar level, the required aqueous extract of *Withania coagulans* is 1106 mg. The anti-diabetic effect produced by 1106 mg of aqueous extract of *Withania coagulans* is equivalent to the anti-diabetic effect produced by 10 mg of glipizide the dose of *Psidium guajava* of aqueous extract was 1000mg/kg P.O. and it reduces blood sugar level by 58.7%. For producing 70% reduction in blood sugar level, the required aqueous extract of *Psidium*

*guajava* is 1193 mg. The anti-diabetic effect produced by 1193 mg of aqueous extract of *Psidium guajava* is equivalent to the anti-diabetic effect produced by 10 mg of glipizide. *Withania coagulans* is more potent than *Psidium guajava* for diabetic patients in controlling their blood glucose level.

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