PHARMACOGNOSTICAL, PHYTOCHEMICAL AND ANTI-DIABETIC STUDIES ON FRUITS OF *Adansonia digitata* Linn.,

A dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

CHENNAI-600 032

In partial fulfilment of the requirements for the award of degree of

MASTER OF PHARMACY

IN

PHARMACOGNOSY

Submitted by

REG. NO: 261520658

Under the guidance of

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MAY 2017

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CERTIFICATE

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INTRODUCTION

1. INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day[1]. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. Herbal medicines are the major remedy in traditional system of medicine have been used in medical practices since antiquity. Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects[2].

"Nature itself is the best physician"

- Hippocrates

The World Health Organization (WHO) has defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. The plant based raw materials are safe, preventive, curative and are particularly useful in achieving the goal of "Health to All" in a cost effective manner. Thus demand of medicinal plant is increasing in both developed and developing countries (Government of India, Planning Commission March, 2000). Undoubtedly nature has all along with the diseases. has created their cure for every diseases that arise on the plant. So, the ancient knowledge coupled with scientific principles can come into the forefront and provide us powerful remedies to treat different diseases[3].

HERBAL MEDICINE IN INDIA:

India stands first among all the Asian countries in having the knowledge on traditional system related to the use of plant species and diversity of higher plant species, which is the reason for the use of herbs in different forms in alternative systems of medicine. India has 12 mega biodiversity centres and about 45,000 plant species. This huge number of medicinal plants species possess important role in health system. Among these, about 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine. Although herbal medicine are used in various ailments, often these drugs are improperly used and only few of them have got scientifically documented. Hence plant drug need a detailed study in the light of modern medicine in bringing new herbal chemical entities[4-13].

TRADITIONAL AND ALTERNATIVE SYSTEM OF MEDICINE:

1. Traditional Chinese medicine and kampoh system
2. Ayurveda- Indian system of medicine
3. Siddha system of medicine
4. Unani system of medicine
5. Homeopathy system of medicine
6. Acupressure and acupuncture
7. Naturopathy and yoga
8. Aromatherapy
9. Hydrotherapy
10. Batch flower therapy
11. Native American healing practices
12. Tibetian system of medicine

DISEASE PROFILE:

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Most of the food we eat is turned into glucose, or sugar, for our bodies to use for energy. In pancreas, cells of Islets of Langerhans, makes a hormone called insulin to help glucose get into the cells of our bodies. Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke[14].

TYPES OF DIABETES MELLITUS: [15,16]

Type 1 Diabetes Mellitus:

It is called Insulin dependent (IDDM) or juvenile-onset diabetes in which the body's immune system destroys pancreatic beta cells, the only cells in the body that make the hormone insulin that regulates blood glucose.

Type 2 Diabetes Mellitus:

It is called non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce insulin.

Type 3 Diabetes Mellitus:

This covers a wide range of specific types of diabetes including various genetic defects in insulin action, and diseases of the exocrine pancreas.

Type 4 Diabetes Mellitus:

Also called as Gestational Diabetes is a form of glucose intolerance that is diagnosed in some women during pregnancy.

Other types:

LADA (Latent Autoimmune Diabetes in Adults):

It is also called as Late-onset Autoimmune Diabetes of Adulthood and Slow Onset Type 1 diabetes. It is a form of autoimmune diabetes which is diagnosed in individuals who are older than the usual age of onset of type 1 diabetes.

MODY (Maturity-Onset Diabetes of Youth):

It is a monogenic form of diabetes with an autosomal dominant mode of inheritance. Mutations in any one of several transcription factors or in the enzyme glucokinase lead to insufficient insulin release from pancreatic ß-cells, causing MODY. Different subtypes of MODY are identified based on the mutated gene.

Secondary Diabetes Mellitus:

Secondary causes of Diabetes mellitus include:

- Acromegaly
- Cushing syndrome
- Thyrotoxicosis
- Pheochromocytoma
- Chronic pancreatitis
- Cancer
- Drug induced hyperglycemia

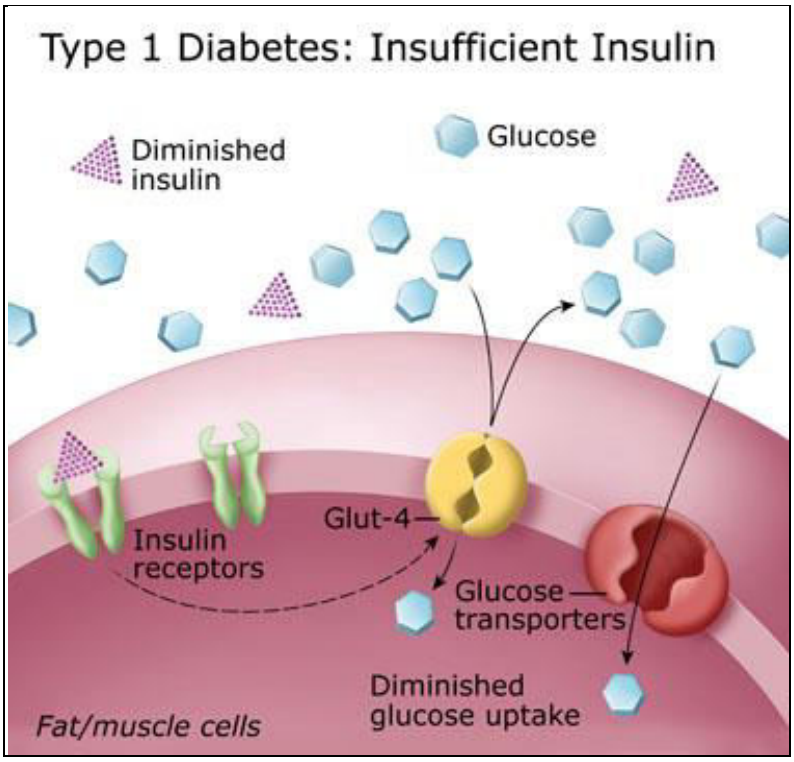


Fig no:1 Type I diabetes mellitus

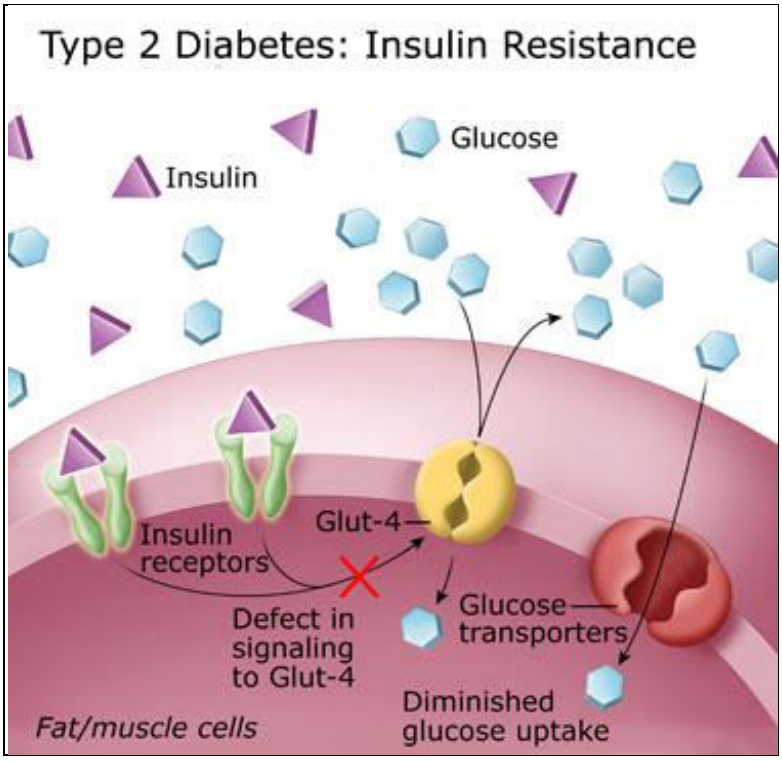


Fig no:2 Type II diabetes mellitus

EPIDEMIOLOGY OF DIABETES MELLITUS:

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) and United States (17.7 million). The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India It is predicted that by 2030 diabetes may afflict up to 79.4 million individuals in India. India currently faces an uncertain future in relation to the potential burden that diabetes may impose upon the country[17].

PATHOGENESIS OF T1DM: [18]

Several features characterize T1DM as an autoimmune disease are,

- Presence of immuno-competent and accessory cells in infiltrated pancreatic islets.
- Association of susceptibility to disease with the class II (immune response) genes of the major histocompatibility complex (MHC; Human Leucocyte Antigens HLA).
- Presence of islet cell specific auto-antibodies.
- Alterations of T-cell mediated immunoregulation in CD4+ T cell compartment.
- Involvement of monokines and TH_1 cells producing interleukins in disease process.
- Response to immunotherapy and frequent occurrence of other organ specific autoimmune diseases in affected individuals or in their family members.

PATHOGENESIS OF T2DM:

In type 2 diabetes, these mechanisms break down, with the consequence that the two main pathological defects in type 2 diabetes are impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance.

Fig no:3 Pathogenesis Of T1DM

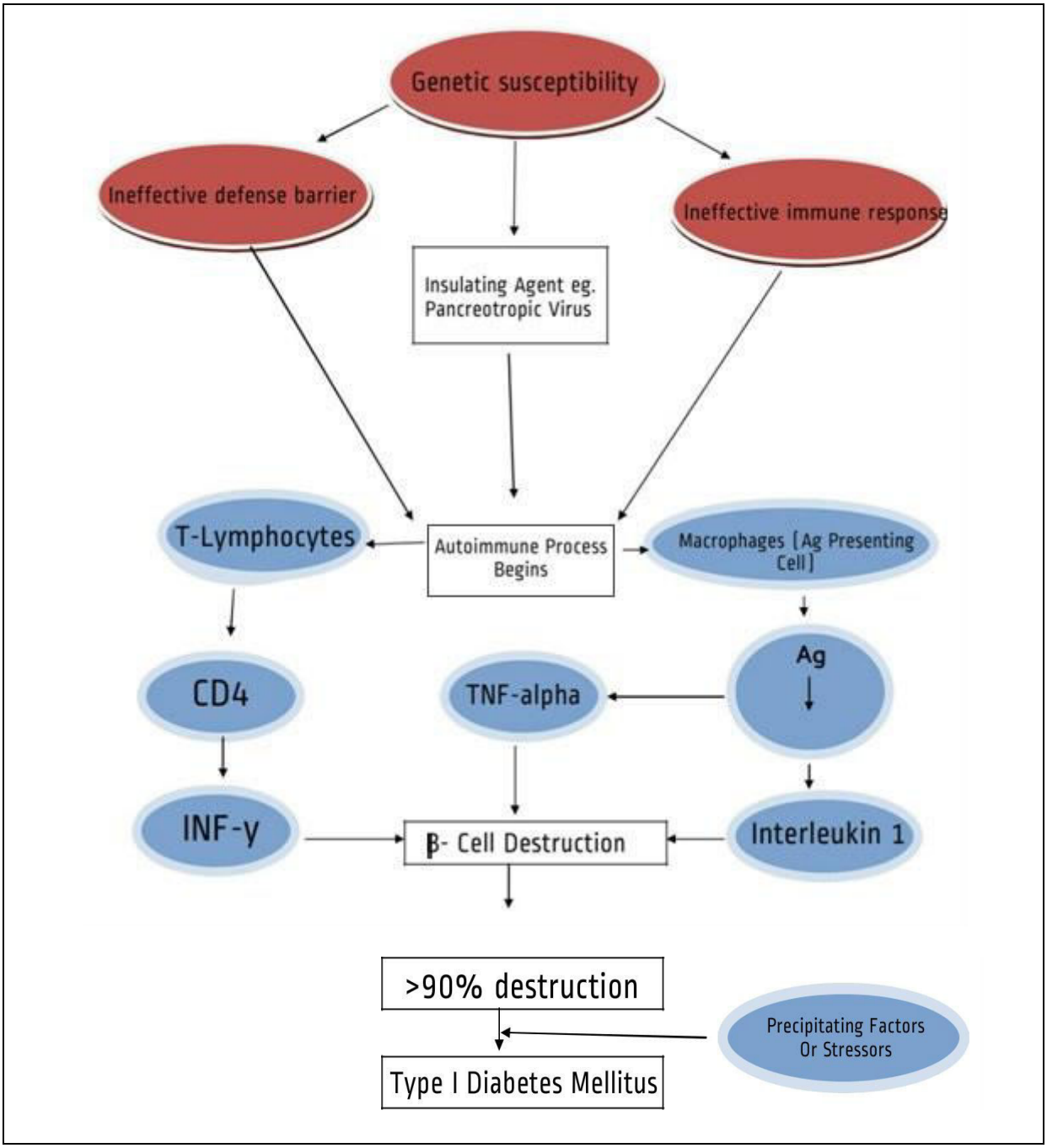
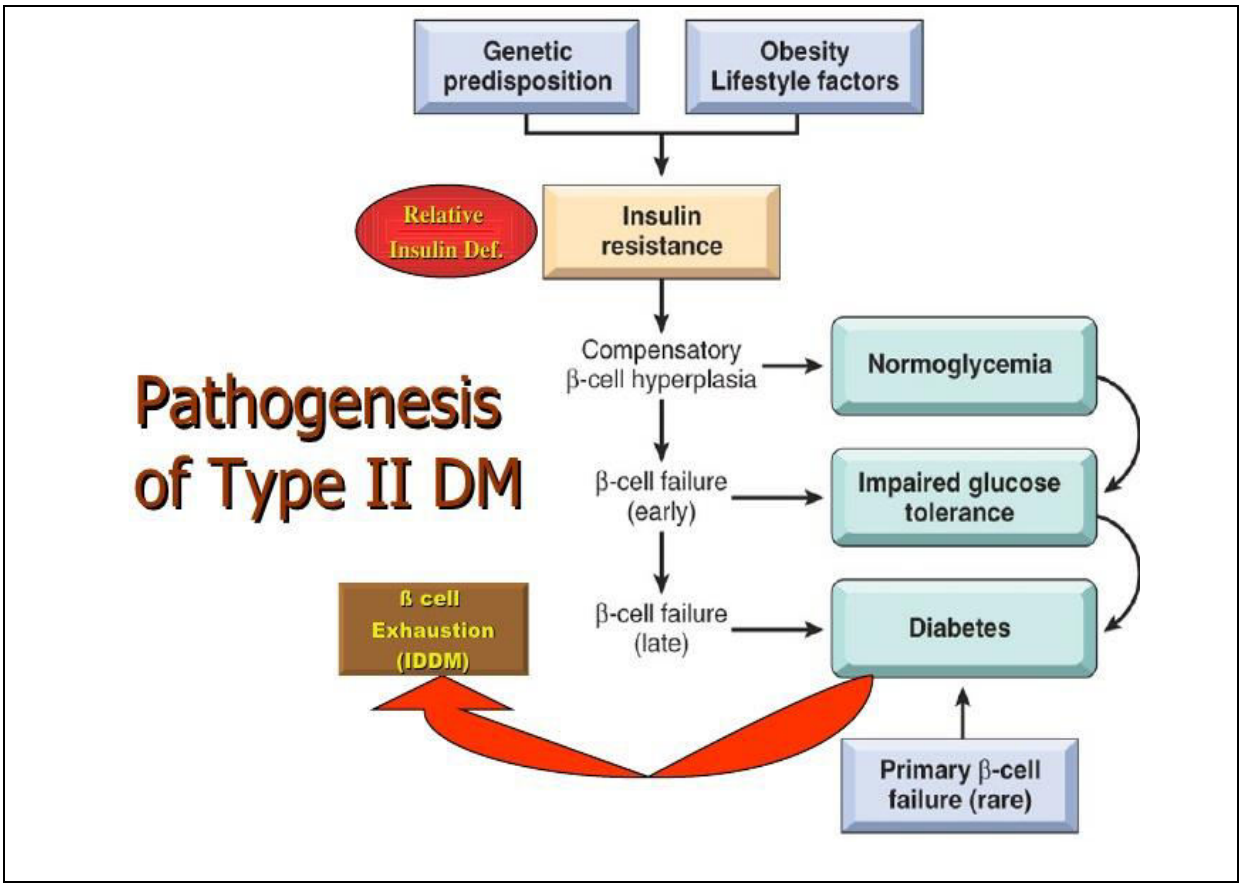


Fig no:4 Pathogenesis Of T2DM



GENES OF DIABETES MELLITUS: [19]

Two genes called "MODY 1" and "MODY II" that appear to contribute to the 2% to 5% of diabetes, that are clearly inheritable.

MODY I GENE:

Mody I gene is located on chromosome 20, makes hepatocyte nuclear factor -4α (HNF, 4α) a cell receptor that plays a role in HNF - 4α production.

MODY II GENE:

Mody II gene is located on chromosome 12, produces hepatocyte nuclear factor -1α (HNF, 1α) a protein found in the liver and β cells of the process.

PATHOPHYSIOLOGY OF TYPE I DIABETES MELLITUS: [20]

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with IDDM. In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessive secretion of glucagon's in IDDM patients. The resultant inappropriately elevated glucagon's levels exacerbate the metabolic defects due to insulin deficiency. The patients with IDDM rapidly develop diabetic **ketoacidosis** in the absence of insulin administration. Deficiency in insulin leads to **uncontrolled lipolysis** and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle. This impairs glucose utilization. Insulin deficiency also **decreases the expression of a number of genes** necessary for target tissues to respond normally to insulin such as glucokinase in liver and the GLUT 4 class of glucose transporters in adipose tissue. This explained that the major metabolic derangements, which result from insulin deficiency in IDDM are impaired glucose, lipid and protein metabolism.

PATHOPHYSIOLOGY OF TYPE II DIABETES MELLITUS:

Individuals with NIDDM have detectable levels of circulating insulin, unlike patients with IDDM. On the basis of oral glucose tolerance testing the essential elements of NIDDM can be divided into four distinct groups:

1. Those with normal glucose tolerance.
2. Chemical diabetes (called impaired glucose tolerance).
3. Diabetes with *minimal fasting hyperglycemia* (fasting plasma glucose less than 140 mg/dl).
4. Diabetes with *over fasting hyperglycemia* (fasting plasma glucose greater than 140 mg/dl).

The individuals with impaired glucose tolerance have hyperglycemia inspite of having highest levels of plasma insulin, indicating that they are resistant to the action of insulin. In the progression from impaired glucose tolerance to diabetes mellitus, the level of insulin declines indicating that patients with NIDDM have decreased insulin secretion. **Insulin resistance** and **Insulin deficiency** are common in the NIDDM patients.

COMPLICATIONS: [21]

The chronic complications of diabetes are classified as follows:

1. MICROVASCULAR (microangiopathic)

a. Diabetic Retinopathy
b. Diabetic Neuropathy
c. Diabetic Nephropathy
d. Diabetic skin problems (the "Diabetic foot")

2. MACROVASCULAR

a. Accelerated propensity to atherosclerosis
b. Peripheral vascular disease/ coronary heart disease
c. Myocardial infarction
d. Arteriosclerosis
e. Hypertension and cerebrovascular disease

3. OTHER ASSOCIATED METABOLIC ABNORMALITIES

a. Hypercholesterolemia

SIGNS AND SYMPTOMS:

- Polyuria: Excessive Frequent urination (Frequent bed-wetting in children)
- Polydipsia: Excessive Thirst
- Polyphagia: Excessive Hunger
- Weakness and fatigue
- Drowsiness
- Irritability
- Blurred vision or any change in sight
- Fruity breath
- Nausea and vomiting
- Sudden unexplained weight loss

TREATMENT:

Treatment typically includes diet control, exercise, home blood glucose testing, and in some cases, oral medication and/or insulin. Approximately 40 percent of people with type 2 diabetes require insulin injections.

1. TABLETS (ORAL HYPOGLYCAEMIC AGENTS)

I. Secretegogues

a) **Sulphonyl ureas:**

1. First generation agents: Tolbutamide, Acetoxamide, Tolazomide, Chlorpropamide.
2. Second generation agents: Glipizide, Glimipridem Glibenclamide, Gliclazide.

b) **Non-sulphonyl ureas:**

1. Meglitinides/Glinides: Repaglinide, Nateglinide, Meglitinides.

II. Sensitizers

a) **Biguanides:** Metformin, Phenformin, Buformin.

b) **Thiazolidinediones:** Pioglitazone, Rosiglitazone.

III. Alpha-glucosidase inhibitors: Miglitol, Acarbose.

IV. Peptide analogs: Incretin mimetics, Amylin analogs.

V. Experimental agents.

2. INSULIN

All type1 diabetics and some type 2 diabetics cannot achieve an acceptable blood sugar level by tablets alone and therefore require insulin therapy instead.

MANAGEMENT OF DIABETES MELLITUS: [22,23]

Glycemic Control:

There are 2 techniques available to assess the quality of a patient's glycemic control.

1. Self - monitoring of blood glucose (SMBG)
2. Interval measurement of haemoglobin A1C (HbA1c).

Self-Monitoring of Blood Glucose:

SMBG is an effective way to evaluate short-term glycaemia control. It helps to monitor the effect of food, medications, stress, and activity on blood glucose levels of patients. The frequency of checking helps to prevent the risk factor and to maintain the medical therapy. For patients with type 1 and type 2 diabetes mellitus, self monitors their glucose at least three times per day. Initially some patients require more frequent monitoring, including both pre-prandial and postprandial readings.

Patients with gestational diabetes who are taking insulin should monitor their blood glucose three or more times daily. But also the inclusion of health-improving behaviour such as diet and exercise maintained. For pregnant women, glucose levels of 60 to 99 mg/dl and peak postprandial levels between 100 and 129 mg/dl.

Haemoglobin A1c:

HbA1c measures no reversible glycosylation of the haemoglobin molecule, which is directly related to blood glucose concentrations. Periodic testing is recommended in all patients with diabetes. The ADA recommends that patients with stable glycaemia control be tested at least twice a year. The frequency of testing depends on the clinical situation and the patient's treatment regimen.

Table no:1 List of some anti-diabetic plants having anti diabetic activity

S.No	Botanical name	Family	Parts commonly used
1	*Areca catechu*	Palmitaceae	Dried ripe seeds
2	*Bidens leucantha*	Asteraceae	Aerial parts
3	*Citrus sinensis*	Rutaceae	Peels
4	*Delonix regia*	Fabaceae	Leaves
5	*Erythrina variegata*	Fabaceae	Leaves
6	*Feronia elephantum*	Rutaceae	Fruits
7	*Glycyrrhiza glabra*	Leguminoceae	Roots, stolen
8	*Hygrophila longifolia*	Acanthaceae	Whole plant
9	*Ipomoea crassicaulis*	Convolvulaceae	Bark
10	*Jatropha glandulifera*	Euphorbiaceae	Tubers
11	*Piper longum*	Piperaceae	Leaves
12	*Swertia chirata*	Gentinaceae	Entire herbs
13	*Tinospora cardifolia*	Menispermaceae	Stems and Roots
14	*Withania somnifera*	Solanaceae	Roots and Leaves
15	*Zingiber officinalis*	Zingiberaceae	Rhizome

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

PHARMACOGNOSTICAL REVIEW:

1. **Sundarambal M *et al.*, (2016)** Reported the Pharmacognostical Studies on the Root Bark of *Adansonia digitata* Linn.[24]
2. **Thiyagarajan V *et al.,* (2015)** Reported the Pharmacognostical Studies on the Seeds of *Adansonia digitata* Linn.[25]
3. **Shri Vijayakiruba T *et al.,* (2004)** Reported Studies of Pharmacognostical Profiles of *Adansonia digitata* Linn.[26]

PHYTOCHEMICAL REVIEW:

4. **Sundarambal M *et al.*, (2016)** Studied the Phytochemical Studies on the Root Bark of *Adansonia digitata* Linn., Department Of Pharmacognosy, College Of Pharmacy, Madras Medical College.[24]
5. **Abiona D.L *et al.,* (2015)** Reported Proximate Analysis, Phytochemical Screening of Baobab (*Adansonia digitata*) Leaves.[27]
6. **Sugantha Singh *et al.,* (2014)** Reported Preliminary Phytochemical Evaluation of in vitro and in vivo parts of *Adansonia digitata* L., An Endangered Medicinal Tree.[28]
7. **Deshmukh, S. S *et al.,* (2013)** Reported Isolation and Evaluation of Mucilage of the *Adansonia digitata* Linn., as a Suspending Agent.[29]
8. **Thiyagarajan V *et al.,* (2015)** Studied the Phytochemical Pharmacological Studies on the Seeds of *Adansonia digitata* Linn.., Department Of Pharmacognosy, College Of Pharmacy, Madras Medical College.[25]
9. **Gahane R. N and Kogje K. K (2013)** Carried out the Phytochemical analysis and reported presence of Anthracene, Bitter principles, Coumarin, Lignin and Tannin in the plant parts.[30]

10. **Parkouda C *et al.,* (2012)** Accessed the nutrient content in the fresh fruits of the *Adansonia digitata* Linn.[31]
11. **Vartunai *et al.,* (2011)** Analysed the total phenol content in the plant and reported 708 ± 14.2 mg/g of the extract.[32]
12. **Compaore W. R *et al.,* (2011)** Studied the Chemical composition in the seeds of the *Adansonia digitata* Linn., Proteins, Carbohydrates, Lipid crude fibres, and Minerals were studied.[33]

PHARMACOLOGICAL REVIEW:

13. **Akinwunmi O. A *et al.,* (2016)** Studied the Suppressive and prophylactic potentials of flavonoid-rich extract of *Adansonia digitata* L. stem bark in Plasmodium berghei-infected mice.[34]
14. **Samreen F *et al.*, (2015)** Reported the Anti-inflammatory and Analgesic study of fibrous part of *Adansonia digitata* fruits using microwave extraction techniques.[35]
15. **Fahmy G *et al*., (2015)** Studied the effects of both seeds and fruit pulp of the plant *Adansonia digitata* L. (Baobab) on Ehrlich Ascites Carcinoma.[36]
16. **Amrish Sharma *et al.,* (2015)** Reported Antibacterial, Antifungal and Antitubercular activity of methanolic extracts of *Adansonia digitata* Linn.[37]
17. **Ngozi Justina Nwodo *et al.,* (2015)** Reported the Anti-trypanosomal activity of the Nigerians plants and their constituents.[38]
18. **Hauwa'u Yakubo Bako *et al.,* (2014)** Reported Lipid profile of Alloxan induced diabetic wistar rats treated with methanolic extract of *Adansonia digitata* fruit pulp.[39]
19. **Ahmed S *et al.,* (2014)** Reported the in vitro Amoebicidal, Antimicrobial and Antioxidant activities of the plants *Adansonia digitata* and *Cucurbita maxima.*[40]

20. **Yihunie Ayele *et al* (2013)** Reported the methanol extract of *Adansonia digitata* L. Leaves inhibits the pro-inflammatory iNOS possibly via the inhibition of NF-KB activation.[41]
21. **Sulaiman *et al., (*2011)** Reported the in vivo evaluation of the Anti-viral activity of Methanolic root bark extract of the African baobab (*Adansonia digitata* Linn).[42]
22. **Yusha'u M *et al.,* (2010)** Reported Antibacterial activity of *Adansonia digitata* L., stem bark extracts of some clinical bacterial isolates.[43]
23. **Vimalanathan K *et al.,* (2009)** Reported the Multiple Inflammatory activities and Anti-viral activities in *Adansonia digitata* (Baobab) leaves, fruits and seeds.[44]
24. **Tanko Y *et al.,* (2008)** Reported Hypoglycemic activity of Methanolic stem bark of *Adansonia digitata* extract on blood glucose levels of Streptozotocin Induced diabetic wistar rats.[45]
25. **Al-Qarawi *et al.,* (2003)** Reported Hepatoprotective influence of *Adansonia digitata* fruit pulp.[46]

ETHNOBOTANICAL SURVEY

3. ETHNOBOTANICAL SURVEY

BIOGRAPHY OF THE PLANT

Fig no:5 ***Adansonia digitata*** **Tree**

Fig no:6 ***Adansonia digitata*** **LEAVES**

Fig no:7 ***Adansonia digitata*** **FLOWERS**

Fig no:8 ***Adansonia digitata*** **FRUITS**

Fig no:9 ***Adansonia digitata*** **SEEDS**

Fig no:10 ***Adansonia digitata*** **FRUIT SECTION**

3. ETHNOBOTANICAL SURVEY

PLANT PROFILE:

Plant name : *Adansonia digitata*

Common name : Baobab , African Baobab

Synonyms : *Adansonia bahobab* L.

Adansonia baobab Gaert.

Baobabus digitata (L.) Kuntze

Family : Bombacaceae

TAXONOMICAL STATUS: [47,48]

Botanical name : *Adansonia digitata*

Family : Bombacaceae

Kingdom : Plantae

Subkingdom : Viridiplantae

Infra kingdom : Streptophyta

Division : Tacheophyta

Sub-division : Spermatophyte

Infradivision : Angiosperm

Class : Magnoliopsida

Super order : Rosanae

Order : Malvaless

Genus : *Adansonia*

Species : *digitata*

VERNACULAR NAMES: [49,50]

Synonym	:	Baobab
English	:	Baobab
Tamil	:	Papparapuli
		Anaipuliyamaram
Bengali	:	Gorak amali
Gujarati	:	Gorak ambli
Hindi	:	Gorak amli
Kannada	:	Anehunese
		Bhramlica
Marathi	:	Gorakh
Telugu	:	Brahmaamlika
		Seemaichinthakaaya
Sanskrit	:	Kuchandana
Malayalam	:	Manjeti

PLANT DESCRIPTION: [51]

Baobab, a tree plant belonging to the Bombacaceae family, is widespread throughout the hot, drier regions of tropical Africa. It is a deciduous, massive and majestic tree up to 25m high, which may live for hundreds of years. The trunk is swollen and stout, up to 10 m in diameter, usually tapering or cylindrical and abruptly bottle-shaped, often buttressed. Branches are distributed irregularly and large. The bark is smooth, reddish brown, soft and fibrous. The tree produces an extensive lateral root system and the roots end in tubers. Leaves are alternate and foliate. Leaves of young tree are often simple. Overall mature leaf

size may reach a diameter of 20 cm. Flowers are pendulous, solitary or paired in leaf axils, large and showy. Flower bud is globose or ovoid. The fruits of the baobab tree hangs singly on long stalks with an ovoid, woody and indehiscent shell 20 to 30 cm long and up to 10 cm in diameter. The shell contains numerous hard, brownish seeds, round or ovoid, up to 15 mm long, which are embedded in a yellowish-white, floury acidic pulp. The fruit pulps appears as naturally dehydrated, powdery, whitish coloured and with a slightly acidulous taste.

HABITAT: [52]

Baobab found in areas of south Africa, Botswana, Namibia, Mozambique and other tropical African countries where suitable habitat occurs. Tree grown various parts of India chiefly in Bombay, Andhra, Bihar, utter Pradesh, Gujarat, Coromandel Coast and Ceylon. *Adansonia* is regarded as the “Queen of all carbon storage trees".

OTHER NAMES:

African baobab is also known as Monkey bread tree, Ethiopian sour gourd, Judas's bag, Lemonade tree, Monkey tamarind, Cream of tartar tree, Senegal calabash (fruit), Upside-down tree, Bamba, Kouka, kuka, Mwambo, Gorakh-imli and hathi-khatiyan.

CHEMICAL CONSTITUENTS: [53]

LEAVES:

The leaves contain more essential amino acids, minerals and vitamin A. It contains (expressed on dry weight basis) 13–15% protein, 60–70% carbohydrate, 4–10% fat and around 11% fibre and 16% ash. Energy value varies between 1180-1900 kJ/100g of which 80% is metabolizable energy. The leaves also contain an important amount of mucilage which on hydrolysis yields galactouronic acid and glucouronic acid with small quantities of galactose, rhamnose, glucose and arabinose.

BARK:

Baobab bark is well-known for its fibres used to make ropes, sacks, clothes, baskets. The alkaloid 'adansonin' in the bark is thought to be the active principle for treatment of malaria and other fevers. Bark also contains fat, calcium, copper, iron, and zinc. In addition, betulinic acid was isolated from the bark whereas the leaf exclusively yielded taraxerone and acetate of lupeol and baurenol. It contains β-sitosterol, Friedelin, lupeol and baurenol (terpenoids). It yields a large quantity of semi fluid white gum, have acidic reaction.

FRUITS:

The baobab fruits are composed of an outer shell (epicarp) (45%), fruit pulp (15%) and seeds (40%). The woody epicarp or pod contains the internal fruit pulps (endocarp) which is split in small floury, dehydrated and powdery slides that enclose multiple seeds and filaments, the red fibres, that subdivide the pulp in segments.

FRUIT PULP:

The dry baobab fruit pulps has high values for carbohydrates, energy, calcium, potassium (very high), thiamine, nicotinic acid and vitamin C (very high). The baobab fruit pulp is rich in mucilage, pectins, tartarate and free tartaric acids. The presence of the tartarate gives rise to the name 'cream of tartar tree'. Pulp sweetness is provided by fructose, saccharose and glucose contents. Fruit pulps are also acidic and this is due to the presence of organic acids including citric, tartaric, malic, succinic as well as ascorbic acid. When eaten raw, the pulp is a rich source of calcium and vitamins B and C. The fruit pulps has a very high vitamin C content, almost ten times that of oranges. However, the vitamin C content of the bulk fruit pulps reportedly varies from 1623 mg/kg in one tree to 4991 mg/kg in another.

SEEDS:

The seeds contain lipids, ash, calcium, protein, vitamin B1, fatty acids (palmitic acid, oleic acid, stearic acid, linoleic acid). The seed contains appreciable quantities of oil (29.7%, expressed on a dry weight basis). Besides, baobab seeds have high levels of lysine, thiamine and iron. Baobab seed can be classified as both protein-rich and oil-rich.

ETHNO MEDICINAL USES:

- Diarrhoea & dysentery
- Promote granulation
- Sickle cell anemia
- Bronchial asthma
- Dermatitis
- To treat Hiccoughs in infants & children
- Diminishing the heat & quenching the thirst
- Substitute for cinchona bark
- Laxative
- Source of cream of tartar
- To treat fatigue and insect bites
- Baobab oil is used on inflamed gums and to ease diseased teeth
- The baobab bark was exported to Europe for use as a fever treatment
- The seeds can also be roasted and used as a substitute for coffee
- In Malawi, where a poison arrow is withdrawn from a killed animal, the juice of baobab is poured into the wound in the belief that it neutralizes the toxin before the meat is eaten.

RATIONALE FOR SELECTION OF THE PLANT

4. RATIONALE FOR SELECTION

- The plant *Adansonia digitata* Linn., belonging to the family Bombacaceae was selected for the present work.
- The traditionally claimed properties associated with the plant were laxative, asthma, insect bites, fever, malaria, hiccoughs in children, wound healing, toothache, gingivitis, skin complaints, dysentery, diaphoretic, kidney and bladder diseases, anti-oxidant, anti-dote, anti-inflammatory and anti-trypanosome uses and cold infusion of leaves is used in diabetes.
- No pharmacognostical study carried out in the fruits so far.
- Phytochemical constituents are not estimated in the fruits so far.
- The anti-diabetic activity was not yet scientifically validated on fruits.
- So, the fruits of the plant *Adansonia digitata* Linn., was selected for evaluation of Anti-diabetic activity.

AIMS AND OBJECTIVES

5. AIMS AND OBJECTIVES

- The present study is to explore the Pharmacognostical, Phytochemical and Anti-diabetic studies on fruits of *Adansonia digitata* Linn.
- Collection and Authentication of the plant material.
- Establishing the pharmacognostical profile of the plant.
- Extraction of plant material by successive solvent extraction by increasing polarity (petroleum ether, ethyl acetate, ethanol).
- Phytochemical screening of the crude powder and various extracts.
- To evaluate the anti-diabetic activity by *in vitro* & *in vivo* methods.

PLAN OF WORK

6. PLAN OF WORK

PHARMACOGNOSTICAL STUDIES:

- Collection of plant material
- Authentication
- Macroscopy
- Microscopy
- Powder microscopy
- Histochemical studies
- Physicochemical constants determination
- Qualitative and Quantitative estimation of inorganic elements and heavy metals

PHYTOCHEMICAL STUDIES:

- Preparation of extracts
- Preliminary phytochemical screening of powder and extracts
- Fluorescence analysis of powder and extracts
- Qualitative and Quantitative estimation of phytoconstituents

PHARMACOLOGICAL STUDIES:

- *In vitro* evaluation of anti-diabetic activity
    1. α- amylase inhibition assay.
    2. Haemoglobin glycosylation inhibition assay.
- *In vivo* evaluation of anti-diabetic activity
    1. Streptozotocin induced diabetes in rats.
    2. Measurement blood glucose level.
    3. Measurement of body weight.
- Histopathological examination of pancreas.

PHARMACOGNOSTICAL STUDIES

7. PHARMACOGNOSTICAL STUDIES

7.1 MATERIALS AND METHODS

Collection of Plant Material:

The fresh fruits of *Adansonia digitata* Linn., was collected from the Madras Medical College Men's Hostel, Chennai, Tamilnadu in July-2016.

Identification and Authentication of Plant Material:

The plant material was authenticated by Botanist Prof.P.Jayaraman Ph.D., Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Tambaram. The fruits were shade dried, coarsely powdered and used for further studies.

MACROSOPY: [54]

The plant material is categorized according to sensory characteristics. Organoleptic evaluation provides the simplest and quickest means to establish the identity, purity and quality of a particular sample. Hence, this observation is of primary important before any further testing can be carried out.

MICROSCOPY: [55-67]

Staining method:

a. Fixation of plant material:

The sample was cut fixed in FAA solution (Formalin 5ml + Acetic acid 5ml + 90ml of 70% Ethanol). The specimen was dehydrated after 24 hours of fixing. The specimen were graded with series of tertiary butyl alcohol, as per the standard method.

b. Infiltration of the specimen:

It was carried out by gradual addition of 58 - 60°C of melting pointed paraffin wax until Tertiary Butyl Alcohol (TBA) solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning:

The paraffin embedded specimens were sectioned with the help of Rotary microtome. The thickness of the sections was 10 - 12µ . Dewaxing of the sections was done by customary procedures. The sections were stained Toludine blue. Since toludine blue is a polychromatic stain, the sections were stained as per the method published by O'Brein *et al*.

The staining results were remarkably good. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to subrein, violet to mucilage and blue to the protein bodies. Whenever necessary sections were also stained with safranin, fast green and iodine for starch.

PHOTO MICROGRAPHS:

Microscopic descriptions were supplemented with photo micrographs whenever necessary. Photographs of different magnifications were taken with Nikon lab photo two microscopic units. For normal observations bright field was used.

For the study of crystals and lignified cells, polarized light was employed. Since, these structures have birefringent property under polarized light they appear bright against dark background. Descriptive terms of the anatomical features are as given in the standard anatomy books.

POWDER MICROSCOPY:

The shade dried, powdered plant material was used for powder microscopic analysis. The Organoleptic characters were observed and to identify the different characteristic features various staining reagent were used. Powder was stained with 1% phloroglucinol in 90% ethanol, concentrated hydrochloric acid and observed through microscope. All the lignified cells stained with pink colour.

HISTOCHEMICAL STUDIES:

Portions of fresh fruits of the plant of *Adansonia digitata* Linn., was used. The fruit pulps were soaked in Formalin, Acetic acid, Ethanol before taking the section. The sections were stained using specific reagents (N/50 Iodine, dilute ferric chloride, phloroglucinol and concentrated hydrochloric acid, Picric acid, Dragendroff's reagent and O- toludine blue) to observe and locate starch, lignin, tannin, protein, alkaloid and flavonoid respectively as per the protocols. The stained sections were then washed in water to remove the excess stain and observed under a microscope (Magnus).

PHYSICO-CHEMICAL EVALUATION:

Shade dried powdered plant material of fruits of *Adansonia digitata* Linn., was used for the determination of physicochemical constants in accordance with WHO guidelines

DETERMINATION OF ASH VALUES:

Ash values are helpful in determination the quality and purity of a crude drug in the powdered form. The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring drug or adhering to it or deliberately added to it, as a form of adulteration.

TOTAL ASH:

Total ash is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from plant tissue itself and non– physiological ash which is the residue of the extraneous matter adhering to the plant surface.

Procedure:

Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccators. About 2gm of powdered sample was weighed accurately and evenly distributed in the crucible. Dried at 100 - 105°C f or 1 hour and ignited to constant weight in a muffle furnace at 600 ± 25°C. The crucible was allowed to cool in desiccators. The percentage of ash with reference to the air dried substance was then calculated by the formula,

$$\text{Total ash value} = \frac{\text{weight of total ash}}{\text{weight of crude drug taken}} \times 100$$

Water soluble ash:

The ash was boiled for 5 minute with 25 ml of distilled water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of the ash and the difference in weight represented the water soluble ash, the percentage of water soluble ash with reference to the air dried substances was calculated by the formula,

$$\text{Water soluble ash} = \frac{\text{weight of total ash} - \text{weight of water soluble ash}}{\text{weight of crude drug taken}} \times 100$$

Sulphated ash:

2-3gm of air dried substance was ignited gently at first in a crucible, until the substance was thoroughly charred. Then the residue was cooled, moistened with 1ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800 ± 25°C, until all the black particles were disappeared. The crucible was allowed to cool, a few drops of sulphuric acid was added and heated. Then it was ignited as before, cooled and weighed. The percentage of sulphated ash with reference to the air dried substance was then calculated.

DETERMINATION OF EXTRACTIVE VALUES:

Extractive values are useful for the evaluation of phytoconstituents especially when the constituents of a drug cannot be readily estimated by other means. Further these values indicate the nature of the active constituents present in a crude drug.

Determination of water soluble extractives:

5gm of the powder drug was weighed and macerated with 100ml of chloroform water (95 ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. 2ml of alcohol was added to the residue and it was dried at 105°C for 1 hour in the hot air oven and cooled in desiccators for 30 minute and weighed. The process was repeated till a constant weight was obtained; the percentage of water soluble extractive value with reference to the air dried drug was calculated.

$$\text{Water soluble extractive value} = \frac{\text{weight of dried extract}}{\text{weight of sample taken}} \times 100$$

Determination of alcohol soluble extractive:

5gm of the powder was weighed and macerated with 100ml 90% ethanol in a closed flask for 24hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105°C for 1 hour in a hot air oven. The dish was cooled in desiccators and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to the air dried drug was calculated.

$$\text{Alcohol soluble extractive value} = \frac{\text{weight of dried extract}}{\text{weight of sample taken}} \times 100$$

Determination of ether soluble extractive:

Transfer 5g of dried powdered drug to an extraction thimble and extract with solvent ether or petroleum ether Boiling Point 40-60ºc in a soxhlet for 6 hrs. The extract was filtered quantitatively into a tarred evaporating dish, evaporated and dried at 105ºc to constant weight. The percentage of ether soluble extractive value with reference to the air dried drug was calculated.

Determination of moisture content (Loss on Drying) :

Specified quantity of the substances was taken in a previously ignited and cooled silica crucible and the substance was evenly distributed by gentle side wise shaking. The crucible with the contents was weighed accurately. The loaded crucible and the lid were placed in the drying chamber (105° C). The substance was heated for a specified period of time to a constant weight. The crucible was covered with the lid and allowed to cool in a

desiccator at room temperature before weighing. Finally the crucible was weighed to calculate the loss on drying with reference to the air dried substance.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{weight of sample}} \times 100$$

Determination of foaming index:

1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml boiling water. The flask was maintained at temperature 80 - 90°C for about 30min. it was then cooled and filtered into a volumetric flask and sufficient water was added through the filtrate to make up the volume to 100ml. The decoction was poured into 10 stopper test tube (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml, 3ml, 4ml up to 10ml and the volume of the liquid in each tube was adjusted with water to 10ml and the volume of the liquid in each tube was adjusted with water to 10ml. The tube were stopper and shake in a length of the foam was measured. The results are assessed as follows,

If the height of foam in every tube is less than 1cm,the foaming index is less than 100. If a height of 1cm is measured in any tube, the volume of the plant material decoction in the (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result. If the height of the foam is more than 1cm in every tube, foaming index is over 1000. In this case repeat the determination using a new series of dilution of the decoction in order to obtain a result. Calculate the foaming index using the following formula:

$$\textbf{Foaming index} = 1000/a$$

Where, a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

Determination of swelling index:

The swelling index is the volume in ml occupied by the swelling of 1gm of plant material under specified conditions. A specified quantity of the plant material were previously reduced to the required fineness was accurately weighed and transferred into a 25ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125mm, marked in 0.2ml divisions from 0 to 25ml in an upward direction. Unless otherwise indicated in the test procedure, add 25ml of water and shake the mixture thoroughly every 10min for 1hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material was measured including any sticky mucilage. Calculate the mean value of the individual determination, related to 1gm of plant material.

QUALITATIVE ANALYSIS OF INORGANIC ELEMENTS AND HEAVY METALS: [68-71]

To the ash of the drug material 50% v/v hydrochloric acid was added and kept for 1 hour. It was filtered and the filtrate was used for the following tests.

Aluminium: White gelatinous precipitate of aluminium hydroxide is formed on addition of ammonia solution. It is slightly soluble in excess of the reagent. The precipitate dissolves readily in strong acid and base, but after boiling it becomes insoluble.

Arsenic: Arsenious salts in neutral solution react with solution of copper sulphate to form green precipitate (scheele's green) which on boiling gives a red precipitate of cupric oxide.

Borate: The mixture obtained by the addition of sulphuric acid and alcohol (95%) to a borate when ignited, burns with flame tinged with green.

Calcium: Solution of calcium salts, when treated with ammonium carbonate solution, yield a white precipitate after boiling and cooling the mixture (it is insoluble in ammonium chloride solution).

Carbonate: Carbonate, when treated with dilute acid effervescence, liberating carbon dioxide which is colourless and produces a white precipitate in calcium hydroxide solution.

Chlorides: Chlorides, when treated with silver nitrate solution, yield a white crude precipitate which is insoluble in nitric acid, but soluble after being well washed with water, in diluted ammonia, from which it is re precipitated by the addition of nitric acid.

Copper: An excess of ammonia, added to a solution of a cupric salt, produces first a bluish precipitate and then a deep blue coloured solution.

Iron: Solution of ferric salts, when treated with potassium ferrocyanide solution, yields an intense blue precipitate which is insoluble in dilute Hcl.

Lead: Strong solution of lead salts, when treated with Hcl, yield a white precipitate. Which is soluble in boiling water and is re deposited as crystals when the solution is cooled.

Magnesium: Solution of magnesium salts, when treated with ammonium carbonate solution and boiled, yield a white precipitate, but yield no precipitate in the presence of ammonium chloride solution.

Mercury: Solution of mercury salts, when treated with sodium hydroxide solution, yields a yellow precipitate.

Nitrate: With solution of ferrous sulphate no brown colour was observed but if sulphuric acid is added (slow from the side of the test tube), a brown colour is produced at the junction of two liquids, indicating the presence of nitrates.

Phosphate: Solution of phosphate when treated with silver nitrate with dilute ammonia solution and in dilute nitric acid yield yellow precipitate of normal silver orthophosphate (distinction from meta and pyrophosphate) solution.

Potassium: Moderately strong potassium salts, which have been previously ignited to remove ammonium salts, when treated with perchloric acid (60%) yield a white crystalline precipitate.

Silver: Solution of silver salts, when treated with potassium iodide solution yield a cream coloured precipitate which is insoluble in dilute ammonia solution and in nitric acid.

Sulphates: Solution of sulphates, when treated with lead acetate solution yields a white precipitate which is insoluble in ammonium acetate solution and in sodium hydroxide.

QUANTITATIVE ESTIMATION OF INORGANIC ELEMENTS AND HEAVY METALS

Inductive coupled plasma-Optical emission spectroscopy (ICP-OES):

It is an excellent multi-element technique with relatively good sensitivity and selectivity when configured correctly. This technique utilizes the plasma as an ion source or light emission source are capable of producing values.

Instrumentation parameters

Instrument name: Inductive coupled plasma-Optical emission spectroscopy

Instrument Model: PE Optima 5300DV ICP-OES; Optical system Dual view-axial or radial.

Detector system:

Charge coupled detector, (UV-Visible detector which is maintaining at -40°C) to detect the intensity of the emission line.

Light source (Torch):

Positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a simple software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Standard alumina injector: 2.0 mm inner diameter.

Spray chamber: Scott type

Nebulizer: Cross flow gem tip.

Preparation of sample by acid digestion method:

50mg of powder was treated with acid mixture of sulphuric acid: water in the ratio of 4:1in the Kjeldahl flask and heated continuously till the solution is colourless. The sample mixture was then transferred in a 25ml volumetric flask and made up to the volume with distilled water. Blank solution was prepared as above without sample. The standards of Arsenic, Lead, Mercury and Cadmium were prepared as per the protocol and the calibration curve was developed for each of them.

Detection:

Samples were analyzed for the detection and quantification of the heavy metals and inorganic elements by Inductive Coupled Plasma Emission Spectrometry.

PHARMACOGNOSY
RESULTS AND DISCUSSION

7.2 RESULTS AND DISCUSSION

ORGANOLEPTIC CHARACTERS:

- **Texture - Smooth**
- **Colour - Fruits are green in colour**

  Seeds are lustrous brown with thick testa
- **Odour - Odourless**
- **Taste - Mucilaginous**
- **Shape - Fruits are large ellipsoid shaped capsule (often >120 mm)**

  Seeds are reniform in shape.

STRUCTURE OF THE SEED:

MORPHOLOGY OF THE SEED:

The plant is a huge tree with very wide, shining trunk and thick curved spines on the branched. The fruit *Adansonia digitata* is a large woody pod, possessing many dry free seeds.

Fig no:11 Seeds of *Adansonia digitata* Linn., fruit

The seeds are elliptic ovate in shape and measure 1× 1.5 cm in size. The seeds are clothed with dense, long soft hairs.

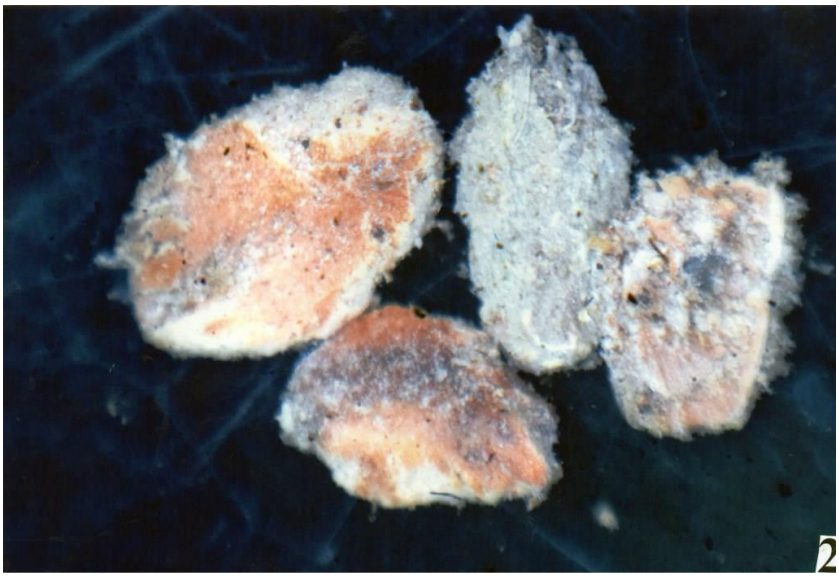


Fig no:12 Seeds showing dense surface hairs

When the hairs are removed, the surface of the seed shows prominent, dense reticulate thickenings.

Fig no:13 Seeds cleaned to show reticulate thickenings

ANATOMY OF THE SEED :

The seed consists of outer single layer of epidermis, the epidermal cells are wide, horizontally elongated and have thick mucilaginous cuticle. Inner to the epidermis is the multi layered hypodermis which is hetrocellular. The outer hypodermis consists of two or three layers of sclerenchyma cells.

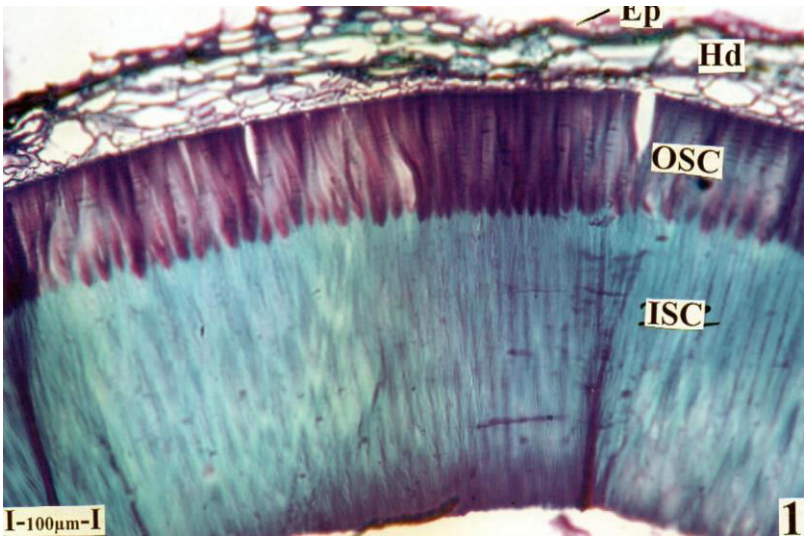


Fig no:14 Transverse section showing outer epidermal and inner sclerotic zones

The inner hypodermal zone includes about five layers of compact parenchyma cells. The inner most layer of the hypodermis has a narrow layer of thick walled cells.

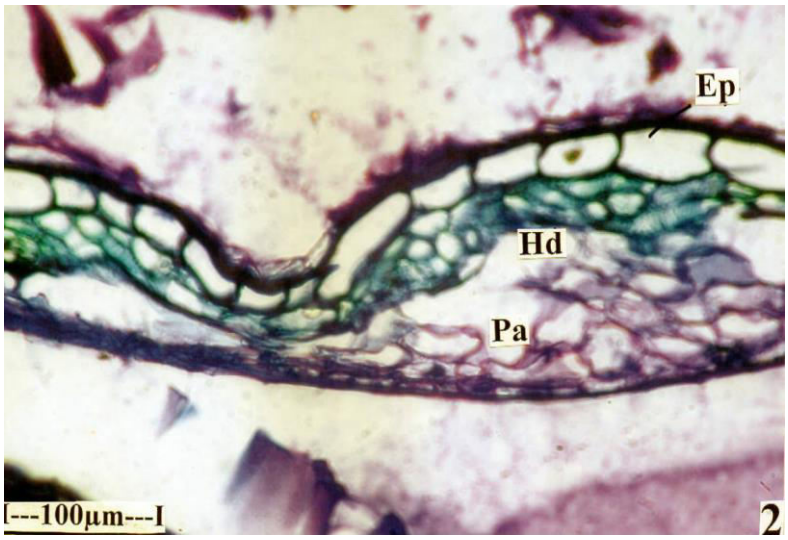


Fig no:15 Hypodermal zone showing compact parenchyma cells

Inner to the epidermis and hypodermis is the sclerotesta which are thick walled sclerotic layers.

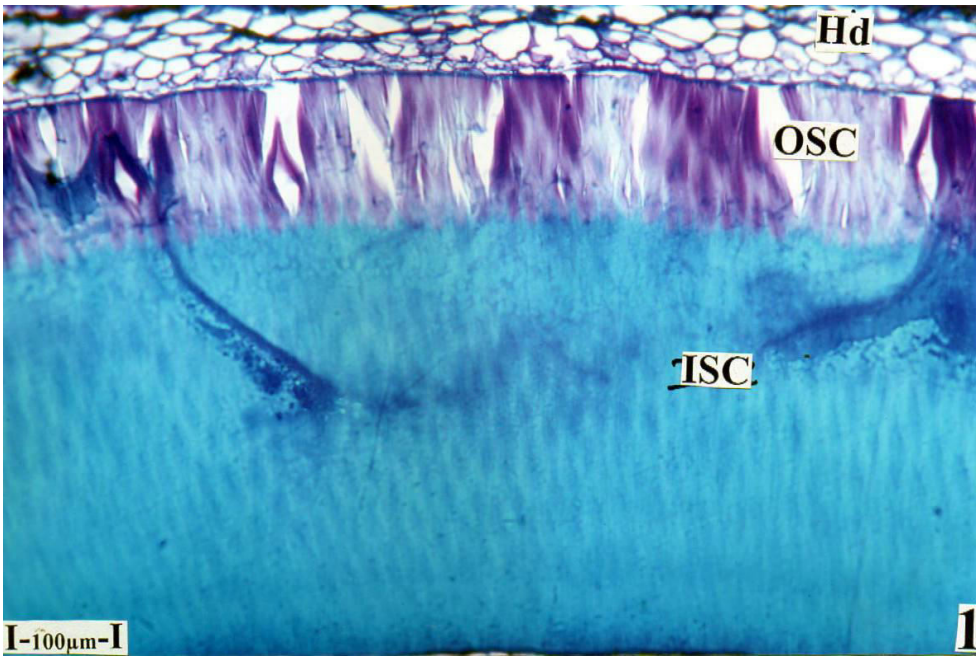


Fig no:16 Transverse section showing hypodermal, outer and inner sclerotic layers

The sclerotesta consists of single layer of osteosclereids where the cells are bone-shaped.

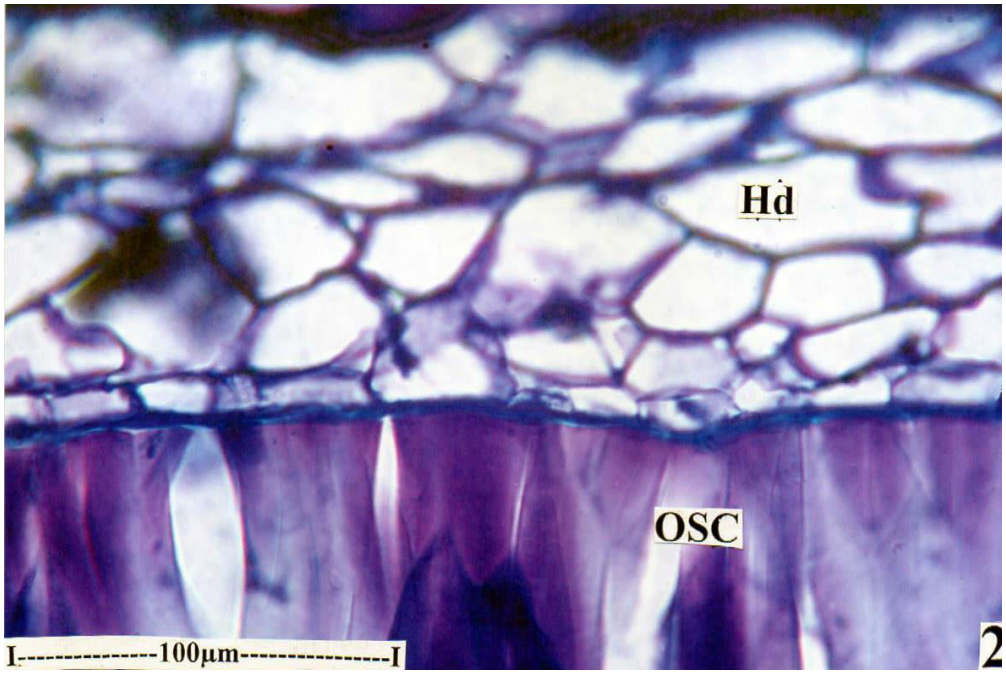


Fig no:17 Hypodermal cells and bone shaped osteosclereids

These sclereids have wide ends of narrow middle portion.

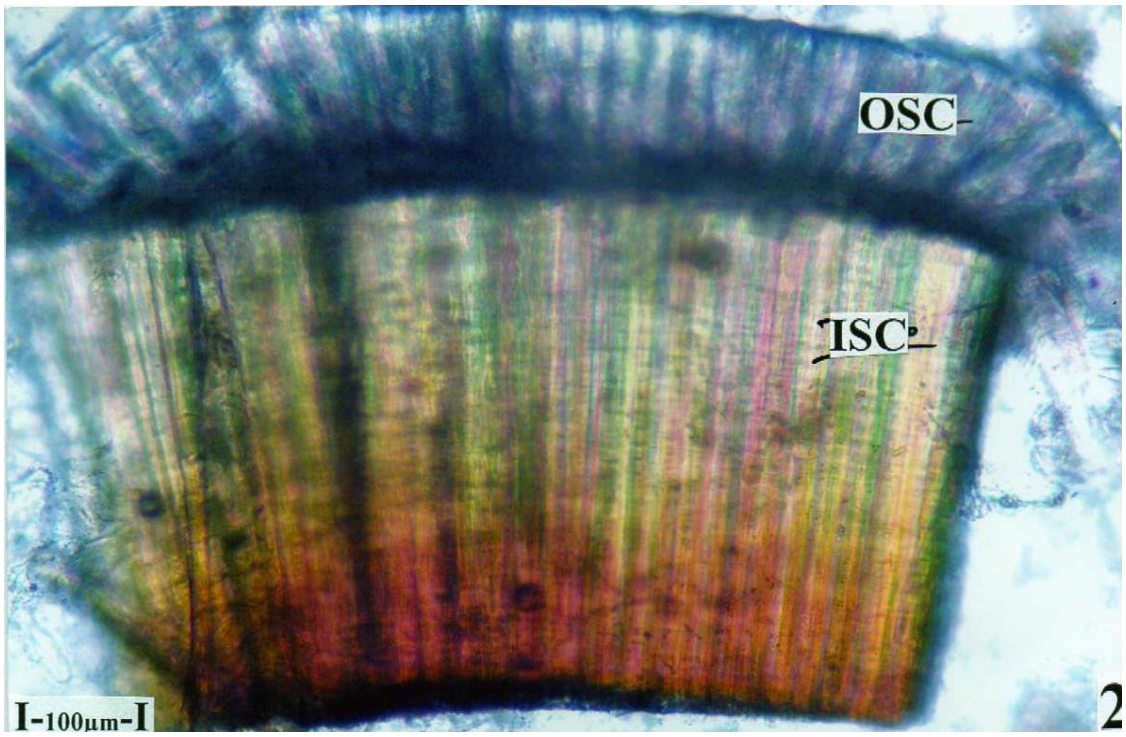


Fig no:18 Cell section showing narrow middle portion of sclereids

Endosperm is present inner to the inner sclerotic cells.

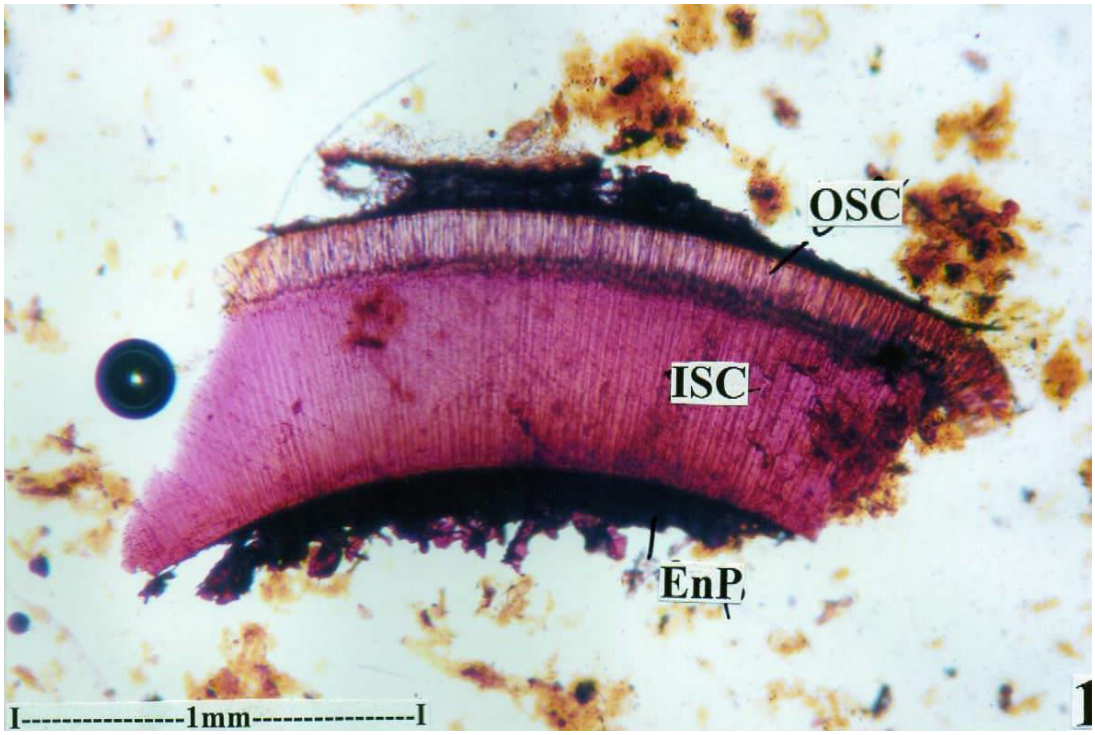


Fig no:19 Transverse section of seed coat showing endosperm

Inner to the osteosclereid zone is a very thick, compact, lignified columnar sclereids or macrosclereids. These solid layer of macrosclereids have deposited thick secondary walls. Each cell is much taller and narrower than the parenchyma cells.

Fig no:20 Osteosclereid zone showing macrosclereids in the inner sclereid zone

The outer sclereid zone consists of osteosclereids (Bone shaped cells) and the inner sclereid zone consists of macrosclereids (Rod shaped cells). The thickness of the entire seed coat is 620 µm. The osteosclereid zone is 140 µm thick and the columnar macrosclereid zone is 480µm thick.

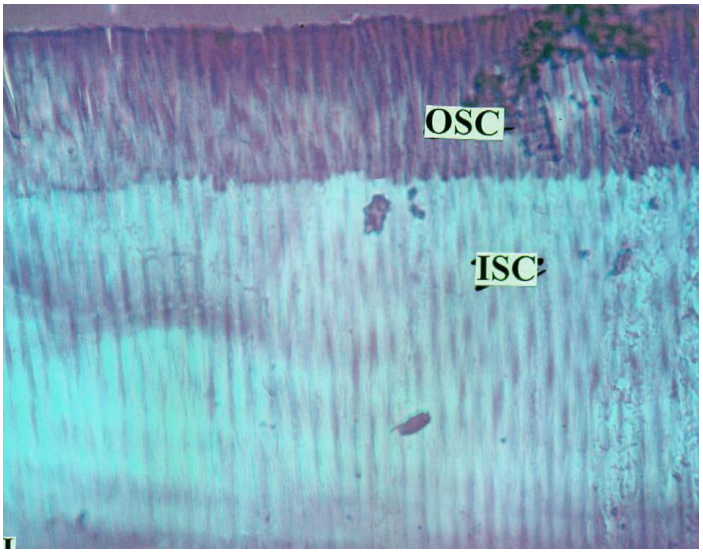


Fig no:21 Transverse section showing sclerotic zones

Inner to the seed coat is a thick zone of wide compact parenchymatous cell layers.

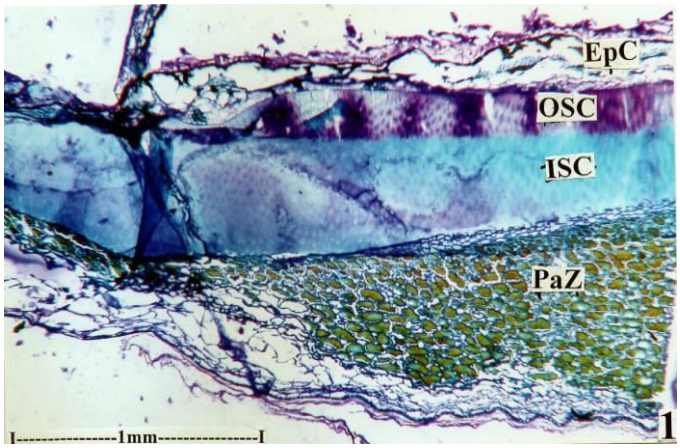


Fig no:22 Transverse section showing epidermal, sclerotic and parenchyma zones

These cells have thick, undulate anticlinal walls.

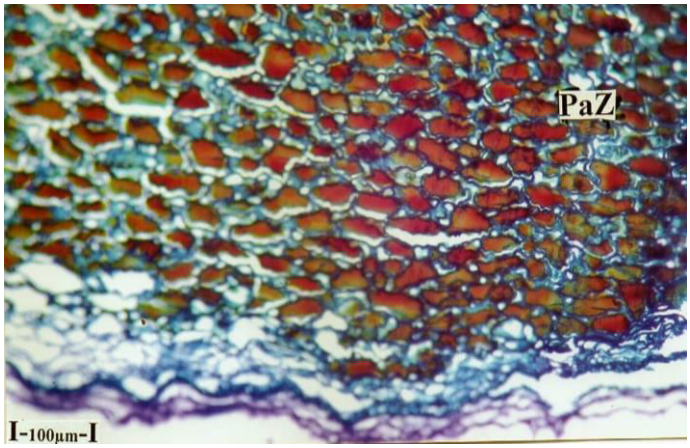


Fig no:23 Transverse section showing parenchyma zone

These parenchymatous cells are filled with dense tannin contents.

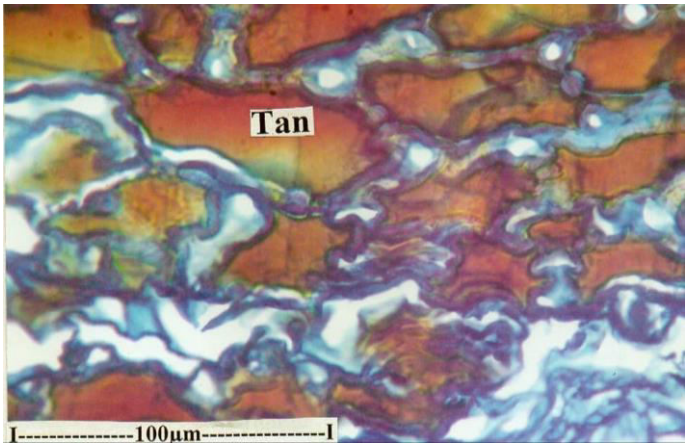


Fig no:24 Parenchymatous cells enlarged showing tannin contents

POWDER MICROSCOPY:

The powder preparation of the seed shows, isolated individual cells of different types.

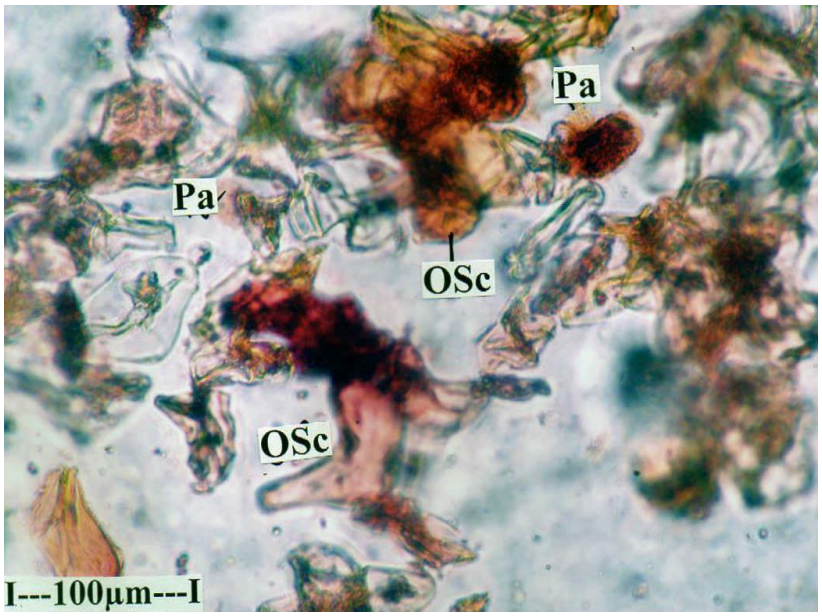


Fig no:25 Different cell types found in the powder

These are bone shaped sclereids called osteosclereids. These sclereids have dilated end and constricted middle part. The sclereid is 210µm long and 130µm thick at the ends.

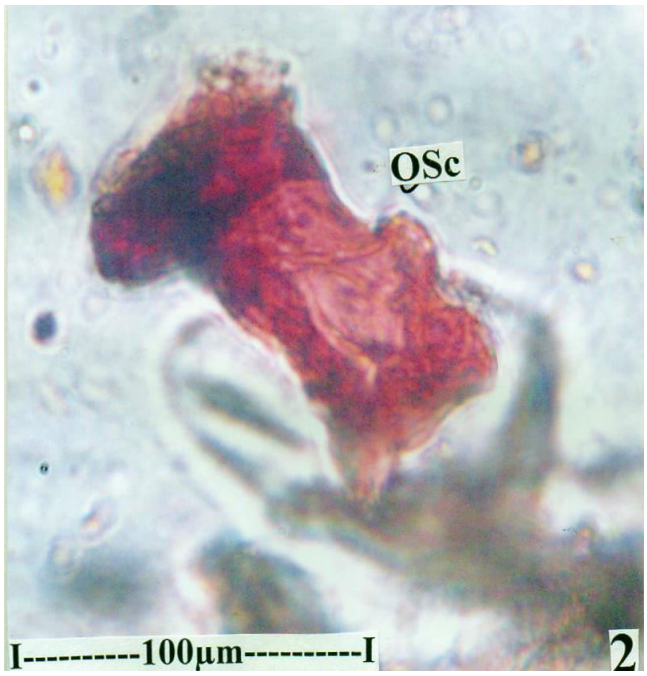


Fig no:26 Bone shaped osteosclereids

Apart from the sclereids, there is also parenchyma cells of polyhedral shape. Some of the parenchyma cells have no cell contents and other have dense mucilage. Tannin containing cells are also seen.

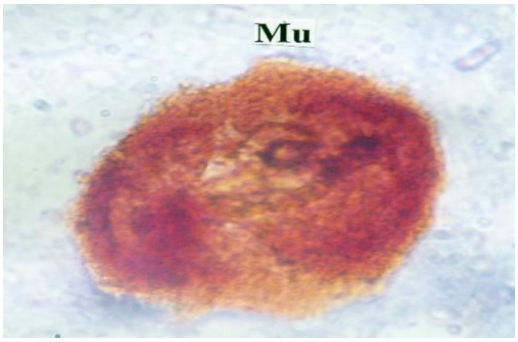


Fig no:27 Parenchymatous cells showing dense mucilage

Small fragments of epidermal layer are common in the powder. These fragmentary peeling of the epidermal tissue shows polygonal thin walled compact parenchyma cells.

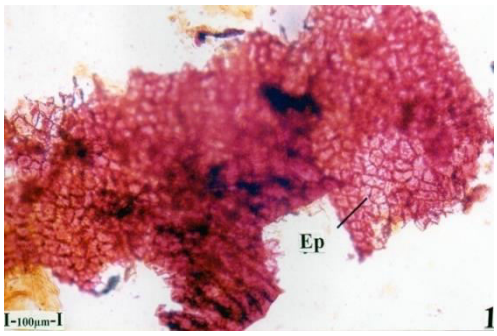


Fig no:28 Fragments of epidermal cells

The anticlinal walls of the cells are straight and smooth. No cell inclusions are seen in the powder.

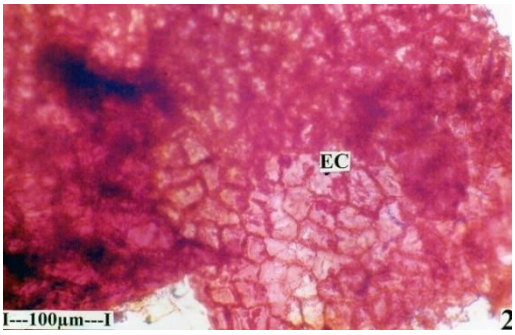


Fig no:29 Enlarged surface view of epidermal cells

The broken pieces of the sclerotic cells of the seed coat are thick walled. These cells are elongated and rectangular in shape.

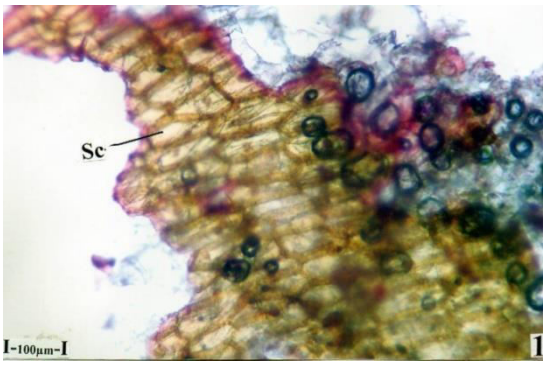


Fig no:30 Sclerotic hypodermal rectangular shaped cells in surface view

The cell walls are highly thick walled and possess prominent and wide canal like simple pits. The cells are 100-200µm long and 20-30µm wide.

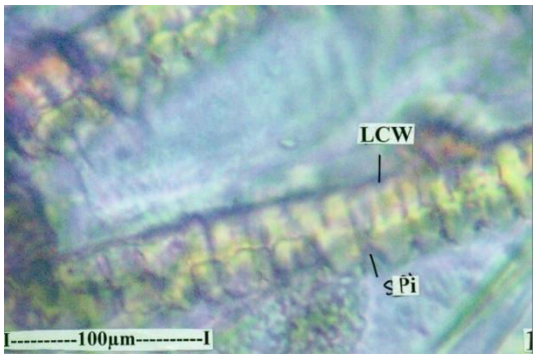


Fig no:31 Lignified cell walls showing canal like simple pits in lumen

Small bundles of sclerotesta comprising osteosclereids and columnar macrosclereids are important component of the powder. These two types are attached with their ends.

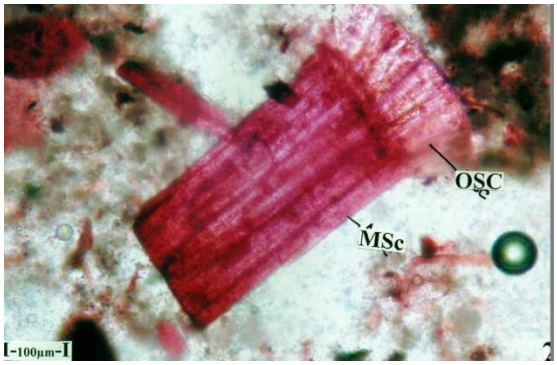


Fig no:32 Osteosclereids and macrosclereids bundle

HISTOCHEMICAL COLOUR REACTION

Table No:2 Histochemical colour reaction of *Adansonia digitata* Linn.

S.NO	CHEMICALS	TEST FOR	NATURE OF CHANGE	HISTOLOGY	DEGREE OF CHANGE
1	Phloroglucinol + Hcl	Lignin	Pink colour	Cell wall	+
2	N/50 Iodine solution	Starch	Blue colour	Endosperm and embryo	+
3	Dilute ferric chloride	Tannin	Bluish colour	Epidermal layer and inner seed coat	+
4	Picric acid	Protein	Yellow colour	Endosperm	+
5	Dragendroff's reagent	Alkaloid	Orange	Inner seed coat	+

PHYSICO-CHEMICAL CONSTANTS

Table No:3 The physicochemical analysis on the fruits of *Adansonia digitata* Linn.

S.NO	PARAMETERS		RESULTS (%w/w)
1	ASH VALUE	Total ash	8.9±0.5
		Water Soluble ash	6.4±0.42
		Acid insoluble ash	7.3±0.3
		Sulphated ash	10.1±0.2
2	EXTRACTIVE VALUE	Water Soluble extractive	3.01±0.02
		Alcohol Soluble extractive	2.55±0.7
		Ether Soluble extractive	1.98±0.15
3	LOSS ON DRYING		6.07±0.32
4	FOAMING INDEX		<100
5	SWELLING INDEX		9.5

Values are expressed as Mean ± SD, n=3

Table No:4 Qualitative analysis of inorganic elements

S.NO	INORGANIC ELEMENTS	OBSERVATION
1	Aluminium	+
2	Chloride	+
3	Copper	+
4	Calcium	+
5	Iron	+
6	Borate	+
7	Potassium	+
8	Silver	+
9	Phospate	-
10	Nitrate	-
11	Sulphare	-

Table No:5 Quantitative estimation of inorganic elements

S.NO	INORGANIC ELEMENTS	TOTAL AMOUNT (%W/W)
1	Aluminium	0.027
2	Chloride	0.051
3	Copper	0.007
4	Calcium	0.09
5	Iron	0.029
6	Borate	0.003
7	Potassium	0.022
8	Silver	0.007

QUANTITATIVE ESTIMATION OF HEAVY METALS BY ICP OES METHOD

The quantification of the individual heavy metals was analyzed for the powdered mixture of *Adansonia digitata* Linn., by ICP-OES technique. The following metals were detected and quantified, results are given in the following table.

Table No:6 Quantitative estimation of heavy metals

S.NO	HEAVY METALS	OBSERVATION (ppm)	STANDARD LIMITS (as per WHO)
1	Arsenic	0.06	Not more than 5 ppm
2	Lead	0.02	Not more than 10 ppm
3	Cadmium	0.003	Not more than 0.03 ppm
4	Mercury	0.04	Not more than 0.5 ppm

The above observation showed that the material is within the limits as per WHO standard and it is safe to consume internally.

PHARMACOGNOSTICAL STUDIES DISCUSSION

The pharmacognostical studies plays a key factor in establishing the authenticity of the plant materials. The botanical identity of the fruit of *Adansonia digitata* Linn., was established by examining its morphological, anatomical features as well as the WHO recommended physiochemical chemical studies. The result of this standardization may throw immense light on the botanical identity of the fruit of *Adansonia digitata* Linn., which may be useful in judging the authenticity of the plant and also differentiate the fruit from its adulterants and substitutes.

The macroscopy of the fruits were examined. The microscopical characters of seed showed the presence of root hairs, osteosclereids, macrosclereids, endosperm, tannins and parenchyma cells.

Powder microscopy showed the presence of mucilage, tannins, osteosclereids, macrosclereids and some calcium oxalate crystals. These features can be employed for the interspecific identity of the drug.

Physicochemical parameters are mainly used in judging the purity and quality of the powdered drug. Ash values of the drug gives an idea of the earthy matter or inorganic elements and the other impurities present along with the drug.

The ash values of the drug are used for detecting low grade product and exhausted drug. Hence, it proves to be an important criteria to judge the purity of the crude drugs. A high ash value is the indication of substitution, contamination and adulteration.

The Total ash usually consists of carbonates, phosphates and silicates. Total ash was found to be 8.9±0.5 % w/w. The acid insoluble ash indicates the contamination with siliceous materials like sand and the value was found to be 7.3±0.3 % w/w.

The Sulphated ash was obtained by treating with dilute sulphuric acid where the oxides are converted to sulphates. The value was found to be 10.1±0.2 % w/w.

The parameters which is useful for prediction of the nature of the constituents is the extractive value.

The alcohol and water soluble extractives was found to be 2.55±0.7 % w/w and 3.01±0.02% w/w respectively. Ether soluble volatile extractive value was found to be 1.98±0.15 % w/w respectively.

The alcohol soluble and water soluble extractive values indicates the presence of considerable amounts of polar compounds. These constants would help to identify and to standardize the plants for future researches. Loss on drying value was found to be 6.07±0.32.

The qualitative analysis of heavy metals and inorganic elements were carried out and it showed only trace amounts of heavy metals (within the limits).

These detailed pharmacognostical studies on fruits of *Adansonia digitata* Linn., provides information on the identification of the drug and also used to differentiate the plant from its adulterants and substituents.

PHYTOCHEMICAL STUDIES

8. PHYTOCHEMICAL STUDIES

8.1 MATERIALS AND METHODS

Phytochemical evaluation is used to determine the nature of phytoconstituents present in the plant by using suitable chemical tests. It is essential to study the pharmacological activities of the plant. It can be done by confirmation with different chromatographic techniques like TLC and HPTLC. Therefore a complete investigation is required to characterize the Phytoconstituents qualitatively and quantitatively.

Preparation of Extracts: [72]

Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites into the extracting solvent depends upon its polarity.

Extraction:

The first step was the preparation of successive solvent extracts. The dried coarsely powdered sample of *Adansonia digitata* Linn., fruits (500gm) was first extracted with Petroleum ether in Soxhlet apparatus and then with solvents of increasing polarity like Ethyl acetate, Ethanol (60-70°C). Each extract was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were recorded and preceded for further detailed phytochemical and pharmacological screening.

PRELIMINARY PHYTOCHEMICAL SCREENING: [73-83]

The chemical tests for various phytoconstituents in the dried powder and extracts of fruits of *Adansonia digitata* Linn., were carried out as described below and the results were recorded.

Detection of Alkaloids:

Small quantity of the extract was treated with few drops of diluted hydrochloric acid and filtered. The filtrate was used for the followings,

a) **Mayer's reagent** (Potassium mercuric iodide solution): Alkaloids give cream colour precipitate with Mayer's reagent.
b) **Dragendorff's reagent (**Potassium bismuth iodide solution): Alkaloids give reddish brown precipitate with Dragendorff's reagent.
c) **Hager's reagent** (Saturated solution of picric acid): Alkaloids give yellow coloured precipitates with Hager's reagent.
d) **Wagner's reagent** (Solution of iodine in potassium iodide): Alkaloids give reddish brown precipitate with Wagner's reagent.

Detection of Proteins:

a) **Biuret test**: The sample was treated with 5-8 drops of 10% w/w copper sulphate solution, violet colour is formed.

Detection of Flavonoids:

a) **Shinoda's test:** Small quantity was dissolved in alcohol to these pieces to magnesium followed by concentrated hydrochloric acid were added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.
b) **With aqueous sodium hydroxide solution:** Small quantity of the extract was dissolved in aqueous sodium hydroxide and appearance of yellow colour indicates the presence of flavonoids.
c) **Zinc hydrochloride test:** Small quantity the extract was mixed a mixture of zinc dust and concentrated Hydrochloric acid. It gives red colour after a few minutes.

Detection of Tannins:

a) **Lead acetate test:** The test solution was mixed with basic lead acetate solution and examined for formation of a white precipitate.
b) **Ferric chloride test:** A few drops of 5% aqueous ferric chloride solution was added to 2ml of an aqueous extract of the drug and examined for the appearance of bluish black colour.

Detection of fixed oils and fats:

a) **Spot test:** Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils and fats.
b) **Saponification test:** Few drops of 0.5 % alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on the water bath for 1-2 hr. Formation of soap with the alkali indicates the presence of fixed oils and fats.

Detection of Glycosides:

a) **Borntrager's test:** The powdered material was boiled with 1ml of sulphuric acid in a test tube for five minutes. Filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.
b) **Modified Borntrager's test:** The test material was boiled with 2ml of the dilute sulphuric acid. This was treated with 2ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 minutes, and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

Detection of Steroids and Triterpenoids:

a) **Libermann Burchard's test:** The powdered drug was treated with few drops of acetic anhydride, boiled and cooled. concentrated sulphuric acid was added from the sides of the test tube.Brown ring is formed at the junction of two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoids.

b) **Salkowski test:** The extract was treated with few drops of concentrated sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

c) **Noller's test:** The extract was warmed with tin and thionyl chloride. Pink colouration indicates the presence of triterpenoids.

d) **Sulfur powder test:** The extract added with small amount of sulfur powder, it sinks at the bottom.

Detection of Carbohydrates:

a) **Molisch's test:** To the test solution few drops of alcoholic solution of α-napthol and few drops of concentrated sulphuric acid were added through the sides of test tube, purple to violet colour ring appears at junction.

b) **Fehling's test:** The test solution was mixed with Fehling's I and II and heated and examined for the appearance of red coloration for the presence of sugar.

Detection of Saponins:

a) A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for 3 minutes. Development of any honey comb like froth was examined.

FLUORESCENCE ANALYSIS: [84]

Many crude drug show the Fluorescence when the sample is exposed to UV radiation. Evaluation of crude drugs based on fluorescence in day light is not much used, as it is usually unreliable due to the weakness of the fluorescent effect. Fluorescent lamps are fitted with suitable filter, which eliminate visible radiation from the lamp and transmits UV radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation.

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS:

TOTAL SAPONIN CONTENT: [85]

Saponin content of the extracts was determined using the Folin–Ciocalteu method. 0.5ml of the extract was mixed with 0.5 ml of 8% (w/v) vanillin solution and 5 ml of 72% (v/v) H_2SO_4 solution. The mixture was incubated at 70° C for 10 min and then rapidly cooled to room temperature using an ice water bath. The absorbance was measured at 560 nm using a UV-VISIBLE spectrophotometer. Escin was used as the standard. Saponin content was expressed as mg of Escin equivalents (EE) per gram of dry extract.

TOTAL FLAVONOID CONTENT: [86]

The total flavonoid content was determined according to the Aluminium Chloride colorimetric method. Plant extract (2 ml, 0.3 mg/ml) were mixed with 0.1 ml of 10% aluminium chloride hexahydrate, 0.1 ml of 1 M potassium acetate and 2.8 ml of deionized water. After the 40 minutes incubation at the room temperature, the absorbance of the reaction mixture was determined spectrophotometrically at 415 nm. Quercetin was chosen as a standard (the concentration range: 0.005 to 0.1 mg/ml) and the total flavonoid content was expressed as milligram of Quercetin equivalents (QE) per gram of dry extracts.

CHROMATOGRAPHY: [87,88]

Chromatographic methods are important analytical tool in the separation, identification and estimation of components present in the plant.

THIN LAYER CHROMATOGRAPHY

Principle:

It is a technique used for the separation, identification and estimation of single or mixture of components present in various extracts. In this technique, solute undergoes distribution between stationary and mobile phase. The separation is based on the differential migration that occurs when a solvent flows along the thin layer of stationary phase. This is achieved by partition or adsorption depending on stationary phase used.

TLC Plate Preparation:

The plates were prepared using Stahl TLC spreader. 40gm of silica gel G was mixed with 85ml of water to prepare homogenous suspension and poured in the spreader. 0.25mm thickness of plates was prepared, air dried until the transparency of the layer disappeared, then dried at 110° C for 30 minutes and kept in desiccators.

Selection of mobile phase:

Solvent mixture was selected on the basis of the phytoconstituents present in each extract. Factors such as nature of components, stationary phase, mobile phase, polarity, influence the rate of separation of constituents. From the vast analysis, best solvent was selected which showed good separation with maximum number of components.

The Retention Factor **(R_f)** is calculated using following formula,

$$Rf\ value = \frac{Distance\ travelled\ by\ the\ solute\ from\ the\ origin}{Distance\ travelled\ by\ the\ solvent\ from\ the\ origin}$$

PHYTOCHEMICAL RESULTS AND DISCUSSION

8.2 RESULTS AND DISCUSSION

PHYTOCHEMICAL STUDIES:

Table No:7 Percentage yield in successive solvent extraction of the various extracts of *Adansonia digitata* Linn.

S.No	EXTRACT	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD (% W/W)
1	Petroleum ether	Continuous hot percolation method using soxhlet apparatus (successive solvent extraction)	Semi-solid	Dark green	2.93
2	Ethyl acetate		Sticky	Brownish green	3.31
3	Ethanol		Solid	Dark Reddish Brown	5.27

TOTAL SAPONIN AND FLAVONOID CONTENT:

The *Adansonia digitata* Linn., was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them the estimation of total saponins and flavonoids content in the ethanol extract were decided to be taken as parameters. Samples were drawn from three random samples and the total saponins and flavonoids content present in them were estimated.

Table No:8 Quantitative Estimation of Phytoconstituents

S.No	Parameters	Values
1	Total Saponin content	3.631 mg EE/gm
2	Total Flavonoid content	5.252 mg QE/gm

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Table No:9 Preliminary phytochemical screening for extracts of *Adansonia digitata* Linn.

S.No	Chemical constituents	Drug powder	Petroleum ether extract	Ethyl acetate extract	Ethanol extract
1	Alkaloids	-	-	-	+
2	Glycosides	+	-	-	+
3	Steroids	+	+	-	+
4	Flavonoids	+	-	+	+
5	Saponins	+	-	-	+
6	Phenolic compounds and tannins	+	-	-	+
7	Triterpenoids	+	+	-	-
8	Carbohydrates	+	-	-	+
9	Protein and amino acids	+	+	-	-
10	Gums and mucilage	+	-	-	+
11	Fixed oil and fats	-	+	-	-

Note: (**+**) indicates presence, (-) indicates absence.

FLUORESCENCE ANALYSIS

Table No:10 Fluorescence analysis of fruit powder of *Adansonia digitata* Linn.

S.NO	TREATMENT	DAY LIGHT	UV LIGHT	
			SHORT UV (254 nm)	LONG UV (365 nm)
1	Powder	Light Yellow	Yellow	Brownish yellow
2	Powder + water	Light Yellow	Pale brown	Brown
3	Powder + NaOH	Greenish yellow	Yellow	Dark Yellow
4	Powder + HCl	Brownish yellow	Light yellow	Dark Brown
5	Powder + Acetic acid	Brown	Light orange	Brown
6	Powder + Picric acid	Yellow	Light brown	Yellowish brown
7	Powder + Sulphuric acid	Yellow	Dark yellow	Dark brown
8	Powder + Nitric acid	Dark brown	Yellow	Brown
9	Powder + Iodine	Light brown	Orange	Dark brown
10	Powder + $FeCl_3$	Dark Yellow	Brownish orange	Dark Brown

Table No:11 Fluorescence analysis of various extracts of *Adansonia digitata* Linn.

S.NO	EXTRACT	DAY LIGHT	UV LIGHT	
			SHORT UV (254 nm)	LONG UV (365 nm)
1	Petroleum ether	Dark green	Orange	Pale Brown
2	Ethyl acetate	Brownish green	Light Brown	Dark brown
3	Ethanol	Dark Reddish Brown	Dark Brown	Brown

THIN LAYER CHROMATOGRAPHY OF EXTRACTS:

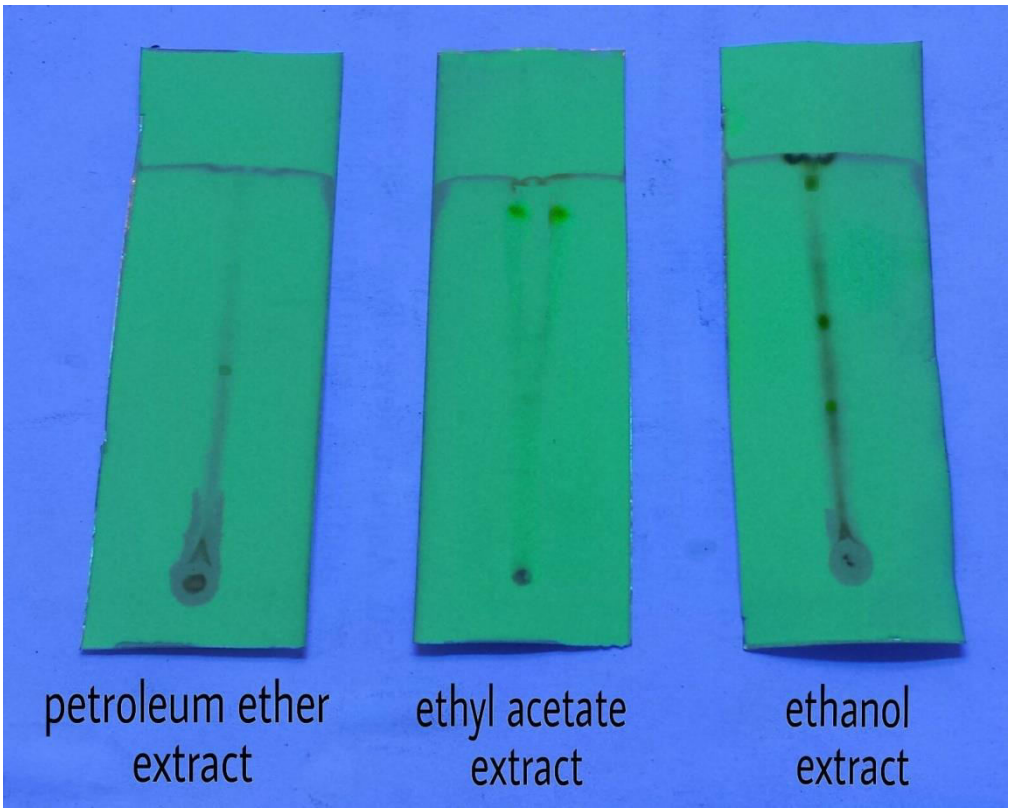


Fig no:33 TLC of different extracts of *Adansonia digitata* Linn.

Table No:12 R_f values of various extract of *Adansonia digitata* Linn.

S.NO	EXTRACTS	SOLVENT SYSTEM	NO. OF SPOTS	R_f VALUE
1	Petroleum ether	Chloroform: Methanol (96:4)	1	0.48
2	Ethyl actetate		2	0.93 0.91
3	Ethanol		3	0.94 0.68 0.43

PHYTOCHEMICAL STUDIES DISCUSSION

A lot of analytical techniques have developed for the quality control of drugs from plant origin. Therefore, it is very important to undertake the phytochemical investigations along with biological screenings to understand therapeutic efficacy of medicinal plants and also to develop quality parameters.

In this analysis, different polarity of phytoconstituents were sorted out from the coarsely powdered fruits of *Adansonia digitata* Linn., by using solvents of increasing polarity like petroleum ether, ethyl acetate, ethanol by using successive solvent extraction.

Successive solvent values revealed the solubility and polarity particulars of the metabolites in the fruit powder. Percentage yield of various extracts were as follows, Petroleum ether (2.93% w/w), Ethyl acetate (3.31% w/w), Ethanol (5.27% w/w). Ethanolic extract shows high extractive yield among the other extracts.

Qualitative preliminary phytochemical analysis was performed initially with different respective chemical detecting agents to detect the nature of phytoconstituents present in each extract. Petroleum ether showed the presence of steroids, triterpenoids and proteins. Ethyl acetate showed the presence of flavanoids. Ethanolic extract showed the presence of alkaloids, glycosides, steroids, flavanoids, saponins, carbohydrates, gums and mucilage.

In quantitative estimation, the total flavonoid content in ethanolic extract was found to be 5.252 mg QE/gm, which showed the presence of Quercetin. The total saponin content in ethanolic extract was found to be 3.631 mg EE/gm, which showed the presence of Escin. Qualitative chromatographic analysis of the extracts were done by using TLC to separate and identify the single or mixture of constituents present in each extract. The Chloroform : Methanol (96:4) solvent system was used to separate the phytoconstituents.

PHARMACOLOGICAL STUDIES

9. PHARMACOLOGICAL STUDIES

9.1 INVITRO ANTI DIABETIC STUDIES

1) α-Amylase Inhibition Assay: [89]

In vitro amylase inhibition was studied by the method of Bernfeld. In brief, 100µl of the test extract was allowed to react with 200µl of α-amylase enzyme (Hi median Rm638) and 100µl of 2mM of phosphate buffer (P^H–6.9). After 20 minute incubation, 100µ of 1% starch solution was added. The sample was performed for the controls where 200µl of the enzyme was replaced by buffer. After incubation for 5minutes, 500µl of dinitrosalicylic acid reagent was added to both control and test. They were kept in boiling water bath for 5minutes. The absorbance was recorded at 540nm using spectrometer and the percentage inhibition of α-amylase enzyme was calculated using the formula,

$$(\%)\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2) Haemoglobin Glycosylation Inhibition Assay: [90]

Anti-diabetic activity of fruits of *Adansonia digitata* Linn., were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4.1 ml each of above solution was mixed. Fruit extracts of *Adansonia digitata* Linn., was weighed and dissolved in DMSO to obtain stock solution and then 1-5 µg/ml solutions were prepared. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm.

Percentage inhibition was calculated as,

$$(\%)\ \text{Inhibition} = \frac{\text{Absorbance of sample} - \text{Absorbance of control}}{\text{Absorbance of sample}} \times 100$$

The IC 50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor.

ACUTE ORAL TOXICITY STUDY (UP AND DOWN METHOD) OECD 425 GUIDLINES FOR THE TESTING OF CHEMICALS: [91]

The organization of economic co-operation and development (OECD) guideline 425 reveals the acute oral toxicity up and down method is a stepwise procedure in which 5 rats of single sex is five steps (one animal per step). Depending upon the mortality and morbidity status of the animal, on average of 2 to 4 steps may be necessary to allow judgement on the acute oral toxicity of the substance. This procedure results in the use of minimal number of animal while allowing for acceptable data based scientific conclusion.

Literature review showed that the acute toxicity study on fruit extracts of *Adansonia digitata* was performed and the extract did not produced toxicity till the dose level of 5000 mg/kg. Hence, a starting dose level of 200 mg/kg of fruits of *Adansonia digitata* was used. After oral administration, animals were observed at an hourly basis for the first 4 hours and periodically for 24 hours to assess the general behaviour and 72 hours for any toxic symptoms and mortality of the animal for 28 days.

The protocol for conducting the *in vivo* study in wistar albino rats was approved by the Institutional Animal Ethical Committee (IAEC) which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Approval no: IAEC/MMC/07/2016

9.2 INVIVO EVALUATION OF ANTI-DIABETIC ACTIVITY OF STREPTOZOTOCIN INDUCED DIABETES: [92,93,94]

EXPERIMENTAL DESIGN FOR STREPTOZOTOCIN INDUCED HYPERGLYCEMIC STUDIES

Animals were randomly divided into 5 groups of rats (n=6)

The animals received the following treatments,

Table No:13 Experimental design for anti-diabetic activity

S.NO	GROUP	NAME OF THE DRUG	DOSE	NO. OF ANIMALS	DURATION OF DOSAGE (days)
1	Group-1	Normal control	Saline	6	28
2	Group-2	Diabetic control (0.9% v/v saline)	2ml p.o	6	28
3	Group-3	Glibenclamide	4mg/kg p.o	6	28
4	Group-4	Extract low dose	200mg/kg p.o	6	28
5	Group-5	Extract high dose	400mg/kg p.o	6	28
			TOTAL	30	28

The experimental animals were fasted for 18 hours and the blood glucose level (BGL) was monitored using a glucometer after streptozotocin injection. Blood samples was collected by tail clipping method. Rats with blood glucose level of greater than 250 mg/dl were considered diabetic and selected for the study (WHO, 1985). Rats were randomly divided into 5 groups of 6 rats per group for screening.

Streptozotocin monohydrate 45 mg/kg body weight was dissolved in 0.9% v/v cold normal saline and injected intraperitoneally to 18 hours fasted rats (24 no's,) group II-IV in order to induce hyperglycaemia in experimental wistar rats (130-180g body weight (b/w) and the six control rats (group-I) received equal volume of 0.9% v/v cold normal saline injected intraperitoneally.

COLLECTION OF BLOOD AND ORGANS:

The treatment was carried up to 28 days and on 1st, 7th, 14th and 21st days 0.5 ml of blood was collected from lateral tail vein using lance or butterfly needle and blood glucose level was checked by using a Glucometer. After 28 days the blood was collected and used to determine haematological parameters. The test animals were anesthetized with ketamine hydrochloride at the dose of 10 mg/kg and sacrificed. Pancreas was isolated and used for histopathological studies.

9.3 HISTOPATHOLOGICAL STUDY: [95]

For histological examinations, small pieces of pancreas were fixed in Bouin's Solution for 24h dehydrated through graded concentration of ethanol, embedded in Paraffin wax, sectioned at 5µm thicknesses and stained with Mayer's haematoxylin and Eosin and observed under light microscope.

STATISTICAL ANALYSIS:

Results were expressed as Mean ± S.E.M. The data was analyzed using One Way of Variable (ANOVA) followed by Dennett's test. P-value <0.05 considered as significant.

PHARMACOLOGY
RESULTS AND DISCUSSION

9.4 RESULTS AND DISCUSSION

INVITRO ANTI-DIABETIC STUDIES

1) α-AMYLASE INHIBITION ASSAY:

Table No:14 IC_{50} values of α-amylase inhibitory assay in standard

S.No	Concentration (µg/ml)	% Inhibition
		Standard (Acarbose)
1	**125**	**78.69** ± 0.17
2	100	58.58 ± 0.24
3	75	40.64 ± 0.43
4	50	29.73 ± 0.56
5	25	23.62 ± 1.24
IC_{50}		371.20

Table No:15 IC_{50} values of α-amylase inhibitory assay in extracts

S.No	Concentration (µg/ml)	% Inhibition		
		Petroleum ether extract	Ethyl acetate extract	Ethanol extract
1	125	38.17 ± 0.01	46.14 ± 0.02	51.38 ± 0.01
2	**250**	41.29 ± 0.01	66.51 ± 0.01	**70.02** ± 0.01
3	500	28.75 ± 0.01	36.67 ± 0.005	47.84 ± 0.01
4	1000	13.78 ± 0.02	22.25 ± 0.02	37.47 ± 0.012
5	2000	1.41 ± 0.02	3.01 ± 0.02	5.39 ± 0.02
IC_{50}		1086.11	556.28	442.26

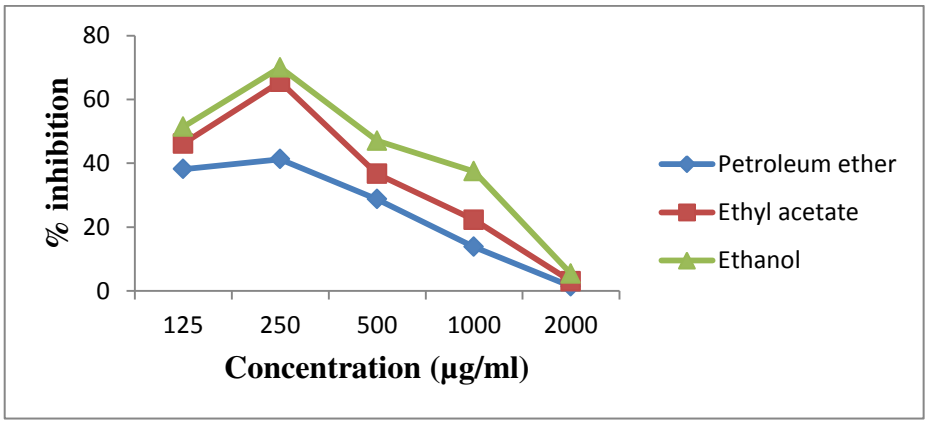


Fig no:34 Graphical representation of the α-amylase inhibition assay

2) HAEMOGLOBIN GLYCOSYLATION INHIBITION ASSAY:

Table No:16 IC_{50} values of haemoglobin glycosylation inhibitory assay in standard

S.No	Concentration (µg/ml)	% Inhibition
		Standard (Acarbose)
1	**125**	**70.15** ± 0.14
2	100	55.14 ± 0.11
3	75	38.67 ± 0.87
4	50	28.72 ± 0.57
5	25	17.68 ± 1.97
IC_{50}		476.41

Table No:17 IC_{50} values of haemoglobin glycosylation inhibitory assay in extracts

S.No	Concentration (µg/ml)	% Inhibition		
		Petroleum ether extract	Ethyl acetate extract	Ethanol extract
1	125	30.47 ± 0.01	40.44 ± 0.02	57.43 ± 0.01
2	**250**	32.29 ± 0.01	48.69 ± 0.01	**61.52** ± 0.01
3	500	15.00 ± 0.04	33.08 ± 0.01	44.90 ± 0.01
4	1000	6.78 ± 0.01	16.18 ± 0.03	27.47 ± 0.02
5	2000	0.53 ± 0.02	1.07 ± 0.02	9.90 ± 0.02
IC_{50}		1567.33	1139.12	568.75

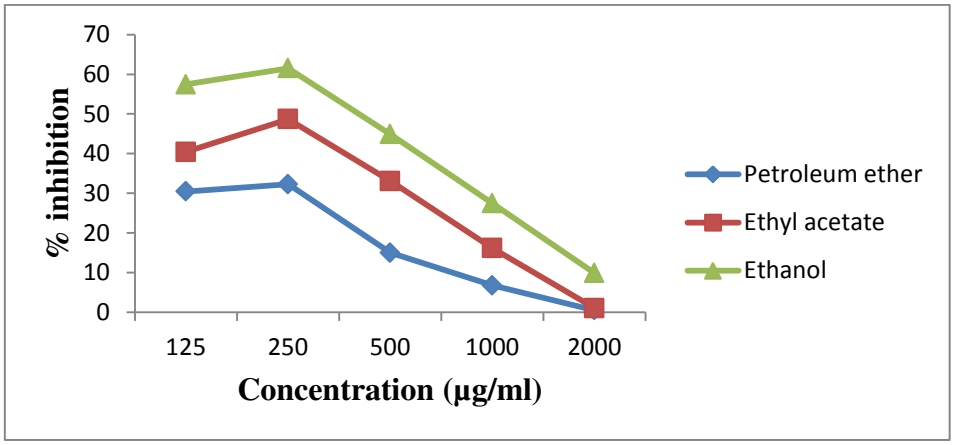


Fig no:35 Graphical representation of the haemoglobin glycosylation inhibition assay

INVIVO ANTI-DIABETIC STUDIES

STREPTOZOTOCIN INDUCED DIABETES MELLITUS IN RATS:

Table No.18 Effects of Ethanolic extract of *Adansonia digitata* Linn., on blood glucose level in streptozotocin induced diabetic rats (mg/dl)

TREATMENT	DAY 0	DAY 1	DAY 7	DAY 14	DAY 21	DAY 28
GROUP I (Normal)	**103** ± 1.4	**96** ± 1.3	**94** ± 1.5	**99** ± 1.3	**101** ± 1.1	**95** ± 1.9
GROUP II (Diabetic)	**108** ± 2.3	**410** ± 1.9	**356** ± 1.5	**347** ± 1.6	**304** ± 2.6	**296** ± 2.2
GROUP III (Standard)	**111** ± 1.6	**397** ± 3.4	**96** ± 2.8	**89** ± 1.5	**84** ± 2.2	**79** ± 3.3
GROUP IV (Low dose)	**105** ± 2.2	**418** ± 2.0	**111** ± 1.5	**107** ± 4.2	**106** ± 1.7	**97** ± 1.6
GROUP V (High dose)	**115** ± 1.5	**405** ± 3.3	**142** ± 1.1	**130** ± 2.7	**126** ± 1.2	**118** ± 2.1

Values are expressed as mean ±SD; n = 6; P < 0.05 compared to diabetic control

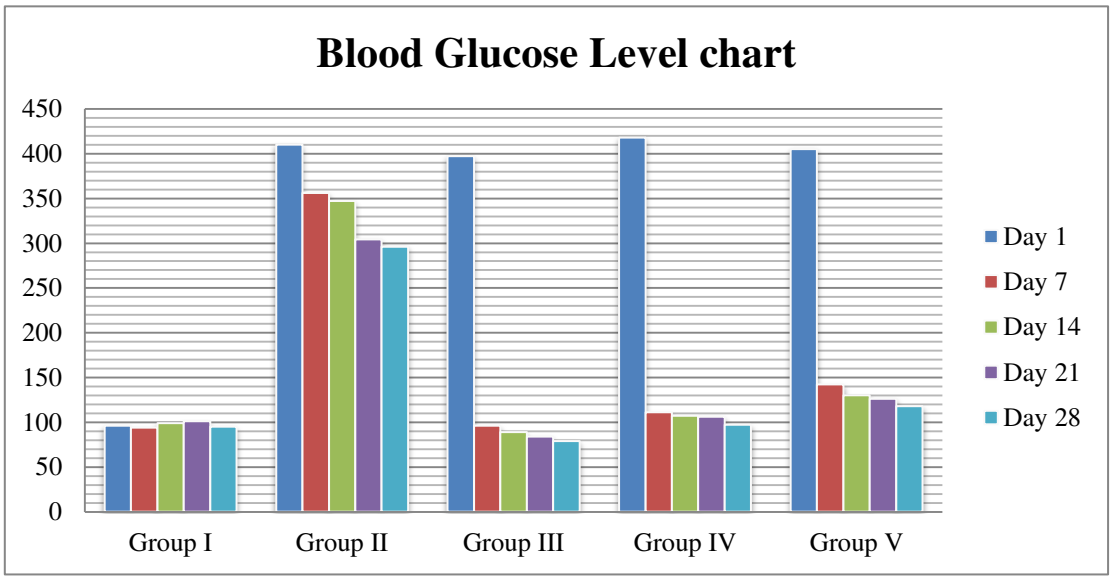


Fig no:36 Graphical representation of blood glucose level in study groups (mg/dl)

Table No.19 Effects of Ethanolic extract of *Adansonia digitata* Linn., on body weight in streptozotocin induced diabetic rats (mg/dl)

TREATMENT	DAY 0	DAY 1	DAY 7	DAY 14	DAY 21	DAY 28
GROUP I (Normal)	**135** ± 5.68	**138** ± 9.43	**143** ± 3.43	**147** ± 3.47	**133** ± 5.32	**146** ± 2.22
GROUP II (Diabetic)	**149** ± 5.75	**141** ± 7.22	**129** ± 6.32	**103** ± 4.33	**97** ± 3.55	**93** ± 1.55
GROUP III (Standard)	**157** ± 7.06	**154** ± 6.48	**137** ± 6.75	**144** ± 5.67	**152** ± 9.76	**166** ± 6.78
GROUP IV (Low dose)	**149** ± 7.75	**145** ± 8.67	**105** ± 6.97	**123** ± 7.55	**145** ± 5.54	**149** ± 2.37
GROUP V (High dose)	**151** ± 1.25	**144** ± 7.77	**101** ± 3.21	**119** ± 3.34	**122** ± 7.64	**136** ± 3.54

Values are expressed as mean ±SD; n = 6; P < 0.05 compared to diabetic control

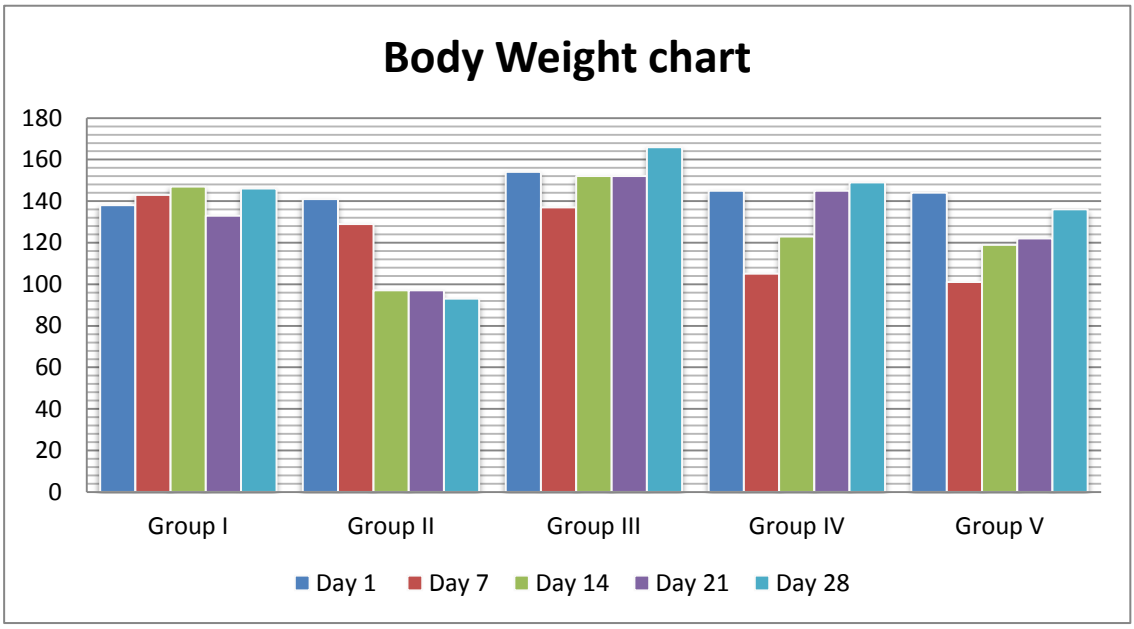


Fig no:37 Graphical representation of body weight level in study groups (grams)

HISTOPATHOLOGICAL EXAMINATION OF RAT PANCREAS:

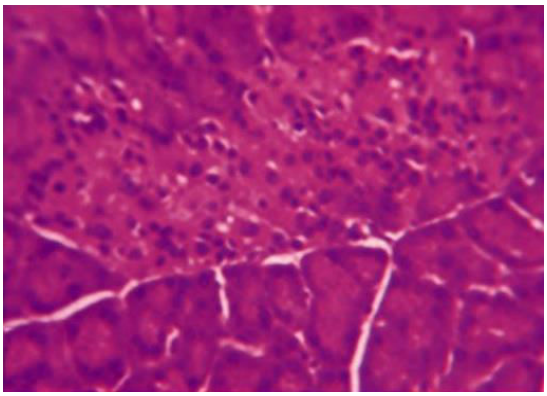


Fig no:38 Normal control group

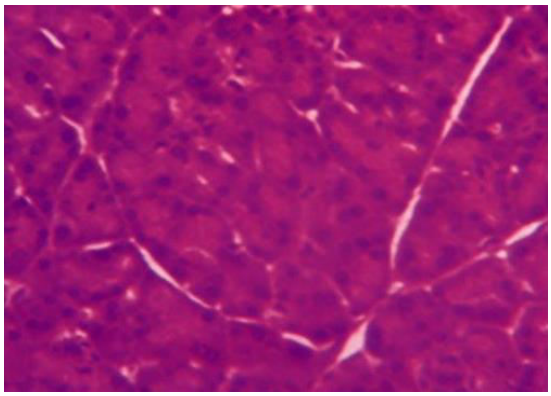


Fig no:39 Diabetic control group

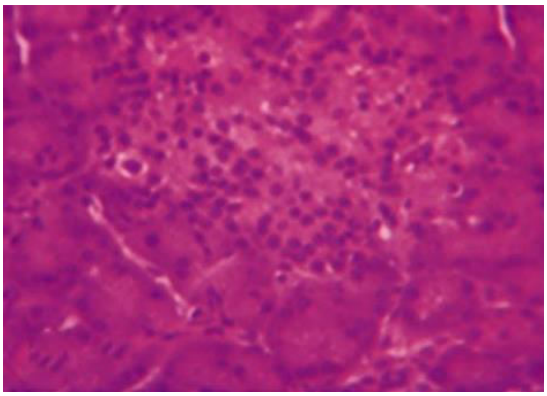


Fig no:40 Standard (Glibenclamide 4mg/kg)

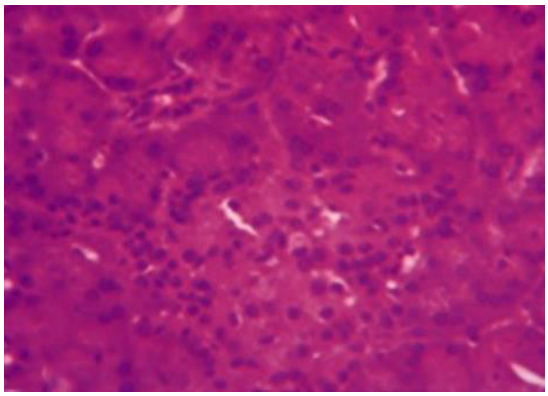


Fig no:41 *Adansonia digitata* Extract (200 mg/kg)

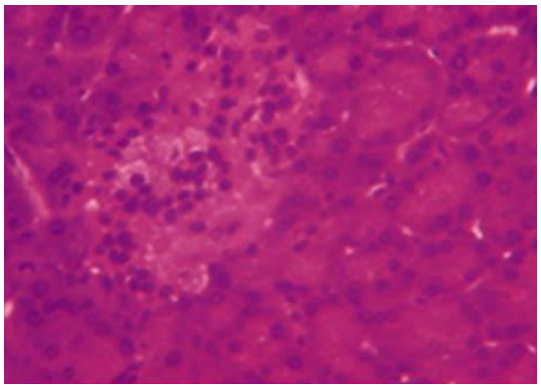


Fig no:42 *Adansonia digitata* Extract (400 mg/kg)

Fig no:38 Presence of normal pancreatic islet cells.
Fig no:39 Reduction in the size of islets, damaged β-cell population and extensive necrotic changes followed by fibrosis and atrophy.
Fig no:40 Restored necrotic and fibrotic changes. Increased number and size of the islets.
Fig no:41&42 Absence of necrosis and fibrotic changes. Increased number and size of the islets and presence of normal pancreatic islet cells.

PHARMACOLOGICAL STUDIES DISCUSSION

The Pharmacological studies were carried out for accessing the anti-diabetic activity on the fruits of *Adansonia digitata* Linn.

The literature review showed that the acute toxicity studies were performed according to the OECD 425 guidelines and it explains that the fruit extracts did not produce any behavioral changes or mortality up to the dose of 5000 mg/kg. These extracts belong to category 5 of the Globally Hormonised classification System (GHS). So, the *in vivo* studies were carried out at a dose of 200 mg/kg & 400 mg/kg.

In vitro studies was carried out on fruits extracts of *Adansonia digitata* Linn. Alpha amylase inhibitory activity of ethanolic extract shown high inhibiting potential. So, the ethanolic extract was used for *in vivo* studies.

Anti-diabetic activity of extract was accessed by the method of streptozotocin induced diabetes in rats. In this method, parameters like blood glucose level and body weight was evaluated. Administration of ethanolic extracts (200 mg/kg and 400 mg/kg) and standard drug glibenclamide (4 mg/kg p.o) on 1^{st}, 7^{th}, 14^{th}, 21^{th}, 28^{th} days was carried out.

Blood glucose level was observed in the animals treated with ethanolic extracts of *Adansonia digitata* Linn., and the blood glucose level was clearly reduced.

The body weight of the standard group and extract treated groups significantly increased when compared to disease control group.

Histopathological study results showed the *decrease in necrosis* and *increase in the β-cell size and number* in the standard and extract treated groups. All the parameters reveal the potent anti-diabetic activity of the Ethanolic extract of *Adansonia digitata* L. fruits.

SUMMARY AND CONCLUSION

10. SUMMARY AND CONCLUSION

The project entitled "Pharmacognostical, Phytochemical and Anti-diabetic studies on fruits of *Adansonia digitata* Linn.," (Bombacaceae) has been achieved by the following results.

Authentication plays a key role in pharmacognostical studies. The fresh fruits of *Adansonia digitata* Linn., was collected from the Madras Medical College Men's Hostel, Chennai and authenticated by Prof.P.Jayaraman Ph.D., Director, Institute of Herbal Botany, Plant Anatomy Research Centre.

The Pharmacognostical parameters such as Macroscopy, Microscopy, Powder Microscopy, Histochemical Studies and Physicochemical Constants were studied to establish the data for characteristics feature of plant and detection of adulterants.

The qualitative and quantitative analysis was carried out to identify Inorganic Elements present in the fruits.

The qualitative and quantitative analysis of toxic heavy metals like Arsenic, Cadmium, Lead and Mercury were within the WHO limits and ensured the safety of the drug.

In Phytochemical analysis, extraction is the first step involved. The coarse powder was extracted by Petroleum Ether, Ethyl Acetate, Ethanol by Successive Solvent Extraction by Hot Percolation Method.

The preliminary phytochemical screening of various extract and powder of the plant has revealed the presence of phytoconstituents like Flavonoids, Saponins, Steroids, Tannins, Alkaloids, Glycosides And Carbohydrates.

Qualitative chromatographic analysis-TLC for various extracts was carried out to identify the phytoconstituents present.

In vitro studies, α-Amylase Inhibition Assay and Haemoglobin Glycosylation Inhibition Assay was performed for the selection of the Bio-active extract.

Accordingly, the ethanolic extract possessed maximum anti-diabetic activity. So, it was selected for *in vivo* studies.

The Acute toxicity studies revealed that the extract was safe up to the dose of 5000 mg/kg. Anti-diabetic activity was assessed by Streptozotocin Induced Diabetic Mellitus model.

The parameters examined were Blood Glucose Level and Changes in the body weight.

The Histopathological study was performed. The inference made from it were that the cells in the diabetic control group were reduced in size, damaged β-cell population and extensive necrotic changes, followed by fibrosis and atrophy.

While in the group that received that the test dose showed, the absence of necrosis, fibrotic changes, increased number and size of the islets and presence of normal pancreatic cells.

These were in the levels comparable with the ones that were administered the standard drug Glibenclamide.

The phytochemical evaluation showed the presence of flavonoids. These compounds might be responsible for the anti-diabetic activity on the fruits of *Adansonia digitata* Linn.

The present study revealed the Ethanolic extract has the significant anti-diabetic activity in the both *In vitro* and *In vivo* models.

Further studies is required to find out the mechanism of action responsible for the anti-diabetic activity.

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ANNEXURES