KUVEMPU UNIVERSITY

Final Report on a Major Research Project

"Enumeration and Investigation of Medicinal Plants for Antidiabetic Activity in Western Ghats of Karnataka"

UGC F.NO. 37-260/2009(SR) dated 12.01.2010

Submitted to

ज्ञान-विज्ञान विमुक्तये

University Grants Commision
Bahadur Shah Zafar Marg, New Delhi – 110002

By

Dr. Yadav D. Bodke
Principal Investigator
Department of P.G.Studies and Research in Industrial Chemistry
Kuvempu University, Jnana Sahyadri, Shankaraghatta-577 451 Shimoga Dist.
Karnataka, India

February -2014

Annexure -VIII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002
Final Report of the work done on the Major Research Project

1. Project report No : Final report

2. UGC Reference No. : UGC F.NO.37-260/2009 (SR) Dated: 12/01/2010

3. Period of report : From 01/022010 to 31/07/2013

4. Title of research project : Enumeration and Investigation of Medicinal Plants for Antidiabetic Activity in Western Ghats of Karnataka

5. (a) Name of the Principal Investigator : **Dr. Yadav D. Bodke**

(b) Dept and University/College where work has progressed : Department of Industrial Chemistry
Kuvempu University, Jnana Sahyadri
Shankaraghatta-577451, Karnataka

6. Effective date of starting of the project : 23/06/2010

7. Grant approved and expenditure incurred during the period of the report

a. Total amount approved : Rs 6, 44,340=00

b. Total expenditure : Rs 6, 44,340=00

c. Report of the work done (Please attach a separate sheet) : Separate sheet attached

i. Brief objective of the project : Details given in **Annexure I**

ii. Work done so far and results achieved and Publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication) : Details given in **Annexure III**

iii. Has the progress been according to original plan of workand towards achieving the objective? if not, state reasons : Yes, as per the plan of work

iv. Please indicate the difficulties, if any, Experienced in implementing the project : After isolation of the compounds from plants extract, we don't have adequate spectroscopic facilities for the characterization of the compounds

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed

A summary of the work done for the period (Annual basis): Completed as per the original plan may please be sent to the Commission on a separate sheet

vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission : Attached (Annexure II)

vii. Any other information which would help in evaluation of work done on the project.

At the completion of the project, the first report should indicate the output, such as

(a) Manpower trained : Yes

(b) Ph. D. awarded : No (Registered for Ph. D. Degree)

(c) Publication of results : 2 papers published and 2 papers communicated

(d) Other impact, if any : No

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

Dr. YADAV D. BOOKE
M.Sc., Ph.D.,
Assistant Professor
Dept. of P.G. Studies and
Research in Industrial Chemistry
Kuvempu University
Shankaraghatta-577 451

3/3/14

REGISTRAR/PRINCIPAL

(Seal)

Registrar
Kuvempu University
Jnana Sahyadri
Shankaraghatta-577 451
Shimoga (Dist.) Karnataka (State)

Annexure –IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Title of the Project	: Enumeration and Investigation of Medicinal Plants for Antidiabetic Activity in Western Ghats of Karnataka
2. Name and address of the principal investigator	: Dr. Yadav D. Bodke Assistant professor, Department of Industrial Chemistry
3. Name and address of the Institution	: Department of Industrial Chemistry, Janna Sahydri, Kuvempu University Shankaraghatta,Shimoga, Karnataka
4. UGC approval no. and date	: Fo. No. 37-260/2009 (SR) dated 12.01.2010
5. Date of Implementation	: 23/06/2010
6. Tenure of the Project	: Three Years six months
7. Total Grant Allocated	: Rs. 7, 07.167/-
8. Total grant received	: Rs. 6, 44,340/-
9. Final expenditure	: Rs. 6, 44,340/-
10. Title of the Project	: Enumeration and Investigation of Medicinal Plants for Antidiabetic Activity in Western Ghats of Karnataka

11. Objectives of the Project

- To prepare an exhaustive list of medicinal plants available in Western Ghats of Karnataka used for diabetes
- To use crude extracts of these plants for diabetic case of different species
- To isolate active components from these extracts.

12. Whether Objectives were achieved : Yes

13. Achievements from the Project:

- The plants with potent antidiabetic activity have been identified
- The active compounds for the antidiabetic activity has been identified and characterized

14. Summary of the Findings : Attached (**Annexure II**)

15. Contribution to the Society : We have come up with specific plants which exhibit potent antidiabetic activity. We have isolated the active components from the plant extracts and are characterized with the help of spectroscopic techniques. The results from the research findings, promoted us to do further study.

16. Whether any Ph.D. Enrolled/produced Out of the Project : Yes, enrolled for Ph.D. Degree

17. No. Of publications out of the project : 2 papers published and 2 paper communicated

PRINCIPAL INVESTIGATOR

Dr. YALAV. D. BODKE
M.Sc., Ph.D.
Assistant Professor
Dept. of PG Studies and
Research in Industrial Chemistry
Kuvempu University
Shankaraghatta-577 451

5/3/14

REGISTRAR/PRINCIPAL

(Seal)

Registrar
Kuvempu University
Jnana Sahyadri
Shankaraghatta-577 451
Shimoga (Dist.) Karnataka (State)

1. Introduction

1.1: Introduction to medicinal plants

The ancient people were depending on nature for their survival, they learned the use of plants by trial and error methods. Traditionally, this treasure of knowledge passed orally from generation to generation without any written document and it still retained by some indigenous tribal people of some part of the world (Perumal Samy R, et al.,2000). The world health organization estimates that about 80% of the world population relies mainly on herbal medicine for primary health care (World Health Organization (WHO) 2003). Ethnobotanical studies have brought to light numerous plants having significant medicinal properties which were earlier unknown to scientific world.Today many drugs that are in markets have come to us from folk use and use of indigenous communities.

Medicinal plants have curative property due to the presence of various complex chemical substances of different composition, which are found as secondary metabolites in one or more part of the plant body. These plant metabolites are grouped according to their chemical compositions as alkaloids, glycosides, steroids, essential oils, terpenoids, etc.

India is known as the "Emporium of Medicinal Plants". The plants were used to treat various diseases in India dates back to the times of Rigveda (3500BC-18000BC). The great saint Athreya, preached Ayurveda at kashi in the Veda's period and its followers Charaka, Sushrutha, and Vaaghbhata wrote the great Indian epics of Ayurveda namely: "CharakaSamhitha", "SushruthaSamhitha", and "AashtangaHrudayaSamhitha". These treatises have incorporated namely 700 herbal drugs for several ailments. It also describes the mode of preparation of drugs, mode of administration and management of health care. In this system of health care each person treated individually in holistic fashion.

Western Ghats of Karnataka is one of the eighteenth biodiversity hot spots of the world. It attracts attention from people mainly due to the presence of large medicinal plants. Kuvempu University is one of the young and fast growing Universities of India. It is situated in the heart of the Malnad region i.e., Shimoga district in the state of Karnataka. The flora around the University has spurred young researchers into action for finding out new medicinal plants for various ailments.

In recent years, extensive research work has been carried out on medicinal plants possessing biological activity. The researchers always aim to bring out cost-effective and efficacious medicines for the benefits of mankind.

Literature survey revealed that plants which possess antioxidant activity have also shows good antidiabetic activity (SaikatDewanjee et al., 1999). Brief literature survey about medicinal plants, which are used for diabetes treatment, was carried out at the library of Kuvempu University and Indian Institute of Science, Bangalore. Name of some medicinal plants which are used for diabetes are given in T**able 1.**

Table 1.Antidiabetic potential medicinal plants found in Western Ghats of Karnataka

Sl No.	**Plant name**	**Sl No.**	**Plant name**
1	*Abutilon indicum*	35	*Imperata cylindrical (Linn.)*
2	*Aeglemarmelos (Linn.)*	36	*Ipomea aquatic*
3	*adhatodavasica*	37	*Jussieuarepens Linn*
4	*Albizialebbeck*	35	*Justiciaadhatoda*
5	*Allium cepa*	36	*Ludwigiaoctovalvis (Jacq.)*
6	*Aloe vera*	37	*Mangiferaindica*
7	*Anacardiumoccidentale*	38	*Mimosa pudica*
8	*Andrographispaniculata*	39	*Momordicacharantia*
9	*Antidesmaacidum Retz*	40	*Morusindica Linn*
10	*Azadirachtaindica A.*	41	*NelumbonuciferaGaertn*
11	*Brassica oleracea*	42	*Ocimumtenuiflorum*
12	*Capparisdecidua*	43	*Piper nigrum*
13	*Cassia alata Linn*	44	*Phyllanthusemblica Linn*
14	*Cassia fistula*	45	*Plumbagozeylanica*

15	*Cassia occidentalis*	46	*Rutagraveolens*
16	*Carica papaya*	47	*Salaciachinensis*
17	*Catharanthusroseus*	48	*Syzigiumcumini (Linn.)*
18	*Centellaasiatica*	49	*Terminalis arjuna*
19	*ClerodendrumindicumLinn.*	50	*Terminalia bellirica*
20	*Cuminumcyminum L*	51	*Terminalischabula*
21	*Cynodondactylon*	52	*Tinosporacordifoli*
22	*CyperusrotundusLinn.*	53	*Terminaliapallida*
23	*Debregeasialongifolia (Burm.f.)*	54	*Trigonellafoenum-graecum*
24	*Embeliaribesburm*	55	*Tridaxprocumbens*
25	*Ficusracemosa Linn*	56	*Vitexnegundo*
26	*Ficushispida Linn*	57	*Vignaunguiculata*
27	*Glycyrrhizaglabra*		
28	*Gymnemasylvestre*		
29	*Hepatica transsilvanica*		
30	*Hibiscus Rosa sinensis*		
31	*HolarrhenaAntidysenterica*		
32	*Hordeumvulgare*		
33	*Lantana camara*		
34	*Leucasaspera*		

1.2 : Introduction to Diabetes Mellitus

Diabetes Mellitus (DM) is an increasingly common, potentially devastating, expensive, treatable but incurable lifelong disease. DM is a prevalent systemic disease affecting a significant proportion of the population worldwide. It is commonly found in all part of the world **(**Kavishankar,Net al., 2011**)** and it is a global health problem. Diabetes is one of the major causes of premature illness and death worldwide (Roglic G et,. al2005).It has been estimated that about 366 million people, corresponding to 8.3% of the world's adult population, lived with diabetes in 2011. The number is expected to grow to 552 million by 2030. An estimated 80% of the current cases of diabetes found in developing countries, China has the world's largest diabetes population, followed by India with 61.3 million (International Diabetes Federation 2011).

DM is a chronic disease characterized by hyperglycemia caused by insufficiency of insulin action **(**Saltiel, A. R and Kahn, R. 2001). In other words, it is a condition in which the body either does not produce enough insulin nor does not properly response to the insulin. Diabetes mellitus is one of the most important metabolic disorder that affecting every part and every system of our body. It is the fact that diabetes cannot be cured and it has been never reported that someone had recovered totally from the diabetes.

The term ‘diabetes mellitus’ describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure and neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction.

Diabetes is fist recorded in English, in the name of diabete, in a medical text written around 1425. In 1675 Thamas Willis added word mellitus from the Latin meaning “honey”, a reference to the sweet taste of the urine. Matthew Dobson in 1776 confirmed the sweet taste was because of an excess of sugar in the urine and the blood of people with diabetes

In the late 1970s both WHO and the National Diabetes Data Group produced new diagnostic criteria and a new classification system for diabetes mellitus. In 1985, WHO slightly

modified their criteria to coincide more closely with the National Diabetes Data Group (NDDG) values. Now there are many data available, and also much more etiological information has appeared. An American Diabetes Association (ADA) expert group was convened to discuss these issues.

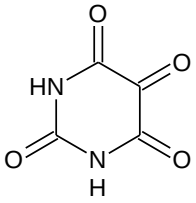
In ayurvedic terminology, diabetes is termed as madhumeha and is characterized by the production of sweet urine. In 6^{th} century, Sushruta identified diabetes and classified it as madhumeha. The ancient people tested for diabetes by observing whether ants were attracted to a person's urine and called the ailment "sweet urine disease."

When people eat, the pancreas automatically produces the right amount of insulin to move glucose from blood into the cells. In the people with diabetes, the pancreas either produces little or no insulin, or the cells do not respond appropriately to the insulin that is produced. Glucose builds up in the blood, overflows into the urine, and passes out of the body in the urine. Thus, the body loses its main source of fuel even though the blood contains large amounts of glucose. The main causative factors for diabetes mellitus include genetic factor as well as environmental conditions. Diabetes is complex in nature and changing life style, food habits and stress have further complicated it. Irregular food habit, lack of exercises, stress and strains are the important environmental factors. Family history also play important role, overweight and mutation in gene also causes diabetes. Several pathogenetic processes are involved in the development of diabetes; these include processes which destroy the β cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. A virus called *Coxsaekie* B4 and Vitamin D3 deficiency develops type-1 diabetes. Some chemicals and drugs are also inducing diabetes by destroying pancreatic cell. Some other pancreatic problem like tranum, pancreatitis is also leads to loss of insulin production. On the other hand, the generation of free radicals in our body is mainly responsible for the development of diabetes (Oberley, L.W., 1998).

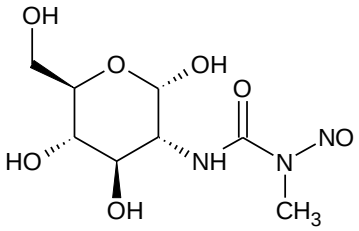
Alloxan and streptozotocin are the most prominent diabetogenic chemicals in diabetes research. Both are cytotoxic glucose analogues. Although their cytotoxicity is achieved *via* different pathways, their mechanisms of β cells selective action are identical.

Alloxan and streptozotocin are toxic glucose analogues that preferentially accumulate in pancreatic β cells *via* the GLUT-2 glucose transporter. In the presence of intracellular thiols,

especially glutathione, alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid. Auto oxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalyzed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the β cells, which have particularly low antioxidative defense capacity, and the ensuing state of insulin-dependent alloxan diabetes. As a thiol reagent, alloxan also selectively inhibits glucose-induced insulin secretion through its ability to inhibit the β cell glucose sensor glucokinase. Following its uptake into the beta cells, streptozotocin is split into its glucose and methylnitrosourea moiety. Owing to its alkylating properties, the latter modifies biological macromolecules, fragments DNA and destroys the β cells, causing a state of insulin-dependent diabetes. The targeting of mitochondrial DNA, thereby impairing the signaling function of β cell mitochondrial metabolism, also explains how streptozotocin is able to inhibit glucose-induced insulin secretion.



Alloxan



Streptozotocin

1.2.1: Types of Diabetes:

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 (Oberley, L.W., 1998). In 1980, expert committee proposed two major classes of diabetes mellitus and named them as Insulin-Dependent Diabetes Mellitus (IDDM) or Type 1, and Non-Insulin Dependent Diabetes Mellitus (NIDDM) or Type 2. In the 1985 Study Group Report, the terms Type 1 and Type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class of Malnutrition–related Diabetes Mellitus (MRDM) was introduced (National Diabetes Data Group. 1979). In both 1980 and 1985 reports, other classes of diabetes included and they are Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM). The 1985 classification was widely accepted and is using Internationally. The recommended classification includes both staging of diabetes mellitus based on clinical descriptive criteria and

a complementary aetiological classification. The classification encompasses both clinical stages and aetiological types of diabetes mellitus.

1. Type 1 diabetes mellitus (IDDM)
2. Type 2 diabetes mellitus (NIDDM)
3. Gestational diabetes

1.2.1.1: Type 1 Diabetes mellitus:

It is formerly known as insulin-dependent diabetes mellitus (IDDM). It accounts 5 % of the cases. It occurs when β-cells of the pancreatic islets of Langerhans are destroyed by autoimmune process (Bottini, N et al., 2006). The rate of destruction is quite variable, being rapid in some individuals and slow in others (Zimmet PZ et al., 1994). The rapidly progressive form is commonly observed in children, but also may occur in adults (Humphrey ARG et al., 1998). Generally, type 1 diabetes mellitus develops in an adult that is before the age of 40. In IDDM, the body doesn't produce sufficient insulin, as a result, the body cell inability to uptake and utilize the glucose from the blood resulting rise in glucose level in blood. Type 1 diabetes often become dependent on insulin for survival eventually and is at risk for ketoacidosis (Willis JA et al., 1996).

Causes for type 1 diabetes

Normally, the immune system protects the body from infection by identifying and destroying bacteria, viruses, and other potentially harmful foreign substances. But in autoimmune diseases, the immune system attacks the body's own cells and destroyed β-cells of the pancreatic islets of Langerhans. Heredity plays an important part in determining who is likely to develop type 1 diabetes. Environmental factors, such as food, viruses and toxins, may play a role in the development of type 1 diabetes, but the exact nature of their role has not been determined. Some theories suggest that environmental factors trigger the autoimmune destruction of β cells in people with a genetic susceptibility to diabetes. Viruses possibly associated with type 1 diabetes include *coxsackie virus* B, *cytomegalo virus, adeno virus, rubella* and *mumps*. Scientists have described several ways these viruses may damage or destroy β cells or possibly trigger an autoimmune response in susceptible people.

Symptoms of type 1 diabetes mellitus

➢ Irritability	➢ Polyuria (frequent urination)
➢ Polydipsia (abnormal thirst)	➢ Polyphagia (unusual hunger)
➢ Nausea	➢ Vomiting
➢ Weakness	➢ Fatigue
➢ Dizzinesss	➢ Sweating
➢ Confusion palpitation	➢ Tingling of libs
➢ Weight loss.	

1.2.1.2: Type 2 diabetes mellitus

It is formerly known as non-insulin dependent diabetes mellitus (NIDDM) or maturity onset diabetes mellitus. It accounts 95 % of the cases. NIDDM is a complex heterogeneous group of metabolic disorders characterized by hyperglycemia and impaired insulin action and/or insulin secretion. The complex nature of NIDDM reflects the multifaceted genetic background and the varied genetic-environmental interaction (Current views on type 2 diabetes. Yi Lin and Zhongjie Sun, 2010. NIDDM has strong genetic link that means type 2 Diabetes tends to run in families and develops after the age of 50 year (Knowler WC et al., 1993). In this type the serum insulin level are normal or elevated so type 2 diabetes mellitus is a disease of insulin resistance and impaired insulin secretion (DeFronzo et al., 1997.Lillioja et al., 1993.Roderick, E.W. 2004). The risk of developing type 2 diabetes increases with age, obesity, stress and lack of physical activity (Zimmet PZ, 1992).

Causes of type 2 diabetes

Physical inactivity and obesity are strongly associated with the development of type 2 diabetes.The genetic factor play important role in NIDDM. An imbalance between caloric intake and physical activity can lead to obesity, which causes insulin resistance and is common in people with type 2 diabetes. As long as β cells are able to produce enough insulin, blood glucose levels stay in the normal range. But when insulin production falters because of β cell dysfunction, glucose levels rise, leading to prediabetes or diabetes.

Symptoms of type 2 diabetes mellitus

- Blurred vision
- Unusual thirst
- Dry red tongue
- Skin infection
- Mental depression
- Fatigue
- Tingling
- Excessive bowel movements.
- Itching
- Inordinate appetite
- Indigestion
- Progressive weakness
- Obesity
- Slow wound healing
- Numbness in feet

1.2.1.3. Gestational diabetes mellitus (GDM)

As the name indicates that the GDM exhibit high blood glucose level during pregnancy. Blood glucose level is usually returns to normal after the delivery. It affects about 3-10 % of the pregnancies. There is no specific reason for GDM, but it is believed that the hormone produced during pregnancy increases a woman's resistance to insulin. In some time it will lead to type 2 DM (Department of Noncommunicable Disease Surveillance Geneva,1999).

1.2.2. Common symptoms of diabetes

- **Fatigue:** In diabetes, the body is inefficient and sometimes unable to use glucose for fuel. The body switches over to metabolizing fat, partially or completely, as a fuel source. This process requires the body to use more energy. The end result is feeling fatigued or constantly tired.

- **Unexplained weight loss:** People with diabetes are unable to process many of the calories in the foods they eat. Thus, they may lose weight even though they eat an apparently appropriate or even excessive amount of food. Losing sugar and water in the urine and the accompanying dehydration also contributes to weight loss.

- **Excessive thirst (polydipsia):** A person with diabetes develops high blood sugar levels, which overwhelms the kidney's ability to reabsorb the sugar as the blood is filtered to make urine. Excessive urine is made as the kidney spills the excess sugar. The body tries to counteract this by sending a signal to the brain to dilute the blood, which translates into thirst. The body encourages more water consumption to dilute the high blood sugar back to normal levels and to compensate for the water lost by excessive urination.

- **Excessive urination (polyuria):** Another way the body tries to get rid of the extra sugar in the blood is to excrete it in the urine. This can also lead to dehydration because excreting the sugar carries a large amount of water out of the body along with it.

- **Excessive eating (polyphagia):** If the body is able, it will secrete more insulin in order to try to deal with the excessive blood sugar levels. Moreover, the body is resistant to the action of insulin in type 2 diabetes. One of the functions of insulin is to stimulate hunger. Therefore, higher insulin levels lead to increased hunger and eating. Despite increased caloric intake, the person may gain very little weight and may even lose weight.

- **Poor wound healing:** High blood sugar levels prevent the function of white blood cells, which are important in defending the body against bacteria and also in cleaning up dead tissue and cells, from functioning normally. When these cells do not function properly, wounds take much longer to heal and become infected more frequently. Also, long-standing diabetes is associated with thickening of blood vessels, which prevents good circulation including the delivery of enough oxygen and other nutrients to body tissues.

- **Infections:** Certain infection syndromes, such as frequent yeast infections of the genitals, skin infections and urinary tract infections, may result from suppression of the immune system by diabetes and by the presence of glucose in the tissue, which allows bacteria to grow well.

- **Altered mental status:** Agitation, unexplained irritability, inattention, extreme lethargy, or confusion can all be signs of very high blood sugar, ketoacidosis, hyperosmolar hyperglycemia nonketotic syndrome, or hypoglycemia (low sugar).

- **Blurry vision:** Blurry vision is not specific for diabetes but is frequently present with high blood sugar levels.

1.2.3. Complications of diabetes

Both forms of diabetes ultimately lead to high blood sugar levels, a condition called hyperglycemia. Over a long period of time, hyperglycemia damages the retina of the eye, kidneys, nerves, and blood vessels. Some complications of diabetes as follows

- Diabetic retinotherapy

- Diabetic nephropathy
- Damage to the nerves from diabetes is a leading cause of foot wounds and ulcers, which frequently lead to foot and leg amputations.
- Damage to the nerves in the autonomic nervous system can lead to paralysis of the stomach and an inability to control heart rate and blood pressure during postural changes.
- It leads to heart attack, stroke, and decreased circulation in the arms and legs lead to peripheral vascular disease.
- Diabetes predisposes people to high blood pressure and high cholesterol and triglyceride levels. These conditions independently and together with hyperglycemia increase the risk of heart disease, kidney disease, and other blood vessel complications.
- Many infections are associated with diabetes, and infections are frequently more dangerous in someone with diabetes because the body's normal ability to fight infections is impaired. To compound the problem, infections may worsen glucose control, which further delays recovery from infection.
- **Hypoglycemia** (low blood sugar) occurs from time to time in most people with diabetes. It results from taking too much diabetes medication.
- **Hyperglycemia** is a serious condition in which the blood sugar level gets very high. The body tries to get rid of the excess blood sugar by eliminating it in the urine. This increases the amount of urine significantly and often leads to dehydration
- **Diabetic ketoacidosis** is a serious condition in which uncontrolled hyperglycemia (usually due to complete lack of insulin or a relative deficiency of insulin) over time creates a buildup in the blood of acidic waste products called ketones. High levels of ketones can be very harmful. This typically happens to people with type 1 diabetes who do not have good blood glucose control. Diabetic ketoacidosis can be precipitated by infection, stress, trauma, missing medications like insulin, or medical emergencies like stroke and heart attack.

2. Collection and identification of plants

Based on the literature survey and discussion with local healers of Shimoga district, four plants were selected. While selecting the plants, due care was taken to select only those plants on which there were no reports or very less reports on antidiabetic activity screening. It was found that lot of scope still remained in the field of antioxidant and antidiabetic studies on the following plants.

1. *Muntingiacalabura*
2. *Terminalia arjuna*
3. *Wendlandiathyrsoidea*
4. *Ficusamplissima*

The plants were collected during their flowering seasons from Bhadra reserve forest and from HaniyaShimoga district. These plants were selected and authenticated with the assistance of faculties of Departments of Applied Botany, Kuvempu University, Shankaraghatta, Shimoga.

2.1. Literature Survey

2.1.1.Muntingiacalabura

The species of *Muntingiacalabura* belong to the family Elaeocarpaceae (Morton, 1987), it is one of the Philippine medicinal plants and widely distributed throughout the world. In southern Taiwan,*Muntingiacalabura* plant was cultivated in gardens and along the road side for edible and ornamental purposes. This is commonly planted in residential area as ornament and shade. It grows vigorously in some street corner, highway and open lands in metropolitan cities. It can tolerate air pollution that prevails in the area. *Muntingiacalabura* is commonly known as Jamaican Cherry Tree and is also known as capulin or capuli in Latin America.

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Malvales

Family : Muntingiaceae

Genus : MuntingiaL.

Species : M. calabura

Common name **:** GasgaseHanninamara (kan), Jamaica cherry(eng)

Key Characters : It is a fast growing medium short- lived evergreen tree. Crown is speeding and has drooping branches; the tree grows from5 to 10 metre in height. Leaves are 8-13 cm long with toothedmargene. Flower is 2 cm in diameter with white color.The number of sepals is 5 and is green, reflexed, lanceolateand about 1 cm long. Petals are 1 cm long, deciduous and the fruit is rounded, about 1.5 cm in diameter,red smooth, very fleshy with sweet taste.

Parts used :Fruits, Leaves,Flowers and Roots

Medicinal use: In traditional medicine, its flowers can be used as an antiseptic and treat abdominal cramps and also used as emollient and to treat spasms and used to relieve colds and headaches. The bark can be used to produce ropes. Bark decoction is mucilaginous and used as emollient. The timber from the Jamaican cherry is reddish-brown. It is compact, durable and lightweight and can be used for carpentry. It could also be used as firewood. Wood is compact, fine-grained, moderately strong and light in weight and durable. Due to its ability to grow in poor soil and its effective propagation by means of bats and birds, it could be used for reforestation projects, in India it is used in urban gardens for its ability to grow fast and attractiveness to small fruit eating birds such as the flower peckers. It is also commonly planted in parking lots.

Phytochemistry

The fruit has sweet taste and hence the children eaten widely. The fruit is also cooked in tarts and made into jam. The leaf infusion is drunk as a tea-like beverage. The nutritional value per 100 g of edible portion of the fruit contain approximately: moisture 77.8 g, protein 0.32 g, fat 1.56 g, fibre 4.6 g, ash 1.14 g, calcium 124 mg, phosphorous 84 mg, iron 1.18 mg, carotene 0.019 mg, thiamine 0.065 mg, riboflavin 0.037 mg, niacin 0.554 mg, and ascorbic acid 80.5 mg. The energy value is 380 kJ/100 g (Morton, 1987). The polyphenolic compounds are found in all

parts of plants such as leaves, fruits, seeds, roots, flower and bark. Scientifically a number of flavonoids and phenolic compounds have been isolated and structures were elucidated by spectroscopic analysis. From the bark of the *Muntingiacalabura*, 8- hydroxy-7,3,4,5-tetramethoxyflavone and 8,4-dihydroxy-7,3,5-trimethoxyflavone were isolated and twelve flavonoids from root were isolated, characterized and screened their cytotoxic activities against A549 and HT-29 cells (Jih-Jung Chenet al., 2004: Noritokanedaet al., 1991).

Pharmacology:

The flowers are said to possess antiseptic properties. An infusion of the flowers is valued as an antispasmodic. It is taken to relieve headache and the first symptoms of a cold. The leaves of *Muntingiacalabura* have potential antibacterial activity against a number of harmful microorganisms.(ZakariaZA et al., 2006). In addition, the *M. calabura* leaves possess free radical scavenging activity (ZainulAmiruddinZakaria et al., 2007), antinociceptive activity (Zakaria ZA, et al., 2007). The aqueous extract of leaves of *M.calabura* possess antipyretic, anti-inflammatory effect (Zakaria Z, et al., 2007) and antistaphylococcal activity (Zakaria ZA et al., 2007). *M. calabura* was able to internally transport of Hg, it will reduces the soil pollution (Patricia Anne G, et al., 2011) and the fruits can be used as carbon source inorder to get very higher yield of glutamic acids (PayalaVijayalakshmi, et al., 2011).

2.1.2. Terminaliaarjuna

Kingdome : Plantae

Division : Magnoliophyta

Class : Mangnolipsida

Order : Myrtales

Family : Combretaceae

Genus : Terminalia

Species : arjuna

Common name **:**Arjuna, Kakubha, Karubha, Kudurekivimara,Torematti, Bilimatti, Holematti, Mattikore (Kan)

Arjuna, Dhavala, Kakubha, Nadisarjaveeravriksha, partha, Indradru(sansrit), Arjun (eng)

Key Characters **:**The bark is grey and smooth on external surface and softand red colour from the inside. Bark is bitter in taste.The leaves are guava, oblong and 4-6 inch long and 2-3 inch wide. White or yellowish flower are found in groups. The fruits are 1-2 inch in diameter with 5-7 longitudinallobes. Flowering occurs in summer and fruit appears in the winter season. The tree is about 60-80 feet height, large and evergreen with a speeding crown and dropping branches.

Parts used : Stem bark, leaves, fruits, roots and flowers.

Medicinal use:

Arjuna bark, nutmeg, tea should be soaked when consumed in the morning gives reduction in fever, loose motions, stomach related problems, to acquire relief from wounds the will bark should be boiled or soaked within water and this water should be used to wash the acute wounds. The powder of the sound off is useful in cleaning the teeth. Smell it to get rest from headache. Apply the stick of arjuna bark and baby to remove ache. Consume the powdered ingredients with honey to strengthen the bones. Oral administration of an aqueous suspension of the bark powder reduces coagulation, bleeding and prothrombin time.

Apart from this, the juice of garden-fresh leaves of arjuna tree can be utilized for managing earache. The ash of the bark can be useful in treating the scorpion sting. Furthermore, its decoction can be also employed for cleansing difficulties like ulcers, syphilitic sores, etc. The bark of this tree is usually found enriched with calcium, magnesium, aluminium and tannins. This also has some crystalline compounds such as lactone, arjunetin, arjunine, sugars and essential oils. It is beneficial in removing stones or calculi from the urinary systems, helps in relieving fever, arrests bleeding or secretion and heals wound, etc. The researchers found that arjuna extract reduced and normalized blood glucose levels much more effectively compared to Glibenclamide. They also noted that arjuna extract significantly decreased cell damage caused by

free radicals. They concluded that arjuna leaf demonstrated remarkable anti-hyperglycemic activity. The researchers speculated that the anti-hyperglycemic action is likely due to arjuna's antioxidant content. It also showed good antidiabetic activity. A number of formulations of arjuna plants are available in the market, Arjunarishta, Arjunaghrita, Arjunadisiddha, KakubhadiKshira, Shankara, etc.

Phytochemistry:

The bark constitutes an important crude drug, which contains tannins, triterpenoidssaponins, esters, flavonoids, sterols, calcium salts, alkaloidal and glycosidal substances, arjunine and arjunglycoside, arjunin, a sapogenin, arjungenin, a flavone, arjunolone, a methylated flavone, arjunone, terpene acids, arjunolic, terminoic and tomentosic acids, a phenolic substance, ellagic acid, sitosterol, β-sitosterol and oxalic acid. The cancer-cell growth inhibitory constituents, gallic acid, ethyl gallate and luteolin have also been found to occur in the bark.It also contains a lactone, essential oil, reducing sugars, arjunalic acid saponin and (+)-leucodelphinidin (padmaa M. 2010).

Pharmacology

Terminalia arjuna is a species of tree native to India. The Indian school of healing called Ayurveda, siddha and unani that first employed arjuna is thought to be at least 5,000 years old. In Indian system of medicine , the bark of the *T. arjuna* are used as a astringet, cooling, aphrodisiac, cardio tonic, tonic in fractures, ulcers, spermatorrhoea, leucorrhoea, diabetes, cough, tumore, excessive perspiration, asthama, inflammatory, and skin disorders etc. (warrier et al., 1994,dwivedi and Udupa, 1989). The bark of this tree is used in Ayurveda with regard to prevention and treatment of lots of heart problems like angina, coronary artery disease, congestive cardiovascular system failure, high cholesterol and blood pressure level. It has been considered by the Ayurvedic physicians as well as by the modern practitioners as a cardiac tonic. Vagbhatta was the first to prescribe use of arjuna's treating cardio vascular diseases the bark in heart diseases. It can be an ancient tree also is known as Nadisarjja in Sanskrit Vedas. Their bark proves to be a heart tonic for those patients struggling with heart diseases (Dwivedi S, et al., 2005).

Arjunic acid , arjungenin and arjunetin are isolated from the stem bark and their antibacterial activity were screened.(Gupta MM, et al., 2008).Evidence suggests that *Terminalia arjuna* may have blood vessel–relaxing properties.(Bharani A, et al., 2004).*T arjuna* may have antimicrobial effects, providing benefits against amoebas and other microorganisms. (Sohni YR, et al., 1995: Silva O, et al., 1997).

2.1.3:Wendlandiathyrsoidea (R & S) Steud.

Family **:** Rubiaceae

Genus **:** Wendlandia

Species **:** Thyrsoidea

Common name: Torani, Thinthulu, Taligi, Neerupale, BettadhakamagaggareNeeru pale, Chndhukare(Kan).Pekanarakam, Vellathalachedi, Puvu, Thovara (Malayalam), Kadamban, Thovarai(Tamil).

Key Characters: It is a large, branched shrub or small trees growing up to 5m tall. Stem is cylindrical 4-angled, densely velvety. Leaves simple, elliptic, 8-10 x 2.5-3.5 cm, are arranged in whorls on the branches, rarely opposite. Leaves sharp tipped or obtusely tipped, with a wedge-shaped base. Leaf stalks 5-10 mm long.Stipulusintrapetiolar, Flowers 4-5 mereus, white, tubular, in panicles at the end of branches. Flower tube is narrow, 4 mm long, with 5 petals to 1 mm long. Stamens remain inserted on the mouth of corolla, ovary usually bilocular, fruit a globose capsule, Flowering: January-April.

Parts used : Whole plant

Medicinal uses : Which is used in ethno medicine for the treatment of constipation, asthma, tooth ache, skin diseases and also this plant is used in the treatment of jaundice.

Pharmacology:

The antimicrobial and analgesic activity of leaf extracts of Wendlandiathyrsoideawas evolved.

2.1. 4:Ficusamplissima, Smith.

Class:	Magnoliatae
Order:	Urticales
Family:	Moraceae
Genus:	*Ficus*
Species:	*amplissima*

Common name **:**Goolli, Juvvi, Dodhabasuri, Basuri, Bilibasuri,Bovinamara, Hebbasuri (Kan).Bat tree, tsiela (Eng).

Key Characters **:**A fast growing, evergreen tree, which grows 6 to 12m in height. Crown is round in shape with widelySpreading branches. Aerial roots are rarely developed from the branches. Bark is smooth and greenish-grey in colour. Leaves are simple, leathery, and smooth and spiral in arrangement, ovate-elliptic to lanceolate-elliptic in shape with acute tip and rounded base. Leaf size ranges from 5 to 12 cm in length and 2 to 6 cm in width. Leaf stalk is long, varies from 2.5 to 8 cm in length. Leaves become grey-green to brownish in colour when dry and dried leaves have the upper surface covered with minute raised dots. Fruit, a fig, is subglobose to obovoid in shape, sessile and axillary in position and located in the twigs below the leaves. Fig is about 1 to 1.2 cm in diameter, green when young and pink to purple when ripened. In Maldives, it is widely grown as ornamental and shade tree.

Parts used :Bark and leaves.

3. *Extraction*

To achieve preliminary separation depending upon polarity of bio-molecules present in plants, successive hot extraction using Soxhlet extractor was carried out with the solvents of increasing polarity starting from pet-ether (60-80°C), chloroform, ethanol and water.

Weighed amount (500gm) of coarsely powdered material was successively extracted with pet ether (60-80°C), chloroform, ethanol and water.Each extraction was carried out for nearly 18 hr. (approx. 45 cycles). After each extraction, the plant material was removed from extractor, dried and reloaded in the extractor for subsequent extraction. The extracts were concentrated by evaporating solvent using Buchi type flash evaporator under reduced pressure and at controlled temperature. The extracts obtained was dried under vacuum, packed and stored in a refrigerator.

3.1. Details of extraction of Muntingiacalabura

Part used : leaves (320 gm)

Solvent used for extraction: Pet-ether (60-80°C), chloroform, ethanol and water.

Table 1.4.1. Yield and physical appearance of extracts of Muntingiacalabura

Solvent used	**Colour and nature of extract**	**Yield in Gm**
Pet ether (60-80o C)	Green Pasty	2.3
Chloroform	Green flake	5.6
Ethanol	Green pasty	15.3
Water	Brown crystals	8.4

3.2. Details of extraction ofTerminalia arjuna

Part used : Stem bark (230gm)

Solvent used for extraction : Pet-ether (60-80°C), chloroform, ethanol and water.

Table 1.4.2. Yield and physical appearance of extracts of Terminalia arjuna

Solvent used	Colour and nature of extract	Yield in Gm
Pet ether (60-80o C)	Yellowish pasty	1.2
Chloroform	White amorphous	2.3
Ethanol	Brown red flake	48.5
Water	Brown crystals	25.8

3.3. Details of extraction of Wendlandiathyrsoidea (R. & S.) Steud.

Part used : leaves (250 gm)

Solvent used for extraction : Pet-ether (60-80° C), chloroform, ethanol and water

Table 1.4.3. Yield and physical appearance of extracts of Wendlandiathyrsoidea

Solvent used	Colour and nature of extract	Yield in Gm
Pet ether (60-80o C)	Light brown Pasty	1.3
Chloroform	Green flake	2.1
Ethanol	Brown flake	10.8
Water	Brown amorphous	4.3

3.4.Details of extraction ofFicusamplissima

Part used : Stem bark (250 gm)

Solvent used for extraction : Methanol andWater

Table 1.4.4. Yield and physical appearance of extracts of Ficusamplissima

Solvent used	Colour and nature of extract	Yield in Gm
Methanol	Light brown Pasty	6.3
Water	Green flake	8.9

4. Phytochemistry

The plant chemistry has developed in recent years. It is concentrated with the enormous variety of organic substances elaborated and accumulated by plant in different parts and deals with chemical structure of these substances. Much of the plant analysis is devoted to isolation and identification of secondary constituents in a particular species or group of species with the expectation, that some of the constituents may be novel with unusual structure. It is important to recognize many of the isolated compounds either commonly presents or universally in occurrence and the most interesting components are often those present in lower amounts. The phytochemical investigation was carried out by Standard procedures and results are tabulated in Tables.

4.1.Qualitative analysis

1) Test for Alkaloids

a) Mayer's test (potassium mercuric iodide)

Mayer's reagent was added to the acidic test solution (small quantity of extract + 2ml of water + 2ml conc. HCL) cream colored precipitate was formed.

2) Test for Flavonoids

a) Ferric chloride test

To the test solution (small quantity extract + 2ml water) few drops ferric chloride solution was added, intense green colour was appeared.

b) Alkaline reagent test

To the test solution(small quantity of extract + 2ml water)sodium hydroxide solution was added the solution mixture showed increase in intensity of yellow color which become colorless on addition of few drops of dilute acids.

3) Test for Glycosides

a) Keller-killiani test

To the test solution (small quantity extract +2ml water) few drops ferric chloride solution was added and mixed well. Then conc. H2SO4 was added slowly, two layers were formed. The upper layer was bluish green in color and lower layer was reddish brown in colored.

b) Bromine water test

Test solution (small quantity of extract + 2ml water) was dissolved in bromine water, yellow precipitate was obtained.

4) Test for proteins

a) Xanthoproteic test

Test solution (small quantity of extract + 2ml water) was treated with conc. HNO_3 and on boiling gives yellow precipitate.

b) Ninhydrin test

Test solution (small quantity of extract + 2ml water) when treated with ninhydrin reagent gives blue colored precipitate.

c) Biuret test

Test solution (small quantity of extract + 2ml water) when treated with biuret reagent gaves red colored precipitate.

5) Test for saponins

a) Foam test

Small quantity of extract was treated with 5ml water and shaked well. It showed formation of froth which was found to be stable for about 15min.

6) Test for Steroids

a) Salkowaski's test

To the test solution (small quantity of extract + 2ml chloroform) few drops of conc. H2SO4 was added, shaken well for some times and allowed to stand. The lower layer color turned red, indicates the presence of steroids.

b) Lieberman'sburchard test

A few drops of acetic anhydride was added to the test solution (small quantity of extract + 2ml water) then conc. H2SO4 was added along the side of the test tube a brown ring was formed at the junction of two liquids and the upper layer color turned to green.

7) Test for Tannins

a) Ferric chloride test

Test solution (small quantity of extract + 2ml water) taken in a test tube, few drops of ferric chloride solution was added and then dark red color was obtained.

The crude extracts were prepared from the selected plantsand the preliminary phytochemical screening of crude extract indicates the presence of flavonoids, glycosides, triterpenoids, saponins, and tannins in the crude ethanolic extract. The preliminary phytochemical screenings of all extracts are tabulated in the following tables.

Table 4.1. Summary of phytochemical investigations ofMuntingiacalabura

Extraction	**constituents**
Pet ether	Phenolics.
Chloroform	Steroids, flavonoids, Phenolics
Ethanol	SaponinsSteroids, Phenolics and
Water	SteroidsFlavonoids, Saponins, Phenolics and tannins

Table4.2.Summary of phytochemical investigations ofTerminalia arjuna

Extraction	**constituents**
Pet ether	Alkaloies, Carbohydrates,
Chloroform	Alkaloids, Carbohydrates, Proteins, Steroids
Ethanol	Alkaloids, Carbohydrates, SaponinsSteroids, Proteins, Phenolics
Water	Alkaloids, Carbohydrates, Proteins, SteroidsFlavonoids, Saponins, Phenolics and tannins

Table4.3.Summary of phytochemical investigations ofWendlandiathyrsoidea

Extraction	**constituents**
Pet ether	Alkaloids and saponins
Chloroform	Alkaloids, Carbohydrates, Proteins and Steroids
Ethanol	Alkaloids, SaponinsSteroids, Proteins, Phenolics and tannins
Water	Alkaloids, Proteins, Steroids, Flavonoids, Saponins, Phenolics and tannins.

Table4.4.Summary of phytochemical investigations of Ficusamplissima

Extraction	**constituents**
Methanol	Alkaloids, phenolics, and saponins
Water	Alkaloids, Carbohydrates, Proteins and Steroids

4.2.Quantitative analysis

4.2.1. Determination of total phenolics content

Principle

Folin-Ciocalteu reagent is used to obtain a crude estimation of the amount of phenolic compounds present in the extract. Phenolic compounds undergo complete redox reaction with the Phosphotungstic and phosphomolybdic acid present in the reagent to gives yellow color, which is measured at 750 nm.

Reagents:

1. Standard gallic acid- 20mg/100ml in distilled water
   Working standard- 10 ml stock made up to 100 ml in distilled water
2. Folin-Ciocalteu's reagent (FCR) - 1:10 v/v in distilled water
3. Sodium carbonate- 7.5% in distilled water
4. Extract (20mg in 100ml of distilled water)

Procedure

The total phenolics content in the water extract was determined with the Folin-Ciocalteu's reagent (FCR) according to the method of Slinkard and Singleton (1977). In brief, 0.5 ml of extract was mixed with 2.5 ml FCR (diluted 1:10 v/v) followed by 2 ml of Na_2CO_3 (7.5 % v/v) solution. The tubes were vortexed and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer (Shimadzu UV-1609, Japan). A calibration curve was constructed using gallic acid as standard and total phenolic content of the extract was expressed in terms of milligrams of gallic acid (mg GAE) per gram of dry weight.

4.2.2. Determination of total flavonoids content

Principle

Flavonoids extracted in water gives characteristic reaction with the 5% Sodium nitrite and 10% Alluminium chloride resulting in the formation colored complex, which is measured at 510 nm

Reagents:

1. Standard catechin hydrate – 20mg/100 ml in distilled water
2. Sodium nitrite – 5% in distilled water

3. Alluminium chloride – 10% in distilled water
4. 1 M NaOH in distilled water
5. Water extract– 20 mg/ 100 ml

Procedure

Total flavonoids content of the water extract was determined according to modified method of Zhishen et al., (1999). Briefly, 1ml of test sample and 4ml of water were added to a volumetric flask (10 ml volume). 5 min after adding 0.3 ml of 5 % $NaNO_2$, 0.3 ml of 10% $AlCl_3.6H_2O$ was added. After 6 min incubation at room temperature, 2 ml of 1 M NaOH was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically (Shimadzu UV-1609, Japan). Results were expressed as catechin equivalents (mg catechin/g dried extract).

5. Antidiabetic activity

5.1.Chemicals &Instruments

Streptozotocins (STZ) were purchased from Sigma-Aldrich (Bangalore, India). Nicotinamide was purchased from Ranbaxy Chemicals Ltd., Mumbai,India. Glucometer and Glucometer strips for measurement of fasting blood glucose were purchased from a local vendor, manufactured by Accu-check Advantage, Roche diagnostics Mannheim, Germany. Carboxy Methyl Cellulose (CMC), Tween-80.Glibenclamide and other chemicals were obtained from local firms and were of analytical grade.

5.2.Animal housing and maintenance

Animals of both sex were procured from the S.S Medical College, Davangere, Karnataka, India. In all the protocols animals was group housed in polypropylene cages as per the respective experimental protocols. Standard laboratory conditions with proper air condition, continuously monitored environment, room temperature of 22±2° C with relative humidity and with 12 h of light/dark cycle was maintained. All the animals were provided with free access of drinking water ad libitum and standard food. Standard rats cages with rice bran bedding were employed to maintain proper hygiene conditions. All the animal experiments were approved by Institutional Animal Ethics Committee and were done as per their guidelines.

5.3.Induction of T2DM in rats

Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide in normal saline solution (0.9% NaCl solution). Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of 60 mg/kg of streptozotocin and 60 mg/kg of nicotinamide in 120 Sprague Dawley rats. Hyperglycemia was confirmed by the elevated non fasting glucose levels in blood, determined 48 hours after diabetes inductionusing Glucometer (One touch ultra-lifescan, Johnson and Johnson, USA) and strips. Animals with blood glucose concentrations more than 150 mg/dL were used for the study.

After the induction of diabetes The rats are selected and assigned to different groups randomly and each group consisting of six animals: Group I served as control; Group II received the vehicle (Celluose in saline); remaining groups were received different plant extracts of dosage 250, 500 and 1000 mg/kg, up to 7 days continuouslyand one group receive Glibenclamide by orally.

The blood sample was collected from caudal vein by the following procedure. The animal tale was cleaned with a cotton swab and the tip was cut using the fine scissor. Tail was gently massaged if required and a drop of blood was placed in the area specified on the blood glucose measuring strip to record the WBG value. Reduction in blood glucose produced by the compound was calculated on 1^{st}, 2^{nd}, 3^{rd}, 5^{th}, 7^{th} and 14^{th} day. Blood glucose concentration was determined by enzymatic glucose oxidase method using a commercial glucometer.

6. Results and Discussions

The phenolics, tannins and flavones constitute one of the major groups of compound acting as primary antioxidants and antihyperglycemic agents. Therefore, it is worthwhile to determine their total amount present in the extracts. The total content of phenolic and flavonoid tend to change with the change in climate. From the observation, it was found that the phenolic and flavonoids contents were high in high polar solvent extracts compared to less polar solvent extracts. The total amounts of phenols were expressed as µg/mg equivalent to Gallic acid and the flavonoid content was expressed as µg/mg equivalent to Catachin.

Table 6.1.Qualitative phytochemical analysis of M.calabura leaves extracts

Extracts	Total phenolic (µg/mg of extract)	Total Flavonoid (µg/mg of extract)
MCPE	15.83±0.12	17.936±0.89
MCCE	22.16±2.3	47.086±0.68
MCEE	33.33±0.13	123.316±0.54
MCAE	25.00±2.52	44.833±0.21

Each value represents mean±SE, Where, n= 3.

Table 6.2. Qualitative phytochemical analysis of T. arjuna stem bark extracts

Extracts	Total phenolic (µg/mg of extract)	Total Flavonoid (µg/mg of extract)
TACE	174.80±0.82	029.33±1.6
TAEE	623.20±1.84	418.06±2.5
TAAE	372.66±0.17	220.00±0.9

Each value represents mean±SE, Where, n= 3.

Table 6.3. Qualitative phytochemical analysis of W. thyrsoidea Leaves extracts extracts

Extracts	Total phenolic (µg/mg of extract)	Total Flavonoid (µg/mg of extract)
WTCE	108.22±0.42	32.85±9.21
WTEE	164.04±1.05	69.01±2.05
WTAE	301.15±1.99	101.56±1.45

Each value represents mean±SE, Where, n= 3.

Table 6.4.Qualitative phytochemical analysis of F. amplissima leaves extracts

Extract	Total Phenolics (mg GAE/g extract)	Total Flavonoids (mg CHE/g extract)
FAME	28.96±1.07	58.09±1.86
FAAE	18.68±2.87	35.98±2.14

GAE, gallic acid equivalents; CHE,chetachin equivalents

Currently-available drug in market regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drugs. So the aim of the study is to analyze the antihyperglycemic activity of oral administration of different extracts of selected plants on blood glucose level in STZ induced diabetic rats. A

significant increase in blood glucose was observed in diabetic rats when compared to control rats and with commercial anti-diabetic drug, Glibenclamide.

The obtained results clearly indicated that the blood glucose level in diabetic control group increased significantly by comparing with normal group, which indicate that the diabetic animal mode was successfully established. In STZ-Nicotinamide induced diabetic rats, the body weight was significantly decreased. 14^{th} days of treatment with extracts and standard drug, the body weight was significantly increased compared to the initial days (table 6.5). All the three doses showed significant improvement in body weight compared to diabetic control (Graphs 1, 2 and 3.). Extracts administered at three different doses of 250mg/kg, 500mg/kg, 1000mg/kg to STZ-Nicotinamide treated diabetic rats caused significant ($P<0.001$) reduction of blood glucose levels which was related to dose and duration of treatment. The MCEE showed promising activity while MCAE and MCCE showed moderate activity by comparing standard.

The effect of repeated oral administration of extract of *T. arjuna* bark on blood glucose levels in STZ-Nicotinamide diabetic rats is presented in graphs 4, 5 and 6 at 250, 500 and 1000 mg/kg concentration respectively. The body weight was significantly increased compared to the initial days (table 6.6)STZ- Nicotinamide treated diabetic rats caused significant reduction of blood glucose levels which was related to dose and duration of treatment of extracts. Among all extracts TAEE showed good hyperglycemic activity followed by TAAE, TACE and TAPE as compare to the standard Glibenclamide in dose dependent manner.

The results of the study of extract of *W.thyrosadia* on the blood glucose levels of normal and STZ- Nicotinamide induced diabetic in rats were showed in graphs 7, 8 and 9 at concentration of 250, 500 and 1000 mg/kg of body weightrespectively. The obtained results clearly indicated that the blood glucose level in diabetic control group increased significantly by comparing with normal group. In STZ-Nicotinamide induced diabetic rats the body weight was significantly decreased. The extract treated rats showed significant improvement in body weight compared to diabetic control (Table 6.7).

The WTAE exhibited promising activity as compare to the standard drug while WTME showed minimal activity. The biological activity of the WTAE may, probably rich in secondary metabolites like alkaloid, flavonoid, polyphenolics and saponinsins, in turns they are suppressed the glucose level and increased their hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets. Thus further isolation of compounds from the aqueous

extracts could well explain the mechanisms involved for its efficient radical scavenging and antihyperglycemic activity.

The effects of oral administration of the *F.amplissima*bark extracts on the blood glucose in STZ induced diabetic rats have been shown in graphs 10, 11 and 12 at the concentration 250, 500 and 1000 mg/kg body weight respectively. The obtained results clearly indicated that the blood glucose level in diabetic control group increased significantly by comparing with normal group, which indicate that the diabetic animal mode was successfully established. In STZ-Nicotinamide induced diabetic rats the body weight was significantly decreased. After 14^{th} days of treatment with extracts the body weight was significantly increased compared to the initial day. All the three doses showed significant improvement in body weight compared to diabetic control (Table 6.8). Extracts administered at three different doses of 250mg/kg, 500mg/kg, 1000mg/kg to STZ-treated diabetic rats caused significant ($P<0.001$) reduction of blood glucose levels which was related to dose and duration of treatment. The FAME showed promising activity while FAAE showed moderate activity by comparing with standard.

The *in vivo* results obtained in STZ–Nicotinamide induced type 2 diabetic rats model indicated that the crude extract of *F. amplissima*bark at a concentration of 1000 mg/kg body weight, has the ability to lower blood glucose levels. The extracts exhibited a significant dose dependent hypoglycemic activity and these results are comparable with standard glibenclamide. In general there is very little biological knowledge on the specific mode of action in the treatment of diabetes, probably secondary metabolites like alkaloid, flavonoid, polyphenolics and saponinsins suppressed the glucose level and increased their hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets i.e., its action to release bound insulin from regenerated β cells by inhibiting ATP sensitive K^+channels like glibenclamide or may be pancreatic secretion of insulin from regenerated β-cells. In STZ–Nicotinamide induced type 2 diabetic rat model, the body weight is reduced due to destruction of muscle tissue and loss of protein contents. The improvement of body weight in rats treated with extract signifies its reversal of gluconeogenesis, in turn reflect its ability to reduce hyperglycemia.

The antihypoglycemic activity of *F. amplissima*s are due to the presence of phytoconstituents, γ-sitosterol, olean-12-en-3-one, lupeol, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Menthol and stearaldehyde. Several studies on these compounds have demonstrated antidiabetic, antioxidant, antimicrobial, anticancer and anti-inflammatory properties. Polyphenols

and flavonoids in the bark extract are well known for their antioxidant properties, they may scavenge the free radicals generated during diabetes and these might have crucial roles in the observed antioxidant and hypoglycemic activity of the bark.

Table 6.5.Effect of extracts of M.calabura leaves on body weight in STZ-Nicotinamide induced diabetic rats.

Group (n=6)	Treatment	Body weight	
		0^{th} day	$14t^{h}$ day
I	Normal control	195.01 ± 4.08	224.09 ± 9.94
II	Diabetic control	190.56 ± 9.45^{c}	168.13 ± 15.83^{c}
III	Diabetic + MCCE (250mg/kg)	204.01 ± 3.42	216.60 ± 11.08
IV	Diabetic + MCCE (500mg/kg)	198.01 ± 5.12	231.45 ± 14.01
V	Diabetic + MCCE (1000mg/kg)	203.03 ± 5.50	234.17 ± 10.76
VI	Diabetic + MCEE (250mg/kg)	199.33 ± 9.78	222.32 ± 20.45^{abc}
VII	Diabetic + MCEE (500mg/kg)	192.02 ± 7.05	232.88 ± 2.77
VIII	Diabetic + MCEE (1000mg/kg)	203.78 ± 16.22^{a}	243.85 ± 4.55^{abc}
XI	Diabetic + MCAE (250mg/kg)	195.00 ± 2.98	217.04 ± 6.01
X	Diabetic + MCAE (500mg/kg)	187.11 ± 14.52	228.06 ± 9.01
XI	Diabetic + MCAE (1000mg/kg)	199.88 ± 12.44^{ab}	240.77 ± 12.05^{abc}
XII	Diabetic + glibenclamide (0.25 mg/kg)	195.65 ±13.73^{ab}	216.88 ± 16.45^{ab}

Values are mean± SEM of 6 animals in each group. a: P<0.05 comparing with the normal; b:P<0.05 comparing with diabetic control: c: P<0.05 comparing with glibenclamide treated grou

Table6.6. Effect of extracts of T. arjuna stem bark on body weight in STZ-Nicotinamide induced diabetic rats.

Group (n=6)	Treatment	Body weight	
		0^{th} day	14^{th} day
I	Normal control	195.01 ± 4.08	224.09 ± 9.94
II	Diabetic control	190.56 ± 9.45c	168.13 ± 15.83c
III	Diabetic + TACE (250mg/kg)	202.01 ± 4.42	211.60 ± 2.07
IV	Diabetic + TACE (500mg/kg)	200.01 ± 1.12	225.11 ± 5.41
V	Diabetic + TACE (1000mg/kg)	212.35± 4.54	229.88 ± 8.44
VI	Diabetic + TAEE (250mg/kg)	201.84 ± 11.15	225.59 ± 12.05abc
VII	Diabetic + TAEE (500mg/kg)	205.54 ± 12.36	228.74 ± 6.24
VIII	Diabetic + TAEE (1000mg/kg)	208.12 ± 9.24a	235.81 ± 12.68abc
XI	Diabetic + TAAE (250mg/kg)	199.35 ± 8.31	222.89 ± 24.35
X	Diabetic + TAAE (500mg/kg)	195.35± 18.05	225.42 ± 10.35
XI	Diabetic + TAAE (1000mg/kg)	200.45 ± 11.04ab	235.12 ± 3.85abc
XII	Diabetic + glibenclamide (0.25 mg/kg)	195.65 ±13.73ab	216.88 ± 16.45 ab

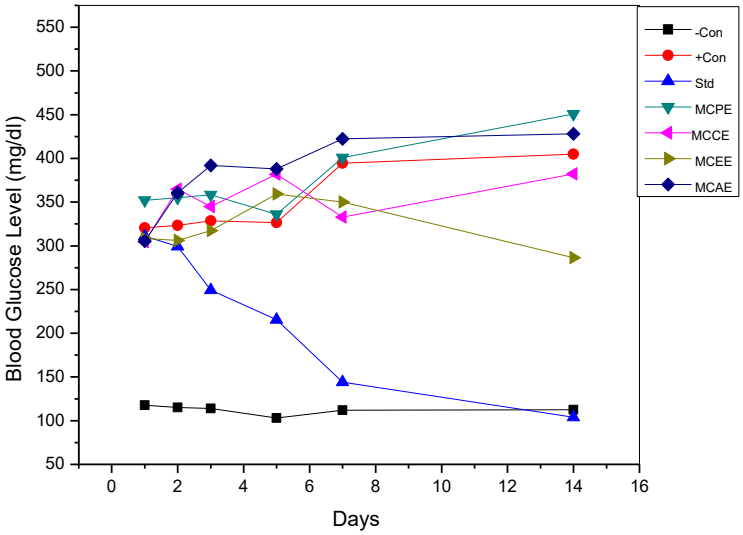
Table 6.7. Effect of extracts of W.thyrosadia leaves on body weight in STZ-Nicotinamide induced diabetic rats.

Group (n=6)	Treatment	Body weight	
		0^{th} day	14^{th} day
I	Normal control	195.01 ± 4.08	224.09 ± 9.94
II	Diabetic control	190.56 ± 9.45c	168.13 ± 15.83c
III	Diabetic + WTCE (250mg/kg)	225.01 ± 1.32	231.32 ± 8.68
IV	Diabetic + WTCE (500mg/kg)	214.08 ± 11.12	235.16 ± 2.41
V	Diabetic + WTCE (1000mg/kg)	232.49± 8.32	234.08 ± 2.04
VI	Diabetic + WTEE (250mg/kg)	212.03 ± 9.56	225.96 ± 5.68abc
VII	Diabetic + WTEE (500mg/kg)	198.02 ± 5.68	202.85 ± 4.35
VIII	Diabetic + WTEE (1000mg/kg)	221.35 ± 5.84a	239.35 ± 11.35abc
XI	Diabetic + WTAE (250mg/kg)	205.54 ± 14.06	224.68 ± 3.45
X	Diabetic + WTAE (500mg/kg)	195.84± 6.45	221.02 ± 5.65
XI	Diabetic + WTAE (1000mg/kg)	206.47 ± 13.84ab	232.48 ± 6.85abc
XII	Diabetic + glibenclamide (0.25 mg/kg)	195.65 ±13.73ab	216.88 ± 16.45 ab

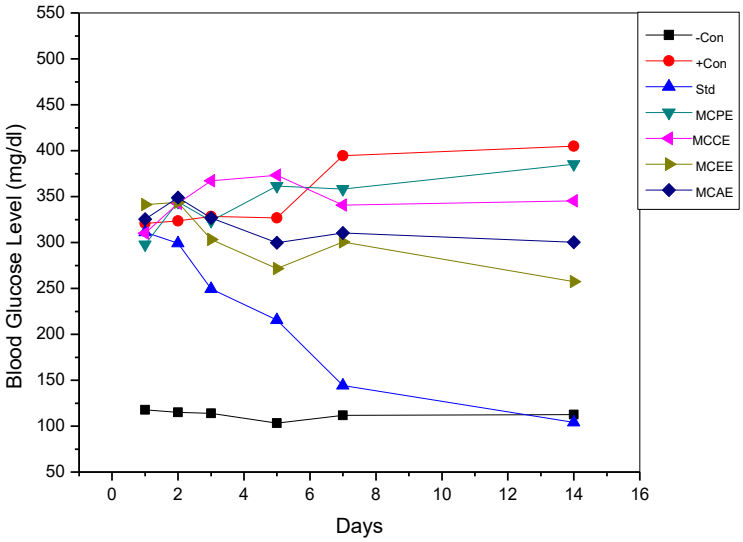
Table 6.8. Effect of extracts of F. amlissima bark on body weight in STZ-Nicotinamide induced diabetic rats.

Group (n=6)	Treatment	Body weight	
		0^{th} day	14^{th} day
I	Normal control	195.01 ± 4.08	224.09 ± 9.94
II	Diabetic control	190.56 ± 9.45	168.13 ± 15.83
III	Diabetic + FAME (250mg/kg)	201.01 ± 8.45	213.68 ± 7.38
IV	Diabetic + FAME (500mg/kg)	195.85 ± 4.08	228.07 ± 15.07
V	Diabetic + FAME (1000mg/kg)	200.98 ± 4.11	231.57 ± 8.76
VI	Diabetic + FAAE (250mg/kg)	196.78 ± 7.05	219.45 ± 10.08
VII	Diabetic + FAAE (500mg/kg)	189.86 ± 5.75	229.86 ± 5.75
VIII	Diabetic + FAAE (1000mg/kg)	200.07 ± 11.75a	240.08 ± 9.81a
XI	Diabetic + glibenclamide (0.25 mg/kg)	197.00 ±9.03a	218.07 ± 10.57 a

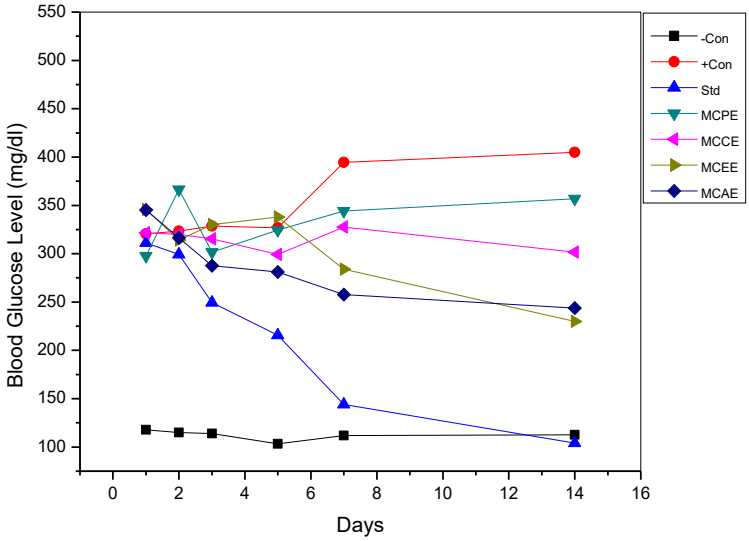
Values are mean± SEM of 6 animals in each group. a: P<0.05 comparing with the normal; b:P<0.05 comparing with diabetic control: c: P<0.05 comparing with glibenclamide treated group.



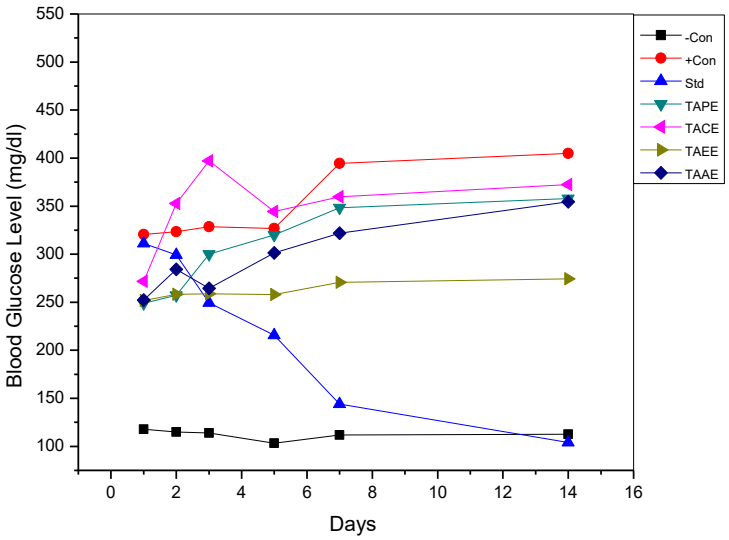
Graph 1. Concentration 250 mg/kg



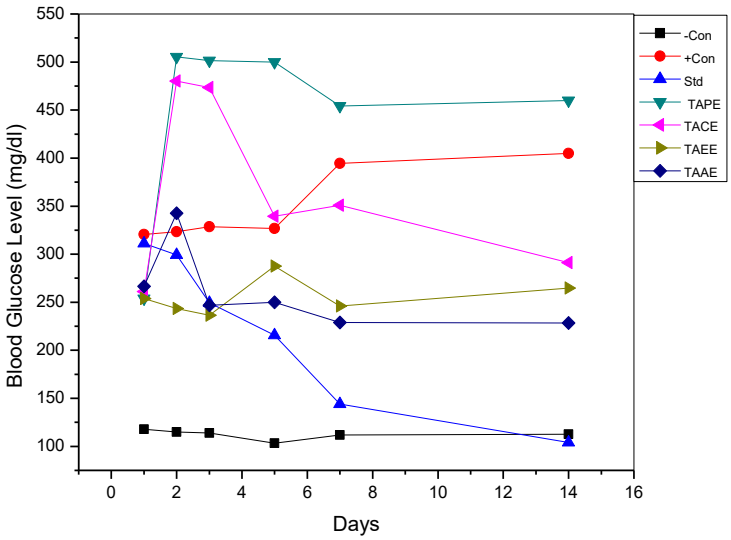
Graph 2. Concentration-500 mg/kg



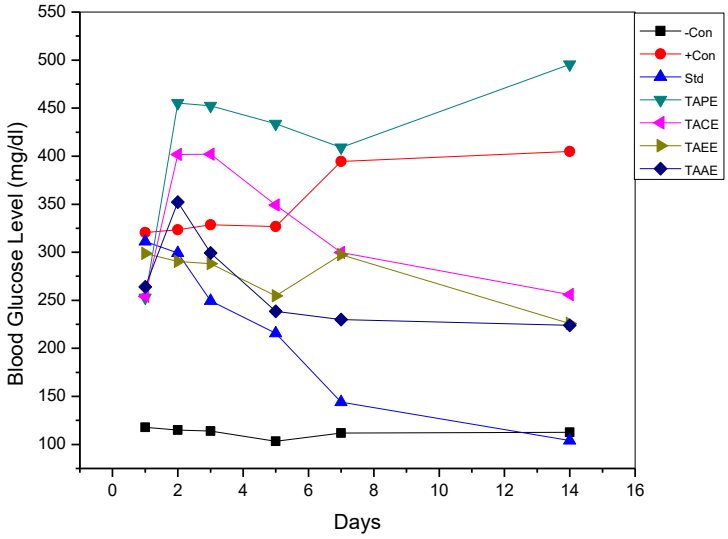
Graph 3. Concentration-1000 mg/kg



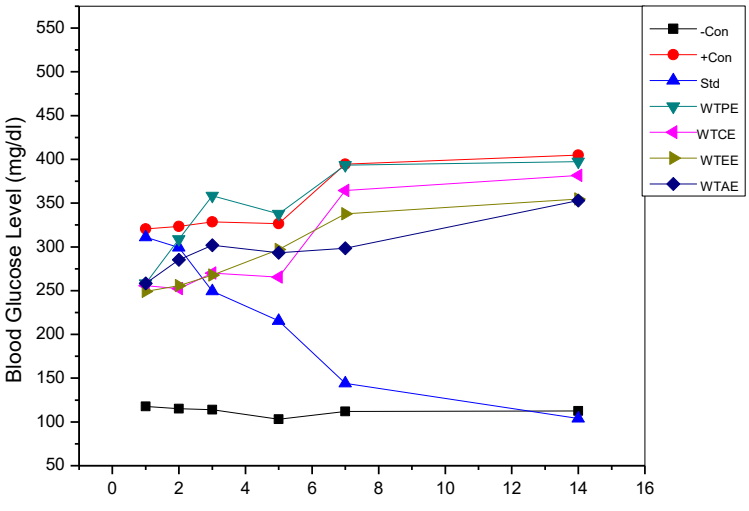
Graph 4. Concentration-250 mg/kg



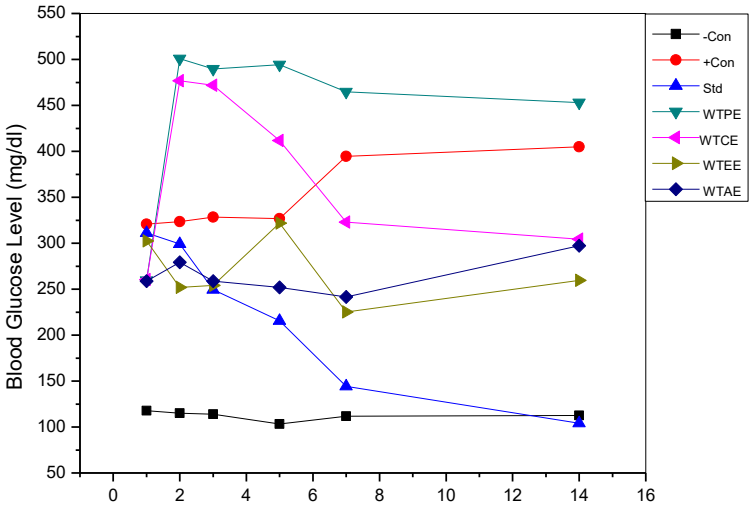
Graph 5. Concentration-500 mg/kg



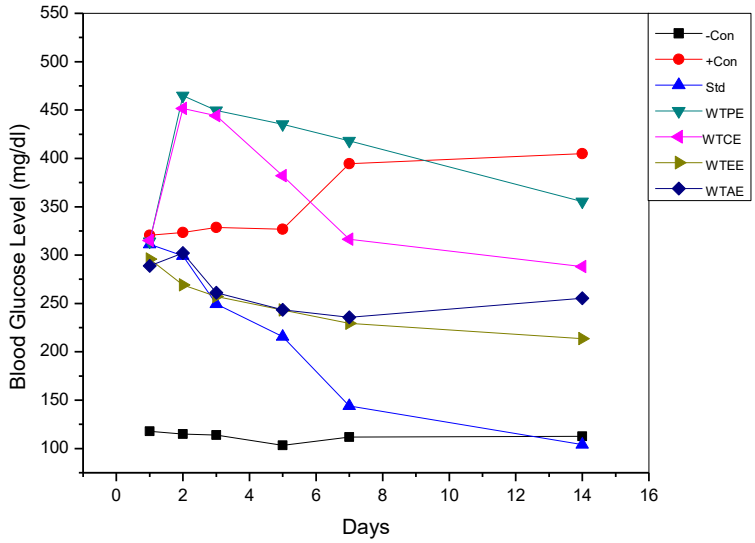
Graph 6. Concentration-1000 mg/kg



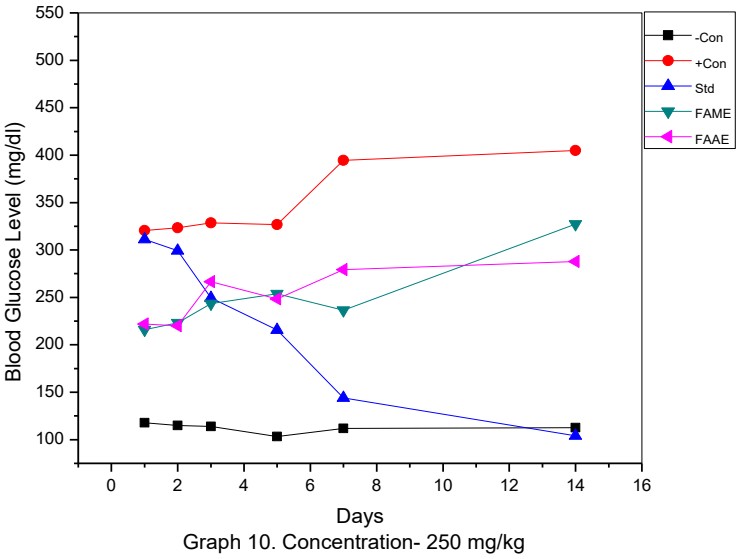
Graph 7. Concentration-250 mg/kg



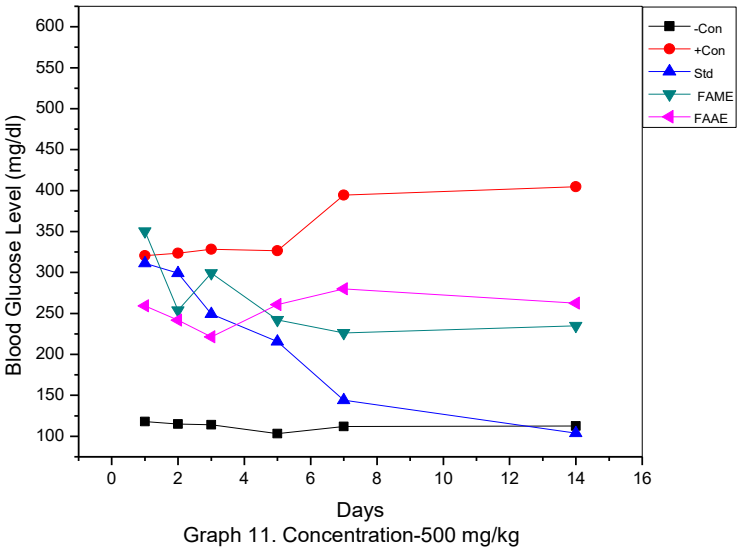
Graph 8. Concentration-500 mg/kg



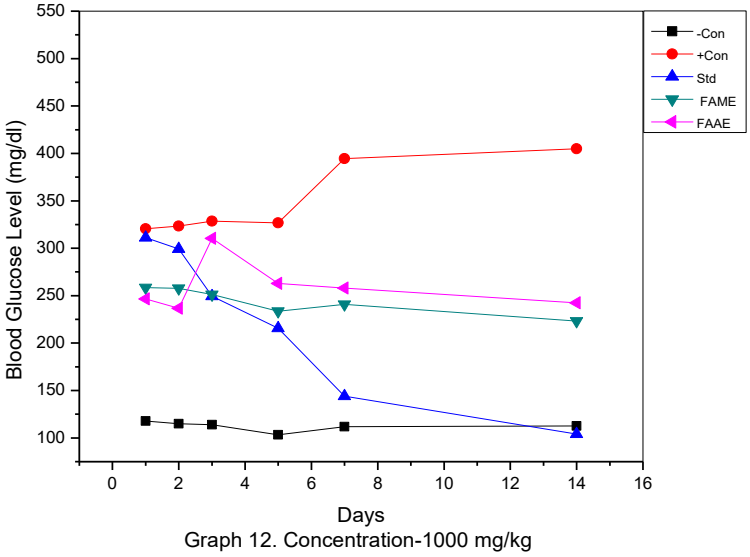
Graph 9. Concentration-1000 mg/kg



Graph 10. Concentration- 250 mg/kg



Graph 11. Concentration-500 mg/kg



Graph 12. Concentration-1000 mg/kg

7. Isolation of phytoconstituents

The extracts of selected plants were subjected to the antidiabatic activity. Among all the extracts methanol extract of leaves of *W. thyrsoidea*and bark extracts of *F.amplissima*showed significant antidiabetic activity. These observations prompted us to take up the isolation and characterization of the active chemical compounds present in plant extracts.

The methanol extract of leaves of*W. thyrsoidea*andmethanolic bark extract of *F.amplissima* plant was found to be significant antidiabetic activity as compare to standard.The showed activity probably due to the presence of alkaloids, saponin, glycosides and other phytochemicals present in the plant body. Further investigations indicated that the extract

possessed significant analgesic, antioxidant and anthelmentic activities. From all these observation we selected methanol extract of leaves of*W. thyrsoidea*and methanolic bark extracts of *F.amplissima* for the isolation and characterization of the active phytoconstituents present in extract.

Isolation of phytoconstituents from methanolic extract of bark of *F. amplissima*

Chromatographic separation using silica gel (100-200 mesh)

The crude methanol extract (30g) was suspended in water; methanol (8; 2) and partitioned successively with hexane, chloroform, ethyl acetate and n-butanol to get fractions which up on concentrated gave of 1g, 3g, 3g and 5g respectively. The chloform fraction was purified over column of silica gel (100- 200) using petether and ethyl acetate eluents.Fractions were collected in 20 ml portions and monitored by TLC (silica gel as adsorbent) solvent system petether: ethyl acetate.The homogeneous fractions were combined based on TLC and divided in to three major fractions FA 1, FA2 and FA 3. The compound FA 3 was again purified on column using same solvent system as mentioned above. The pure compounds were spectrally analyzed.

The Chromatographic details of methanolic bark extract of chloroform fraction of *F. amplissima* plant

Fractions	Eluent	Colour and Nature	Yield
1	Petroleum ether: Ethyl acetate 90:10 V/V	No residue	--
2	Petroleum ether: : Ethyl acetate 80:20 V/V	No residue	--
3	Petroleum ether: Ethyl acetate 70:30 V/V	No residue	--
4	Petroleum ether: : Ethyl acetate 60:40 V/V	No residue	--
5	Petroleum ether: : Ethyl acetate 50:50 V/V	Brown pasty	0.5 g **(FA 1)**
6	Petroleum ether: : Ethyl acetate 40:60 V/V	Reddish Brown powder	0.8 g **(FA 2)**
7	Petroleum ether: : Ethyl acetate 20:80 V/V	Brown powder	0.1 g **(FA 3**

8	Petroleum ether: : Ethyl acetate 10:90 V/V	No residue	--
9	Ethyl acetate 100 V/V	No residue	--

Isolation of pure components form chloroform leaves extracts of *W. thyrsoidea*

The methanol leaves extract (10 g) of *W. thyrsoidea*plant was chromatographed over silica gel (100-200 mesh) on column 55 cm length and 6 cm diameter. Elution was carried out with solvent mixtures of increasing polarities. Fractions were collected in 20 ml portions and monitored by TLC (silica gel as adsorbent, solvent system petether: ethyl acetate) and the fractions showing similar spots are pooled together. The pure compounds were spectrally analyzed.

The Chromatographic details of chloroform leaves extract of *W. thyrsoidea* plant

Fractions	**Eluent**	**Colour and Nature**	**Yield**
1	Petroleum ether: Ethyl acetate 90:10 V/V	Brown pasty	0.08g WT-1
2	Petroleum ether: : Ethyl acetate 80:20 V/V	White powder	0.12g **WT-2**
3	Petroleum ether: Ethyl acetate 70:30 V/V	No residue	--
4	Petroleum ether: : Ethyl acetate 60:40 V/V	No residue	--
5	Petroleum ether: : Ethyl acetate 50:50 V/V	White power	0.2g WT-3
6	Petroleum ether: : Ethyl acetate 40:60 V/V	No residue	--
7	Petroleum ether: : Ethyl acetate 20:80 V/V	No residue	--
8	Petroleum ether: : Ethyl acetate 10:90 V/V	No residue	--
9	Ethyl acetate 100 V/V	No residue	--

Spectral analysis of compound FA-3

Name of the compound: 2-(3,5-dimethylphenyl)-7-hydroxy-4H-chromen-4-one

Nature : Light yellow solid

Solubility : Chloroform, Ethanol, Methanol.

Melting point : 294-296^{o} C

Light yellow amorphous solid, m.p. 294-296^{o} C. UV: IR (KBr): max = 3279 (br, OH), 2915 (CH saturated stretch), 1656 (C=O), 1598, 1536 cm-1 (aromatic ring C=C stretch); MS (LCMS): m/z = 266.2912; 1H-NMR (400 MHz, DMSO, δ ppm) - 2.48 (6H, s, Me), 6.952 (1H, s), 7.07 (1H, s), 7.09 (2H, s), 7.332 (2H, s), 7.554 (1H,s), 7.783 (2H, d), 10.14 (OH, s); 13C-NMR (CDCl3, 125 MHz)- 40.17 (Me-6_), 120.74,125.81, 129.02, 133.25, 135.48, 137.98, 139.74 (Aromatic CH).

Spectral analysis of compound WT-2

Name of the compound: Hexadecanoic acid

Nature : white amorphous solid

Solubility : chloroform, Ethanol, Methanol.

Melting point : 58-61^{o} C

Amorphous white solid, m.p. 58-61^{o} C. UV: IR (KBr): max = 3918 (br, OH), 2915 (CH saturated stretch), 1703 (COO st); MS (LCMS): m/z = 256.42; ^{1}H-NMR (400 MHz, DMSO, δ ppm) – 0.80 (3H, m, Me), 1.207 (25H, m), 1.45 (2H, m), 2.13 (2H, m); ^{13}C-NMR (CDCl3, 125 MHz)- 14.22 (Me-3_), 22.61, 24.99, 29.55, 34.10, 40.16 (CH), 174.71(C=O).

Conclusion

It’s clear that the plants play a very important role in pharmacology. The hypothesis of obtaining plant based medicine is beneficial to human health. Based on the *in vivo* experimental study and the active profile exposed through various biochemical parameters it can be concluded that the extracts of selected plants showed significant antidiabetic activity. Further investigations on the isolation and identification of Bio active components on the plant would help to ascertain its potency.

8. References

Benavente-Garcia O, Castillo J, Marin FR, Ortuño A, Del-Rio JA. Uses and properties of *Citrus* flavonoids. J Agric. Food Chem.**1997**:45:4505-4515..

Bharani A, Ahirwar LK, Jain N, et al. Terminalia arjuna reverses impaired endothelial function in chronic smokers. Indian Heart J . **2004**:56:123–8.

*Bottini, N., Vang, T., Cucca, F., Mustelin, T.,. Role of PTPN22 in type 1 diabetes and other autoimmune diseases. Seminars in Immunology, **2006:** 18: 207–213.*

*Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications, Part 1: Diagnosis and Classification of Diabetes Mellitus, World Health Organization Department of Noncommunicable Disease Surveillance Geneva,**1999**.*

*DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. In: Alberti KGMM, Zimmet P, DeFronzo RA, eds. International Textbook of Diabetes Mellitus. 2nd edn. Chichester: John Wiley, **1997**:635–712.*

*Diabetes and medicinal plants-A review. G.B. Kavishankar,N. Lakshmidevi, S. Mahadeva Murthy, H.S. Prakash3, S.R. Niranjana. Int J Pharm Biomed Sci**2011**:2(3):65-80.*

Dwivedi S, Aggarwal A, Agarwal MP, et al. Role of Terminalia arjuna in ischaemic mitral regurgitation. IntJCardiol . **2005**:100:507-508.

*Humphrey ARG, McCarty DJ, Mackay IR, Rowley MJ, Dwyer T, Zimmet P. Autoantibodies to glutamic acid decarboxylase and phenotypic features associated with early insulin treatment in individuals with adult– onset diabetes mellitus. Diabetic Med **1998**:15:113–19.*

Jih-Jung Chen, Ru-Wei Lin, Chang-Yih Duh, Hung-Yi Huang and Ih-Sheng Chen. Flavones and Cytotoxic Constituents from the Stem Bark of Muntingiacalabura. Journal of the Chinese Chemical Society, **2004**: 51:665-670.

*Knowler WC, Nelson RG, Saad M, Bennett PH, Pettitt DJ. Determinants of diabetes mellitus in the Pima Indians. Diabetes Care **1993**:16:216–27.*

*Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E et al. Insulin resistance and insulin secretory dysfunction as precursors of non–insulin– dependent diabetes. Prospective Study of Pima Indians. N Engl J Med **1993**:329:1988–92.*

M. M. Gupta, T. R. Santhakumar, dharmendrasaikia S. P. S. Khanuja, Antibacterial principles from the bark of Terminalia arjuna, Current Science, 2008:94(1).

Marinova, E. M., &Yanishlieva, N. VI. Antioxidative activity of extracts from selected species of the family Lamiaceae in sunflower oil. Food Chemistry, **1997:**245–248.

*National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes **1979**:28:1039–57.*

Noritokaneda, john m. Pezzuto, d. Doelsoejarto, a. Douglas kinghorn,. Norman r. Farnsworth, Plant Anticancer Agents, XLVIII.

Oberley, L.W.,. Free radicals and diabetes. Free radical biology& medicine 5, 113–124.

padmaa M. Paarakh, Terminalia arjuna (Roxb.) Wt. and Arn.: A review, international journal of pharmacology, ***1998:*** 6 (5): 515-534.

PayalaVijayalakshmi, DhurjetiSarvamangala. Production of L-Glutamic acid by Corynebacteriumglutamicum DSM 20300^{T} and Arthrobacterglobiformis MTCC 4299 using fruits of Muntingiacalabura Linn. International Research Journal of Microbiology, **2011:**2(4):116-121.

PerumalSamy, R. &Ignacimuthu, S. Antibacterial activity of some of folklore medicinal plants used by tribal in Western Ghats of India. J. Ethnopharmacol., **2000:**69:59-77.

Roderick, E.W.,. The stepwise approach to the management of type 2 diabetes. Diabetes Research and Clinical Practice, ***2004:*** *65:53–58.*

SaikatDewanjee, AnupMaiti, RanabirSahu, Tarun K. Dua and Vivekananda Mandal, Effective Control of Type 2 Diabetes through Antioxidant Defense by Edible Fruits of Diospyros peregrine, eCAMAdvance Access published,**2009.**

Saltiel, A. R: Kahn, R. Insulin signaling and regulation of glucose and lipid metabolism. Nature ***2001****:414:799-806.*

Silva O, Duarte A, Pimentel M, et al. Antimicrobial activity of Terminalia macroptera root. J Ethnopharmacol . **1997**:57:203–207.

Sohni YR, Kaimal P, Bhatt RM. The antiamoebic effect of a crude drug formulation of herbal extracts against Entamoebahistolytica in vitro and in vivo. J Ethnopharmacol . **1995**:45:43–52.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care ***1997****:20:1183–97.*

WHO Expert Committee on Diabetes Mellitus. Second Report. Geneva: WHO,Technical Report Series646, ***1980****.*

*Willis JA, Scott RS, Brown LJ, Forbes LV, Schmidli RS, Zimmet PZ et al. Islet cell antibodies and antibodies against glutamic acid decarboxylase in newly diagnosed adult–onset diabetes mellitus. Diabetes Res ClinPract****1996****:33:89–97.*

World Health Organization. Diabetes Mellitus: Report of a WHO Study Group. Geneva: WHO, Technical Report Series 727, ***1985****.*

Z Zakaria, N Nor Hazalin, S Zaid, M Ghani, M Hassan, H Gopalan and M Sulaiman, Antinociceptive, anti-inflammatory and antipyretic effects of Muntingiacalabura aqueous extract in animal models, J Nat Med, **2007:** 61(4):443-448.

Z.A. Zakaria, H. Zaiton, E.F.P. Henie, A.M. Mat Jais, D. Kasthuri, M. thenamutha, F.W. Othman, R. Nazaratulmawarina and C.A. Fatimah. The in vitro antimicrobial activity of Corchorusolitorius and Muntingiacalabura extracts. Journal of pharmacology and toxicology, **2006:** 1 (2):108-114,.

ZainulAmiruddinZakaria, Free Radical Scavenging Activity of Some Plants Available in Malaysia , Iranian Journal Of Pharmacology & Therapeutics, **2007:** 6:87-91.

Zakaria ZA, Mat Jais AM, Mastura M, Mat Jusoh SH, Mohamed AM, Mohd NS,Jamil MS, Rofiee, Sulaiman MR . In vitro antistaphylococcal activity of the extracts of several neglected plants in Malaysia. Int. J. Pharmacol. **2007:** 3(5): 428-431.

Zakaria ZA, Safarul M, Sulaiman MR, Mat Jais AM, Somchit MN, Fatimah CA. The antinociceptive action of aqueous extract from Muntingiacalabura leaves: The role of opioid receptors. Med. Prin. Pract. **2007:**16:130-136.

Zimmet PZ, Tuomi T, Mackay R, Rowley MJ, Knowles W, Cohen M et al. Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. Diabetic Med ***1994****:11:299 303*

Zimmet PZ. Kelly West Lecture 1991: challenges in diabetes epidemiology: from West to the rest. Diabetes Care ***1992****:15:232–52.*

International Diabetes Federation. IDF Diabetes Atlas, 5th edn. Brussels, Belgium: International Diabetes Federation, ***2011****.*