PHYTOCHEMICAL CHARACTERIZATION OF ANTI-DIABETIC PLANTS IN MIZORAM

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Submitted in partial fulfillment of the requirements of the Degree of Doctor of Philosophy in Forestry, Department, Mizoram University, Tanhril, Aizawl.

CERTIFICATE

This to certify that the thesis entitled "**Phytochemical characterization of anti-diabetic plants in Mizoram**" submitted by **Mr. Jerry Laldingngheta** (Ph.D Regn No **MZU/Ph.D/646 of 02.05.2014**) in partial fulfillment of the requirements for the award of Doctor of Philosophy in Forestry of Mizoram University, Aizawl embodies the record of his original investigations under my supervision. He has duly registered and the thesis is presented is worthy of being considered for the award of the Doctor of Philosophy (Ph.D) Degree. The work has not been submitted previously for any degree to this or any other university.

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DECLARATION

I Jerry Laldingngheta, hereby declare that the subject of the matter of the thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to do the best of my knowledge to anybody else, and that the thesis has not been submitted by me or any research degree in any other University/Instituted.

This is being submitted to the Mizoram University for the degree of Doctor of Philosophy in Forestry.

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TABLE OF CONTENTS

LISTS OF TABLES

LISTS OF FIGURES

LISTS OF PHOTOPLATES

LISTS OF ABBREVIATIONS USED

1. IDDM - Insulin-Dependent Diabetes Mellitus
2. NIDDM - Noninsulin-Dependent Diabetes Mellitus
3. GDM - Gestational Diabetes Mellitus
4. MODY - Maturity Onset Diabetes Of the Young
5. HIV - Human Immunodeficiency Virus
6. GLUT4 - Glucose Transporter Type4
7. mRNA - Messenger RNA
8. mg/dl - Milligrams Per Deciliter
9. mmol/L - Millimoles Per Liter
10. mol/l - Moles Per Liter
11. g/kg - Gram Per Kg
12. HbA1c - Glycated Hemoglobin
13. HDL - High-density Lipoprotein
14. ACEIs - Angiotensin-Converting Enzyme Inhibitors
15. ARBs - Angiotensin Receptor Blockers
16. NPH - Isophane Insulin
17. GLUT 2 - Glucose Transporter 2
18. ATP - Adenosine Triphosphate
19. GLP-1 - Glucagon-Like Peptides
20. GIP - Glucose-Dependent Insulinotropic Peptide
21. WHO - World Health Organization
22. BCE - Before Common Era
23. HPLC - High-Pressure Liquid Chromatography
24. MS - Mass Spectrum
25. TLC - Thin Layer Chromatography
26. ROS - Reactive Oxygen Species
27. LDL - Low-density Lipoprotein
28. SOD - Superoxide Dismutase
29. RNS - Reactive Nitrogen Species
30. AIDS - Acquired Immune Deficiency Syndrome
31. DNA - Deoxyribonucleic Acid

32. SOD - Superoxide dismutase
33. CAT - Catalase
34. GSHR - Glutathione reductase
35. GST - Glutathione transferase
36. SDFDA - The Chinese State Food and Drugs Administration
37. AYUSH - Ayurveda, Yoga, and Naturopathy, Unani, Siddha and Homeopathy
38. *et al.,* - *et alii*: and others
39. mg/kg - Milligram Per Kilogram
40. G6P - Glucose 6-phosphate
41. PTP-1B - Protein-Tyrosine Phosphatase 1B
42. TBARS - Thiobarbituric Acid Reactive Substances
43. t-DCTN - Trans-Dehydrocrotonin
44. TNFα - Tumour Necrosis Factor Alpha
45. IC_{50} - The Half Maximal Inhibitory Concentration
46. STZ - Streptozotocin
47. µg/ml - Micrograms Per Milliliter
48. Mm - Millimeter
49. DPPH - 2, 2-Diphenyl-1-picrylhydrazyl
50. Gm - Gram
51. µl - Microliter
52. Nm - Nanometer
53. Na_2CO_3 - Sodium Carbonate
54. v/v - Volume Per Volume
55. GAE - Gallic Acid Equivalent
56. DW - Dry Weight
57. M - Molar
58. UV - Ultraviolet
59. ABTS - 2,2`-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
60. GC-MS - Gas Chromatography-Mass Spectroscopy
61. w/w - Concentration of a Solution

62. IAEC - Institutional Animal Ethics Committee
63. mg/kg b.w - Milligram Per Kilogram Body Weight
64. R_f - Retention factor
65. NIST - National Institute of Standards and Technology
66. LCMS - Liquid Chromatography Coupled to Mass Spectrometry
67. µg/g - Microgram Per Gram
68. ESP - Ethanol extract of the leaves of *Scurrula parasitica L.*
69. SGPT - Serum Glutamic Pyruvic Transaminase
70. SGOT - Serum Glutamic Oxaloacetic Tranaminase
71. ALP - Alkaline Phosphatase
72. ml/min - Milliliters Per Minute

CHAPTER 1
INTRODUCTION

1.1 DIABETES MELLITUS

The term diabetes is from a Greek word, which means "a siphon" because patients suffering from diabetes "passed water like a siphon", it is a syndrome described by chronic hyperglycemia. Diabetes mellitus or simply diabetes can be termed as an escalation of glucose level in the blood because insulin is not produced in a sufficient amount in the human body or because of the unresponsiveness of the cells to the insulin that is produced. It results in hyperglycemia, retinal difficulties, abnormalities in lipid production and protein metabolism, and complications arising in the kidney and nervous systems. The deficiency of insulin in the liver cells during diabetes results in proteolysis, glycogenolysis, and lipolysis which lead to reduced weight of the liver and increased weight of the kidney due to enhanced secretion of glucose and over the synthesis of glycogen, protein, and lipogenesis. If hyperglycemia persists for a long period in the human body, it will be accompanied by long-term problems and inappropriate functioning of the organs leading to damage of the heart, kidney, blood vessels, and eyes.

1.2 GLUCOSE METABOLISM

Glucose is a simple sugar that belongs to the group of monosaccharide carbohydrates and is one of the main energy sources for both plants and animal cells. The storage of glucose occurs in the liver as glycogen and the biochemical pathways in the liver release glucose in response to plasma glucose concentrations by several hormones.

1.2.1 Glycolysis

Glycolysis is the first pathway; it takes place in the cytoplasm of the cell virtually in all tissues. Glycolysis is the pathway that severs six-carbon glucose molecules into two molecules of the three-carbon pyruvate. The end product of

glycolysis is two molecules of ATP and two molecules of NADH+H+. The process occurs in both anaerobic and aerobic conditions.

1.2.2 Gluconeogenesis

It is the formation of glucose from non-carbohydrate sources like glucogenic amino acids, glycerol, and lactate. Gluconeogenesis takes place mostly in the liver with the kidneys subsidizing during a prolonged phase. When the extracellular fluid glucose content decreases, glycogen is mobilized providing a short-term supply of endogenous glucose. Then, the supply is complemented by gluconeogenesis, the other source of endogenous glucose. Gluconeogenesis substrates originate from anaerobic glycolysis (lactate) and breakdown of muscle protein (alanine) or adipose tissue triglycerides (glycerol). In contrast to the liver, muscle handles carbohydrates differently, it cannot release glucose into circulation due to the absence of glucose-6-phosphatase (G-6-Pase), and so it uses glycogen for its energy requirements. Nevertheless, muscles release lactate and contribute to endogenous glucose production which is transported to the liver and enters gluconeogenesis. (Baynes and Dominiczak, 2004; Ashcroft and Ashcroft, 1992; Atkinson and Maclaren, 1995; Kahn and Weir, 1994).

1.2.3 Glycogenolysis

It is the process of catabolism of glycogen by removal of a glucose monomer and addition of phosphate to produce glucose-1-phosphate. The derivative of glucose is then converted to glucose-6-phosphate, which is an intermediate in glycolysis. Glycogenolysis is stimulated by the hormones epinephrine and glucagon and the process transpire in the muscle and liver tissues, where glycogen is stored as a hormonal response to epinephrine/ glucagon, a pancreatic peptide triggered by low blood glucose concentrations (Baynes and Dominiczak, 2004; Ashcroft and Ashcroft, 1992; Atkinson and Maclaren, 1995; Kahn and Weir, 1994).

1.2.4 Lipogenesis

It is the process by which simple sugars such as glucose are converted into fatty acids. Lipogenesis starts with acetyl-CoA and builds up by the addition of two-carbon

units. Fatty acids are consequently esterified with glycerol to form triglycerides that are packed in very-low-density lipoprotein (VLDL) and secreted from the liver (Szablewski, 2011).

1.2.5 Regulation of plasma glucose levels

Glucose homeostasis is controlled primarily by the anabolic hormone insulin and also by several insulin-like growth factors. Several catabolic hormones (glucagon, catecholamines, cortisol, and growth hormone) oppose the action of insulin; they are known as anti-insulin or counter-regulatory hormones. (Baynes and Dominiczak, 2004; Ashcroft and Ashcroft, 1992; Atkinson and Maclaren, 1995; Kahn and Weir, 1994; Mizock, 1995).

1.2.5.1 Insulin

It is secreted in response to the increase in plasma glucose following a meal. Insulin decreases the plasma glucose concentration by promoting the uptake of glucose into tissues, intracellular glucose metabolism, and glycogen synthesis. Insulin is secreted from the beta cells of pancreatic islets of Langerhans. Glucose stimulates the secretion of insulin.

1.2.5.2 Glucagon

It is synthesized by the alpha cells of the pancreatic islet of Langerhans. Its secretion is stimulated by low and inhibited by high concentrations of glucose and fatty acids in the plasma. Glucagon stimulates glycogen breakdown and gluconeogenesis and inhibits glycogen synthesis and glucose oxidation. Its metabolic actions on target tissues are thus the opposite of those of insulin. The fine balance between insulin and glucagon action is a key factor in the control of fuel metabolism. The glucose level acts as a signal that initiates the islet hormonal response (Baynes and Dominiczak, 2004; Mizock, 1995; Rang *et al*., 2003).

1.2.5.3 The metabolic effects of insulin

Insulin promotes the storage of carbohydrates and lipids and the synthesis of protein. It acts on three main target tissues - the liver, muscle, and adipose tissue. In the

liver, insulin stimulates both glycolysis and glycogen synthesis. It also suppresses lipolysis and promotes the synthesis of long-chain fatty acids (lipogenesis). The lipids are then packaged into very-low-density lipoproteins (VLDL), which are secreted into the blood. In the peripheral tissues, insulin induces lipoprotein lipase, an enzyme that offloads triglycerides from either hepatic VLDL or dietary chylomicrons by hydrolyzing them into glycerol and fatty acids. Insulin also stimulates triglyceride synthesis from glycerol and fatty acids in adipose tissue. In muscle, insulin increases glucose transport, glucose metabolism, and glycogen synthesis (Baynes and Dominiczak, 2004; Moller and Flier, 1991; Turner *et al*., 1995; Rang *et al*., 2003; Guyton and Hall, 2005).

1.2.5.4 Stimulation of insulin secretion by glucose

The glucose concentration in the vicinity of the beta-cell is sensed by the beta-cell glucose transporter GLUT-2. Glucose is carried into the cell by GLUT-2, where it is phosphorylated into glucose 6-phosphate (G-6-P) by glucokinase which also is a part of the glucose-sensing mechanism. Increased availability of G-6-P increases the rate of glucose utilization and ATP production in the beta-cell. This changes the flux of ions across the cell membrane, depolarizes the cell, and increases the concentration of cytoplasmic free calcium. The final result is insulin exocytosis. Insulin secretion from the beta-cell after glucose stimulation is biphasic. The first phase of insulin secretion occurs within 10-15 min of stimulation and is the release of preformed insulin. The second phase, which lasts up to 2 hours, is the release of newly synthesized insulin. Insulin secretion is also stimulated by gastrointestinal hormones and some amino acids, such as leucine, arginine, and lysine, a pancreatic peptide triggered by low blood glucose concentrations (Guyton and Hall, 2005; Turner *et al*., 1995).

1.2.6 Metabolism in diabetes

Persons with type 1 diabetes do not have any or have only trace amounts of, insulin in plasma. They also have an increased plasma glucagon concentration. Lack of insulin results in the inability of glucose to enter insulin-dependent tissues, such as adipose tissue and muscle. It also contributes to the relative excess of glucagon. As a consequence, glycolysis and lipogenesis are inhibited, and glycogenolysis, lipolysis,

ketogenesis, and gluconeogenesis are stimulated. The key event in diabetes is that the liver becomes a producer of glucose. Increased endogenous glucose production, together with impaired glucose transport, lead to fasting hyperglycemia. Simultaneously, unopposed lipolysis produces an excess of acetyl CoA. Ketogenesis is stimulated. In a grossly decompensated patient, ketonemia and ketonuria develop. Overproduction of acetoacetic and beta-hydroxybutyric acids decreases the pH of blood, which is normally between 7.37 and 7.44, and causes metabolic acidosis. In type 1 diabetic patients, ketoacidosis can develop very quickly, even after missing a single insulin dose. In type 2 diabetes, ketoacidosis is relatively rare but may be precipitated by major stress, such as myocardial infarction. As glucose is osmotically active, renal excretion of a large amount of glucose leads to osmotic diuresis. Poorly controlled diabetic patients complain of having polydypsia and of polyuria. The resulting fluid loss eventually leads to dehydration. Diabetic ketoacidosis is a life-threatening condition (Baynes and Dominiczak, 2004; Ashcroft and Ashcroft, 1992; Guyton and Hall, 2005).

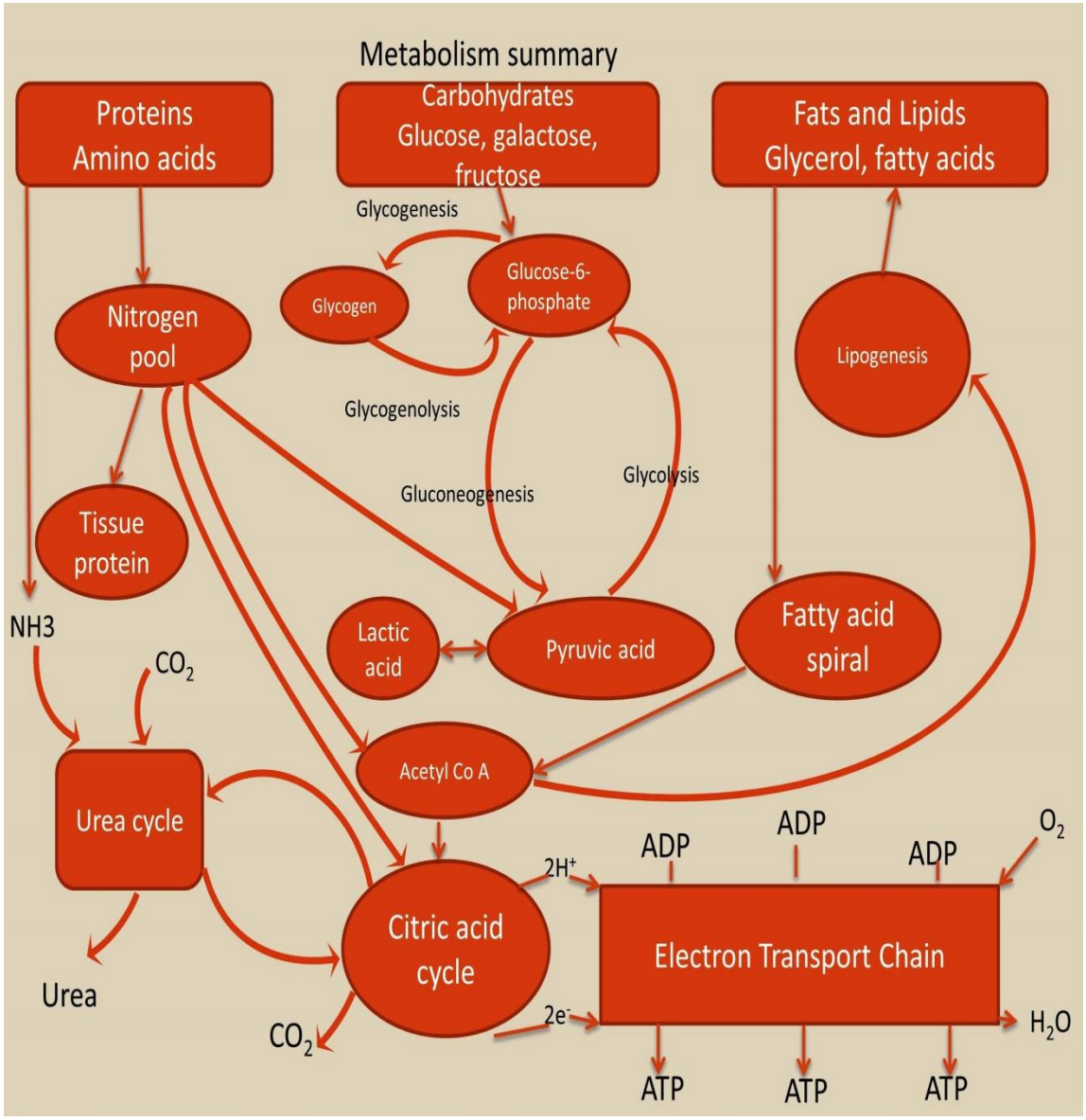

Figure 1: Glucose metabolism

1.3 CLASSIFICATION OF DIABETES MELLITUS

In 1980, The WHO committees classify diabetes into two major classes: Insulin-dependent diabetes mellitus (IDDM) and noninsulin-dependent diabetes mellitus (NIDDM). They are also termed Type-1 and Type-2 diabetes, and then in 1985, the names were changed to only IDDM and NIDDM.

The two types of diabetes are

1.3.1 Type-1 diabetes:

Type-1 results in complete insulin deficiency, which is caused by dysfunction in the β cells. It is further grouped into two types

1. Idiopathic
2. Immune-mediated

Type-1 occurs genetically, with the real causal of the disease still unknown and known to occur most commonly among young adults and children.

1.3.2 Type-2 diabetes:

Type-2 is the common type of diabetes, which accounts for more than 90 % of all cases. Type-2 is caused by the deficiency in the production of insulin or due to a defect in the secretion of insulin. This type mostly affects adults but rarely in children.

1.3.3 Gestational diabetes mellitus:

Gestational diabetes mellitus (GDM) resembles type-2 diabetes in several aspects and develops during the third trimester of pregnancy. GDM are groups of heterogeneous disorders that involve inadequate insulin secretion and responsiveness and the condition might get better or disappear once the baby is delivered. Gestational diabetes can cause damage to the mother or the fetus and it was reported that GDM occurs in 2 to 5% of all pregnancies, 3 to 65% of women suffering from gestational diabetes have an affinity of developing type-2 diabetes after several years. The secretion of mixed insulin and impaired insulin is a result of glucose alterations at a time of pregnancy (Whitelaw and, Gayle 2010).

Gestational diabetes requires proper care with medical supervision if it remains untreated; complications may arise during pregnancy which may result in hyperglycemia, macrosomia, heart diseases, and fetal abnormality. GDM accompanied by fetal malformation can lead to problems in the nervous systems, cardiac and cause skeletal malformations. Poor placental profusion might arise due to vascular impairment which may lead to the fatality of the person.

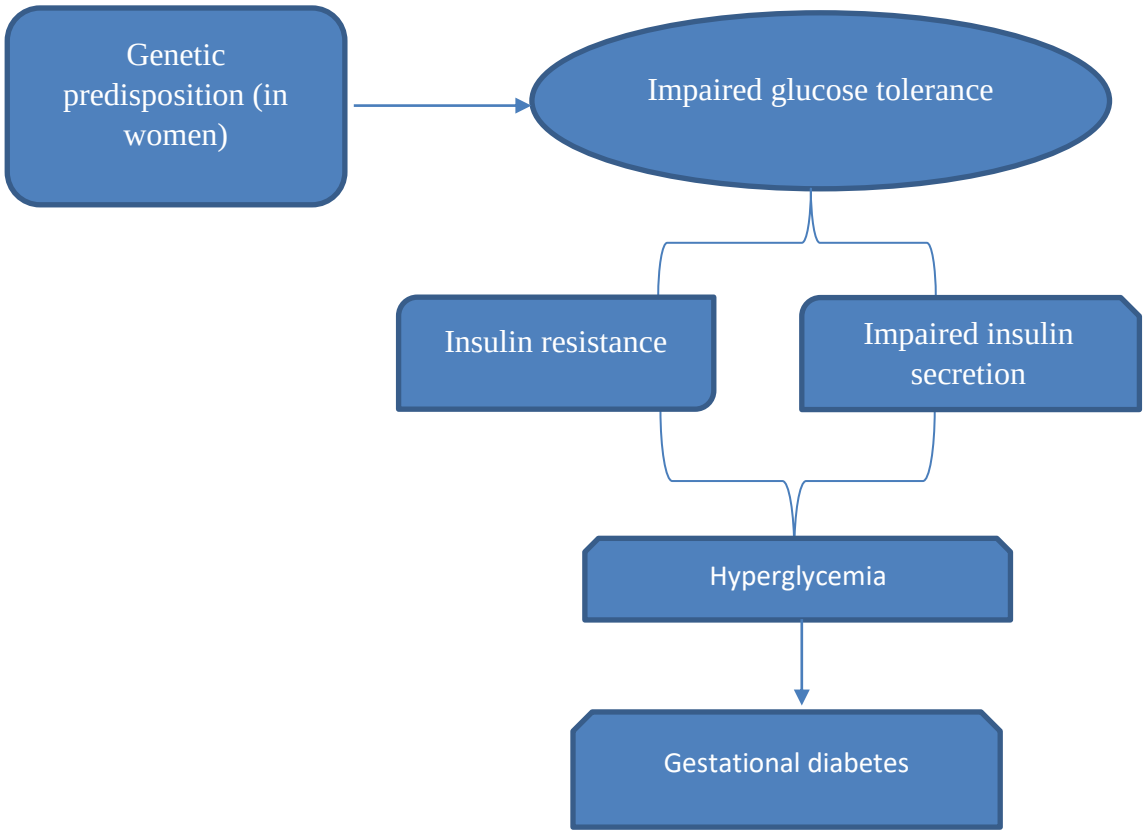

Figure 2: Gestational diabetes

1.3.4 Secondary types of diabetes:

It is further classified into the following:

1.3.4.1 Maturity onset diabetes of the young (MODY)

The cause of MODY is due to the defect in DNA methylation of genes in the pancreas for the development of β- cells. They are characterized as neonatal diabetes and the maturity-onset of the young. The failure of pancreatic β-cells within the first six months leads to the elevation of blood glucose. In MODY, there arise problems in the secretion of insulin with a lesser amount of or no changes in the function of insulin (Thomas and Philipson, 2015).

1.3.4.2 Cystic fibrosis-related diabetes

People suffering from cystic fibrosis are more susceptible to this type of diabetes. The syndrome is caused by the secretion of impaired insulin due to partial fibrotic reduction of the β- cells in the pancreas (ADA, 2015). In cystic fibrosis-related diabetes, the sensitivity of insulin is generally normal or partially impaired (Ode and Moran, 2013). The disorders in the pancreases also lead to pancreatitis, pancreatic neoplasia, and hereditary hemochromatosis.

1.3.4.3 Drugs associated with diabetes

Synthetic drugs were reported of interfering with complex metabolic processes in a physiological system which causes alterations in the secretion and functions of insulin (Thomas and Philipson, 2015). This may induce diabetes or create resistance to insulin in susceptible individuals.

For instance, glucocorticoids were reported to cause the resistance of insulin and hyperglycemia also interfering at the various phases of insulin signaling pathways (Ferris and Kahn, 2012). Antiretroviral drugs which are known as HIV protease inhibitors are known to induce insulin resistance and inhibit glucose disposal via GLUT4 in the cellular system by impairing glucose tolerance (Koster *et al.,* 2003). It is evident from the creation of newer types of diabetes is affecting the overall frequency of prevalence of diabetes.

1.4 PHYSIOPATHOLOGY

1.4.1 Type-1 diabetes:

The deficiency in the secretion of insulin causes the auto-immune damage of pancreatic β-cells which results in the metabolic irrationalities related to type-1 diabetes. Due to the loss of insulin secretion, there is excessive secretion of glucagon and abnormalities in the function of pancreatic α-cells in patients with type-1 diabetes. Glucagon secretion is not suppressed by hyperglycemia which results in improper

elevation of glucagon levels due to deficiency of insulin (Raju and Raju, 2010). Without proper administration of insulin, patients tend to develop diabetic ketoacidosis.

1.4.1.1 Effects on glucose metabolism

If type1 diabetes remained uncontrolled, it enhances the output of hepatic glucose, which causes the mobilization of liver glycogen stores and produces glucose through hepatic gluconeogenesis. The non-hepatic tissue utilization of glucose is due to lack of insulin, this is particular in adipose tissue and skeletal muscles and insulin stimulates the uptake of glucose by movements of glucose transporters proteins to the plasma membranes of the tissues. Reduction in the uptake of glucose by peripheral tissues lowers the rate of glucose metabolism. The increase in hepatic glucose production and reduction in peripheral tissue metabolism elevate the plasma glucose levels. When there is suppression in the kidney to absorb glucose, glucosuria occurs. The escalation in renal loss of glucose results in loss of water and electrolyte which triggers the start of polydipsia (an increase of thirst). The increase in appetite and food intake resulting from the negative caloric balance of glucosuria is known as polyphagia (Raju and Raju, 2010).

1.4.1.2 Effects on lipid metabolism

Insulin stimulates the storage of food energy during a meal in the form of glycogen in hepatocytes and skeletal muscles, moreover, insulin stimulates hepatocytes to manufacture and store triglycerides in adipose tissues. In uncontrolled type-1 diabetes, there is an increase in the plasma free fatty acids due to the rapid mobilization of triglycerides which usually results in hypertriglyceridemia.

1.4.1.3 Effects on protein

The regulation of insulin synthesized numerous genes that have a major effect on the decreased or increased levels of protein synthesis. Insulin insufficiency will cause an enhancement in protein catabolism. The elevation in the concentration of amino acids in the plasma is due to the increased rate of proteolysis (Raju and Raju, 2010). Glucogenic

amino acids serve as pioneers for renal and hepatic glyconeogenesis which causes hyperglycemia in type-1 diabetes.

1.4.2 Type-2 diabetes

The insulin deficiency endangers an increase in hepatic net extraction of glucogenic amino acids, glycerol, lactate, and their conversion to glucose, but also the measurement and action of gluconeogenesis enzymes like fructose-1, 6-biphosphatase, pyruvate carboxylase, glucose-6-phosphatase (Taunton *et al.,* 1974). Glucose-6-phosphatase catalyzes the steps in both gluconeogenic and glycogenolytic pathways and is a key factor in the production of glucose by the liver. When there is an elevation of insulin, the mRNA levels and actions of glucose-6-phosphatase are low in the fed and re-fed stages. Insulin functions well inside the central nervous systems, like regulating meal size, food intake, and body weight (McGowan *et al.,* 1990).

Two main actions of insulin counterbalance each other, as the weight gain is due to the peripheral anabolic effect of insulin yet insulin's central catabolic action would suppress the appetites (Schwartz *et al.,* 1994). Adipose tissue hormones like Insulin and leptin were believed to control energy homeostasis which includes a change in food intake and body weight in the mind (Woods *et al.,* 1998).

Insulin promotes the storages of both exogenous and endogenous derived triglycerides; they also stimulate lipoprotein lipase activities (Eckel and Yost, 1987). It also inhibits hormone-sensitive lipase in adipose tissues and obstructs the hydrolysis of triglycerides stored in the adipocytes. The alterations in triglycerides and cholesterols are evident in diabetic animals, which is relatable to the decrease in actions of insulin-dependent lipoprotein lipase and also the protein contents of lipoproteins (Sparks *et al.*, 1992), required for the acknowledgment and effective lipolysis of triglyceride-rich particles during uptake.

1.4.3 Symptoms of Type-2 Diabetes

The main symptoms of type-2 diabetes include an increase in thirst (Polydipsia), an increase in hunger (Polyphagia), frequent urination (Polyuria), and weight loss. Additional symptoms include peripheral neuropathy, vaginal infections, fatigue, blurred visions, and itchiness. Type-2 diabetic patients infrequently have hyperosmolar hyperglycemia (high blood sugar escorted with low blood pressure and reduction of consciousness levels).

1.5 COMPLICATIONS

Diabetes mellitus is known to cause long-term complications, the consequences resulting from type-1 and type-2 diabetes are almost similar and these are due to uncontrolled hyperglycemia which causes either acute or chronic complications (Monnier *et al.*, 2006). Acute complications include hypoglycemia, hyperosmolar non-ketotic, and ketoacidosis while chronic problems comprise of complications arising in macrovascular diseases include peripheral vascular diseases, atherosclerosis, and myocardial infarction. The microvascular problems of diabetes include damage to the eyes, nerves, and kidneys. Nephropathy is also known as damage to the kidney, it causes chronic kidney diseases, urine protein loss, and tissue scarring occasionally leading to the need for kidney transplants. Diabetes also causes damage to the retina of the eye, these results in vision loss and potential blindness, this is known as retinopathy. Oxidative stress arises due to the failure of conversion of glucose into sorbitol which causes injury to the cells and increases the glycated end product during diabetic retinopathy (Jiang, 2000). Diabetic neuropathy is the damage to the nerves of the body, the symptoms are alteration of a sensation of pain, numbness, and tingling, which harm the skin. Moreover, diabetic neuropathy also causes painful muscle wasting, fatigue, and foot ulcers which in which amputation might be required. Long-term diagnosis with diabetes damaged the blood vessels, enhances the risk of cardiovascular diseases, and causes coronary artery diseases which amount to 75% of mortality.

1.6 DIAGNOSIS ASSESSMENT OF DIABETES MELLITUS

1.6.1 Urine Tests

Analysis of ketone bodies, proteins, and glucose is done by urine tests. For rapid semi-quantitative measures of ketone, the colorimetric reaction among ketones and nitroprusside (Sodium nitroferricyanide) technique is used. Clinistix and Diastix papers are dipped in urine and observed for color changes, and then the strips are compared to a chart that illustrates the amount of glucose in the urine based on the color changes. Urine tests with a paper strip, test stick gives quick and simple results, but they are not as accurate as a blood test. Dipstick tests with Acetest or Ketostix are used to detect ketones in the urine. It is wise to have a simple test to detect ketones because if it persists for a long period, ketoacidosis can occur, so precautions are important. Detection of proteins or albumin by dipstick is also effective as protein in the urine creates complications with kidney functions which can lead to renal failures.

1.6.2 Blood Tests

1.6.2.1 Fasting Glucose Test

This method comparatively shows the severity of diabetes mellitus, the patients haven't eaten and are kept on an empty stomach for at least eight hours typically in the morning. From the patient's vein, blood is then withdrawn. The red blood cells are separated from the sample and the estimation of glucose levels is done in the remaining plasma, fasting is continued the next day for confirmation. Patients with diabetes exhibit a wide variation in the concentrations of their glucose plasma level.

Table 1: Diagnostic principles for Diabetes mellitus by Paula Ford-Martin with Ian Blumer, M.D.

Tests	Regular standard	Prediabetes	Diabetes
Fasting glucose	<100 mg/dl (6.1 mmol/L)	100 to 125 mg/dl (5.6 to 6.9 mmol/L)	≤126 mg/dl (7.0 mmol/L)
Oral glucose tolerance	<140 mg/dl (7.8 mmol/l)	140 to 199mg/dl (7.8 to 11.0 mol/l)	≤200 mg/dl (11.1 mmol/L)
Casual plasma glucose	--	--	≤200 mg/dl (11.1 mmol/L)

1.6.2.2 Postprandial Plasma Glucose Test

The metabolic carbohydrate capacity is detected by stressing the system with a defined glucose load is done for diabetes mellitus. The detection of impairments in glucose metabolism is measured by comparing the amount of glucose entry in a healthy person to the glucose load that is cleared from the body. A 75 gm glucose drink is preferred over a regular meal and is used as the carbohydrate load. Glucose is ingested in the body via a glucose drink and blood is drawn after two hours. The abnormal levels of glucose are 140 mg/dl; uncertain glucose levels are 10 to 140 mg/dl and normal glucose levels are below 120 mg/dl.

1.6.2.3 Oral Glucose Tolerance Test

Committee on Statistics of the American Diabetes Association standardized this assessment to evaluate the circulation clearance subsequently loading of glucose in both

defined and controlled conditions. Fasting is done for 8 to 14 hours with the withdrawal of blood at zero time, and then glucose solution is given to the patient within 5 minutes. For estimating blood sugar and occasionally the level of insulin, blood is drawn at intervals, the intervals and sample numbers may vary according to the diabetes test. The zero and two-hour samples might be the only samples collected in which the 2-hour sample is the most important.

1.6.2.4 Intravenous Glucose Tolerance Test

This type of tolerance test is performed for individuals with mal-absorptive disorders or intestinal or gastric surgery. 20% glucose, with a glucose load of 0.5 g/kg body weight is administered for 30 minutes. Termination of glucose loading causes a reduction in plasma levels within 90 minutes of fasting. Another process called "the Soskin" method, in which the glucose load is 0.3 g/kg of body weight, uses 50% glucose delivered within 3 to 5 minutes. Infusion of glucose is discontinued and in less than 60 minutes and there is non-diabetic re-establishment of fasting levels. For diabetic patients, fasting levels are recognized in about an hour.

1.6.2.5 O' Sullivan Test

This test, in particular, is used to identify gestational diabetes, in which the plasma levels are above 150 mg/dl. The fasting patients are given a 50 load of glucose, and then blood is drawn after an hour.

1.6.2.6 Glycated Hemoglobin and Plasma Albumin Test

In diabetes, glycosylation produced minor hemoglobin called HbA1c, due to the spontaneous reaction and erythrocytes are entirely permeable to glucose, the quantity of HbA1c formed is directly proportional to the concentration of average plasma glucose that the erythrocytes are exposed to during their 120 days life span (4 to 6 weeks before sampling). Glycated hemoglobin is used for monitoring diabetes, but is not sensitive enough to efficiently detect borderline cases of diabetes. Likewise, serum albumin is also glycosylated to a comparative degree to plasma glucose levels. Albumin act as a

good monitor of short-term blood plasma glucose levels, because of their small life span of 15 days.

1.6.2.7 Plasma Insulin Test

Type-1 diabetics are low on plasma insulin levels when the fasting plasma levels exceed 250 mg/ dl or else they are normal. For separating type-1 and type-2 diabetics, a glucose challenge is performed. The results of glucose loading show no substantial response for type-1 diabetics but an extravagant response for type-2 diabetics.

1.7 PREVENTION AND MANAGEMENT OF DIABETES MELLITUS

Type-1 diabetes cannot be managed, but type-2 can be prevented by maintaining normal body weights and exercising, which is beneficial for the heart and lungs as it helps insulin work better and lower blood glucose levels. Physically active people have shown improved blood glucose levels because it improves insulin resistance and lipid profile as it improves high-density lipoprotein (HDL), reduces triglyceride levels, and lowers blood pressure. A change in diets has been recognized to be effective in the control of blood sugar levels, making healthy food choices, and choosing good fats like polyunsaturated fats found in nuts, fish, vegetable oils. Foods that have low salt, sugar, and high in fiber like beans, fruit, and grains help in the maintenance of the weight of the body. Consumption of sugary beverages, eating less red meat, and foods with saturated fats must be limited. Active smoking can lead to the development of diabetes, so preventive measures should be taken as well.

For medication, metformin has proved to be efficient for type-2 diabetes, as the mortality rate is low. The use of aspirin regularly is effective, although, in uncomplicated diabetes, there are signs of no improvements. Angiotensin-converting enzyme inhibitors (ACEIs) were found to improve the condition of diabetes while related drug angiotensin receptor blockers (ARBs) proved to be inefficient. Type-1 diabetes patients are given mixtures of regular and NPH insulin, whereas for type-2 diabetic patients, a long-lasting formulation is added, during oral medications and the

doses of insulin are increased according to the condition of diabetes. Pancreatic transplantation is usually considered for people with severe complications because of type-1 diabetes, like the end phase of renal diseases which requires kidney transplantation.

During hyperglycemia, the pancreatic β- cells that are present in the islets of Langerhans produced a polypeptide hormone called Insulin. To decrease the problems of micro and macrovascular complications because of hyperglycemia, clinical trials/ therapy are performed on insulin, however intensive insulin therapy has major restrictions with hypoglycemia and weight gain.

Insulin is a polypeptide hormone produced by the pancreatic β-cells of the islets of Langerhans in response to hyperglycemia (Harvey and Ferrier, 2011). The β-cells are freely permeable to glucose via type 2 glucose transporter (GLUT 2) which are immediately phosphorylated to glucose 6-phosphate by glucokinase enzyme. This stimulates the rise in metabolic flux through glycolysis, the citric acid cycle, and the generation of ATP (Murray *et al.,* 2003). Elevation of ATP concentration down-regulates the ATP-sensitive K+ channel, leading to depolarization of the pancreatic *β*-cell membrane. This may eventually increase Ca2+ influx via voltage-sensitive Ca2+ channels and stimulates the exocytosis of insulin (Kieffer *et al.,* 1997). Hence, the level of insulin in the blood is proportionate to that of the blood glucose. Therefore, insulin stimulates glucose transport into adipose tissue and skeletal muscles via type 4 glucose transporter (GLUT 4) which are utilized as metabolic fuel and stored as well (Kieffer *et al.*, 1997).

Numerous classes of pharmacological agents like biguanides (phenformin), sulfonylureas (glibenclamide), dipeptidyl peptidase-4 inhibitors (vildagliptin), *α*-glycosidase inhibitors (acarbose), and thiazolidinediones (rosiglitazone) have shown to reduce diabetic levels in clinical trials through diverse mechanisms. Insulin secretagogues, commonly known as Sulfonylureas enhanced the release of insulin from the pancreatic *β*-cells (Aguilar-Bryan *et al.,* 1995). Thiazolidinediones also have been

known to improve the sensitivity of insulin in adipose tissues, liver, and muscle, which escalate the expression of glucose transporters by binding to a PPARγ receptor (Kintscher and Law, 2005; Lehrke and Lazar, 2005). The breakdown of peptides like glucagon-like peptides (GLP)-1 and glucose-dependent insulinotropic peptide (GIP) due to the inhibition of an enzyme by dipeptidyl peptidase-4 inhibitors causes a reduction in glucose levels (Hung *et al.,* 2012). Enzyme inhibitors inhibit the actions of carbohydrates digestive enzymes like α-amylase and α-glucosidase and lower blood glucose levels (Krentz *et al.,* 1994). Biguanides inhibit hepatic glucose production and improve the sensitivity of insulin to the muscle and liver cells (De Fronzo, 1999). But, these oral drugs are costly, especially for people in developing countries and they also have certain side effects resulting in liver and heart failure, hypoglycemia, weight gain, nausea, hypersensitivity (Michael *et al.,* 2005). Consequently, the need for plant-based formulations without any adverse effects to control diabetes is getting more attention from researchers.

1.8 RATE OF PREVALENCE OF DIABETES MELLITUS

Diabetes mellitus is one of the most prevailing diseases worldwide, according to WHO, it was estimated that people suffering from diabetes amounts to 3% of the world's population and the rate of occurrence is projected to be double by 2025 up to 6.3% (Andrade-Cetto and Heinrich, 2005; Attlele *et al.,* 2002). Around 285 million adults are suffering from diabetes in 2010 and will rise to 7.7% of 439 million adults globally by 2030. According to the International Diabetes Federation, the rate of adults suffering from diabetes is 382 million worldwide, and in India, 65.1million people and 382 and is expected to rise globally beyond 592 million over the next 25 years. Type-2 diabetes is the most prevailing type of diabetes mellitus, as it constitutes about 90 to 95 % of the world (Deewnjee *et al.,* 2009), the rate prevalence of diabetes is projected to be around 2 to 3% and rising at 4 to 5% per year globally. (Deore *et al.,* 2011; ADA, 2002; Shaw *et al.,* 2010). As the rate of diabetes rises significantly each year, the search for pharmacologically efficient drugs increases and researches in herbal medicines in

controlling diabetes is proving effective as medicinal plants are easily accessible and have no side effects.

The rapid growth in the prevalence of diabetes shows that, in 2019, 488 million adults aged 20-99 years (9.5%) live with diabetes, and the epicenters of the prevalence in the last decade have been Brazil, Pakistan, China, India, and the United States of America (Saeedi *et al.,* 2019). There have been major shifts in the epidemiology of diabetes for people that have aged over 60 years where illness can be represented by non-specific symptoms and atypical presentations of diabetes (Sinclair *et al.,* 2019). In China, the rate of prevalence of gestational diabetes mellitus is estimated to be around 14.8% (Gao *et al.,* 2019). Li *et al.,* (2018) engagingly conducted a meta-analysis of 64 studies reporting 90 prevalence estimates in India in which they reported that a significantly higher prevalence of 19.2% using the IADPSG principles when compared with 7.4% of DIPSI and 10.13% by WHO in 1999. A systemic review conducted by Nguyen *et al.,* (2018) reported a rate of 10.1% across 48 sites from WP and SEA region countries of Singapore, Taiwan, Thailand, Korea, Japan, Malaysia, Vietnam, and China. Likewise, Macaulay *et al.,* (2014) reported that the prevalence of diabetes mellitus in six countries of Africa is estimated to be between 0% for Tanzania to 13.9% for Nigeria. Diabetes in pregnancy (DIP), hyperglycemia in pregnancy (HIP), and GDM have risen considerably and were reported in the 8th edition of IDF Diabetes Atlas which was released in 2017. It was estimated that the prevalence of DIP to be 2.2% and GDM 14.0% with a total prevalence of HIP at 16.2% (IDF Diabetes Atlas, 2017). However, in the 9th edition released in 2019, there was a minor reduction in the overall prevalence of GDM with 12.8% and HIP with 15.8% and a rise of DIP with 2.6% made up diabetes detected of both pre-existing and during pregnancy both at 1.3% (IDF Diabetes Atlas, 2019). In 2019, it was estimated that 11.3% of diabetes leads to death and the mortality of adults was projected to be more than 4 million. People suffering from diabetes die due to the complications in the human body caused by diabetes. Furthermore, the death rate associated with diabetes is higher in women 2.3 million as compared to men 1.9 million. Moreover, deaths due to diabetes are significantly higher in numbers in middle-income

countries (3.4 million) than the total number of deaths in both low and high-income countries combined (786,800). Also, the proportion of people suffering from diabetes in middle-income countries is expressively higher when compared with low and high-income countries (76.3% v 23.7%). Diabetes-related deaths of age 20-79 years are proportionally high in the MENA Region (16.2%) and lowermost in the AFR Region (6.8%). The increase in the prevalence of diabetes has increased significantly over the past 13 years and should be noted about one-third of countries (35%) are still lacking high-quality country data for estimating the prevalence of diabetes. According to IDF Diabetes Atlas (2006) and Saeedi *et al.,* (2019), the total number of individuals suffering from diabetes has increased from 246 million in 2006 to 463 million in 2019.

1.9 MEDICINAL PLANTS

The plants which possess therapeutic properties in certain parts contain substances against certain diseases or which serve as the origin of useful drugs are known as medicinal plants (Ebadi, 2006; Lewis, 1981). Medicinal herbs remain the source of modern drugs and have served as a global basis for the health care system for many years. The substances derived from plants are receiving much attention as they remain a basis for a large proportion of the commercial medicines and their therapeutic value in the treatment of pain, asthma, heart diseases, high blood pressure, etc. The bioactive compounds in plants are used for the treatment of diseases and ailments while they also act as the framework for the synthesis of modern conventional drugs, therefore plants remain an important source for drug discovery (Mendonça-Filho, 2006; Geldenhuys and Mitchell, 2006).

1.9.1 Medicinal plants in future drug discovery

The use of plant parts and their formulations in the treatment of diseases is as old as time, it was practiced way back during 2600 BCE (Cragg and Newman, 2013; Soladoye *et al.,* 2012), natural products with remarkable capacities are used by the people in their conventional ways to cure a wide variety of diseases. More than 1000 plants with traditional formulations were recorded which were used for the treatment of

numerous infections including communicable and non-communicable diseases. The majority of the modern medicines found today are mostly derived from plants and due to their easy accessibility; they are used by the local inhabitants for the same purposes. For example, *Galega officinalis L*. is used to treat diabetes and an anti-diabetic agent galegine was isolated from the plant (Heinrich, 2010). Likewise, from *Artemisia annua L*. and *Calophyllum lanigerum* var. *austrocoriaceum* (Whitmore) P.F. Stevens, anti-malarial drug artemisinin and anti-HIV drug, calanolide A were isolated. These plants were traditionally used for the treatment of fevers and microbial infections (Gurib-Fakim, 2006).

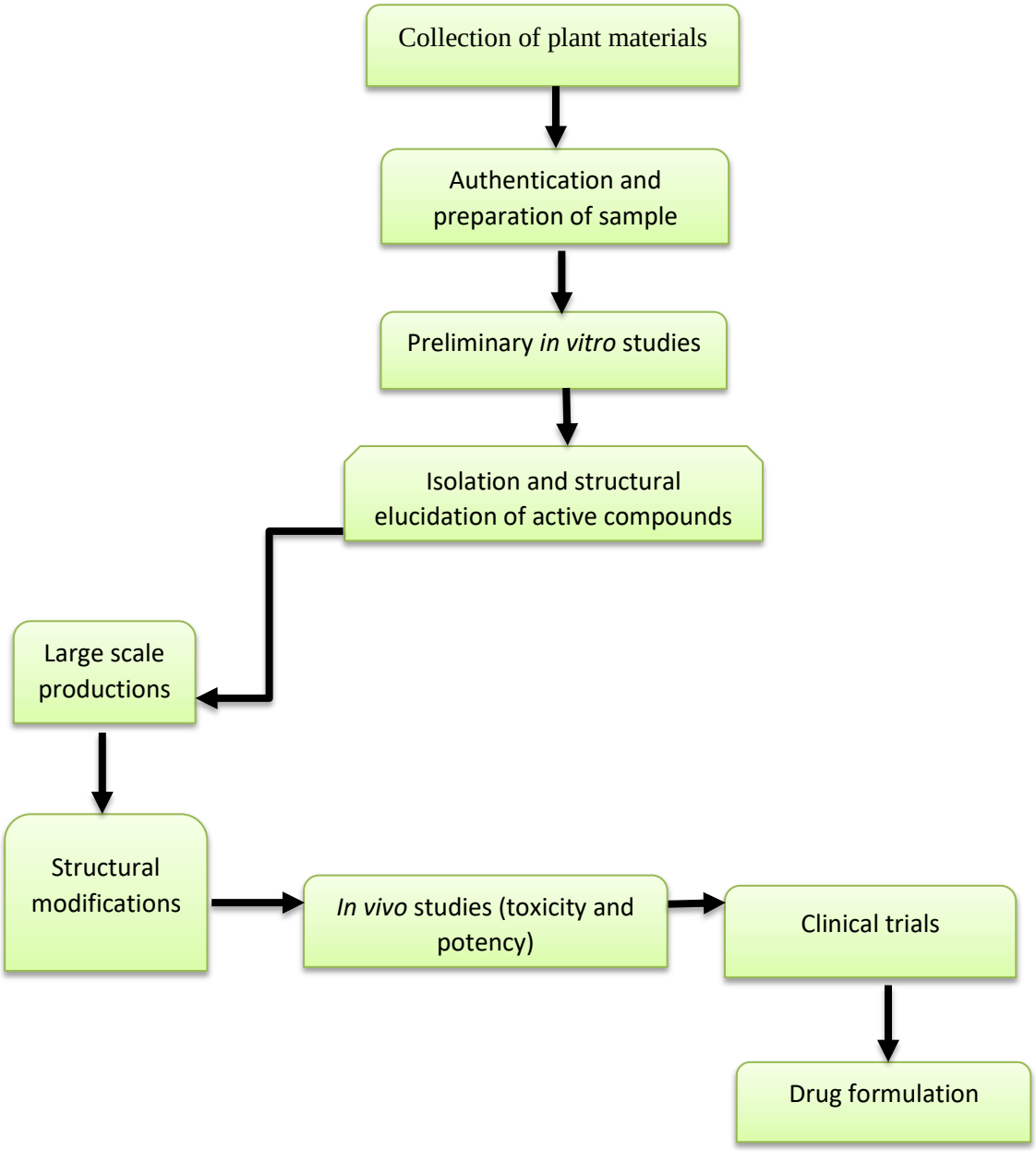

Figure 3: Drug formulation from plants as modified by Balunas and Kinghorn in 2005.

1.9.2 Selection of plant for drug discovery

According to their pharmacological activities, scientists made numerous approaches to map out plants for drug discovery (Heinrich *et al.,* 2004; Fabricant and Farnsworth, 2001). Through ethnobotanical investigations on the usage of a plant by the local people, candidate plants were selected for the research (Shizen and Xiwen, 2004). The selection is based on the phytochemistry of active compounds present in plants, with no knowledge of a target compound responsible for their pharmacological activities (Brusotti *et al.,* 2014). Random selection of target biomolecules with bioactive reports has also been performed (Heinrich *et al.,* 2004; Harborne, 1998).

1.9.3 Authentication and preparation of plant sample

The collected plant samples are properly authenticated by a botanist or taxonomist (Satyajit *et al.,* 2006). The herbarium is deposited and given a specific voucher specimen number for future references (Harborne, 1998).

The preparation of extracts from plant samples is the central part of drug formulation (Brusotti *et al.,* 2014). Bioactive ingredients are extracted by several methods of which solid-liquid solvent extraction technique is frequently used, which are based on the polarity of the solvents and the frequency of solubility of the bioactive compound (Hostettmann *et al.,* 1991). This includes methods like hot and cold maceration, infusion, percolation, decoction, and sequential extraction using the soxhlet apparatus (Harborne, 1998). The plant materials are dried under shade in a well-ventilated room to avoid contamination and alterations of the plant compounds. Subsequently, after drying, the plant materials are made into fine powder form and subjected to different extraction procedures. The extracts are evaporated to their crude form and used to perform *in vitro*, *ex-vitro*, or *in vivo* studies.

1.9.4 Preliminary *in vitro* studies

In vitro bio-assays are used to determine the active potential of the plant extracts. The assays are quick and specific with the only little amount of extracts required

(usually in micro or milligram amount) and the analysis of the chemical and enzymatic processes is carried out using spectrophotometric methods (Beretta and Facino, 2010; Cos *et al.,* 2006). Evaluation of anti-diabetic activities with their mode of action is performed using *in vitro* models, these include models like glucose uptake bioassays (via cell lines such as C2C12 myocytes, human Chang liver cells, and 3T3-L1 pre-adipocytes), enzyme inhibition assays (e.g. α- amylase, glucose 6-phosphatase inhibition, and α-amylase) and the stimulation of insulin released usually conducted in the perfused pancreas, clonal pancreatic *β*-cell-lines or islets cells in the pancreas (Van de Venter *et al.,* 2008; Bhandari *et al.,* 2008; Hannan *et al.,* 2007).

1.9.5 Fractionation of active compounds

It is a bio-assay-guided technique to separate and identify the bioactive compound present in plants. It is a separation method based on repetitive fractionation to acquire a pure compound of certain biological properties using thin layer chromatography and column chromatography (Gurib-Fakim, 2006). Then structural elucidation of the pure compound was done with High-Pressure Liquid Chromatography (HPLC) and Mass Spectrum (MS) with confirmation by TLC.

1.9.6 *In vivo,* clinical and toxicological research

The efficacy and active ingredients of plant extracts are analyzed by *in vivo* trials, in which animals are used to carry out the experiments (Eddouks *et al.,* 2012). From the results based on *in vitro* studies *in vivo* experiments are performed, but the requirements of plant extracts are high in the case of *in vivo* studies. To enhance the bioactivity of the target compound, sometimes a structural modification is employed (Rates, 2001). This pre-clinical investigation has more complications as it demands more resources and takes a longer time.

The human body has complex heterogeneity in disease conditions, thus in the case of diabetes mellitus, to signify the true potency of the bioactive compound, several models are employed. For instance, chemically induced animal models like alloxan and

streptozotocin-induced diabetic animals are used in the case of type-1 diabetes (Wang *et al.,* 2009). Animal models of type-2 diabetes include experimentally induced or non-spontaneous (high-fats died-fats and fructose-fed/ streptozotocin) and genetically induced or spontaneous (Zucker diabetic fatty model) (Eddouks *et al.,* 2012; Islam and Loots, 2009). In developing countries, scientists and researchers have preferred experimentally induced models over genetically induced models due to their ease in the induction of diabetes with its minor cost of maintenance and wider availability (Eddouks *et al.,* 2012; Islam and Loots, 2009). If an adverse effect arises in the animal model, due to the nature of the candidate drug, the compound swiftly undergoes structural modification to reduce the severity (Lin and Lu, 1997). Pharmacological experiments like *in vivo* and *in vitro* are performed to study the toxicological effects of the compounds in plants, to ensure their use before clinical trials. The clinical trials of active compounds in plants are done in animals after which they can be given to humans. Before the commencement of such research, protocols with strict ethical approvals in animals are prepared according to the subjects to be performed. After successful trials with the candidate drug, they are given to humans and if there are no adverse effects, the drug will be marketed and subsequently be available at a general pharmaceutical store.

1.9.7 Free radicals, oxidative stress on diabetes mellitus

For proper health, maintaining a balance between antioxidants and free radicals is important, but when there is an imbalance it leads to the formation of oxidative stress (Sies, 1991). Oxidative stress plays a crucial role in the development of type 2 diabetes and frequent studies reveal the occurrence of oxidative stress in diabetic patients (Haffner, 2000; Bonnefont-Rousselot *et al*., 2000; Pham-Huy *et al*., 2008). Free radicals are created in diabetic patients due to increased lipid peroxidation and non-enzymatic glycation of proteins which leads to increased risk of insulin resistance which is a phenomenon of oxidative stress (Maritim *et al*., 2003). Further reactive oxygen species trigger pathogenesis of inflammatory response and ischemic reperfusion that promote diabetes. Hyperglycemia also increases the production of free radicals through numerous mechanisms. Diabetic patients are diagnosed with chronic and acute oxidative stress that

enhanced the possibilities of macrovascular complications (Oberley, 1988). In diabetes mellitus, oxidative stress occurs mainly in mitochondria, the metabolism in which a molecule of oxygen is reduced to water and the additional oxygen molecule is converted to oxygen free radicals that finally generate reactive species such as H 2 O 2, ONOO-,OH (Moussa, 2008). Regulation of insulin signaling by reactive oxygen species (ROS) and reactive nitrogen species (RNS) is done in two ways, the first one is that ROS/RNS displayed its effect when it is stimulated by insulin and the second is that ROS and RNS showed negative control on insulin signaling, demonstrating them to reveal insulin resistance which may lead to type 2 diabetes (Erejuwa, 2012). The relationship between diabetes mellitus and oxidative stress is ambiguous; at the same time, undeniable clear that oxidative stress leads to diabetes mellitus as the generation of free radicals plays a key role in the pathogenesis of diabetes.

1.9.8 Role of antioxidants in diabetes

These are the substances that prevent the oxidation of cellular oxidized substrates by scavenging ROS (reactive oxygen species) thus preventing the generation of ROS and activating a battery of detoxifying products (Halliwelli *et al.*, 1992). Antioxidant defends the beta-cell against oxidative stress and preserves the function of the beta-cell. Furthermore, antioxidants lower/ diminish complications caused by diabetes and the recovery of insulin. Numerous researches have revealed a strong association between intakes of dietary antioxidants and protection against diabetes. In recent years, the need for finding plants with natural antioxidants has increased, as they constitute a broad range of compounds including carotenoids, phenolic, and nitrogen compounds and protect the body from free radicals and lowers the risk of development of chronic diseases like cancer, diabetes, eye and heart diseases. Enzymes like superoxide dismutase (SOD), glutathione peroxidase, and catalase remove oxidants by transforming ROS and RNS into stable compounds and generate reactive oxygen (Tiwari, 2001). A natural antioxidant with anti-diabetic properties includes the following:

1.9.8.1 Vitamin E

These are naturally occurring antioxidants that exist as tocotrienol and tocopherol. It is believed that vitamin E defends the cells against oxidative damage, controls hyperglycemia, and in the prevention of diabetic complications. During the diabetic condition, extra glucose is attached to hemoglobin to produce glycosylated hemoglobin and Vitamin E was treated in rats to prevent the diabetic condition (Je *et al*., 2001). Vitamin E associated also minimizes the macrovascular and microvascular complications in diabetic patients (Milman *et al*., 2008). Vitamin E has been revealed to control hyperglycemia and obstructing oxidative stress by lowering the HbA1c level in diabetic rats (Ihara *et al.,* 2004). The mechanism by which antioxidants decrease glucose levels is ambiguous, however, there is a decrease in plasma glucose level by enhancement of glucose metabolism in peripheral tissues (Roldi *et al.,* 2009).

1.9.8.2 Vitamin C

It is powerful antioxidants which are capable of scavenging free radicals in the aqueous phase which provides stability to the cell membrane and they can convert Vitamin E free radicals to Vitamin E which is essential for hydroxylation reaction in human. During diabetes, there is an increased level of Vitamin C, which may be due to increased utilization in trapping oxyradicals. Previous researches also have reported that diabetes may result in decreased Vitamin C and E due to increased oxidative stress (Hisalkar *et al*., 2012). The mechanism of how Vitamin C can treat diabetes is not clear, but it plays a key role in diminished microalbuminuria, erythrocyte sorbitol levels, and improving insulin resistance due to its antioxidant function (Paolisso *et al.,* 1994; Cunningham *et al.,*1994). Tanko *et al.,* (2013) reported that administration of Vitamin C and E (100 mg/kg of body weight of rat) significantly reduced the blood glucose level. In a population-based study, it was found that frequent intake of Vitamin C dietary sources was found to decrease the risk of Type 2 diabetes (Williams *et al*., 1999).

1.9.8.3 Alpha-lipoic acid

It is a potent antioxidant also known as thioctic acid/ 1, 2-dithiolane-3-pentanoic acid. It is capable of restoring endogenous antioxidants such as Vitamin E, Vitamin C, and glutathione. It an effective antioxidant against many pathological conditions like diabetes mellitus, liver, and cardiovascular diseases (Wollin and Jones, 2003; Bustamante *et al*., 1998). This antioxidant has been reported to improve glucose metabolism in type-2 diabetes by activating lipid, tyrosine, and serine/threonine kinases in target cells, which stimulate glucose uptake and glycogenesis. Alpha-lipoic acid has been found to increase the translocation of GLUT1 and GLUT4 to the plasmatic membrane of adipocytes and skeletal muscle, which is related to enhanced activity of proteins of an insulin signaling pathway (Lester and Enrique, 2011). In another research, oral supplementation ALA (600 mg twice daily for 4 weeks) treatment enhances the plasma insulin activity (Kamenova, 2006). ALA scavenged ROS produced during lipid peroxidation and guards the cell structure against damage and continued supplementation in diabetic rats reduces hyperglycemia and diabetic nephropathy (Packer *et al*., 2001).

1.9.8.4 Selenium

It is a significant trace element, naturally found in many food products. It existed in both organic and inorganic forms. Selenate and selenite are inorganic forms while selenomethionine and selenocysteine belong to organic form. Selenium plays a crucial role in immune functions and thyroid hormone metabolisms. Due to their antioxidant activity, selenium has various pathological activities (Sunde *et al*., 2006). In the experimental model, supplementation of selenium with a low dose has beneficial effects on glucose metabolism by mimicking insulin-like actions while the process is unclear, previous report denotes that selenium activates the key protein responsible for insulin signal cascade (Stapleton, 2010). By activating kinases, sodium selenite is also involved in the insulin signaling cascade. In experimental animal studies, oral administration/ intraperitoneal injection of selenite for daily doses up to 3/4 weeks in streptozotocin-

induced diabetic rats, shows a reduction in glucose levels (McNeill *et al*., 1991; Battell *et al*., 1998).

1.10 THE USE OF MEDICINAL PLANTS AGAINST DIABETES MELLITUS

The use of herbal products against diabetes was dated back centuries ago and has been an important source in the development of drugs against diabetes. Documentation of ethnobotanical plants reported about 800 plants with anti-diabetic activities. For the control of hyperglycemia, synthetic drugs like insulin and other oral hypoglycemic agents are currently used, but these drugs have certain side effects and failed to reduce the complications of diabetes leading to the persistence of hyperglycemic conditions in the body for a longer period which increases the risk of stroke and heart diseases with prominent problems to the kidney, blood vessels, heart, nerves, and eyes. Patients suffering from diabetes find it tough to maintain control of hyperglycemia due to the progressive reduction in the function of β –cells. Investigations of medicinal plants from 2005 to 2010 highlighted around 100 plant-derived preparations with anti-diabetic actions. During this period, some of the plant formulations were marketed as a remedy for diabetes and a total of 85 bioactive compounds with anti-diabetic activity were isolated (Hung *et al.,* 2012). It was also reported that from 2000 to 2005, about 106 compounds from plants with possible anti-diabetic effects were published (Jung *et al.*, 2006).

Even in India, especially among the tribal inhabitants, the use of herbal medicines against diabetes is a major avenue as more exploration of the plants can be done through works of literature and interaction with the local people. Herbal remedies are the most effective therapy available for rural people as they are easily accessible, low in cost, and with no side effects (Arya *et al.,* 2011). Traditional practitioners prescribed many herbal products in the form of plant extract or their folk preparations for persons suffering from diabetes, especially in the rural areas in India. The country has a rich heritage of medicinal plants in the Ayurveda, Siddha, and Unani system, around 15000 medicinal plants have been documented which include 7000 plants in Ayurveda, 700 in

Unani, 600 in Siddha, 450 in Homeopathy, and 30 in modern medicines (Das, 2008). The plant parts and their extracts play a critical role in the treatment of diseases. These plants have been tested for their biological and hypoglycemic activity (Glombitza *et al.*, 1994) along with antiulcer, hepatoprotective, analgesic, and antihelminthic activities. (Mahasnesh, 1986; Nadir *et al.*, 1986; Alkofahi *et al.*, 1997).

The antioxidant properties of plants might be the reason for their capabilities of reducing blood glucose levels in the body during diabetes (Hongxiang *et al.*, 2009). From previous research on experimental models, it was established that terpenoids, coumarins, flavonoids possess hypoglycemic activity along with other secondary metabolites like arginine and glutamic acid (Ross, 2001). Medicinal plant's phytochemical constituents and their hypoglycemic potential have been demonstrated in animal models (Hwang *et al.,* 2005). However, screening for biologically active constituents has not been done in the majority of the species of higher plants; therefore the challenge is to exploit the phytochemical constituents of the medicinal plants with insulin-based properties for diabetes. Medicinal plants serve as an important dietary supplement and they are the source of oral hypoglycemic agents for drug developments, thus plant parts are extensively used in the treatment of diabetes globally (Bailey and Day, 1989).

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CHAPTER 2

AIMS AND OBJECTIVES

AIM:

The study aims to carry out the phytochemical analysis of the anti-diabetic plants of Mizoram.

OBJECTIVES:

1. Documentation of medicinal plants used by the tribal people of Mizoram for the treatment of Diabetes mellitus.
2. Phytochemical screening of 15 plants for potential secondary metabolites effective against diabetes mellitus.
3. Determination of the antioxidant activity of one selected plant using DPPH and ABTS methods.
4. Evaluation of the anti-hyperglycemic activity of one selected plant with streptozotocin-induced diabetic rats.
5. Isolation of active anti-diabetic compound from one selected plant using chromatographic techniques.

CHAPTER 3

REVIEW OF LITERATURE

Medicinal plants and their important phytoconstituents are getting renowned for their pharmacological and clinical values especially in the case of diabetes mellitus all over the world. The surveys for anti-diabetic plants through ethnobotanical explorations are gaining a reputation for the development of new synthetic drugs for the treatment of diabetes mellitus. Medicinal herbs have been used since primitive times for treating diabetes, but the efficacy and the chemical constituents for which they possess anti-hyperglycemic activities remain a mystery. Therefore, it is essential to document important plant species for research in their secondary metabolites contents and formulation of important drugs for diabetes.

3.1 POTENTIAL ETHNOBOTANICAL PLANTS FOR SCREENING OF ANTI-DIABETIC COMPONENTS

Various investigations have been done through ethnobotanical explorations by scientists and researchers all over the world for the identification of medicinal plants with potential compounds present in them for the treatment of diabetes mellitus for pharmacological and phytochemical studies. Ethnobotanical explorations of medicinal plants, which are used for the treatment of diabetes, have been done by numerous ethnobotanists over the world. A few substantial contributions are mentioned below

3.1.1 Worldwide

Al-Aboudi and Fatma (2010) reported 69 plant species, all native floras from Jordon. It was found that 65% of the 69 plants have been studied for their *in vivo* hypoglycemic activity, out of which 54% were found to be effective against diabetes. The remaining 34.5% have not been scientifically analyzed.

Abu *et al.,* (2015) investigated the Ayurvedic plants with pharmacological anti-diabetic potential in Bangladesh. They reported 25 species belonging to 25 families and the different parts used by the people for managing diabetes.

Brown (1995) investigated *Viscum album* (mistletoe) and reported that the plant possesses important pharmacological properties like slowing the rate of heartbeat, stimulation of immune system, lowering of blood sugar levels along with its anti-cancer and diuretic effects on the human body. Peters (1957) also reported that a traditionally made tea in the West Indies which is prepared from *Viscum album* is used for treating diabetes. The treatment with mistletoe has been known to relieve the symptoms of diabetes in streptozotocin-induced diabetic mice, increase body weight, and in controlling polydipsia and hyperphagia (Swanston-Flatt *et al.,* 1989).

Bailey and Flatt (1990) reported that during the early 1920s, before the development of oral hypoglycemic drugs and insulin discovery, diabetic patients were subjected to therapies with medicinal plants involving starvation and dietary management. Ethnobotanical explorations have documented more than 400 plants, which are frequently used for treating diabetes (Bailey and Day, 1989; Swanston-Flatt *et al.,* 1989; Gray and Flatt, 1997).

Miyake *et al.,* (1986) reported that *Bauhinia forficata* which belongs to the family Caesalpiniaceae is the most frequently used plant for the management of diabetes in Brazil. It is traditionally known as Pata de Vaca (cows hoof) in Brazil in which the leaves display hypoglycaemic activities (Viana *et al.,* 1999). Juliani (1941 and 1931) performed the preliminary report of the anti-diabetic activity of *Bauhinia forficata* in diabetic patients.

According to Xie *et al.,* (2011) two or more herbs are mixed to make a formula to suppress the symptoms created by diabetes mellitus in China. In Chinese medicinal plants, the most common bioactive compound used against diabetes is Berberine (Lee *et al.,* 2006) and the major source for this compound is found in *Rhizoma coptidis* (Tang *et al.,* 2006). The Chinese traditional plants have many common plants concerning diabetes

with the Ayurveda system in India, the plants include onion, garlic, peepal, blackberry, etc. (Patwardhan, 2005). The Chinese State Food and Drugs Administration (SDFDA) approved 30 herbal formulations for the treatment of diabetes.

According to Mahomoodlly (2013), the traditional medicinal system in Africa recorded almost 45000 species of plants that are used for the treatment of numerous diseases, and the system is ritually followed in Guinea (Balde *et al.,* 2006). Tsabang *et al.,* (2016) surveyed 116 individuals diagnosed with diabetes in Cameroon and reported that *Momordica charantia, Phyllanthus amarus, Persia americana,* and *Allium cepa* were the major plants used frequently amongst the people of Cameroon.

The majority of the people in Nigeria still depend on herbal medicines for treatment against diseases and infections and in the North-western region of Nigeria *Combretum Micranthum* also commonly called 'geza' in Hausa, is frequently used for controlling diabetes and for treating several other diseases in West Africa (Muhammad and Amusa, 2005; Inngjerdingen, 2004). *Parinari excels* is one of the most extensively used medicinal plants to manage diabetes mellitus in Africa. It belongs to the family Chrysobalanaceae and the bark is used for the treatment of diabetes mellitus. Kerharo and Adam (1974) reported that the plant is found growing in Guinea, Congo, Senegal, and Casamance.

Asman *et al.,* (2018) investigated that the most potent anti-diabetic plants come from Rubiaceae which is the main group of angiosperms (flowering plants). They reported 34 plants from the family Rubiaceae which possess anti-diabetic potential in which the plants have chemical compositions rich in alkaloid, flavonoid, terpenoids, glycosides, phenols which generally act as anti-diabetic.

Ethnobotanical explorations by Souad *et al.,* (2019) reported 30 plant species belonging to 18 families, which were used for the treatment of diabetes mellitus in Rabat, Morocco. Out of the 30 species 13 were wild species, 4 imported species, 13 cultivated species, and 2 uncertain mixtures of powdered plants.

Ocvirk *et al.,* (2013) documented 37 anti-diabetic plants from the urban regions of Dhaka, Bangladesh out of which *Trigonellafoenum-graceum, Centella asiatica, Azadirachta indica, Momordica charantia*, *Syzygium cumini, Catharanthus roseus, Centella asiatica, Ocimum tenuiflorum,* and *Cynodondactylon* are frequently used by the people of Dhaka.

Puja and Nirmala (2018) documented 52 traditionally used anti-diabetic plants belonging to 35 families from 5 places in the Kaski district of Nepal.

He *et al.,* 2005; Jung *et al.,* 2006; Ji *et al.,* 2009 reported that important constituents of plant products like flavonoids, terpenoids, coumarins, and phenolic compounds are proficient in reducing blood glucose levels.

3.1.2 India

In India, Ayurveda is the main traditional system and around 800 plant species with anti-diabetic activities have been described. Ayurveda comprises 3 elemental substances like Vata, Kapha, and Pitta, the disproportion in these elements would result in diseases. In this system, plants and their products are used for the treatment of diseases. The government of India created a separate ministry of Ayurveda, Yoga, and Naturopathy, Unani, Siddha, and Homeopathy (AYUSH) for research in the indigenous medicinal system. Due to the advancement of indigenous medicinal systems, the government of India has established the All India Institute of Ayurveda in Delhi.

Medicinal plants which are rich in antioxidants and phytochemical constituents like *Jamun (Eugenia jambolana), Lahsun (Allium sativum), Neem (Azadirachta indica), Peepal (Ficus religiosa), Kawar (Aloe barbadensis), Karela (Momordica charantia), Babul (Acacia arabica),* etc. are used for management of diabetes mellitus (Modak *et al.,* 2007). The phytochemical constituents reduce high glucose levels, enhance enzymes like glutathione and catalase and increase the production of insulin in the body (Rizvi and Mishra, 2013).

Nutraceutical products like beverages and capsules like Diabeta, Health kart Karela and Neem tea are commercially available in India to conquer complications arising due to diabetes like renal and hepatic problems along with a report on anti-stress properties. But despite the availability of these nutraceutical products, *hakims/vaids* still widely have more faith in traditional herbs and their formulations as their source of medicine.

In India, bitter melon is one of the main plants used for the preparations of anti-diabetic medicines by *hakims*. Abascal and Yarnell (2005) reported that the juice of bitter melon is more effective than any other formulations. Alam *et al.,* (2015) reported that bitter melon helps in maintaining the structural integrity of the pancreatic cells in islets of Langerhans and regulating the synthesis of pancreatic hormones, and also maintain blood cholesterol. Due to the high hypoglycemic nature of bitter melon, consumption with other medicines of similar activity should be avoided as it can cause immediate lowering of glucose levels which will create more health problems (Bhowmik *et al.,* 2007).

The Unani medicinal system was introduced in India during the 18^{th} century by Arabs and Iranians (Subbarayappa, 2001). The system is based on the 4 fluids of the body like yellow bile, black bile, blood, and phlegm which are related to the emotional, mental, spiritual, and physical sources of diseases. The proper instructions on the dosage of the medicine are given by the Unani system and the herbs which are used by the Unani system for the treatment of diabetes mellitus include marshmallows, licorice root, spiny gourd, malabar nut, hisawarag, cape lilac, bitter apple and virgin's mental (Said, 1983).

Aadhan and Anand (2017) reported the medicinal plants used for the treatment of diabetes by the Paliyar’s Tribe in Sadhuragiri hills of Tamil Nadu. Ethnobotanical explorations documented 65 anti-diabetic plant species belonging to 58 genera and 38 families from this area along with 34 plant species of 29 genera and 22 families were recorded to be used by the Thirumoorthy hill region of Western Ghats of Tamil Nadu

(Vijayalakshmi *et al.,* 2014). Thirumalai *et al.,* (2012) recorded 40 traditional plants with anti-diabetic activity used by the people of Javadhu hills of Tamil Nadu. Formulations of important drugs have been done from these plants due to their potent phytochemical constituents. Upendra *et al.,* (2010) documented the aromatic plant *Elephantopus scaber* in the moist deciduous forests of central Western Ghats. It belongs to the family Asteraceae and is commonly used by people for treating diabetes. The plant also possesses antipyretic, cardiotonic, and diuretic activities and is also convenient against stomach pain, dysentery, and diarrhea (He *et al.,* 2005).

In Odisha, Mishra *et al.,* (2019) documented the folklore medicines through ethnobotanical investigations in the Khurda district and recorded 50 species belonging to 45 genera and 34 families which were used in the management of diabetes, among which 40 species belonging to 36 genera and 25 families were widely used by local practitioners in Khurda district of Odisha for treatment of diabetes. The anti-diabetic activities of these plants are due to the presence of important secondary metabolites.

Mhasker and Caius (1930) reported that in the late 1920s, the hypoglycemic activity of *Gymnema sylvestre* was first investigated. It is evolving as a potent medicinal plant especially in India and parts of Asia in which the leaves are used in treatment against diabetes.

Upendra *et al.*, (2010) reported the use of *Ricinus communis* or commonly known as castor oil, which is widely used in India for the management of diabetes. It belongs to the family *Euphorbiaceae* and is cultivated all over the country for extracting its seed oil. In Hindi, it is known as Arandi, Amudam in Telugu and Erandah in Sanksrit. *Ricinus communis* were noted in Susruta and Ayurved in the sixth century and have been used as herbal medicine during the early Greek and Egyptian era (Olsnes *et al.,* 1976).

Modak *et al.,* (2007) reported the Indian herbs used for the treatment of diabetes of 33 plants having anti-diabetic properties with their mode of actions were documented and 12 anti-diabetic drugs which were formulated from different ingredients of plants were also recorded.

Priti *et al.,* (2011) reported the anti-diabetic plants which were used by the people in Almora districts, Uttarakhand, India. They documented 16 species belonging to 134 families and the study was done on 100 peoples suffering from diabetes through surveys. The study revealed that 65% of people are relying on herbs and their products for the management of diabetes.

Aswini Kumar *et al.,* (2011) documented 58 anti-diabetic species, 52 genera comprising 36 families along with their therapeutic uses were recorded from Puducherry Coromental coasts of India.

Sidhu and Sweta (2015) surveyed in Mandi district of Himachal Pradesh and reported a total of 25 plant species belonging to 23 genera and 17 families, most of which are from Cucurbitaceae and Lamiaceae for treatment of diabetes.

Sangeeta *et al.,* (2016) reported that ethnomedicinal plants like *Melia azedarach* (Meliaceae), *Butea monosperma Lam.* (Papilionaceae), *Curcuma longa L.* (Zingiberaceae), *Cassia fistula* (Caesalpiniaceae), *Hibiscus rosa sinensis Linn* **(**Malvaceae), *Mallotus philippinesis* **(**Euphorbiaceae), *Ficus religiosa Linn* **(**Moraceae) are widely used for the treatment of diabetes in Bhopal region of Madhya Pradesh.

Charuta and Gupta (2018) reported 53 plants with anti-diabetic activity used by the tribal people and traditional healers from Rajasthan.

3.1.3 North-Eastern states

Mondal *et al.,* (2013) investigated the herbal products used for the treatment of diabetes by North East India. The indigenous tribal people of Northeast regions like Manipur, Mizoram, Meghalaya, Nagaland, Tripura, Arunachal Pradesh, and Assam still mainly rely on plant products for the management of diabetes. A total of 141 plants were collected from North-east India that was frequently used for anti-diabetic purposes. Venkat *et al.,* (2010) reported that the leaves decoction of *Cassia alata L.* (Caesalpiniaceae), *Bryphytum sensativum* (Oxalidaceae), *Cinnamomum tamal T.* (Lauraceae); root decoction of *Ichnocarpus frutescens* (Apocynaceae), and the bark

infusion of *Cassia occidentallis* (Caesalpiniaceae) are used by the inhabitants of Mizoram, Arunachal Pradesh and Manipur for treatment of diabetes.

Inaocha (2015) reported the commonly used traditional herbs that are found in the hill stations of Manipur used for the treatment of diabetes. The plants include *Clerodendrum viscosum, Portulaca oleraceae, Lantana camara Linn, Adiantum capillur, Fagopyrum esculantum, Mangifera indica, Ardisia crenata,* and *Aegle marmelos.* Over the Imphal east district of Manipur, 50 plant species belonging to 26 families were documented which are used for the treatment of various diseases including diabetes, skin diseases, cough, fever, asthma (Leishangthem *et al.,* 2014). Premila (2011) documented 51 anti-diabetic plants that were used by the Meitei community of Manipur. The different plant parts were taken and prepared in their traditional ways and given to diabetic patients. Devi *et al.,* (2011) reported that *Antidesma diandrum Retz.* of the family Euphorbiaceae and *Lysima chiaobovata* of the family Primulaceae are used as traditional medicines by the Meitei community for the treatment of diabetes. Khan and Yadava (2010) also reported that among the Meitei and Meitei-Pangal communities, boiled extracts of the leaves of *Ardisia colorata Roxb* (Myrsinaceae), *Artemisia maritima Linn.* (Asteraceae), and the decoction of the leaf of *Leucaena leucocephala* (Fabaceae), the tuber of *Cyperus esculentus Linn.* (Cyperaceae) are mainly used for the treatment of diabetes. The leaf of *Quercus serrata Murray* (Fagaceae) is used by traditional practitioners for curing diabetes (Khumbongmayum *et al.,* 2006). The leaf, seed, and fruit of *Euryale ferox Salisb.* (Nymphaeaceae) contains phytosterols which help in lowering plasma and LDL cholesterol levels and act as proteinuria inhibitors of diabetic nephropathy (Singh and Singh, 2011).

Pratul (2011) investigated the ethnic anti-diabetic plants used by different tribes of Assam including the Karbi and Dimasa of Karbi Anglong districts, Tiwa and Kachari of Nagaon and Morigaon district, Rabha, Hazang, Deori, Garu, Boro of lower Assam districts, Kachari, Motok, Mishings of Upper Assam districts, Chorei, Reangs and Hmar of Barak valley. 105 plant species of 51 families were used to treat diabetic patients.

Chakravarty and Kalita (2012) also documented the anti-diabetic plants from Namdhonga, Guakusi, Bhungkusi, and Larkusi villages of Nalbari districts in Assam. A total of 35 plant species belonging to 28 families were recorded which are used for the management of diabetes mellitus by the tribal inhabitants of these areas. Bhuyan (2015) investigated the medicinal plants used for the treatment of diabetes by the people of Garo and Rabha tribes in different villages from Bongaigaon districts, Assam. From 10 villages from the Boitamary block, 27 plant species belonging to 21 families were recorded which were extensively used for the management of diabetes.

Chhetri *et al.,* (2005) investigated the anti-diabetic medicinal plants in Sikkim and Darjeeling by interacting with traditional healers, Jhankris, Fedangwas, Bongthings, Mon-Bongthings, Lamas, and Bijuwas, and the elderly village people. They found that 37 species belonging to 28 families were commonly used by the people in the treatment of diabetes. Neelam *et al.,* (2014) documented the anti-hypoglycemic plants from the western part of Sikkim, Himalaya. They recorded 36 plants along with the parts used and their traditional mode of preparation. The anti-diabetic plants were collected by interaction with the tribal communities of Tamang, Sunwar, Limboo, Sherpa, Lepcha, Bhutia, Chhetri, Newar, Gurung, Rai, and Bhaun. Shrestha *et al.*, (2015) documented the medicinal plants used by traditional healers in three communities' viz. Lepcha, Bhutia, Nepali of Sikkim. The roots and the whole plant of *Costus speciosus Sm.* (Costaceae) and the tubers of *Stephania hernandifolia Willd.* (Menispermaceae) are prepared in their traditional system and used for the treatment of diabetes.

Manish *et al.,* (2018) reported that the rootstock slices of *Potentilla fulgens Hk.* (Rosaceae) is used for treating diabetes by the Khasi people of Meghalaya. Syiem and Phibangipan (2011) performed the anti-hyperglycemic and hypoglycemic activity of *Ixeris gracilis* at a dose of 450mg/kg body weight on albino rats and significantly reduce the blood sugar level to normal. The plant is found in the Khasi Hills of Meghalaya which is directly consumed in their traditional treatment of diabetes. Carey *et al.,* (2016) investigated the hypoglycemic and anti-hyperglycemic activity of aqueous-methanol

extract of leaves of *Olax acuminata* and *Bauhinia acuminate* in normoglycemic and alloxan-induced diabetic mice. The anti-hyperglycemic study revealed *O. acuminata* leaves extract at a dose of 250 mg/kg b.w and *B. acuminata* leaves extract at a dose of 500 mg/kg b.w shows a significant reduction of blood glucose. Leaves of *O. acuminate* were found to contain Cu, Cr, Mn, Zn, Fe, V, Mg, K, C, whereas *B. acuminata* leaves were found to contain all of the above elements except Pb and V. These elements are known for regulation of blood glucose. Syiem and Khup (2006) reported that the aqueous-methanol extracts of the roots of *Osbeckia chinensis L.* (Melastomaceae) show a substantial reduction in glucose level in normal and alloxan-induced diabetic mice. The effects were observed after administration of a maximum of 4 hours in normal rats and 6 hours in diabetic mice, which shows both anti-hyperglycemic and hypoglycemic activities.

Ramananda *et al.,* (2015) reported 39 plant species of 37 genera belonging to 28 families used among the different ethnic communities in Unakoti district, Tripura. The plants are given by their traditional system for the treatment of diabetes. Manoj *et al.,* (2017) reported that the rhizome of the herbaceous perennial herb, *Amomum aromaticum Roxb.* is useful in the treatment of diabetes in the Khowai district of Tripura (Naidu and Pullaiah, 2003).

Nima *et al.,* (2011) reported that the seeds and fruits of *Ficus glomerata Roxb.* (Moraceae) and the leaf *Momordica charantia* (Cucurbitaceae) are used by the Monpa ethnic groups of Arunachal Pradesh in treating diabetes. Among the medicinal plants used by the Galo community of West Siang district, Arunachal Pradesh the bark juice of *Syzygium cumini* (Myrtaceae) and the leaves of *Catharanthus roseus* (Apocynaceae) are used in the control of diabetes (Gode *et al.,* 2018). Murtem and Pradeep (2016) also reported that the rhizome of *Homalomena aromitica Linn.* (Araceae) is used in the treatment of diabetes in the upper Subansiri district of Arunachal Pradesh.

Bipul *et al.,* (2017) reported a total of 30 anti-diabetic plants belonging to 25 families which were used by the Apatani tribe of Ziro. Cucurbitaceae was the dominant

family with 5 anti-diabetic plants along followed by Lamiaceae and Amaryllidaceae with 2 species each.

Alino and Kimiyekato (2018) reported 50 ethnomedicinal plants used by Sumi Nagas of Nagaland as medicines; out of which *Bauhinia variegate Linn, Dioscorea alata Linn, Passiflora edulis Sims, Allium sativum Linn* are commonly used by the people of Zunheboto districts – Philimi, Ashkhomi, Khrimtomi and Rotomi for treatment of diabetes mellitus. The ethnic people of Chungtia village, Nagaland uses the infusion of the roots of *Cissampelos pareira Linn.* (Menispermaceae) and the decoction of the leaves of *Gynura crepidioides Benth.* (Asteraceae) for control of diabetes (Kichu *et al.,* 2015). Temsutola *et al.,* (2019) investigated the medicinal plants used by the Chang Naga tribe of Teusang and reported that the tuber of *Discentra scandens Walp.* (Fumariaceae), the leaves and bark of *Juglans regai L.* (Juglandaceae), the fruits of *Morus alba* (Moraceae), and the fruits and seeds of *Momordica balsamina L.* (Cucurbitaceae) are commonly used for the treatment of diabetes.

3.1.4 Mizoram

Laha *et al.,* (2016) identified a total of 53 plant species distributed in 49 genera and belonging to 32 families used by the tribal people of Mizoram for treatment of diabetes.

Lalmuanpuii *et al.,* (2013) reported the medicinal plants used by the people in Lunglei districts among which decoction of the leaves of *Thunbergia grandiflora Roxb* (Acanthaceae) and *Eucalyptus sp.* (Myrtaceae) were mainly used for the treatment of diabetes mellitus.

Bidens pillosa is commonly used by the tribal inhabitants of Mizoram for the treatment of diabetes. It belongs to the family Compositae and the plant has been known to possess other important pharmacological properties including anti-inflammatory, anticancer, antitumor, anti-malarial, and anti-influenza. (Geissberger and Sequin 1991; Jager *et al.,* 1996; Andrade- Neto *et al.,* 2004; Steenkamp and Gouws, 2006).

Sawmliana (2003) reported that *Carica papaya* is widely used in Mizoram for treatment against dog bites, jaundice, diabetes, and food poisoning.

Lalfakzuala *et al.*, (2007) documented the medicinal plants used in western Mizoram, out of which infusion of the bark of *Ficus Benghalensis*, the decoction of leaves of *Macaranga denticulate* were widely used by the people for the management of diabetes in the state.

According to Venkat *et al.*, (2010), the tribal people of Mizoram use the decoction of the leaf of *Lepionurus sylvestris* (Opiliaceae) and the decoction of the bark of *Dillenia pentagyna* (Dilleniaceae) are given to diabetic patients for 6 to 8 weeks to lower the blood glucose level.

Lalramnghinglova (2016) documented the medicinal plants of the Indo-Burma hotspots region of Mizoram, the decoction of the stem and bark of *Syzygium cumini L.* (Myrtaceae) and the decoction of the whole plant of *Phyllanthus fraternus* (Euphorbiaceae) are taken internally for treatment of diabetes.

Hemanta *et al.*, (2001) investigated the medicinal plants of Mizoram and reported that for diabetes, the decoction of leaf and stem of *Plantago major L.* (Plantaginaceae), *Orthosiphon aristatus* (Lamiaceae), *Coccinia indica* (Cucurbitaceae), *Clerodendrum colebrookianum* (Verbenaceae), *Abrus precatorius L.* (Papilionaceae) are used. Decoctions of the root bark of *Helicia robusta* (Proteaceae) and the rhizome of *Colocasia esculenta* Linn. (Araceae) along with the infusion of powdered bark and fruit of *Eugenia jambolana* Lam. (Myrtaceae) are also commonly used by the tribal people.

Lalrinzuali *et al.*, (2015) reported that the decoction of the leaves of *Centella asiatica* (Apiaceae), *Eryngium foetidium* (Apiaceae), and the bark, leaves of *Careya arborea* (Lecythidaceae) are taken orally in the treatment of diabetes.

3.2 ANTI-DIABETIC COMPOUNDS FROM IMPORTANT ETHNO-MEDICINAL PLANTS

3.2.1 Worldwide

Bressler *et al.,* (1969) isolated a derivative of aminopropylpropionic acid, hypoglycins from the fruits of *Blighia sapida,* which belongs to the family Sapindaceae. They observed that hypoglycins encourage the use of glucose and impede gluconeogenesis in both diabetic animals and humans (Kean and Pogson, 1979). The people of Central America and Africa frequently used the unripe fruit of *Blighia sapida* for the treatment of diabetes.

Abdallah *et al.,* (2011) examined the bark of *Carya illinoinensis (*Wangenh*)* in streptozotocin-induced diabetic rats at 10 mg/kg body weight which shows both anti-hyperglycemic and antioxidative properties and he isolated a Flavonoid/ Phenolic compound called Azaleatin from *Carya illinoinensis.*

Yoshikawa *et al.,* (1995) isolated alpha-glucosidase inhibitor, kotalanol from the aqueous extract of *Salacia reticulate*. The plant belongs to the family Celastraceae. The aqueous extract was found to lower plasma glucose levels significantly, which was attributed to the presence of kotalanol in the plant. The results also showed a higher potent inhibitory effect against sucrose than that of acarbose and salacinol.

Saito *et al.,* (1994) reported that the rhizome of *Anemarrhena asphodeloides* contains a xanthone compound, mangiferin. The effect of mangiferin was analyzed on blood lipids and performed on KK-ay mice, which are type-2 diabetic animal models. After two weeks of oral administration of mangiferin on KK-ay mice with regular exercise, there was a reduction of cholesterol and triglyceride when compared to the control group. The study proved accurate in the anti-diabetic activity of mangiferin in animal models (Toshihiro *et al.,* 2001).

Hii and Howell (1985) described important flavonoids like quercetin, naringenin, and chrysin considerably enhanced the release of insulin from the islets of Langerhans in

rats in the company of 20 mmol glucose/1. Quercetin was found to utilize its stimulatory effect on the release of insulin moderately by altering the Ca2+ metabolism.

Manickam *et al.,* (1997) investigated the anti-hyperglycemic activity of 3 flavonoids like marsupsin, pterosupin, and pterostilbene, which were isolated from the heartwood of *Pterocarpus marsupium Roxb.* in diabetic rats. The results were observed that both marsupsin and pterostilbene were effective and the levels of hyperglycemia in diabetic rats were comparatively lower when compared to standard drug metformin.

Pinent *et al.,* (2004) reported that procyanidins, a grape seed derivative possesses anti-diabetic by its insulin-mimetic activity in streptozotocin-induced diabetic rats. Other compounds of flavonoids like proanthocyanidins were found to enhance the oxidative state of diabetes and stimulate glucose uptake in insulin cells via *in vitro* studies.

Before the discovery of insulin, the leaves of *Vaccinium myrtillus L.* were frequently used for the control of diabetes mellitus. Allen and Wilder (1927) extracted an active glycoside; neomyrtillin from the leaves of *Vaccinium myrtillus L.* Neomyrtyllin was proved to be effective in the reduction of glycosuria and postprandial hyperglycemia in adult-onset diabetes but not much in juvenile-onset diabetes.

Ng *et al.,* (1986) inspected the action of insulin-like molecules of *Momordica charantia* and establish that the seeds possess the insulin-like activity and have hypoglycemic properties due to the presence of a steroidal saponin called charantin (a mixture of β-sitosterol-D-glucoside and stigmadine glucoside). Charantin motivates the release of insulin and hinders glucose formation in the bloodstream, which helps in the control of diabetes mellitus, especially in non-insulin-dependent diabetic patients.

Yoshikawa *et al.,* (1997) investigated the saponin constituents of *Kocia scoparia L.* and the two compounds of saponin- momordin Ic and 2'-O-beta-Dglucopyranoside isolated from the methanol extract of *Kocia scoparia L.* reveal anti-diabetic activity as they inhibit glucose and absorption of ethanol in diabetic rats.

Nadkarni (1976) examined the seeds of *Syzygium cumini* and confirmed that it possesses hypoglycemic activities due to a glycoside compound known as Jambolin. Jambolin has anti-diabetic actions by reducing thirst, prevents the conversion of starch into sugar, and moderating the sugar contents in urine (Sagrawat *et al.,* 2006; Yarnell *et al.,* 2009).

According to Crowley and Bennett (1928), *Catharanthus roseus* is a renowned anti-diabetic plant used widely across the globe, especially in Nigeria, West Indies, and India for treating diabetes, the plant contains important dimeric alkaloids like vincristine and vinblastine which act against diabetes mellitus. Chattopadhyay (1999) also reported that compounds like vindolinine, catharanthine, vindoline and leurosine isolated from *Catharanthus roseus* reduces blood sugar levels in alloxan-induced diabetic rabbits.

Vegetables, oilseeds, cereals, and legumes contain condensed tannins which are reported to have significant antioxidant and anti-diabetic properties. Kunyanga *et al.,* (2011) performed the antioxidant and anti-diabetic experiments on food ingredients from Kenya and extracted condensed tannins from sunflower seed, grains of α-amaranth, beans, amaranth leaves, and finger millet. The anti-diabetic activity is due to the inhibition of active α-amylase and α-glucosidase.

Jayasree *et al.,* (2011) reported the hypoglycemic activity of the stem extract of *Benincasa hispida* in normal Wistar rats and compare it with the control group and glibenclamide as the standard drug. They found that the extract of the plant at a high dose of 400mg/kg body weight and a low dose of 200 mg/kg was significant in lowering blood glucose levels. The anti-diabetic activity of the stem of *Benincasa hispida* is due to the presence of alkaloid, carbohydrate, and saponin which have hypoglycemic properties.

An anti-hyperglycemic study of *Polygonatum kingianum* reveals that saponins from the plant can efficiently improve hyperglycemia in diabetic rats by enhancing the expression of glucose transporter type-4 (GLUT4) which decreases the regulation in the expression of G6P in insulin pathways (Lu, 2015).

Bisht *et al.,* (2013) reported that the polysaccharide from *Acacia tortilis* possesses anti-diabetic activity as it increases serum glutamic pyruvate transaminase for acarbose, reduced high-density lipoprotein (HDL), and prevent constipation.

According to Iwasa *et al.,* (2001) alkaloids constitute the main classes of secondary metabolites that are pharmacologically active. Tiong *et al.,* (2015) investigated the hypoglycemic property of *Catharanthus roseus (L.)* and reveals that an indole alkaloid Vindogenatianine increases the uptake of glucose and PTP-1B inhibition, thus implying its hypoglycemic potential, especially against type-2 diabetes.

A flavonoid compound known as hesperidin is responsible for the anti-diabetic activity of *Citrus aurantium* as it releases pro-inflammatory cytokines by decreasing the generation of free radicals (Visnagri *et al.,* 2014) and (Shi *et al.,* 2012). According to Akiyama *et al.,* (2010) and Yo *et al.,* (2014), the oral administration of hesperidin in diabetic rats causes regulation of lipids and adiponectin which alters the metabolic glucose enzymes which cause an increase in the activities of lactate dehydrogenase (LDH) and decrease in thiobarbituric acid reactive substances (TBARS).

Silva *et al.,* (2005) reported transdehydrocrotonin (t-DCTN), a diterpene compound from *Croton cajucara* possesses hypoglycemic and hypolipidemic activities in alloxan-induced diabetic rats. Dehydroabietic acid was demonstrated to suppress and enhance the anti-inflammatory agents like adiponectin and the pro-inflammatory agents including (TNFα) on the same strain (Kang *et al.,* 2009).

Habicht *et al.,* (2011) from his quantification of compounds in bitter gourd reported the anti-diabetic activity of saponins from the fruits of *Momordica charantia* by inhibition of disaccharidase. Zheng *et al.,* (2012) also verified that saponins from *Enteda phaseoloides* possess anti-diabetic properties as it decreases lipid levels, improves hyperglycemia and raises the serum insulin levels.

El-Baz *et al.,* (2014) revealed that flavonoid glycoside which was isolated from *Jatropha curcus* exhibits anti-diabetic properties in streptozotocin-induced diabetic rats.

The bioactive compound decreases the activities of lactate dehydrogenase and serum α-amylase and the extract of the plant was found to moderates oxidative stress like malondialdehyde.

Patisaul and Jefferson (2010) reported that an isoflavone flavonoid, genistein which is present in leguminous plants like *Sophora subprostrala* and *Genista tinctoria* has significant anti-diabetic activity. After administration with genistein in streptozotocin-diabetic mice, there were substantial improvements in glucose tolerance and enhancement of insulin levels without affecting the body weight and the renal TBARS were also reduced (Elmarakby, 2011).

Isorhamnetin It is an anti-diabetic principle isolated from *Hippophae rhamnoides, Oenanthe javanica,* and *Ginkgo biloba*. Its administration reduces oxidative stress, inhibits sorbitol accumulation, and interferes with lipid metabolism (Yokozawa *et al.,* 2002; Lee *et al.,* 2005; Rodriguez, 2015).

Wansi *et al.,* (2007) experimented that Ellagic acid, a compound of Tannin isolated from *Terminalia superba* (Combretaceae) displays anti-diabetic activity as the stem bark at a dose of 800 μM inhibit α-glucosidase at IC50: 194.1 ± 0.2 μM.

Somova *et al.,* (2003a) described Oleanolic acid, a compound of terpenoid extracted from the leaf of *Olea europaea* (Oleaceae) at 60mg/kg body weight on albino rats demonstrate anti-hyperglycemic, hypoglycemic, antioxidant, and anti-hypersensitive activities.

Rajasekaran *et al.,* (2004) suggested that 5 phytosterols – 24-ethyl-lophenol, 24-methylene-cycloartenol, lophenol, cycloartenol, and 24-methyl-lophenl from *Aloe vera* exhibit anti-diabetic activities in streptozotocin-induced diabetic rats. The 5 phytosterols decrease the blood Hb1A1c levels by 15 to 18 % and due to the quick excretion of glucose in the urine, the rats did not lose weight. Thus phytosterols can synthesize the release and control of insulin levels and serves as glycemic control agents in type-2 diabetes mellitus.

Sheela *et al.,* (1995) revealed that the anti-diabetic principles of *Alium cepa L*. and *Alium sativum L.* were S-methylcysteinesulfoxide and S-allycysteinesufloxide. The experiment was performed with alloxan-induced diabetic rats and the bioactive principles were found to cause stimulation of insulin, while also competing with insulin for inactivating sites in the liver for insulin. Gluconeogenesis was inhibited and they obstruct lipid peroxidation as they possessed antioxidant and secretagogue activities.

Krenisky (1999) isolated a monoterpene compound- bakuchiol from the extract of *Ortholobium pubescent* and found it to possess anti-diabetic activity as oral administration of bakuchiol reduced glycemia in diabetic mice.

Aigaonkar (1979) investigated *Ficus benghalensis*, from an aqueous extract of the stem bark he isolated a flavonoid glycoside compound- bengleonside. The compound produced a slight hypoglycemic result in healthy and alloxan-induced diabetic mice (Brahmachari, 2012).

Chattopadhyay (1993) investigated the hypoglycemic activity of *Polygala senega* in normal and streptozotocin-induced diabetic rats. There was a reduction of blood glucose in normal rats but no significant changes in glucose levels were observed in the STZ-induced diabetic rats even after administration of the plant extract. This denotes that *Polygala senega* possesses hypoglycemic properties without changing the concentration of insulin levels. *Polygala senega* contains two anti-diabetic compounds- saponins and triterpenoid glycoside called Senegin-2 which is the chief agency responsible for its hypoglycemic activity.

Hsu *et al.,* (1997) investigated the anti-diabetic activities of paeoniflorin and 8-debenzoylpaeoniflorin, isolated from the roots of *Paeonia lactiflora,* and observed that they are found to be efficient in lowering blood glucose levels in streptozotocin-induced diabetic rats. The hypoglycemic activity was also observed in normal glycemic rats.

Abhullahi and Olatunji (2010) investigated the anti-diabetic potential of *Anacardium occidentale* L. The stem bark comprises important polyphenols which show

anti-hyperglycemic activity against type-1 and type-2 diabetes and helps in stimulating the uptake of muscle glucose.

Shittu *et al.,* (2010) isolated an Alkaloid compound known as Akuammicine from *Picralima nitida (*Stapf*)* which belongs to the family Apocynaceae. The seeds of the plant were used for the experiment and it functions to stimulate the glucose uptake in completely differentiated 3T3-L1 adipocytes.

Iwu *et al.,* (1990) investigated *Garcinia kola* which belongs to the family Clusiaceae. The seeds of the plant are given at 100 mg/kg body weight to rats which display anti-hyperglycemic activities and inhibit the rat lens aldose reductase activity at IC50: 5.4 μM. The chief anti-diabetic compound isolated from this study is Kolaviron.

Deutschländera *et al.,* (2011) isolated a compound Epicatechin from *Euclea undulata*. The plant belongs to the family Ebenaceae and the root bark at a dose of 200 μg/ml shows inhibition of α-glucosidase (IC50: 5.86 ± 4.28 μg/ml) and at a dose of 10 μg/ml, it enhances the glucose uptake of C2C12 myocytes.

Rengasamy *et al.,* (2013) reported that *Ecklonia maxima* possess anti-diabetic activity as the brown alga shows inhibition of α-glucosidase at IC50: 33.693 ± 0.61 Mm and DPPH at EC50: 0.012 ± 0.001 Mm. The compound responsible for this anti-hyperglycemic activity is known as Dibenzo [1, 4] dioxine-2,4,7,9-tetraol.

Pen and Fang (2003) reported the hypoglycemic activity of *Swertia punicea* in alloxan-induced diabetic mice. The two main constituents present for the hypoglycemic action present in *Swertia Punicea* are Methylswertianin and Bellidifolin. The ethanol and ethyl acetate extracts of *Swertia Punicea* displays hypoglycemic activities in streptozotocin-induced diabetic mice and are used to improve the resistance level of insulin (Wen and Chen, 2007).

3.2.2 Mizoram

Lalhlenmawia *et al.,* (2007) investigated the anti-hyperglycemic activity of *Mallotus roxburghianus* in streptozotocin-induced diabetic rats. The rats were treated with Glibenclamide for 12 days with methanol extract of *Mallotus roxburghianus* at a dose of 200 mg/kg and 400 mg/kg. The results showed a significant reduction in fasting glucose, triglycerides, and cholesterols and enhancement in body weights, the glycogen content of liver and skeletal muscles was observed. It was accomplished that *Mallotus roxburghianus* proved to be effective in the treatment of type-2 diabetes because of its capability to decrease insulin resistance.

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CHAPTER 4

METHODOLOGY

4.1 ETHNOBOTANICAL EXPLORATIONS FOR ANTI-DIABETIC MEDICINAL PLANTS OF MIZORAM FOR PHYTOCHEMICAL ANALYSIS

Ethnobotanical surveys were done to identify anti-diabetic plants used by the tribal inhabitants for the management of diabetes along with the habitat and distribution of the plants, which was carried out through interactions with local practitioners and villagers. Medicinal plant species that were frequently used for the treatment of diabetes were documented and the plants with the highest efficacies will proceed for phytochemical analysis of important secondary metabolites. Secondary metabolites make up the basis for the anti-diabetic potential of plants and each of the plants was screened with different phytochemical methods. From the phytochemical report, one plant will be considered as a candidate plant which will be proceeded for numerous pharmacological properties relating to diabetes mellitus.

4.2 COLLECTION AND IDENTIFICATION OF PLANTS

The potential anti-diabetic medicinal plants were collected from their habitats, identified, and stored. Authentication of the ethnomedicinal plants was done before carrying out the phytochemical analysis. Identification was carried out with assistance from taxonomists, Mizoram, and other important floras like

1. Flora of Lushai Hills (Fischer 1938)
2. Flora of Tripura state (Deb 1981 and 1983)
3. Botanical tour in the Lushai Hills (Gage 1889)
4. The Book of Mizoram plants (Sawmliana 2003)
5. Indian trees (Brandis 1906)
6. Forest Flora of British Burma (Kurz 1877)
7. Forest Flora of Meghalaya (Haridasan & Rao 1985 and 1987)
8. Flora of Mizoram Vol 1 (Singh *et al.,* 2002)

Another important method for confirmation and identification of unidentified plants is to take the plant specimen/ herbarium to the Botanical Survey of India (Kolkata) and Botanical Survey of India (Shillong). The department of Environmental Science, Mizoram University, was also consulted for the identification process.

4.3 PHYTOCHEMICAL ANALYSIS FOR ANTI-DIABETIC CONSTITUENTS

The phytochemical investigation of plants includes different techniques like extraction of plant materials, *in vitro* analysis of antioxidant activities, *in vivo* studies using streptozotocin-induced diabetic rats, and isolation and characterization of pure compounds.

For the investigations, fresh plants are collected from their natural habitats and dried in controlled conditions or in shade to avoid contaminations or alterations in their chemical structures. The samples of the plant materials, the parts of which were commonly used for the treatment of diabetes were selected and dried properly to make it free from diseases, as unexpected changes and unpredicted products from the plants could be obtained. Sometimes contaminations by fungi and mosses may occur while obtaining roots or tubers from the plants, thus making it problematic for the collection of plant samples free of infections for the analysis. The plant samples are washed thoroughly with distilled water and shade dried in a ventilated room. Then they are pulverized into fine concentrated form by a grinder and passed through a 60 mesh sieve to get a fine powder.

4.3.1 Extraction of plant materials

The extraction of the plant materials was done by hot air extraction in the Soxhlet apparatus, using petroleum ether/ benzene (removal of fats), chloroform (removal of chlorophyll), and finally ethanol/ methanol to get a pure extract. This successive extraction is the ideal method to obtain an extract free of impurities. The extracts of each sample are evaporated in a rotary evaporator to obtain crude extracts and stored in a refrigerator at 4 ^{o}C. Then, the extracts are subjected to preliminary

phytochemical screening to detect the presence of important anti-diabetic phytoconstituents.

4.3.2 Phytochemical screening for potential anti-diabetic components of the plant extracts

Preliminary phytochemical testing for important anti-diabetic constituents is carried out to detect the presence/ absence of the following components:

1. Alkaloids
2. Flavonoids
3. Glycosides
4. Terpenoids
5. Tannins
6. Saponins
7. Phytosterols
8. Reducing sugar
9. Phenols

4.3.3 Reagents used for phytochemical screening

1. ***Mayer's reagent*****:** For the preparation, 1.36 gm of mercuric iodide is added to 60 ml of distilled water which is mixed with a solution containing 5 gm of potassium iodide in 20 ml of distilled water.

2. ***Libermann-Burchard reagent*****:** For this reagent, 5 gm of acetic anhydride is cautiously mixed with 5 ml of concentrated sulfuric acid and allowed to cool. Then 50 ml of absolute ethanol is added to the mixture and allowed to cool down.

3. ***Dragendorff's reagent*****:** For this reagent, 1.7 gm of bismuth nitrate and approximately 20 gm of tartaric acid are dissolved in 80 ml of distilled water. Then 16 gm of potassium iodide dissolved in 40 ml of distilled water is added to the solution.

4. *Fehling's solution A*: 35 gm of copper sulfate is dissolved in a solution, containing a mixture of 0.5 ml sulfuric acid and water for this reagent.

5. *Fehling's solution B*: 175 gm of sodium-potassium tartrate and 77 gm of NaOH are added to distilled water to produce 500 ml of solution. Then, equivalent volumes of the solutions are added together.

6. *Benedict's reagent*: 1.73 gm of cupric sulfate with 1.73 gm of citrate solution and 10 gm of anhydrous sodium carbonate are dissolved in distilled water and the volume is completed up to 100 ml with distilled water.

7. *Molish's reagent*: For this reagent, 25 ml of ethanol is mixed with 2.5 gm of pure α-naphthol and is allowed to settle down.

4.3.4 Phytochemical screening for anti-diabetic components:

The following preliminary tests were performed for identifying different chemical groups as reported by Trease and Evans, 1983.

(A). Alkaloids:

(1). Mayer's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added.

(2). Dragendorff's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendorff's reagent was added.

(B). Glycosides:

(1). A small quantity of the ethanol extract of the plant was taken in 1 ml of water. Then a few drops of aqueous sodium hydroxide were added.

(2). A small quantity of the ethanol extract of the plant material was taken in water and alcohol and boiled with Fehling's solution.

(3). A small quantity of the ethanol extract of the plant material was dissolved in water and alcohol and boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution, and boiled with Fehling's solution.

(C). Reducing sugar:

(1). Benedict's test 0.5 ml of an aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict's solution was added to the test tube, boiled for 5 minutes, and allowed to cool spontaneously.

(2). Fehling's test (Standard test) 2 ml of an aqueous extract of the plant material was added to 1 ml of a mixture of equal volumes of Fehling's solutions A & B and was boiled for a few minutes.

(D). Tannins:

0.5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% ferric chloride solution was added.

(E). Flavonoids:

A few drops of concentrated hydrochloric acid were added to a small amount of the ethanol extract of the plant material. Immediate development of red color indicates the presence of flavonoids.

(F). Saponins:

5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. The formation of foam indicates the presence of saponins.

(G). Terpenoids:

2 ml of extract was added to 2 ml of acetic anhydride and the concentration of H2SO4. The formation of blue, green rings indicates the presence of terpenoids as done by Watson and Dallwitz, 1992.

(H). Phenols:

Extracts were treated with few drops of ferric chloride solution; the Formation of bluish-black color indicates the presence of phenols.

(I). Phytosterols

(1). Salkowski test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow color indicates the presence of triterpenes.

(2). Libermann Burchard test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled, and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tubes. The formation of a brown ring at the junction indicates the presence of phytosterols.

4.4 DETERMINATION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF SELECTED PLANT USING DPPH AND ABTS METHODS.

4.4.1 Total phenolic content

The phenolic content of one selected plant was determined by Folin-Ciocalteu's phenol reagent, which was reported by (Kim *et al.*, 2009; Singleton and Rossi, 1965). Firstly, 200 µl of appropriately diluted sample/ gallic acid standard was added to 2.6 ml of distilled water. Then, 200 µl of Folin-Ciocalteu's phenol reagent was added and mixed properly. After 6 minutes, 2 ml of 7% (w/v) Na_2CO_3 solution was further added and mixed. Incubation was done at room temperature for 90 minutes and the absorbance was considered at 750 nm versus a prepared blank. The blank consisted of 200 µl 50% (v/v) ethanol as an alternative to the sample. Gallic acid in 50% (v/v) ethanol solution in standard concentrations of 0.1, 0.3, 0.5, and 0.8 mg ml-1 was used as a calibration curve; this was drawn each day of study. The total phenolic content was expressed as gallic acid equivalent (GAE)/ g of the dry weight of plant extract (DW). The results obtained were performed in triplicate.

4.4.2 Total flavonoids content

The determination of the flavonoid content of one selected plant was measured according to the method specified by (Zhishen *et al.*, 1999). The plant extract was added with 0.3 ml of 5% sodium nitrite and mixed properly. Then it is incubated for 5 minutes,

after which 0.3 ml of 10% aluminum chloride solution was added. It is kept for 6 minutes and then 2 ml of 1M sodium hydroxide was added and which make up the volume to 10 ml with water. The absorbance was considered at 510 nm with a UV-visible spectrophotometer. The content of flavonoids was measured from the quercetin (0-0.3 mg) standard curve furthermore expressed as mg catechin equivalents/g of the dry weight of the plant extract. The samples were made in triplicate.

4.4.3 Antioxidant Activity

1. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay:

The antioxidant action of the ethanolic extract of one selected plant polyphenolic extract/ascorbic acid to scavenge DPPH radical as described by Leong and Shui in 2002 with some modifications. Briefly, a 1 ml ethanol solution of 0.1 Mm DPPH was added to the different concentrations of the ethanol extracts (5-100 µg/ml). The prepared mixture was vortexed and incubated for 30 minutes at room temperature and then the reading was recorded using a spectrophotometer at 523 nm. 80% (v/v) ethanol was used as the blank. Ascorbic acid (Vitamin C) was used for comparison with the plant extracts. The measurements were performed in triplicate. DPPH scavenging activity was performed using the equation: Scavenging (%) = (A control – A sample)/A control x 100. Where A = absorbance of the test sample and Control = absorbance of the control.

2. 2, 2`-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS assay):

ABTS scavenging activity of one selected plant was measured by a reported method given by (Re R *et al.,* 1999) with minor modification. Approximately, 37.5 mg of potassium persulfate was dissolved in 1 ml of distilled water. On preparation of the ABTS solution, a 44 µl of the solution was added to 9.7 mg of ABTS dissolved in 2.5 ml of distilled water. The ABTS solution was then kept at room temperature for around 15 hours. Then, 88 ml of 50% ethanol is mixed with 1 ml of ABTS solution to prepare the working solution. An entire amount of 50 µl of ethanol extract of special concentration ranging from 5-100 µg/ml was mixed with 100 µl of ABTS working solution. It was

allowed to stand for 4 minutes and the absorbance was examined at 734 nm. The readings were expressed as ascorbic acid equivalent, which was used as a standard.

4.5 EVALUATION OF *IN VIVO* ANTI-HYPERGLYCEMIC ACTIVITY OF SELECTED PLANT WITH STREPTOZOTOCIN INDUCED DIABETIC RATS

4.5.1 Introduction

Over the years, numerous publications have been reviewed; focusing on herbal products and ethnomedicinal plants with anti-diabetic action (Hays *et al.,* 2008). The current investigation was aimed to carry out the *in vivo* anti-diabetic activities of a specific plant with streptozotocin-induced diabetic rats and GC-MS analysis to categorize the compounds which are effective in lowering blood glucose level, which may be useful in the production of anti-diabetic drugs and for its therapeutic values. The effects of *Scurrula parasitica L.* on body weight, blood glucose level, the lipid profile of rats were considered for the *in vivo* anti-diabetic research. Mass spectrometry, together with chromatographic separations like Gas chromatography (GC-MS) is generally used for direct investigation of components present in ethnomedicinal plants and traditional drugs. Important constituents like fatty acids, nonpolar components, volatile essential oil, and lipids in plants are analyzed by using the GC-MS technique (Jie and Choi, 1991; Betz *et al.,* 1997).

4.5.2 Preparation of plant extract

The parts of the selected plant were shade dried and prepared into coarse powder and stored in a beaker. Approximately, 200 gm of the plant powder was weighed and subjected to continuous hot extraction using Soxhlet apparatus. The extraction was carried out successively using petroleum ether, chloroform, and ethanol. Subsequently, the extracts were evaporated under pressure using a rotary evaporator until all the solvents have evaporated to give pure crude extracts. The ethanol extract will be used to experiment. The percentage yield of ethanol extract was 3.8% w/w per one extraction.

4.5.3 Animals

Male Albino Wistar rats of body weight 190 to 200 gm were selected for this research. The animals were kept in an animal house at the Department of Pharmacy (RIPANS), (IAEC approval. No. IAEC/RIPANS/18, dated 14th November 2017) with a12 hours dark: 12 hours light cycle. The animal was fed a pellet diet (Pranav Agro-industries, Vadodara, Gujarat), water and *ad libitum* were also provided (Upwar *et al.*, 2010).

4.5.4 Induction of streptozotocin on experimental diabetes

STZ-induced diabetes has been illustrated as a constructive investigational model to study the action of hypoglycemic activity (Junod *et al.,* 1969). The induction was done using the technique given by Upwar *et al.*, (2010) with a slight alteration. Following overnight fasting (deprived of food for 16 hours had been allowed free access to water). The induction of diabetes in rats was completed by intraperitoneal injection of STZ dissolved in 0.1 M sodium citrate buffer pH 4.5 at a dose of 40 mg/kg body weight. Then, after 72 hours, rats with moderate diabetes (fasting blood glucose >250 mg/dl) were employed for the study. Testing by urine test strips (One-touch select, Bayer diagnostics Ltd, India) were considered as diabetic.

4.5.5 Experimental design

For the study, the rats were divided into 5 groups with 6 animals in each group.

Group I: Normal control rats.

Group II: Diabetic control rats

Group III: Diabetic rats given Metformin (250 mg/kg b.w./Rat/day) for 21 days.

Group IV: Diabetic rats given MLS (200 mg/kg b.w./Rat/day) for 21 days.

Group V: Diabetic rats given MLS (100 mg/kg b.w./Rat/day) for 21 days.

Blood samples were collected at 0 hr (before the administration of the extract) on the 5th, 10th, 15th, and 20th day, for estimation of blood glucose level with the help of Glucometer (One Touch Select), and readings were tabulated. The weight and serum

biochemical parameters were monitored on the first and final days of the treatment. After the 21st day, the rats were sacrificed under mild anesthesia. The biochemical parameters were monitored using Auto-analyzer (EM 200).

4.5.6 Blood collection and serum separation

The tip of the tail was cut and few droplets of blood were collected for estimation of glucose level (Maurya *et al.,* 2012) and for serum biochemical parameters estimation, the blood samples were collected from 8 hours fasted animals from the retro-orbital plexus in capillary tubes (Micro Hemocrit capillary, Mucaps) on the 1st day via retro-orbital and serum was separated within 30 minutes after collection using a centrifuge at 2000 rpm for 2 minutes Upwar *et al.,* (2010) and on the 21st day to measure the lipid profiles. A one-touch select glucometer was used for measuring blood glucose levels.

4.5.7 Statistical analysis

Results were expressed as Means±SD and the variations between the groups were tested by two-way analysis of variance (ANOVA) followed by the Tukey multiple mean comparison test using the software "GraphPad Prism v6". The $p<0.05$ were considered statistically significant.

4.5.8 GC-MS Analysis

Gas Chromatography-Mass Spectrometry using JEOL GCmate™ II GC/MS Double-Focusing Mass Spectrometer. An HP-5 MS capillary column (28 m × 0.25 mm × 0.25 μm) with helium as carrier gas (1.0 ml/min) was used for the gas chromatographic separation. The injection mode was split (split ratio: 20:1), injection volume was 2 μL, and the temperature of the vaporization chamber ranged from 50-250 °C. The column was eluted with ethanol at a flow rate of 0.3 ml/min with an increase of 10 °C every minute. The mass detector was EI-Detection (70 eV). The mass spectra of the compounds compared with the NIST08 GC-MS database library with 346,757 Kovats retention index values for 70,835 compounds (38,648 in the EI library), covering

both polar data. GC-MS was carried out to detect the important volatile compounds present in the selected plant which might be the reason for its anti-diabetic property.

4.6 ISOLATION OF ACTIVE COMPOUND WITH ANTI-DIABETIC POTENTIAL FROM SELECTED PLANT

4.6.1 Thin layer chromatography

4.6.1.2 Introduction:

Thin layer chromatography (TLC) is one of the universal separation techniques useful for the analysis of organic chemical reactions and assessment of the purity of organic compounds in phytochemistry. It is a method of separation in which differential migration of solvents occurs along with a fine powder spread on a glass plate. Thin layer chromatography is based on the principle of adsorption affinity and takes advantage of different empathy of the analyte with the stationary and the mobile phases for separating complex organic compounds. Several compounds are spotted on a thin layer of solid adsorbent, then the compounds along with the mobile phase travel to distances varying on the particle coefficient of each molecule.

The solvent system or mixture for different plants may differ in chromatography, to determine the mixture of solvents; solvents of different polarities are tested in different trials and are carefully observed. The result of the increase in the polarity of the solvent in chromatography was that all components of the mixture move quicker and thus lowering the polarity the mixture will move slower, depending on the plant extract. The mixture in which the best separation is observed is considered the ideal solvent system. The elution procedure of TLC is carried over to the outlines of column chromatography.

4.6.1.3 TLC plate preparations:

Readymade aluminum-coated silica gel plates (Merck and Co. Ltd.) were used for the research. The plate is cut into smaller pieces into a 4x10cm dimension. The plates are kept in an oven for 120 °C for half an hour before use (Stahl, 1969).

4.6.1.4 Development of a Solvent system for chromatogram

With the help of capillary tubes, 2.5 µl of plant extracts are spotted carefully on the silica gel plate. Spotting was done 1 cm above the bottom of the plate and kept in a development chamber. The lid of the development chamber was closed to avoid tailing or edging of the spots on the chromatogram. The solvent front was then allowed to travel upwards up to a distance of $3/4^{th}$ from the baseline and then the lid of the chamber is opened and the plate is removed and allowed to dry in the air. The plate is then kept in an iodine chamber for a few minutes. The solvent system is based on the order of elusive power because the rate of migration of compounds on a given adsorbent depends on the solvents being used (Skoog, 1988; Fried *et al.*, 1994). Through numerous trials and errors, an ideal solvent system is selected for thin layer chromatography.

The following solvent systems were used in the preliminary test for the determination of the mobile phase of the plant extract in various ratios:

1. Methanol
2. Chloroform
3. Methanol: Chloroform
4. Ethyl acetate
5. Ethyl acetate: Chloroform
6. Ethyl acetate: Chloroform: Methanol

4.6.2 Column chromatography

4.6.2.1 Introduction:

Chromatographic techniques were used for the isolation of compounds from the fractions. The column chromatographic technique is most commonly used for the separation of compounds into several fractions according to the affinity or solvating capacity of the compounds to the solvent used. The study involves in fractionation and isolation of compounds from pharmacologically active ethanol extract. The structure of the compound was tried to establish by spectroscopic methods. In 1941, Martin and Synge packed a column containing water and silica gel and developed a mobile phase from a solvent mixture of butanol and chloroform (Agrawal and Paridhavi, 2007).

4.6.2.2 Column:

A glass column of a 20 mm dimension was taken and holds firmly with the help of a stand and fitted with a stopcock.

4.6.2.3 Solvent system:

Ethyl acetate: chloroform (7:3, 8; 2 and 9:1).

4.6.2.4 Absorbent:

The stationary phase was made with Silica gel (60-120 mesh size), which was heated at a maximum temperature of 120 °C and placed in the column.

4.6.2.5 Column preparation:

Cotton is inserted into the column and the bottom portion is plugged and the silica gel (60-120 mesh size) is packed into the column which is done by the wet package method. Then the stationary phase is allowed to settle down without entrapment of air bubbles (Lala, 1981). The column is allowed to stand uniformly without disturbances for two hours.

Fractionation of extract was carried out using the above solvent system to obtain the bioactive compound. Based on the color, the fractions were collected and TLC was performed then similar fractions were pool together. The maximum quantity and single spot on TLC were selected for further analysis.

4.6.2.6 Separation of compounds by column chromatography:

40 gm of methanol extract of the plant was dissolved in a small quantity of methanol, then silica gel (60-120 mesh size) was added to make a slurry and packed over the silica gel inside the column and successively elute using the solvent systems of ethyl acetate and chloroform of different polarities (7:3, 8:2 and 9:1). The solvent elution was collected at a rate of 45 drops per minute. The elutes were collected in 50 ml beakers with a time gap of 5 minutes per fraction. The eluted fractions are tested with TLC on a readymade silica gel plate with the mobile phase. The TLC plates are monitored in the iodine chamber and the R_f values were calculated by the formula: R_f = distance traveled by the sample (cm)/distance traveled by the solvent (cm). Around 200 to 250 fractions were collected and recorded, in which fractions of the elutes namely F 1 – 37, F 38 – 76, F 77 – 108 F109 – 146, F 147 – 168, F 169 – 204 all shows single spots and are combined and transferred to a small beaker evaporated to dryness. The fractions were pooled based on the R_f values and visible color from TLC plates. The pooled fractions were named A, B, C, D, E, and F respectively. The bioactivity of the fraction was selected arbitrarily. The elutes were then kept in a refrigerator for 12 hours to enhance purification.

4.6.3 Preparative thin layer chromatography:

4.6.3.1 Introduction:

It is a liquid chromatographic method. PPC or Preparative planar (thin-layer) chromatography is used for the isolation/ separation of compounds. The main purpose is the production of 10 to 100mg of the separated compounds for structural identification

like mass spectrum, Infrared/ Ultraviolet, and for analysis of other activities. It is an important technique for the purification of samples for preparative purposes.

4.6.3.2 TLC plate preparations:

Glass plates were used, in which the glasses were cut into 6x4 inch dimensions. A total of 10 slides were made for the experiment. The glasses were washed properly with water and dried in the oven. Then, Silica gel 'G' as 30 gm of silica gel was weighed and made to a homogenous suspension with 60 ml distilled water for two minutes, this suspension was layered over a glass plate which was air-dried until the transparency of the layer disappeared. The plates were dried in a hot air oven at 110 °C for 30 minutes and then stored in a dry atmosphere and used whenever required.

4.6.3.3 Development of Chromatogram:

TLC profiling was performed as described by Biradar *et al.,* (2013). From the elutes of column chromatography, solvent systems were prepared according to the results of separation observed. Around 2.5-10 µl of the plant, the extract is spotted by a capillary tube to the origins of the TLC plate 2 cm above its bottom at several spots horizontally to produce more than one separation. The ideal solvent system from thin layer chromatography was prepared and poured into the developing chamber and the lid was closed. Then the plates were kept in the developing chamber (solvent saturated) then the mobile phase was allowed to move through the adsorbent phase up to 3/4th of the plate. The plate was removed and allowed to dry in the air. The different layers were separated by scrapping off the layer sections of the silica gel carefully with a spatula and then they are collected in different beakers. Precautions must be taken while separating the layers so no layers are intermixed. The silica powder from each layer collected is mixed with methanol separately and the molecules from the silica are now contained in the solvent. The solvents from the silica gel are filtered using Whatman`s filter paper and evaporated to crude form and stored. The pure compounds from the chromatogram were then sent for identification.

4.6.4 Identification of the Isolated Compound

Identification of the isolated compound was done by Mass spectral analysis, HPLC, and TLC along with the NIST database; it was the endpoint to the isolation of the conclusive compound. In the extracts of natural products after undergoing extractions and phytochemical analysis, the structure of specific compounds responsible for their anti-diabetic activity was defined by spectroscopic techniques.

4.6.4.1 Mass spectral analysis

The mass spectrum of the isolated bioactive compound was analyzed using LCMS Accucore (C18, 150 x 2.1, 2.6 um), SAIF, CSIR-CDRI, Lucknow, India. Then the molecular weight of the peak compound given by the spectrum was integrated with the NIST database online library for confirmation of the specific compound. LC-MS on a specific plant was carried out to detect a non-volatile compound with anti-diabetic activity.

4.6.4.2 HPLC for confirmation of isolated bioactive compound

HPLC was performed with Quaternary LC Pump Model 200Q/410 with series 200 Autosampler. The mobile phase was determined with an isocratic system using appropriate solvents to detect the wavelength, and then it was incorporated with a standard compound. Both the standard and isolated compounds were eluted together. The peak and retention time were observed. The final result obtained was then overlapped.

4.6.4.3 Thin Layer chromatography analysis for isolated compound

Lastly, the isolated compound was subjected to TLC analysis for confirmation of the compound. Both the standard drug and isolated compounds were eluted and movement was observed up to a certain point. It was then visualized under Iodine dye in a closed chamber.

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CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 ETHNOBOTANICAL EXPLORATIONS FOR ANTI-DIABETIC MEDICINAL PLANTS OF MIZORAM FOR PHYTOCHEMICAL ANALYSIS

A total of 36 plants were identified through a literature survey, interaction with homeopathic practitioners, and villagers for treatment of diabetes in the state of Mizoram. From the 36 plants, 32 of them were most frequently used and 4 of which were irregularly or occasionally used by the tribal inhabitants. They prepare the plants through their traditional modes like decoction, consumption of the parts of the plant as raw, or taking it with other dietary products. The natural habitats were recorded for verifying the distribution of these plants throughout the state. The sources of these anti-diabetic plants were located through the information given by the tribal people of specific areas of Mizoram. The most popular anti-diabetic plants are recorded through the response of the villagers, natural growing stock, their means of cultivation in home gardens, the parts which are used specifically and given to diabetic patients, conservation in ex-situ, availability during the seasons, and their mode of formulations and preservations of these plants. All the plants were ranked by the responses given by 5% of the total population of the study area and medicinal practitioners.

A total of 32 plants belonging to 26 families were frequently used, out of which 15 plants were considered as the most effective in treatment against diabetes mellitus. The most dominating families were Cucurbitaceae (3), Euphorbiaceae (3) followed by Verbebaceae (2), and Musaceae (2). The usage of plant parts are Leaves – 9, Bark – 6, Whole plant – 4, Fruit – 4, Tuberous root – 2, Stem and leaf – 2, Seeds – 2, Stem – 1 are shown in figure 2. 15 plants were subjected to phytochemical screening to demonstrate the presence of important secondary metabolites which possesses anti-diabetic activities (Figure 3).

Table 2: Anti-diabetic medicinal plants used by the Mizo ethnic groups in Mizoram.

Sl no	Botanical name	Family	Local name	Mode of application
1.	***Annanus comosus L. Merr***	Bromeliaceae	Lakhuihthei	The decoction of leaves is taken orally, twice a day.
2.	***Bauhinia variegate L. Benth.***	Caesalpiniaceae	Vau-be	Powdered bark infusion is used.
3.	***Benincasa hispida Thunb.***	Cucurbitaceae	Maipawl	Fruit juice is taken orally.
4.	***Callicarpa arborea Roxb.***	Verbenaceae	Hnahkiah	The bark is grinded and the infusion is taken
5.	***Carica papaya L.***	Caricaceae	Thingfangma	The leaves are boiled and taken twice a day.
6.	***Catharanthus roseus L.***	Apocyaceae	Kumtluang par	The decoction of root, stem, and leaves are taken thrice a day for one month.
7.	***Centella asiatica L.***	Apiaceae	Lambak	Whole plant juice is taken on an empty stomach.
8.	***Cinamomum verum J. Presl.***	Lauraceae	Thakthing	Bark powder infusion is taken orally.
9.	***Citrus maxima Merr.***	Rutaceae	Sertrawk	Seeds are peeled and eaten twice a day.
10.	***Clerodendrum colebrookianum Walp.***	Verbenaceae	Phuihnam	Leave paste is taken orally.
11.	***Colocasia esculenta L.***	Araceae	Dawl	Leaves are boiled and taken

				orally
12.	***Costus speciosus (J.Konig) Sm.***	Costaceae	Sumbul	Tubers boiled with water or root juice is used.
13.	***Cucurmis sativus L.***	Cucurbitaceae	Fang-hma	The decoction of leaves is taken orally.
14.	***Emblica officinalis L.***	Euphorbiaceae	Sunhlu	Bark grinded into powder and used.
15.	***Glinus oppositifolia L.***	Aizoaceae	Bakhate	Boil extract of the plant is used.
16.	***Ipomea batata (L). Lam.***	Convolvulaceae	Kawl-ba-hra	Leaves are boiled and juice is taken orally.
17.	***Jasminum laurifolium Var.***	Oleaceae	Maufimhlo	The decoction of stem and leaves is taken twice a day.
18.	***Lagerstroemia speciosa (L.) Pers.***	Lythraceae	Chawnpui	The infusion of the bark is used.
19.	***Lepionurus sylvestris Blume.***	Olacaceae	Anpangthuam	The leaves are boiled, extracted, and taken.
20.	***Mallotus roxburghianus Mull Arg.***	Euphorbiaceae	Zawngte-nawh-lung	The leaves and bark are used for diabetes.
21.	***Mangifera indica L.***	Anacardiaceae	Theihai	The decoction of the young shoots is taken twice a day.
22.	***Mirabilis jalapa L.***	Nyctaginaceae	Artukhuan	Tuberous roots are boiled and taken.
23.	***Momordica charantia***	Cucurbitaceae	Changkha-rek	The fruits are boiled and

	L.			taken with food.
24.	***Musa acuminate Colla.***	Musaceae	Vai-bal-hla	Unripe fruit juice is taken orally.
25.	***Musa glauca Roxb.***	Musaceae	Saisu	The water inside the bark is taken twice a day.
26.	***Phaseolus vulgaris L.***	Fabaceae	Bean	Cooked fruits are taken.
27.	***Phyllanthus fraternus GL. Webster.***	Euphorbiaceae	Mitthi sunhlu	A boiled extract of leave is taken orally.
28.	***Plantago major L.***	Plantaginaceae	Kel-ba-an	The decoction of the whole plant is taken orally.
29.	***Scurrula parasitica L.***	Loranthaceae	Thlik-thli-ek-bawm	The decoction of leaves is taken twice a day.
30.	***Senecio scandens L.***	Asteraceae	Sai-ek-hlo	The whole plant is boiled and taken orally.
31.	***Syzygium cumini L.***	Myrtaceae	Lenhmui	The seed is grinded into powder and taken for diabetes.
32.	***Tinospora sinensis Lour. Merr.***	Meninspermaceae	Vankaihrui	The decoction of the stem is taken orally.

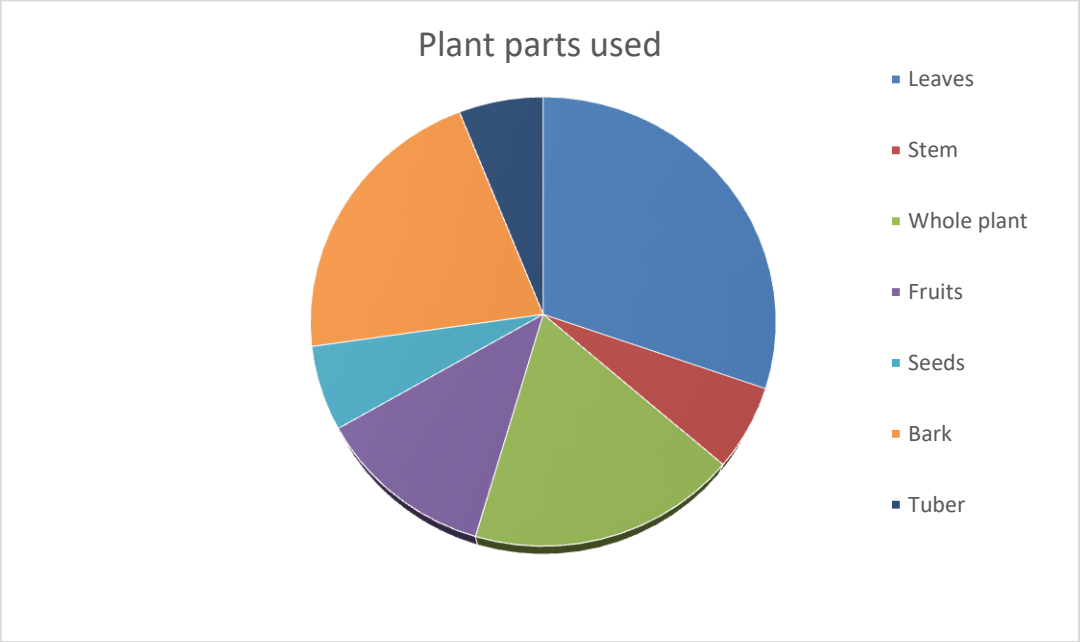


Figure 4: Number of plant parts used for the treatment of diabetes.

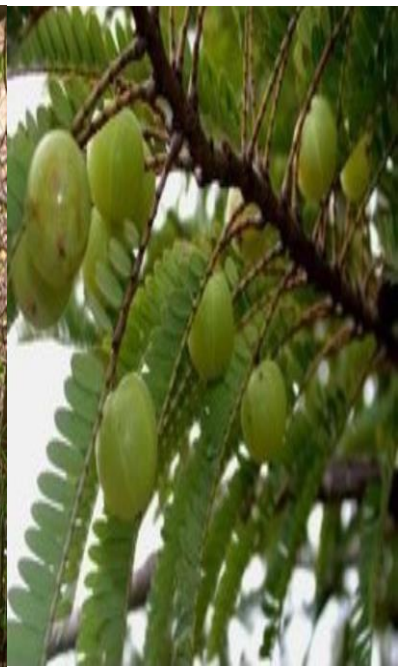
Jusminum laurifolium Var.

Senecio scandens L.

Mangifera indica L.

Colocasia esculenta L.

Mallotus roxburghianus Arg.



Emblica officinalis L.

Benincasa hispida Thunb. ***Ipomea batata (L).Lam.*** ***Tinospora sinensisLour. Merr.***

Centella asiatica L. ***Clerodendrum colebrookianum Walp.*** ***Musa glauca Roxb.***

Glinus oppositifolia L. *Catharanthus roseus L.* *Costus speciosusSm.*

Mirabilis jalapaL. *Annanus comosus (L.)* *Cucurmis sativus L.*

Photo 1: Some important anti-diabetic plants used in Mizoram.

Photo 2: Discussion with Traditional practitioners

Photo 3: Interaction with local people

5.2 PHYTOCHEMICAL ANALYSIS

5.2.1 Phytochemical screening of anti-diabetic plants

The results of phytochemical screening 15 of anti-diabetic plants reveal the presence of alkaloids, flavonoids, terpenoids, glycosides, tannin, saponin, reducing sugar, proteins, phytosterols in almost all of the plants. The curative properties of the selected plants are due to the presence of these secondary metabolites and are the main reason they demonstrate therapeutic action against diabetes mellitus. *Mangifera indica L., Jasminum laurifolium Var., Mallotus roxburgianus Mull Arg., Citrus maximus Merr.* and*Scurrula parastica L.* possesses accurate potential in their secondary metabolites constituents for diabetes.

The highest efficacy with the occurrence of most of the important metabolites for diabetes is *Scurrula parastica L.* and was selected as a candidate plant for further phytochemical analysis including *in vitro* antioxidant analysis, *in vivo* analysis using streptozotocin-induced diabetic rats, and isolation of anti-diabetic compound from the particular plant.

Table 3: Phytochemical screening of anti-diabetic medicinal plants of Mizoram.

Sl. No.	*Scientific Name*	Alkaloid	Terpenoid	Flavonoid	Glycoside	Tannin	Saponin	Reducing Sugar	Phenols	Phytosterols
1.	*Annanus comosus L. Merr*	+	+	–	+	+	–	+	–	+
2.	*Bauhinia variegata L. Benth.*	+	+	+	+	–	–	–	+	–
3.	*Carica Papaya L.*	+	–	–	+	+	+	+	–	+
4.	*Centella asiatica L.*	+	+	–	–	+	+	+	–	+
5.	*Citrus Maximus Merr.*	+	+	–	+	–	–	+	–	+
6.	*Curcumus sativas L.*	+	–	–	+	+	+	–	–	+
7.	*Glinus oppositifolia L.*	+	+	–	–	+	+	+	–	–
8.	*Jusminum laurifolium Var.*	+	+	+	–	+	+	+	+	–
9.	*Lepionurus sylvestres Blume.*	–	+	–	+	+	–	+	——	+
10.	*Mallotus roxburgianus Mull Arg.*	+	+	+	+	–	+	+	+	–
11.	*Mangifera indica L.*	+	–	+	–	+	+	+	+	+
12.	*Mirabilis jalapa L.*	+	+	–	+	+	+	+	–	+
13.	*Phyllanthus fraturnus*	+	+	–	–	+	+	+	–	+
14.	*Scurrulla Parasitica L.*	+	+	+	–	+	+	+	+	+
15.	*Senecio scandens L.*	+	+	–	–	+	–	+	–	+

Note: + = Presence of the metabolite

\- = Absence of the metabolite

Photo 4: Extraction with soxhlet apparatus and purification of extract

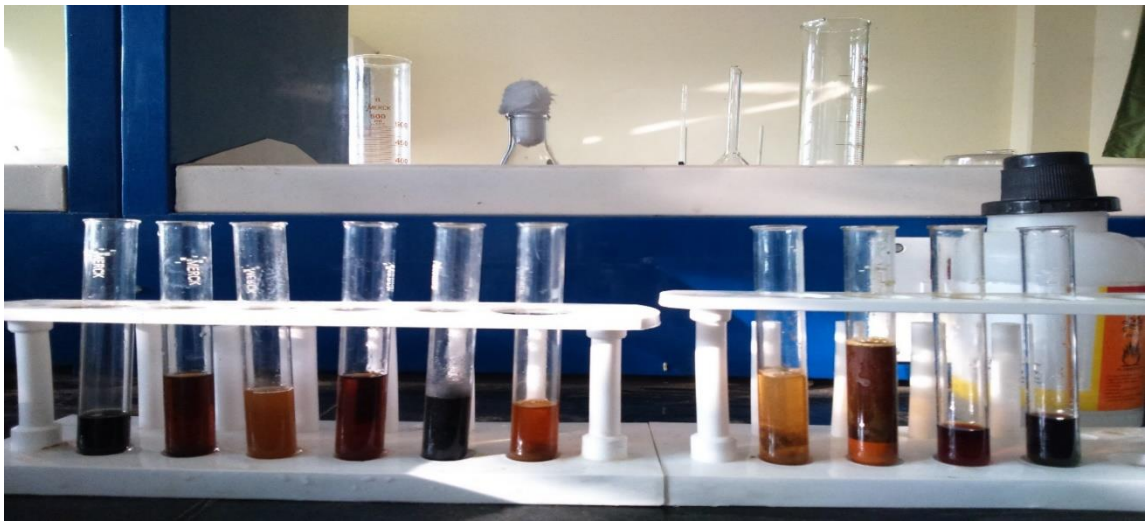


Photo 5: Preliminary phytochemical screening

Candidate Plant: *Scurrula parasitica L.*

Identification:

The identification of the plant was done at the Botanical Survey of India, Shillong (No: BSI/ ERC/ Tech/ 2017/ 43), and the herbarium was deposited and authenticated as voucher number MZU 742 in the Department of Environmental science, Mizoram University.

Medicinal properties:

The decoction of the plant is used for the treatment of cancer. The flowers/ fruits are visited by birds. The plant is used as a traditional medicinal herb as an antioxidant and against microbial infections, hypertension and, cancer in Asia (Lim *et al.,* 2016). Infusion of *Scurrula parasitica L.* is used to relieve fatigue and in the treatment of cancer in Java and Indonesia (Puneetha and Amruthesh 2016). In southern China, the leaves and stem of *Scurrula parasitica L.* are used as antineoplastic, cardiotonic, as an antioxidant (Xiao *et al.,* 2008), and for the treatment of Schizophrenia (Soheil *et al.,* 2014). Manhajan *et al.,* (2013) also reported that the plant shows anti-diabetic, cytotoxic, anticancer, anti-hepatoxic, and immunomodulatory activity. Phytochemicals like aviculin, quercetin, catechin, rutin, oleanolic acid, terpenoids, flavonoids, alkaloids, viscotoxins, lectins, 4-O-acetylquercitrin, lupeol, and amines are found present in *Scurrula parasitica L.* (Nilesh *et al.,* 2013; Manhajan *et al.,* 2013).

Distribution

World: It is found in Sri Lanka, Nepal, Bhutan, Bangladesh, Myanmar, Thailand, Taiwan, Vietnam, Indonesia, Philippines, Moluccas, and Timor. *Scurrula* consists of about 91 species, the majority of which are found in South East Asia, Malaysia, and China, and minor species are also found in regions of Australia and India.

India: *Scurrula parasitica L.* is distributed all over the Western Ghat regions of Maharashtra and parts of Uttar Karnataka and Tamil Nadu.

Mizoram: Pangbalkawn, Bilkawthlir and Kolasib

Place of collection: Kolasib district

1. Bilkhawthlir: 24º 20’ 12.1164” N and 92º 42’ 56.8840” E
2. Kolasib: 24º 13’ 48” N and 92º 40’ 48” E

Description:

Parasitic shrubs found growing on *Dendrophthoe falcate, Myrica esculenta* and, *Mangifera indica.* It belongs to the family Loranthaceae. The branches are erect; young parts tomentose. Leaves are 5 to 9 cm, ovate, rounded at the base and apex; golden tomentose below; petiole to 1cm long. Flowers are in axillary fascicles/shortly peduncled cymes, 16 to 20. The bracts are ovate with dense tomentose. The calyx is cupular and the corolla is 15 mm long and slender. 4 lobes are acute and ovate. The stamens consist of 4 which are glabrous filaments and stigma is clavate. The fruit or berry is obovoid, club-shaped, and densely hairy with a narrowly tapering base.

Habitat:

Habitat variable. Found at humid subtropical and tropical monsoon climate at areas up to 1,300 m from sea levels

Phenology: **Flowering:** November to December

Fruiting: February to April

Photo 6: ***Scurrula parasitica L.***

Photo 7: Flower buds of ***Scurrula parasitica L.***

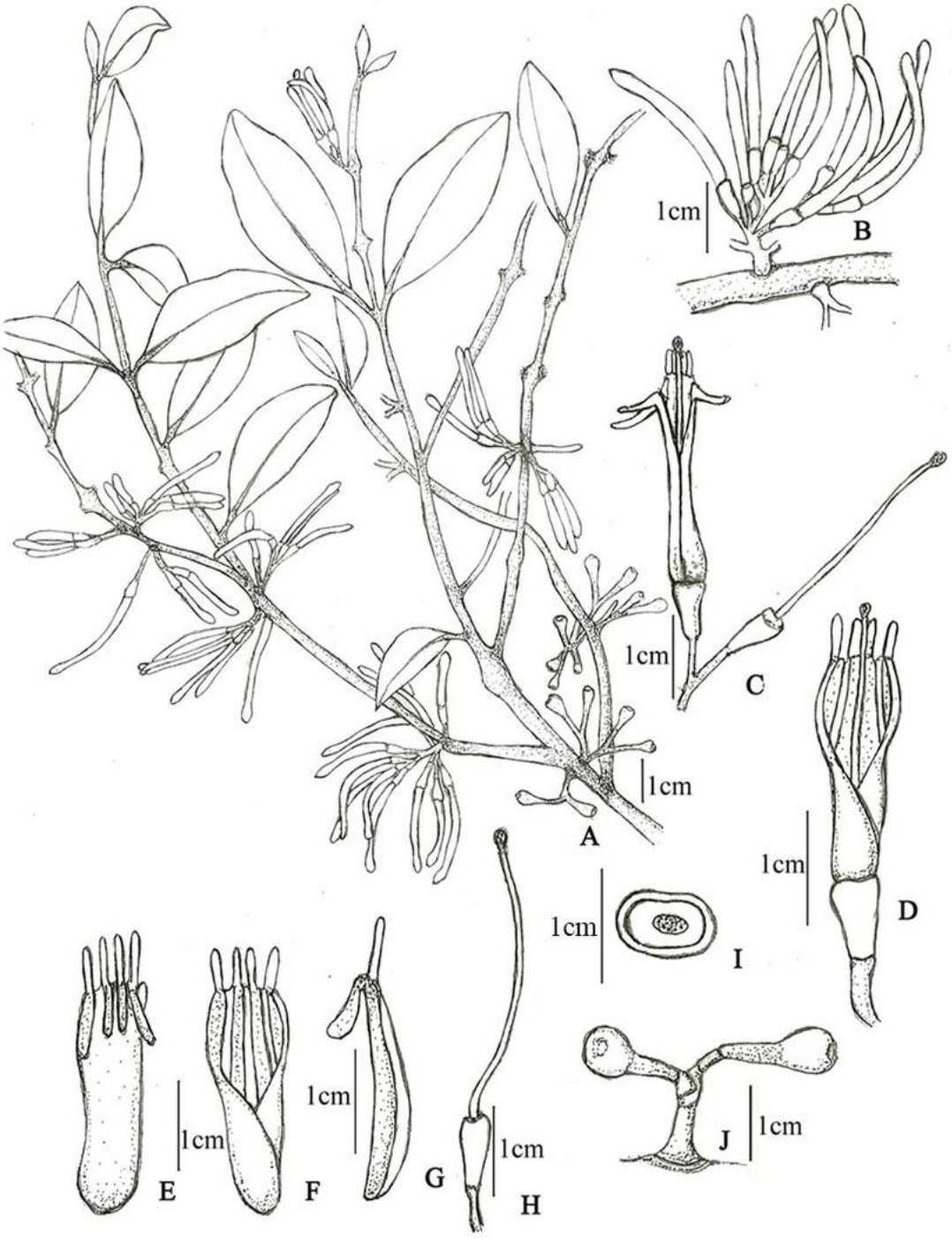


Figure 5: ***Scurrula parasitica L.*** **by LJ Singh (2015)**

5.2.2 Antioxidant activities of *Scurrula parasitica L.*

5.2.2.1 Total phenol and flavonoids contents:

The activity of *Scurrula parasitica L.* might be due to the occurrence of polyphenolic compounds, which are recognized to possess antioxidant activity according to the research done by (Okudu *et al.,* 1994) and (Tepe *et al.,* 1988). Phenols are plant metabolites that are denoted by the occurrence of several phenol groups, several of which are extremely reactive in chelating metal ions in aqueous solutions and neutralizing free radicals by donating a hydrogen atom or an electron (Petti and Scully, 2009). Furthermore, the phenolic compounds acquire numerous pharmacological properties like antimutagenic, antibacterial, and antitumor properties, all of which might be related to their antioxidant property (Shui and Leong, 2002). Flavonoids are exceedingly effective antioxidants and considered as the most significant and broadly distributed single group of phenols present in plants (Yanishkieva, 2001). Flavonoids are forming complexes with metal ions by inhibiting metal-initiated lipid oxidation (Lee *et al.,* 2008). The result of the research revealed the level of phenol and flavonoids compounds in ethanol extract of the leaves of *Scurrula parasitica L.* as shown in (Table 4). The overall phenolic contents of all the formulations of the extract are found to be around 101.9 to 379.1 (µg/g tissue) at concentrations from 5 to 100 µg/ml plant extracts. The entire flavonoid content of the extract is found to be around 72 at 5 µg/ml to 174.1 (µg/g tissue) at 100 ml concentration.

Table 4: Total phenol and flavonoids contents in ethanol extract of *Scurrula parasitica L.*

Plant extract Conc. (µg/ml)	Phenol content (µg/g tissue)	Flavonoid content (µg/g tissue)
5	101.907456	72
10	143.112384	87.9
20	206.528328	99.83333333
40	284.047536	120.7666667
80	329.038248	154.0333333
100	379.130832	174.1333333

This activity in ethanol extract of *Scurrula parasitica L.* is assumed to be largely due to their redox properties in decomposing peroxides, adsorbing and neutralizing free radicals, quenching singlet, and triplet oxygen (Zheng and Wang, 2001) Medicinal plants contain important metabolites like polyphenols, which are acting as antioxidants. In this regard, flavonoids and phenolic acids in plants were reported to possess the considerable antioxidant capacity and consist of several biological properties, together (Gil *et al.,* 1999). The results from the study strongly suggest that polyphenols are essential components of *Scurrula parasitica L.* and the presence of these important constituents should be credited to their pharmacological properties.

5.2.2.2 Antioxidant activity:

DPPH and the ABTS radical scavenging process is an important and comprehensively used antioxidant assay for performing on plant extracts. The frequently useful assays, differing within their effective principles were employed as a part of our research. Specifically, the antioxidant activities of the inspected plant were determined as free radical scavenging capacity. The DPPH and ABTS scavenging activity exhibited

an increase in concentration manner up to a certain concentration and the 50% inhibitory concentration (IC_{50}) was calculated. The optimum concentration for both DPPH and ABTS was obtained at 100µg/ml with an IC_{50} value of 53.28100 µg/ml and 160.8100 µg/ml, respectively. The scavenging action of *Scurrula parasitica L.* is given below:

Table 5: DPPH and ABTS scavenging action of *Scurrula parasitica L.*

Conc. (µg/ml)	**ABTS (µg/ml)**	**Ascorbic acid(µg/ml)**	**DPPH (µg/ml)**	**Ascorbic acid(µg/ml)**
5	0.460337	25.30457	10.92739	29.04385
10	2.129638	29.06166	15.74407	40.00719
20	2.613224	65.02371	38.40762	67.14594
40	3.022412	90.16089	53.01941	67.8289
80	17.92523	95.03394	54.36736	68.2243
100	25.63471		57.09921	
IC_{50}	160.8	2.723	53.28	2.805

5.2.2.3 DPPH radical scavenging activity:

The DPPH radical scavenging activity was performed with the ethanol extract of the leaves of *Scurrula parasitica L.* and compared with ascorbic acid. Its activity increases with increased concentration manner and maximum activity was observed at 57.09% at 0.1 mg/ml concentration. The value of IC_{50} was found to be 53.28 µg/ml. The ascorbic was a potent antioxidant and its IC_{50} value was 2.8 µg/ml. Free radicals are evaluated using DPPH (Porto *et al.,* 2000). The scavenging activity is expressed as percent inhibition and compared with ascorbic acid as a standard compound. It further showed the potential of the plant extract to scavenge diverse free radicals in different systems and could serve as an important therapeutic agent for controlling radical pathological damage.

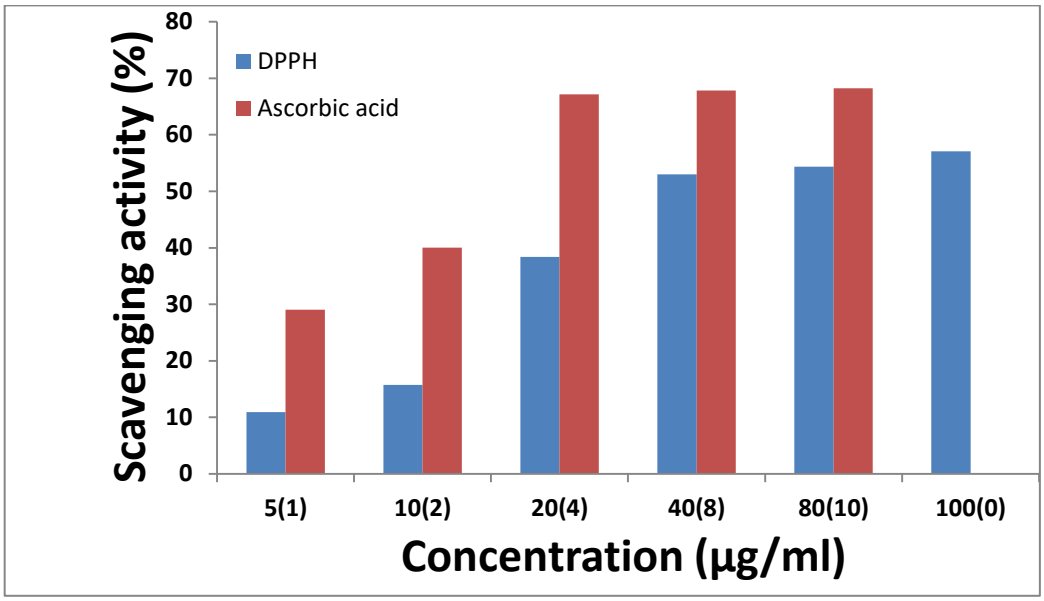


Figure 6: DPPH activity of ethanol extract of *Scurrula parasitica L.* and ascorbic acid

5.2.2.4 ABTS radical scavenging activity:

The assessment of the scavenging activity of *Scurrula parasitica L.* was performed by the ABTS assay method in which the ethanol extracts of the leaves were assessed compared to ascorbic acid. The activity exhibited a rise with an increase in concentration manner and the maximum was obtained at 25.63% (100µg/ml). Again, the scavenging activity is expressed as percent inhibition and compared with ascorbic acid as a standard compound**.** The results of the ABTS scavenging capacity of the plant of the extracts were lower than those of ascorbic acid (100%). These indicate that the plant has the proton-donating capability and might serve as potential free radical inhibitors.

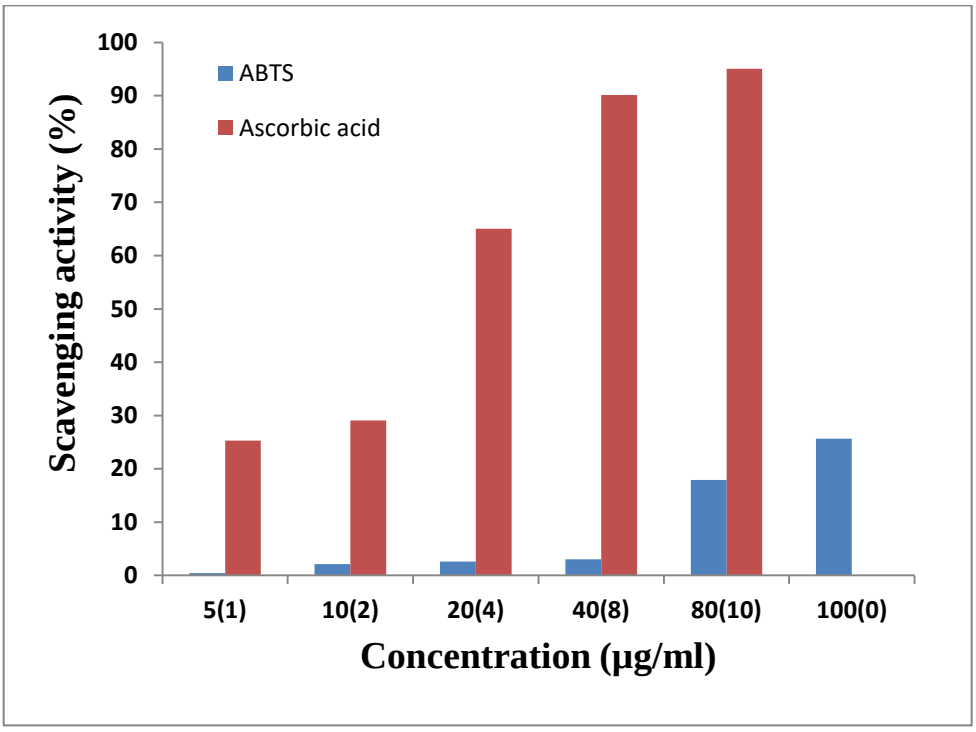


Figure 7: ABTS action of the ethanol extract of *Scurrula parasitica L.* and ascorbic acid

5.2.3 *In vivo* analysis of the anti-hyperglycemic activity of *Scurrula parasitica L.* using streptozotocin-induced diabetic rats

5.2.3.1 Selection of Doses

Acute toxicity studies were carried out following the guidelines of OECD by different doses of leaves of ethanol extract *of Scurrula parasitica* (ESP). For the evaluation of hypoglycemic activity, two doses were selected 100 mg/kg and 200 mg/kg as per acute toxicity test. The streptozotocin-induced rat revealed an increase in the level of blood sugar. All the treatment groups showed a decline in the level of blood glucose from the 5^{th} day until the 21^{st} day when compared to streptozotocin alone. All the treatment groups showed no statistical difference on 10^{th} and 15^{th} and 21^{st} day ($p<0.001$) post-treatment.

Table 6: Effect of ESP on blood glucose levels in STZ-induced diabetic rats

Time Day)	Group 1 (Normal rats)	Group 2 (Diabetic rats)	Group 3 (Metformin)	Group 4 (High dose)	Group 5 (Low dose)
0	88.3 ± 2.55	311.8 ± 2.42	312.8 ± 2.30	318.1±2.66	320.0±2.88
5	85.0 ± 4.48	354.5 ± 2.96	237.0± 1.57[b]	262.5±2.33[b]	338.3±4.42[a]
10	85.5 ± 1.86	393.7 ± 3.53	214.8± 2.14[b]	176.0±3.56[b]	290.0±4.30[b]
15	83.7 ± 2.69	392.0 ± 3.92	179.0 ± 3.53[b]	171.8±3.71[b]	190.6±2.40[b]
21	88.7 ± 2.93	395.5 ± 2.63	124.8 ± 2.39[b]	159.8±2.77[b]	169.8±2.91[b]

All values are Mean ± SEM; N=6. [a]*P*<0.01 when compared with diabetic control. [b]*P*<0.001 when compared with diabetic control.[1]*P*<0.05 when treatment group III & IV compared with standard (metformin).

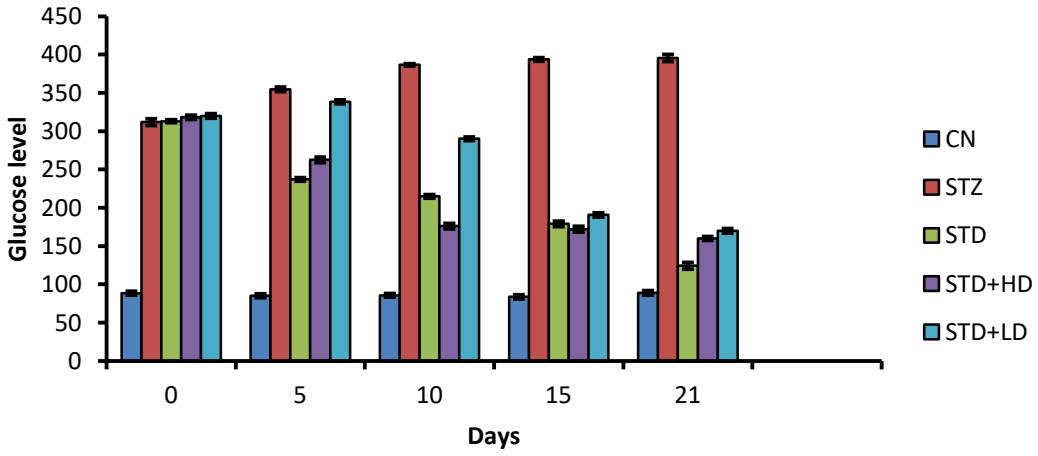


Figure8: Effect of ESP on blood glucose levels in STZ-induced diabetic rats

Table 7: Effect of ESP extract on changes in body weight in normal and experimental rats

TIME (Day)	Group1 (Normal)	Group 2 (Diabetic)	Group 3 (Metformin)	Group 4 (High dose)	Group 5 (Low dose)
0 Day	199.33 ±6.21	193.83 ± 3.25	195.33 ± 5.00	192.50±4.18	198.16 ±3.31
21st Day	207.16 ± 4.42	183.50 ± 4.84	204.16 ± 3.86	197.66±4.50	201.83 ±3.65

All values are Mean ± SEM; N=6. There is no statistical difference in the body weight between 0 days and a 21st day or among groups.

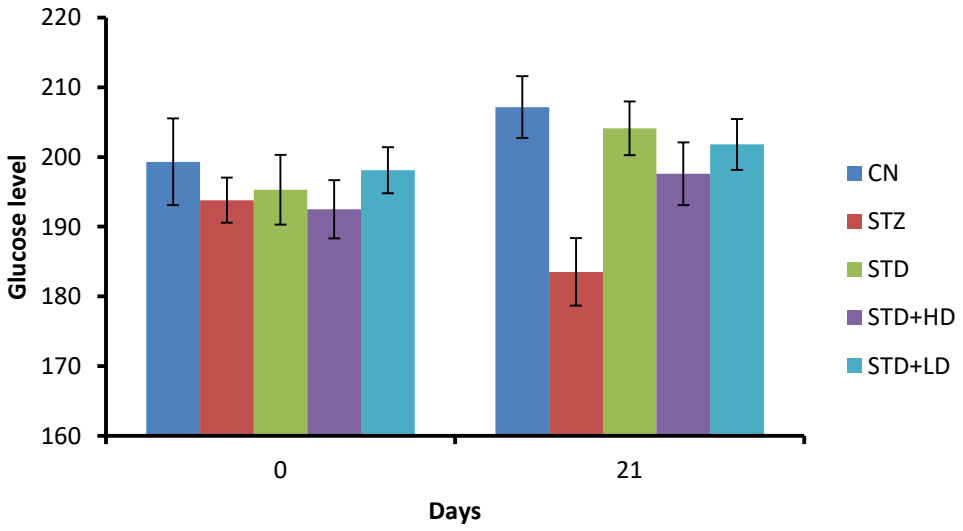


Figure 9: Effect of ESP on body weights on STZ-induced diabetic rats

Table 8: Effect of ESP on Triglyceride, Cholesterol, HDL-Cholesterol, and LDL-Cholesterol

Group	Triglyceride (mg/dl)		Cholesterol (mg/dl)		HDL-Cholesterol (mg/dl)		LDL-Cholesterol (mg/dl)	
	0 Day	21st Day	0 Day	21st Day	0 Day	21st Day	0 Day	21st Day
1	90.9±2.67	95.7±0.49	76±3.77	84.2±3.25	30.4±1.04	33.4±1.44	11.4± 2.57	10.3±2.80
2	152.6±0.59	187.2±4.12	75.8±4.08	123.6±4.06	36.4±3.72	37.8±1.73	22.2±1.08	25.3±3.54
3	104.8±1.16	76.9±2.42[a]	95.5±3.76	73.8±4.58[a]	62.7±2.26	65.7±1.83	16.9±3.71	13.6 ±2.41[c]
4	120.2±2.28	98.3±2.34[a]	118.6±2.81	102.3±3.34[a]	63.5±2.83	62.9±2.91	15.8±2.21	14.7±1.54[c]
5	140.5±4.70	122.8±3.83[a]	128.7±3.43	118.4±4.26[a]	61.4±3.32	64.4±2.94	16.0±1.14	15.1±2.73[c]

All values are Mean ± SEM. [a]$P<0.001$significantly decrease when compared with control and compared with 0 day and 21st day among all treatment groups. [b]$P<0.01$ compared with 0 day and 21st day among all treatment groups. There was no statistically significant change among groups in HDL-Cholesterol level but there was significantly declined in the LDL-Cholesterol among groups ([c]$P<0.05$), N=6.

Figure 10: Effect of ESP on serum Triglyceride

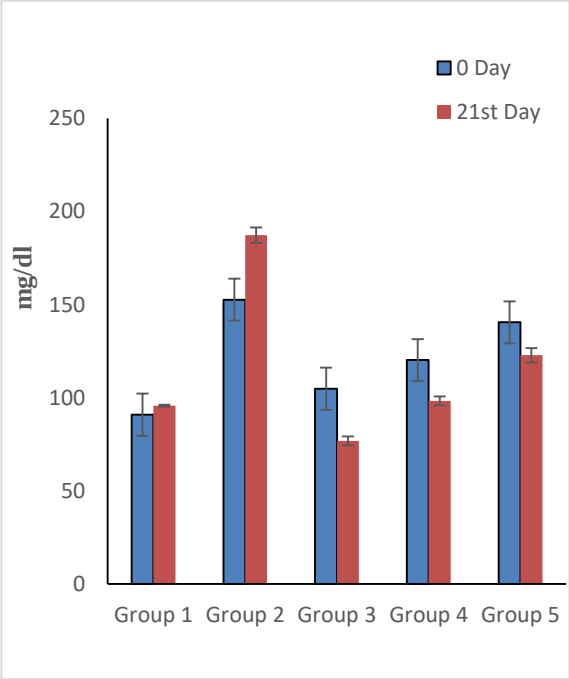


Figure 11: Effect of ESP on Serum Cholesterol

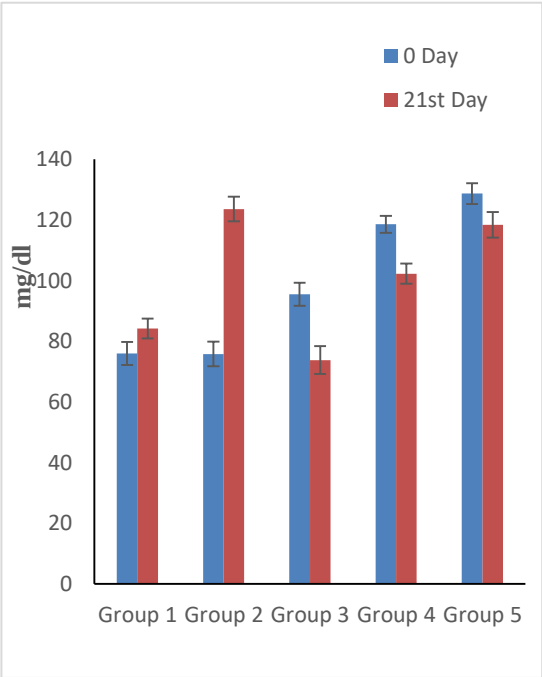


Figure 12: Effect of ESP on serum HDL

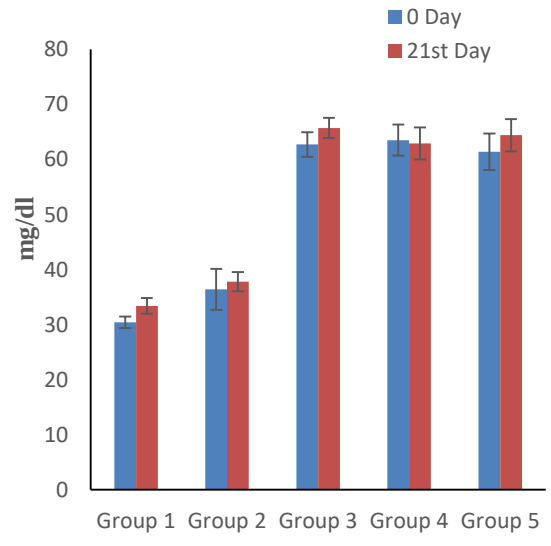


Figure 13: Effect of ESP on serum LDL

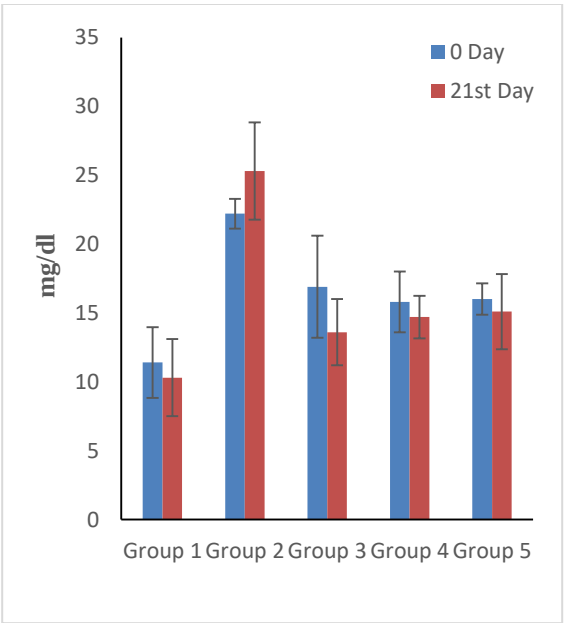


Table 9: Effect of ESP on serum SGPT, SGOT and ALP

Group	SGPT		SGOT		ALP	
	0 Day	21st Day	0 Day	21st Day	0 Day	21st Day
1	55.9 ± 2.33	48.1 ± 2.79	95.7 ± 1.75	93.8 ± 2.14	91.8 ± 4.09	89.4 ± 1.25
2	54.1 ± 3.68	99.3 ± 1.33	103.3 ± 2.38	123.5 ± 4.05	84.7 ± 3.34	117.3 ± 4.65
3	57.5 ± 1.74	31.6 ± 1.23[a]	101.8 ± 0.95	95.4 ± 1.31[b]	98.4 ± 1.52	93.7 ± 1.17[a]
4	71.48±4.7	70.7±4.46[b]	105.9±4.43	97.3 ±2.75[b]	95.0 ±4.38	85.6±3.75[b]
5	68.73±1.73	65.17±1.38[b]	107.5 ± 3.30	99.4 ± 1.41[b]	91.7 ± 4.73	84.8 ± 3.96[b]

All values are Mean ± SEM. [a]P<0.001 significantly decreases when compared with control and compared with 0 day and 21st day among all treatment groups. [b]P<0.01 compared with 0 day and 21st day among all treatment groups .[c]P<0.05,N=6.

Figure 14: Effect of ESP on serum SGPT

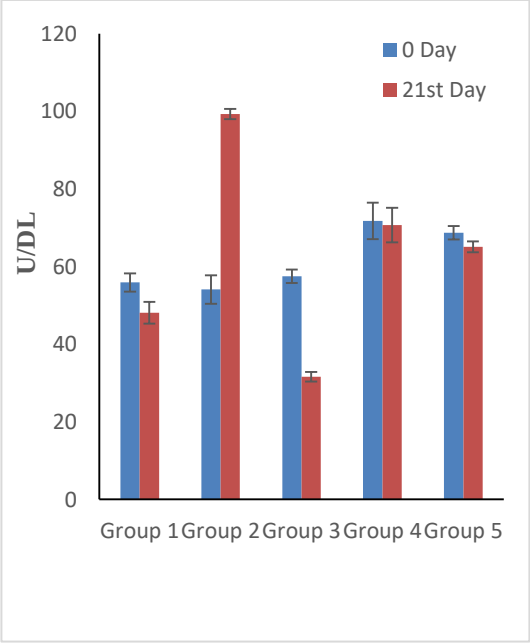


Figure 15: Effect of ESP on serum SGOT

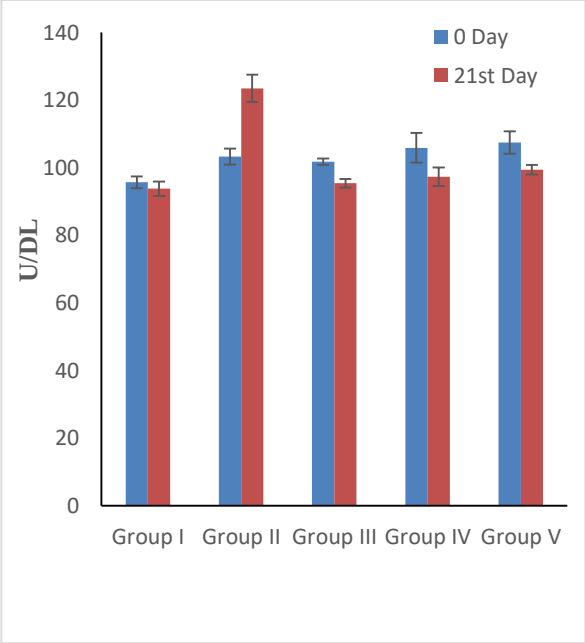
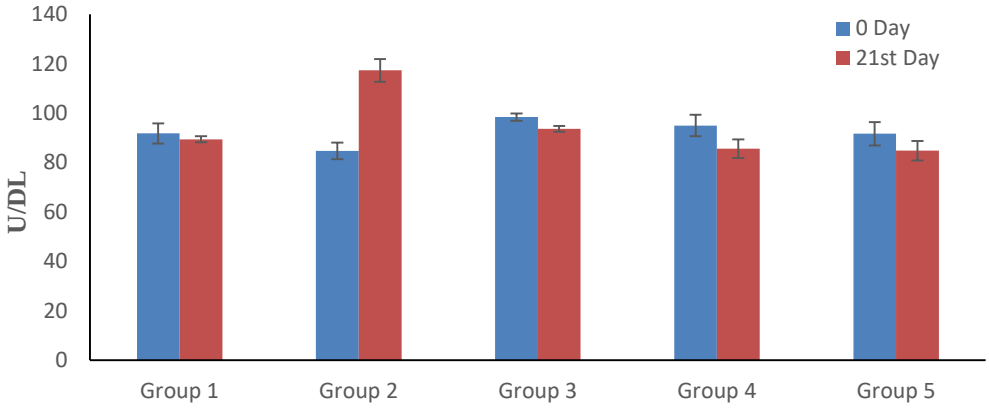


Figure 16: Effect of ESP on serum ALP



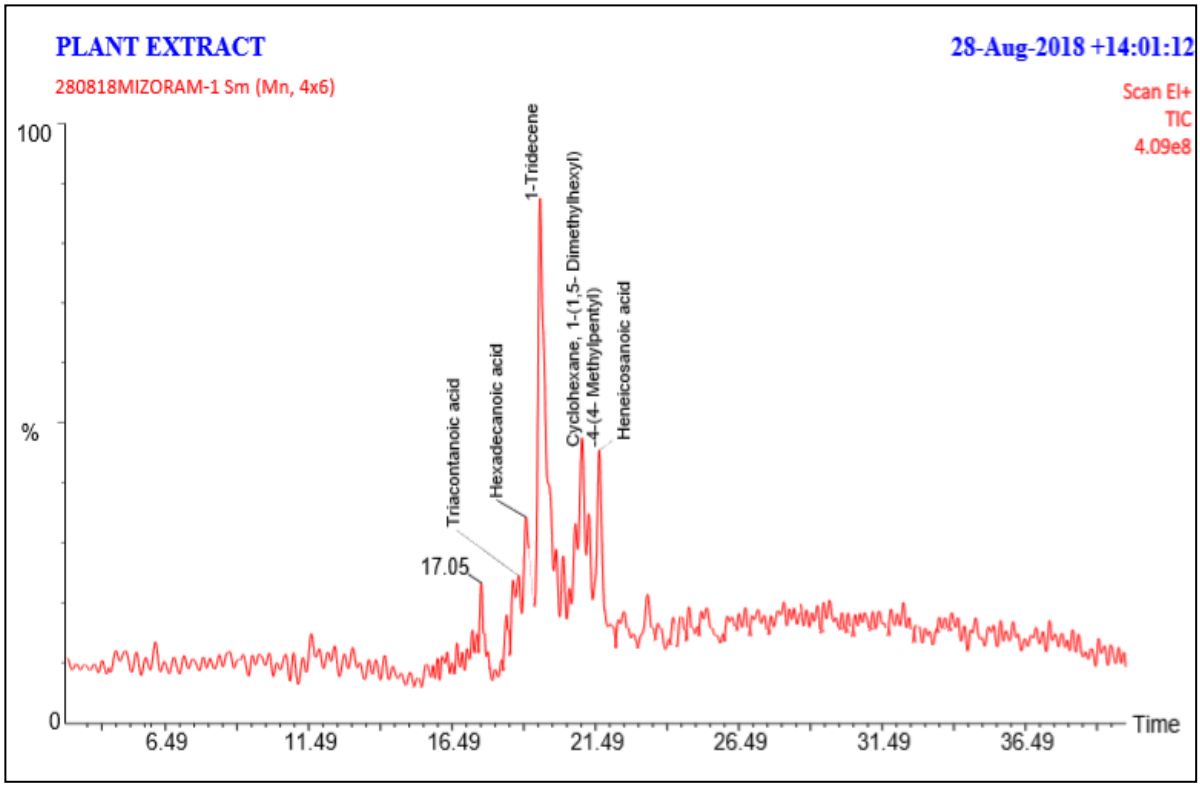
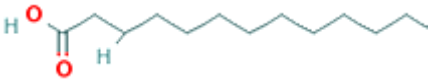
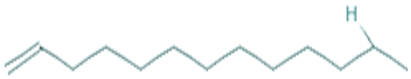
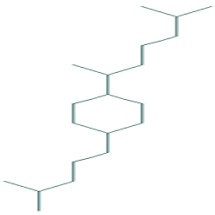
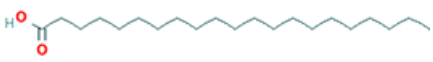


Figure 17: Detection of anti-diabetic compounds by GC-MS

GC-MS can only detect volatile compound Therefore, it is necessary to screen all bioactive compounds present in *Scurrula parasitica L.* (Lists of GC-MS compound presented below in the table 10)

Table 10: List of anti-diabetic compounds in *Scurrula parasitica L.* detected by the GC-MS method.

Sl. No	Compound	RT	Area %	Structure & MW	Reference
1	Triacontanoic acid	18.855	14.87	**$C_{30}H_{60}O_2$** 452.808g/mol 	Eddouks *et al.*, 2005
2	Hexadecanoic acid	19.296	52.91	**$C_{16}H_{32}O_2$** 256.43g/mol 	Ahmad *et al.*, 2012
3	1-Tridecene	20.606	11.44	**$C_{13}H_{26}$** **182.351 g/mol** 	FAOU, 1997. Chien *et al.*,2009
4	Cyclohexane, 1-(1,5-Dimethylhexyl) -4-(4-Methylpentyl)	21.146	12.24	 **$C_{20}H_{40}$,** **280.54g/mol**	Mohammad Nadeem Akhtar and Gayathri, 2015
5	Heneicosanoic acid	21.562	8.54	**$C_{21}H_{42}O_2$** **326.565 g/mol** 	D` Souza *et al.*, 2014

5.2.4 Isolation of active compound for diabetes

5.2.4.1 Thin layer chromatography:

The results of thin layer chromatography of methanol extract of *Scurrula parasitica L.* are presented in table 3. A definite spot showing yellowish-red was detected in the solvent system of Ethyl acetate 7: Chloroform 3 and Ethyl acetate 7: Chloroform 2: Methanol 1 after keeping it inside an iodine chamber. But Ethyl acetate 7: Chloroform 3 display the best result. Thus, it was selected to be used as the solvent system.

Table 11: the Solvent system used in thin layer chromatography

Sl. No	Solvent system	Results
1	Methanol	No definite spot
2	Methanol 6: Chloroform 4	Spots detected, tailing
3	Ethyl acetate	A single spot, tailing exists
4	Chloroform	No definite spot
5	Ethyl acetate 7: Chloroform 3	Single spot with R_f value of 0.44 mm
6	Ethyl acetate 7: Chloroform 2: Methanol 1	Definite spots, tailing exists

5.2.4.2 Column chromatography:

According to fractions from the column chromatography observed, the best fraction was chosen for further analysis. Fraction No. F-200 with TLC profile of Ethyl acetate 7: Chloroform 3 obtaining a single spot with an R_f value of 0.44 mm was selected for Mass spectral analysis for identification. Both HPLC and TLC analysis were done for isolated compound confirmation.

Photo 8: Column chromatography

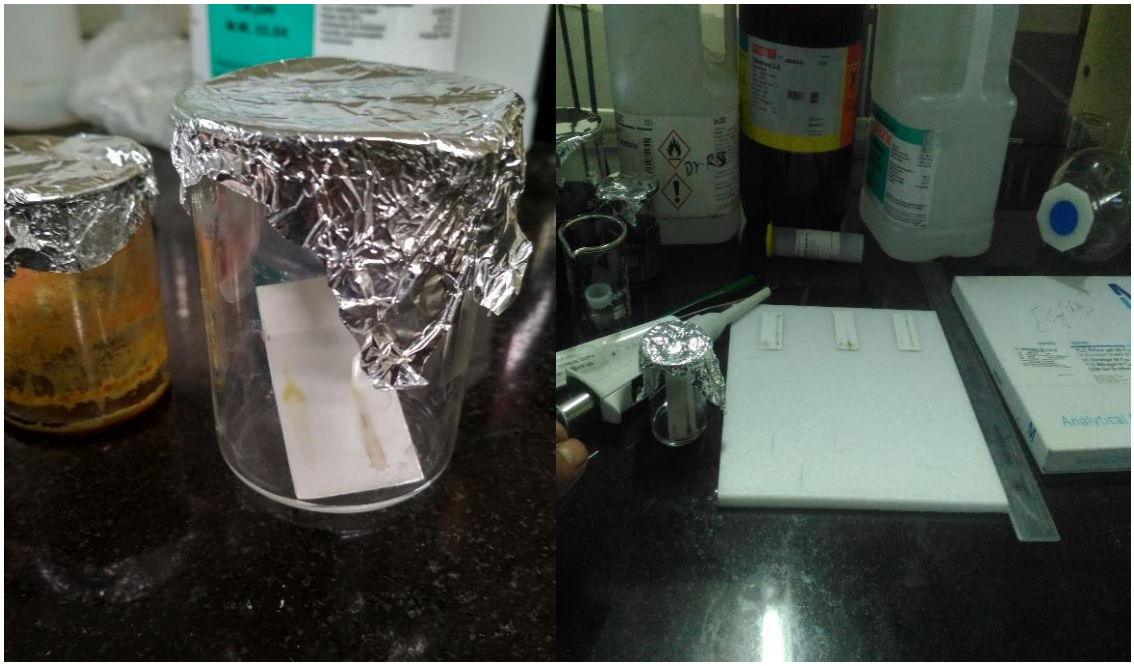
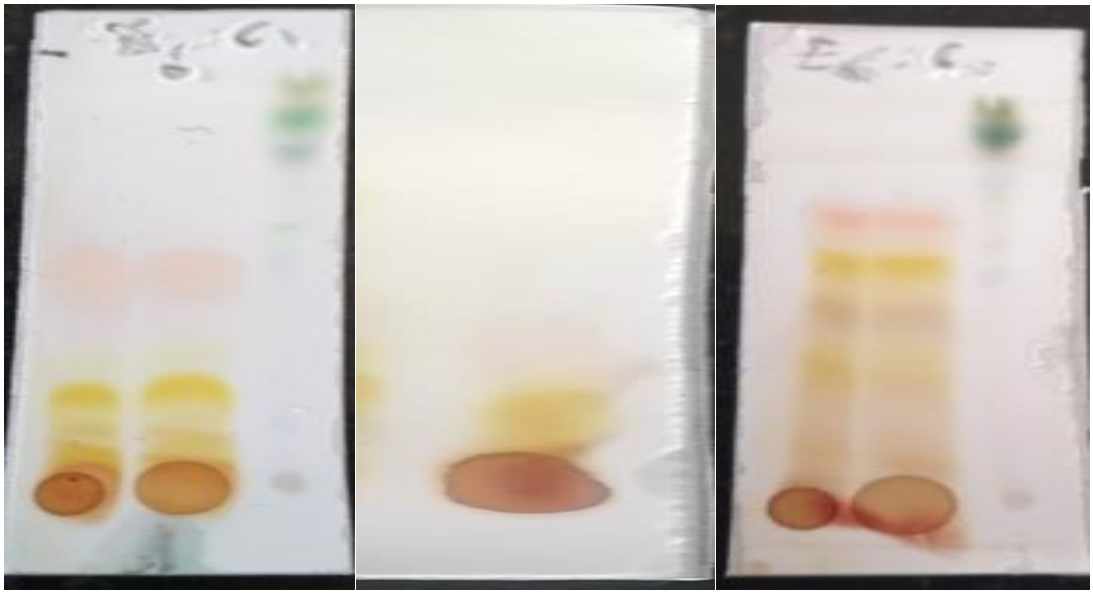


Photo 9: Thin layer chromatography

Methanol **Methanol 7: Chloroform 3** **Chloroform**



Ethyl acetate 7: Chlroform 3 **Ethyl acetate** **Ethyl acetate 6: Chloroform3: Methanol 1**

Photo 10: Thin layer chromatography of *Scurrula parasitca L.* using different solvents

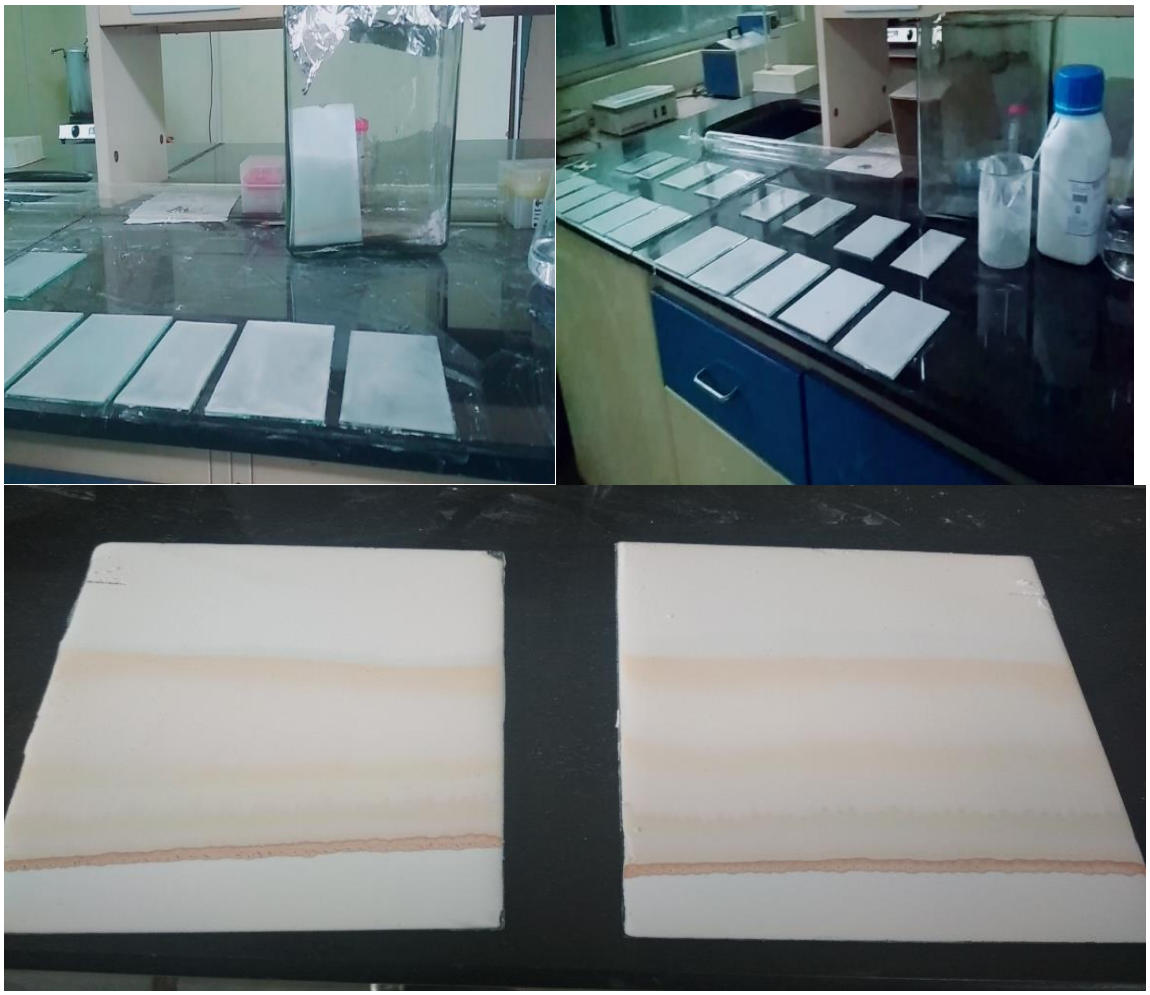


Photo 11: Preparative Thin layer chromatography

Photo 12: Separation and collection of each layer

5.2.4.3 Identification of the isolated bioactive Compound

From the TLC plates of *Scurrula parasitica L.* extract of the solvent system (Ethyl acetate 7: Chloroform 3), each layer was collected separately and purified. The 1st layer which displays the finest observable pattern and vibrant appearance was selected for LC-MS and HPLC for a bioactive compound.

Mass spectral analysis

Mass spectral analysis is used for the detection of non-volatile, pure compounds. There were many peaks, however, all the peaks did not support the isolated compound, moreover, those peaks were minor peaks, scattered from the major peak (302.2). The ESI-Mass spectrum of the sample (full positive scan mode) indicates the molecular peak at *m/z* 302.2 $[M+H]^+$. Further, the molecular weight of the isolated compound was incorporated with the NIST database and found to be 302.23 which is Quercetin. The compound was further confirmed by matching with NIST Database online library.

HPLC for confirmation of isolated bioactive compound

HPLC was accomplished with an Isocratic system (A) 60% Acetonitrile and (B) 40% Methanol (60: 40). The detection wavelength was 254 nm. The isolated compound was incorporated with standard Quercetin obtained from HiMedia (RM6191-25g). The compound was further confirmed by HPLC with standard Quercetin at a wavelength of 254 nm (RT 2.63). This was followed by TLC using a similar solvent system.

Thin Layer chromatography analysis for isolated compound

Finally, Quercetin and the isolated compound were eluted by the TLC method and the movement was observed up to a certain point. Both appeared at the same level with the identical R_f value and travelled up to 0.911 mm and had the same color display thus confirming the isoalted compound is Quercetin.

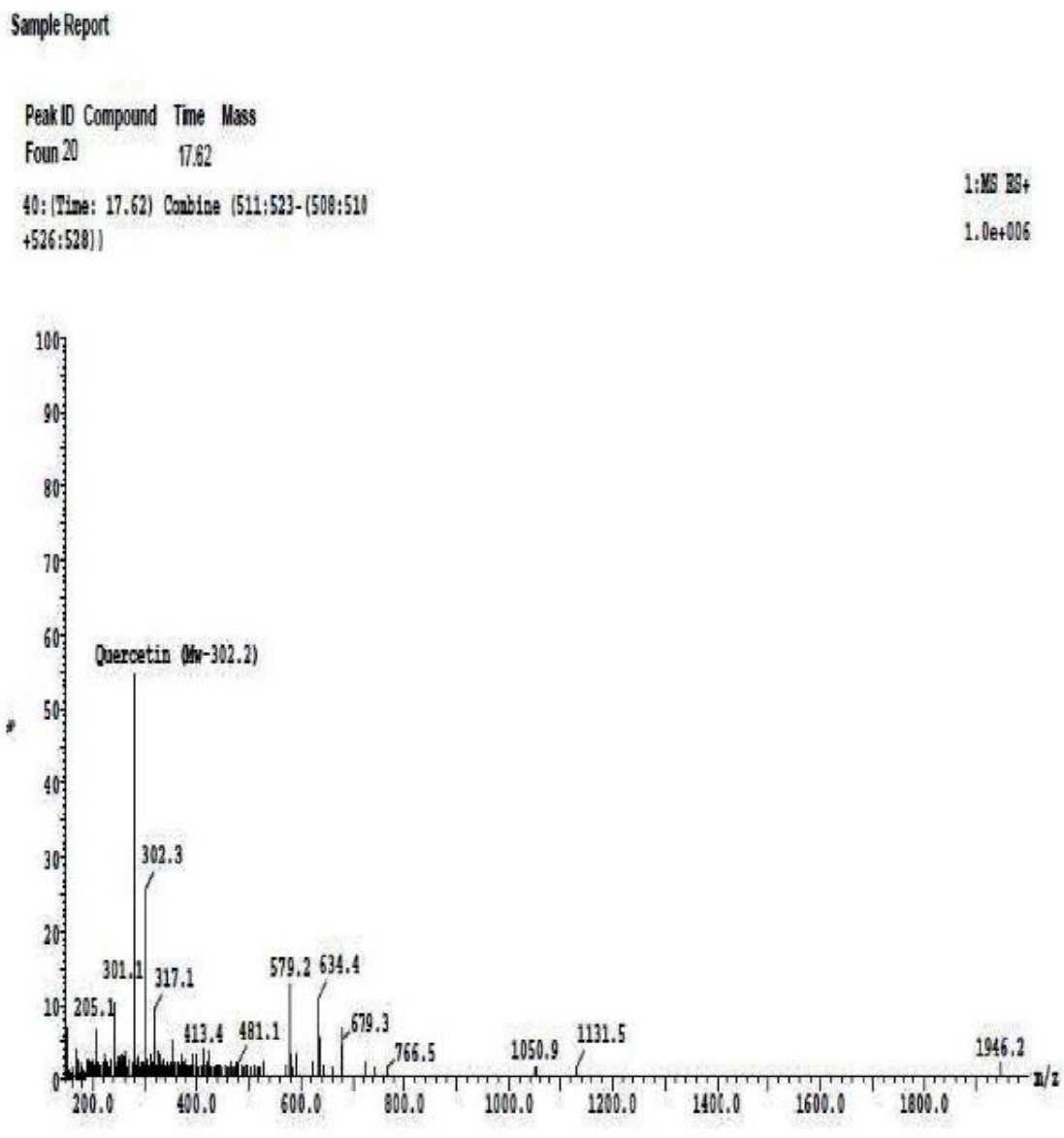


Figure 18: LC-ESI-MS/MS spectrum of the isolated compound from *Scurrula parasitica L.*

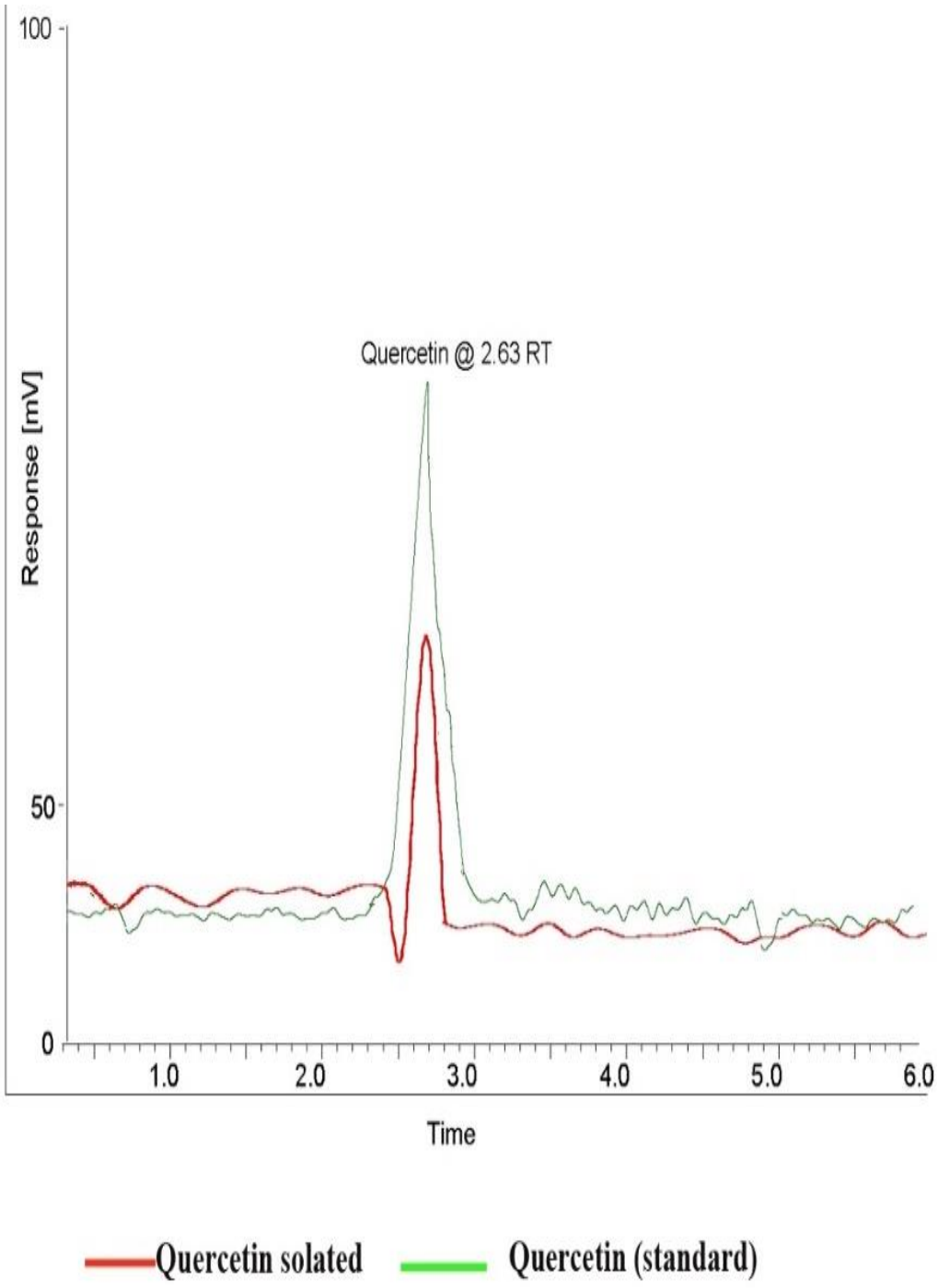


Figure 19: HPLC analysis of isolated compound from *Scurrula parasitica L.*

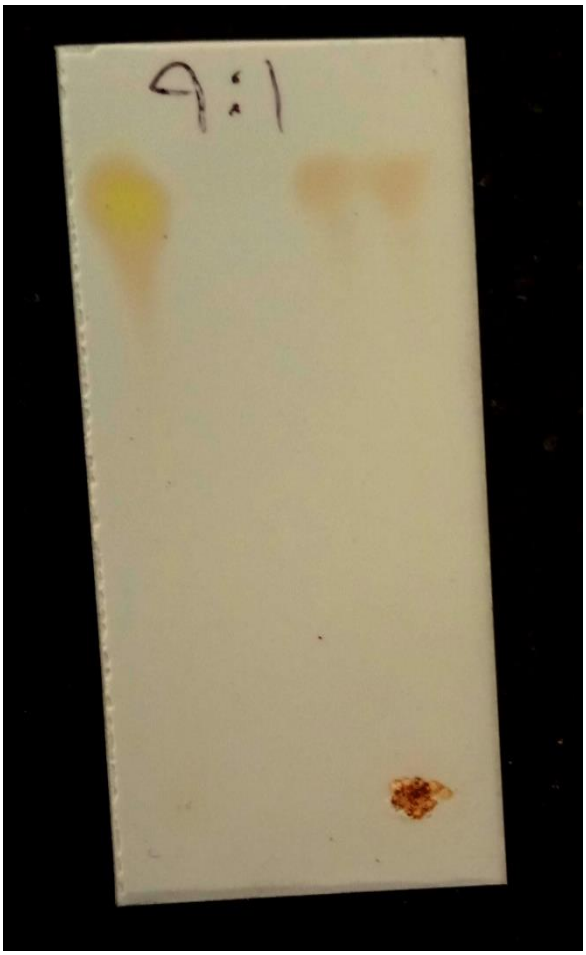


Photo 13: TLC analysis of isolated compound from *Scurrula parasitica L.*

DISCUSSION:

A family of plant-derived compounds that share similar flavone backbones (3 ringed molecules with hydroxyl groups attached to it) is called flavonoids. Substitutions of classes of flavonoids can occur giving rise to other subclasses and different compounds within the subclasses of flavonoids (Table 12).

Flavonoids are divided into the following subclasses:

Subclasses of flavonoids	Dietary flavonoids	Food sources
Flavanones	Eriodictyol, Naringenin	Oranges, lemons, grapefruits.
Flavonols	Myricetin, Quercetin	Apples, berries, teas, kale, broccoli, yellow onions, and scallions.
Isoflavones	Genistein	Legumes, soy foods, soya beans.
Anthocyanidins	Cyanidin	Blue, red and purple berries, red and purple grapes, red wines.
Flavanols	Dimers and polymers: Proanthocyanidins, Monomers (Catechins): Catechin, Epicatechin, Epigallocatechin gallate	Thearubigins: Tea (particularly black and oolong) Catechins: Green and white teas, grapes, apples, chocolate, berries Proanthocyanidins: Red grapes and wine, apples, chocolate, berries.

Table 12: Major subclasses of flavonoid

The functions of flavonoids include the coloration of flowers, protection of plants against insects and microbes (Griesbach, 2005; Bohm 1988; Yao *et al.*, 2004). Flavonoids also exist as aglycones (absence of attached sugar) or as glycosides (with glycosyl groups or sugar attached) (Ross and Kasum, 2002). These compounds have substantial roles in the growth of plants and their existence in environmental stresses. Flavonoids can endlessly fight against the polluted atmosphere and the reason why they

are considered significant reactive oxygen species scavengers. They also act as signal molecules to prevent microbial attacks from the plants. Flavonoids are operational in conditions like infrequent salinity, temperature stress, drought, and freezing injuries of the cell membranes. Flavonoids are bioactive and encourage the transport of important hormones like auxin, but they are subsequently not required for the survival of plants (Buer *et al.*, 2010). Flavonoids are found to improve adipose tissue metabolism and oxidative stress along with the signaling pathways and other inflammatory processes, reduce hyperglycemia, dyslipidemia, and insulin resistance, and in the modulation of carbohydrate and lipids (Bahadoran *et al.*, 2013; Jung *et al.*, 2004; Johnston *et al.*, 2005).

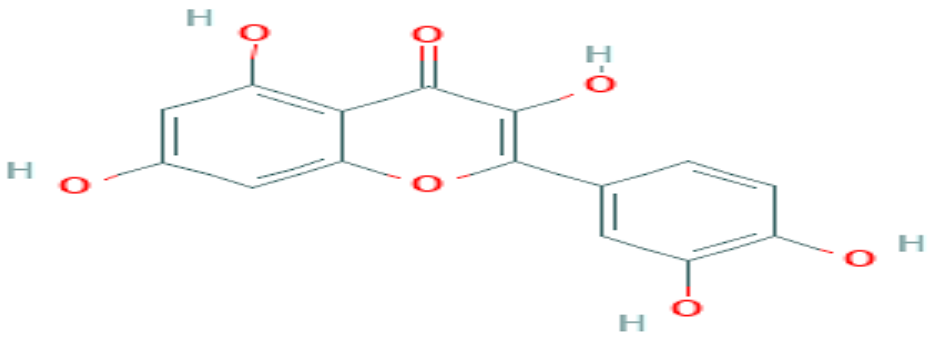


Figure 20: Chemical structure of Quercetin

IUPAC Name: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-Chromen-4-one

Molecular formula: $C_{15}H_{10}O_7$

Molecular Weight: 302.23 g/mole

Quercetin is characterized as a flavonol that belongs to one of the subclasses of flavonoid compounds. Quercetin is aglycone lacking an attached sugar. It is yellowish, hardly soluble in hot water, insoluble in cold water, and quite soluble in alcohol

solvents. Flavonols are present in a variety of fruits and vegetables and quercetin type flavonols are found in foods such as tomatoes, apples, berries, grapes, onions, tea, vegetables, capers, shallots. It is also found to be present on flowers, barks, leaves, and in many nuts and seeds. Quercetin is found to be present on well-known medicinal plants like *Hypericum perforatum, Sambucus canadensis*, and *Ginkgo biloba.* (Hakkinen *et al.,* 1999; Williamson and Manach, 2005).

Differential placement of attached sugar and phenolic -OH groups form several dietary flavonols. Quercetin along with the rest of flavonols possesses a common 3-hydroxy flavone backbone as shown in figure 19. The determination of quercetin as opposition to kaempferol or myricetin is based on the location of phenolic -OH groups. The nomenclature for quercetin as given by The International Union of Pure and Applied Chemistry (IUPAC) is 3, 3', 4', 5, 7 -pentahydroxyflavanone or its synonym 3,3',4',5,7-pentahydroxy-2-phenylchromen- 4-one. This signifies that quercetin has an OH group attached to it at 3, 3', 4', 5, and 7. Quercetin differs from kaempferol as the latter lacks the OH group at 3' position. Quercetin also differs from myricetin because the latter has an extra OH group attached to it at position 5'.

During diabetes mellitus, there is a reduction of antioxidants due to the increased production of free radicals (Bray and Bettger, 1990). The radical scavenging activities of diabetic patients are lower when compared to healthy individuals, which might be due to the lower concentrations of antioxidants (Anderson *et al.,* 2001). Investigations show that numerous plant products like flavonoids might serve as natural antioxidants to ameliorate the effects of hyperglycemia (Dias *et al.,* 2005). Flavonoids display strong antioxidant activity due to the presence of aromatic hydroxyl groups (Du Thie and Crozier, 2000) and therefore inhibit peroxidation as they scavenge nitrogen and reactive oxygen. Flavonoids help maintain glutathione in its reduced state to protect macrophages from oxidative stress (Fuhrman and Aviram, 2001) and inhibit enzymes like cyclooxygenases and protein kinases during cell proliferation and apoptosis (Estany *et al.,* 2007). Flavanoids also regulate the blood glucose levels, protect normal rat's islets

of Langerhans cells, and promote regeneration of â-cell of islets of Streptozotocin treated rats (Un *et al.*, 2006).

Quercetin has been recognized to lower blood glucose levels, preserve the integrity of pancreatic β-cells, standardize glucose tolerance tests and protect against several diabetes-declines in mood, cognition, and functions of renal in diabetic rats (Adewole *et al.,* 2006; Anjaneyulu *et al.,* 2003; Anjaneyulu and Chopra, 2004; Bhutada *et al.,* 2010; Khaki *et al.,* 2010). Quercetin given at a dose of 15 to 50 mg/kg body weight in rats was found capable of maintaining blood glucose level to normal, thus enhancing liver glycogen and reducing cholesterol and LDL concentrations in alloxan-induced diabetic rats (Zapolska-Downar *et al.,* 2006). Hii and Howell (1984) reported that the introduction of flavonoids to the islets of rats increases the release of insulin by 40 - 70% by altering calcium fluxes and in cyclic nucleotide metabolism. Quercetin efficiently blocks the accumulation of polyol in rat lenses incubated in a medium with high sugar concentration and oral administration of quercetin in the rodent Octodon degus results in a decrease of sorbitol accumulation and delayed the onset of diabetic cataracts (Varma *et al.,* 1977). In streptozotocin-induced diabetic rats, quercetin was also found to improve the condition of diabetic neuropathy and neuropathic pain (Anjaneyulu and Chopra, 2003; 2004).

This bioflavonoid has shown significant results in lowering blood pressure (Edwards *et al.,* 2007), endothelial functions (Loke *et al.,* 2008), and inflammations (Garcı´a-Lafuente *et al.,* 2009). Quercetin also displays beneficial effects in protein oxidation-reduction and LDL-C (Egert *et al.,* 2009; Pandey and Rizvi, 2010). Numerous investigations of quercetin in guinea pigs through oral administration or inhalation have shown anti-asthmatic activity (Joskova *et al.,* 2011; Jung *et al.,* 2007; Moon *et al.,* 2008). Intake of non-tea quercetin reduces the risk of developing colon however not rectal cancer (Kyle *et al.,* 2010). Supplementation of quercetin reduces the development of cardiac hypertrophy in rats (Han *et al.,* 2009). Quercetin induces a progressive and sustained reduction in blood pressure in rat models of hypertension and metabolic

syndrome (Perez-Vizcaino *et al.,* 2009). *In vitro* antibacterial activity of quercetin proved to be effective against microorganisms associated with the start and progression of periodontal diseases like *Actinomyces naeslundii* wvl, *Actinomyces viscosus, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum* and *Porphyromonas gingivalis* (Geoghegan *et al.,* 2009; Li and Xu, 2008). Quercetin demonstrates *in vitro* antiviral activity against Para influenza virus type 3, poliovirus type 1, HIV transcriptase, hepatitis C (Gonzalez *et al.,* 2010).

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CHAPTER 6
SUMMARIZATION AND CONCLUSION

The documentation and collection of medicinal plants were done through an ethnobotanical approach in selecting plants that were traditionally used by the tribal people of Mizoram for the treatment of diabetes. In this research through ethnobotanical explorations and responses from local correspondents, anti-diabetic medicinal plants were documented. The plant parts used such as leaf, root, stem, bark, fruit, seeds, or the whole plant for the herbal formulation were also recorded. It was reported that the mode of application of these pants was administered in the form of powder, paste, or decoction. The tribal inhabitants of Mizoram still largely depend on traditional herbal medicines for the treatment of diabetes due to their poor socioeconomic status and the high cost of modern medicines.

Plant extracts have been used for centuries as they contain all the active ingredients of plants, clinical trials have been carried out and are considered safe for human use and environmentally friendly. It is essential to investigate medicinal plants that are traditionally used for the management of diabetes. The phytochemical testing of these medicinal plants can help us signify the phytoconstituents present within them along with their mode of action against certain diseases and infections. These important constituents in plants play a key role in the development of synthetic drugs. Phytochemical screening reveals the presence of alkaloids, flavonoids, terpenoids, glycosides, tannin, saponin, reducing sugar, proteins, phytosterols in almost all of the 15 plants. *Scurrula parasitica L.*has the highest score amongst all the plants and was further processed for in-depth phytochemical studies in the current research.

Scurrula parasitica L. possesses ethnomedicinal properties confirmed by phytochemical screening and as a capable antioxidant agent, scavenging stable free radicals like DPPH and ABTS cations. Those compounds are widely used and essential ingredients in the pharmaceutical and development of new drugs. Therefore, *Scurrula parasitica L.* might provide such important resources for the development of new drugs

or be used as ethnomedicine locally. The antioxidant activity of plant extracts is likely due to the presence of polyphenolic compounds.

The extract of *Scurrula parasitica L.* shows significant results in lowering blood glucose, triglyceride, cholesterol, LDL, ALP, SGOT, and SGPT and increases the body weight and level of HDL after the 21-day experiment. The two doses of the plant extract substantially restored the blood glucose, bodyweight along with liver and kidney enzymes to normal level. 5 volatile compounds having anti-diabetic activities were detected by the GC-MS analysis and thus the plant was proceeded for isolation of compound to get a pure, non volatile compound.

Thin layer chromatography results indicate that the solvent system of Ethyl acetate 7: Chloroform 3 is the best solvent mixture for column chromatography. The peak of the isolated compound was identified by the mass spectrum with reference to the NIST Chemistry web book. Then confirmation was done with the HPLC method by calculating the retention time and peaks of the standard compound with the isolated compound. Verification of the isolated compound was done by thin layer chromatography along with the standard compound. The isolated compound was found to be quercetin, a flavonoid which has numerous pharmacological properties.

The use of medicinal plants serves as an alternative for the treatment of diabetes in Mizoram, as they are readily available from nearby surroundings and forests. The phytochemical constituents of these plants have proved their efficacy and the reason that they are frequently used by the tribal inhabitants of Mizoram. The state is blessed with a rich diversity of flora, and the medicinal plants which are recorded in this thesis have been effectively used for treatment of diabetes by the Mizo people and many more plants remains yet to be discovered. With the isolation of an active compound from *Scurrula parasitica L.* for the management of diabetes and associated with abnormalities in lipid profiles, this research will pave the way for future formulation of important drugs from plants for pharmaceutical and therapeutically important purposes especially in case of diabetes mellitus.

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2. National Symposium on Ethnobotanical importance in North East India held at Mizoram University, Aizawl on 13th to 15th October 2015.
3. National seminar on conservation and sustainable use of medicinal and aromatic plants in the Department of Forestry, Mizoram University, Aizawl, Mizoram.

List of publications :

1. Jerry Laldingngheta and Lalnundanga (2019). Phytochemical Analysis of Anti-diabetic plants of Mizoram, Northeast India. *In Medicinal plants of India: Conservation and sustainable uses*. Eds. Tripathi *et al.,* Today and Tomorrow`s Printers and Publisher, New Delhi. Pp.333-342.

2. Jerry Laldingngheta, Lalnundanga, Malsawmzuala and Lalhlenmawia H (2019). Evaluation of the anti-diabetic activity of ethanol extract of leaves of *Scurrula parasitica L.* in streptozotocin-induced diabetic rats. *Journal of Pharmacognosy and Phytochemistry*. 8(3): 2206-2212.

3. Jerry Laldingngheta, Lalnundanga and Awadhesh Kumar (2019). Ethnobotanical explorations of anti-diabetic plants used by the tribal inhabitants of Mizoram, India. *Environment and ecology*. 37 (B): 1032-1036.

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5. Jerry Laldingngheta, Lalnundanga and M Vabeiryureilai (2020). Phytochemical analysis and isolation of flavonoid compound from methanol extract of the leaves of *Scurrula parasitica L. International Journal of Pharmaceutical Sciences and Research*. 12(9): September issue (ACCEPTED).

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ABSTRACT

PHYTOCHEMICAL CHARACTERIZATION OF ANTI-DIABETIC PLANTS IN MIZORAM

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ABSTRACT

Diabetes mellitus is a syndrome described by chronic hyperglycemia, it is an escalation of glucose level in the blood because insulin is not produced in a sufficient amount in the human body or because of unresponsiveness of the cells to the insulin that is produced. It is one of the most prevailing diseases in the world. In 2010, around 285 million adults were suffering from diabetes and estimated to rise 7.7% of 439 million adults globally by 2030. It results in proteolysis, glycogenolysis, and lipolysis which lead to reduced weight of the liver and increased weight of kidney due to enhanced secretion of glucose and over the synthesis of glycogen, protein, lipogenesis and complications arising in the kidney and nervous systems. If hyperglycemia persists for a long period in the human body, it will be accompanied by long term problems and inappropriate functioning of the organs leading to damage of the heart, kidney, blood vessels and the eyes. In 1980, The WHO committees classify diabetes into two major classes: Insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). They are also termed as Type-1 and Type-2 diabetes, then in 1985, the names were changed to only IDDM and NIDDM. Type-1 diabetes cannot be managed, but type-2 can be prevented by maintaining normal body weights and exercising, which is beneficial for the heart and lungs as it helps insulin work better and lower blood glucose levels. Metformin has proved to be efficient for type-2 diabetes, as the mortality rate is low. The use of aspirin regularly is effective, although, in uncomplicated diabetes, there are signs of no improvements. Angiotensin-converting enzyme inhibitors (ACEIs) were found to improve the condition of diabetes while related drug angiotensin

receptor blockers (ARBs) proved to be inefficient. Type-1 diabetes patients are given mixtures of regular and NPH insulin, whereas for type-2 diabetic patients, a long-lasting formulation is added, during oral medications and the doses of insulin are increased according to the condition of diabetes.

Medicinal herbs remain the source of modern drugs and have served as a global basis for the health care system for many years. The bioactive compounds in plants are used for the treatment of diseases and ailments while they also act as the framework for the synthesis of modern conventional drugs, therefore plants remain an important source for drug discovery. Herbal remedies are the most effective therapy available for the rural people as they are easily accessible, low of cost and comes with no side effects. Investigations of medicinal plants from 2005 to 2010, highlighted around 100 plant-derived preparations with anti-diabetic actions. During this period, some of the plant formulations were marketed as a remedy for diabetes and a total of 85 bioactive compounds with anti-diabetic activity were isolated. This research on anti-diabetic plants is based on the phytochemistry of active compounds present in plants, with no knowledge of a target compound responsible for their pharmacological activities.

Ethnobotanical survey was done to identify anti-diabetic plants used by the tribal inhabitants of Mizoram for the management of diabetes along with the habitat and distribution of the plants, which was carried out through interactions with local practitioners and villagers. With the help of taxonomists, authentication was done and proceeded for phytochemical studies. A total of 36 were documented, out of which 15 plants were considered as the most effective in treatment against diabetes

by the local people. The most frequently used plants to control diabetes by the local people of Mizoram include *Mangifera indica L., Jasminum laurifolium Var., Glinus oppositifolia L., Scurrula parasitica L. and Citrus maxima Merr.* The plant parts used were mostly the leaves, barks and fruits in which they are prepared as decoction or infusion and are given to diabetic patients.

15 commonly used anti-diabetic plants were collected, dried and extracted successively using petroleum ether/ benzene for removal of fats, chloroform for removal of chlorophyll contents and ethanol/ methanol to create a pure crude extract. Then, phytochemical screening was carried out to detect the presence or absence of important metabolites like alkaloids, flavonoids, glycosides, tannin, saponin, reducing sugar, phytosterols, terpenoids and phenols. The preliminary screening reveals the presence of alkaloids, flavanoids, terpenoids, glycosides, tannin, saponin, reducing sugar, proteins, phytosterols in almost all of the plants. Out of the 9 metabolites with anti-diabetic potential tested for screening, the plants with good efficacies are *Mangifera indica L.* (7), *Jasminum laurifolium Var.* (7), *Mallotus roxburgianus Mull Arg.* (7), *Scurrula parasitica L.* (8) *and Citrus maxima Merr.* (6). The number of metabolites tested positive is highest in *Scurrula parasitica L.*, so the particular plant was proceeded to evaluate its antioxidant activity, anti-hyperglycemic activity and isolation of important anti-diabetic compound.

Scurrula parasitica L. was identified at Botanical Survey of India, Shillong (No: BSI/ ERC/ Tech/ 2017/ 43) and the herbarium was deposited and authenticated as voucher number MZU 742 in the Department of Environmental science, Mizoram

University. It belongs to the family Loranthaceae. The plant displays anticancer, antidiabetic, antihepatotoxic, antioxidant, immunomodulatory and cytotoxic activity.

Estimation of total phenols, flavonoid contents of *Scurrula parasitica L.* along with Antioxidant Activity with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and Free radical-scavenging capability by the use of ABTS•+ radical cation (ABTS assay) were performed. The total phenolic contents were found to be 101.9 to 379.1 (µg/g tissue) at 5 to 100 µg/ml concentration and the flavonoid contents were around 72 to 174.1 (µg/g tissue) at 5 to 100 µg/ml concentration. DPPH radical scavenging activity showed increases of activities with increased concentration manner and maximum activity was observed at 57.09% at 0.1 mg/ml concentration. The value of IC 50 was found to be considerably higher compared to ascorbic acid. The ABTS radical scavenging activity reported that the maximum was obtained at 25.63% and the IC 50 value was significantly lower than those of ascorbic acid. This indicate that the plant have proton-donating capability and might serve as potential free radical inhibitors.

The anti-diabetic activity of ethanol extract of the leaves of *Scurrula parasitica L.* was evaluated using streptozotocin-induced diabetic rats. White albino rats were induced with diabetes through intraperitoneal injection of streptozotocin at a dose of 40mg/kg body weight. After 72 hours, rats with moderate diabetes (fasting blood glucose >250 mg/dl) were employed for the study. The ethanol extract of *Scurrula parasitica L.* was given at 100mg/kg and 200mg/kg body weights and testing was done by urine test strip. *Scurrula parasitica L.* shows significant results in lowering blood glucose, triglyceride, cholesterol, LDL, ALP, SGOT, and SGPT

and increases the body weight and level of HDL after the 21-day experiment. The extract was also able to restore the body weight, blood glucose along with the kidney and liver enzymes back to normal level. The gas chromatography-mass spectroscopy analysis of the extract of *Scurrula parasitica L.* showed a total of 5 anti-diabetic compounds present in the plant and substantially be the reason for its anti-diabetic activities.

Finally isolation of anti-diabetic compound for methanol extract of *Scurrula parasitica L.* was performed using column chromatography and thin layer chromatography. Chromatographic techniques involves fractionation and isolation of compounds from pharmacologically active ethanol extract. Thin layer chromatography of methanol extract of *Scurrula parasitica L.* were performed with solvents like methanol, chloroform, methanol: chloroform, ethyl acetate, ethyl acetate: chloroform and ethyl acetate: chloroform: methanol to determine the mobile phase. A definite spot showing yellowish-red was detected in the solvent system of (Ethyl acetate 7: Chloroform 3) and (Ethyl acetate 7: Chloroform 2: Methanol 1) after keeping it inside an iodine chamber. But Ethyl acetate 7: Chloroform 3 displays the best result. Thus, it was selected to be used as the solvent system. Column chromatography was carried out and around 200 fractions were collected among which fraction no F-200 with TLC profile of ethyl acetate: chloroform (7:3) obtaining a single spot with an R_f value of 0.44 mm was selected. From the results of column chromatography, ethyl acetate 7: chloroform 3 was subjected to preparative thin layer chromatography for quantification of the compound.

The isolated compound were identified with refined analysis using HPLC, mass spectroscopy and the NIST database to help in clarifying structures of plant metabolites. First, the ESI-Mass spectrum of the sample (full positive scan mode) indicates the molecular peak at *m/z* 302.2 [M+H] +. Further, the molecular weight of isolated compound was incorporated with the NIST database and found to be 302.23 which is Quercetin. The compound was further confirmed by matching with NIST Database online library. Then, HPLC with an Isocratic system (A) 60% Acetonitrile and (B) 40% Methanol (60: 40) was performed. The detection wavelength was 254 nm. The isolated compound was incorporated with standard Quercetin obtained from HiMedia (RM6191-25g). The compound was further confirmed by HPLC with standard Quercetin at a wavelength of 254 nm (RT 2.63). Finally, Quercetin and the isolated compound were eluted by the TLC method and the movement was observed up to a certain point, both appeared at the same level. The molecular formula of quercetin is $C_{15}H_{10}O_7$ and it has been used as an anti-diabetic drugs for several years.

The phytochemical constituents of these commonly used anti-diabetic plants of Mizoram and the identification of the active compound will prove to be beneficial for the tribal inhabitants in formulation of drugs for treatment of diabetes and other diseases as well.