Phytochemical Evaluation of Some Potential Ethnomedicinal Plants of Nagaland (India) with Anti-diabetic Properties

Submitted By

Takhelchangbam Ibeeka Sharma

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DEPARTMENT OF BOTANY

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Nagaland University

(A Central University Estd. By the Act of Parliament No. 35 of 1989)

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30/10/2024

DECLARATION

I, Ms. Takhelchangbam Ibeeka Sharma, bearing Ph.D. Registration No. Ph.D./BOT/00376 Dated: 15/08/2019, hereby, declare that the subject matter of my Ph.D. thesis entitled **"Phytochemical Evaluation of Some Potential Ethnomedicinal Plants of Nagaland (India) with Anti-diabetic Properties"** is the record of original work done by me and that the contents of this thesis did not form the basis for award of any degree to me or anybody else to the best of my knowledge. This thesis has not been submitted by me for any Research Degree in any other University/Institute.

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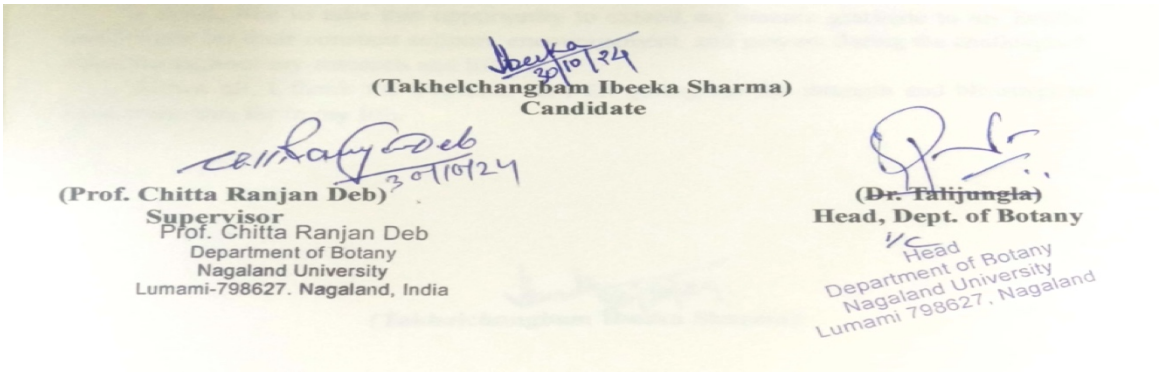

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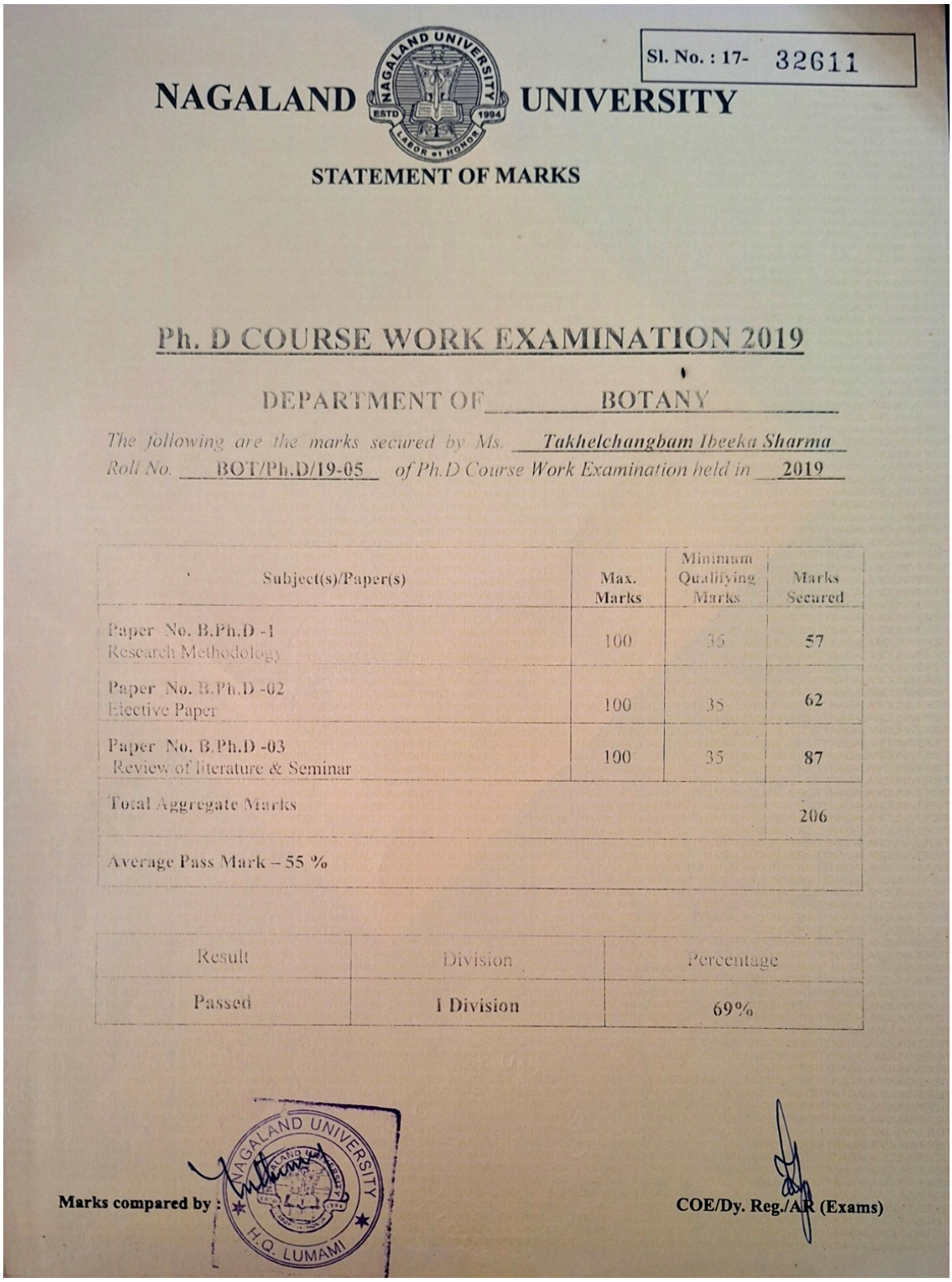
20/10/24

(Takhelchangbam Ibeeka Sharma)

Dedication

This Ph.D. thesis is dedicated to my family. To my parents and brother, thank you for your unwavering belief in me and constant support. Your encouragement has meant everything, and I am deeply grateful for your patience and for always pushing me to be my best, even during the most challenging moments during my research. I truly couldn't have achieved this without you. LOVE YOU ALL!

Ph.D. Coursework Marksheet



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Subject(s)/Paper(s)	Max. Marks	Minimum Qualifying Marks	Marks Secured
Paper No. B.Ph.D -1 Research Methodology	100	35	57
Paper No. B.Ph.D -02 Elective Paper	100	35	62
Paper No. B.Ph.D -03 Review of literature & Seminar	100	35	87
Total Aggregate Marks			206
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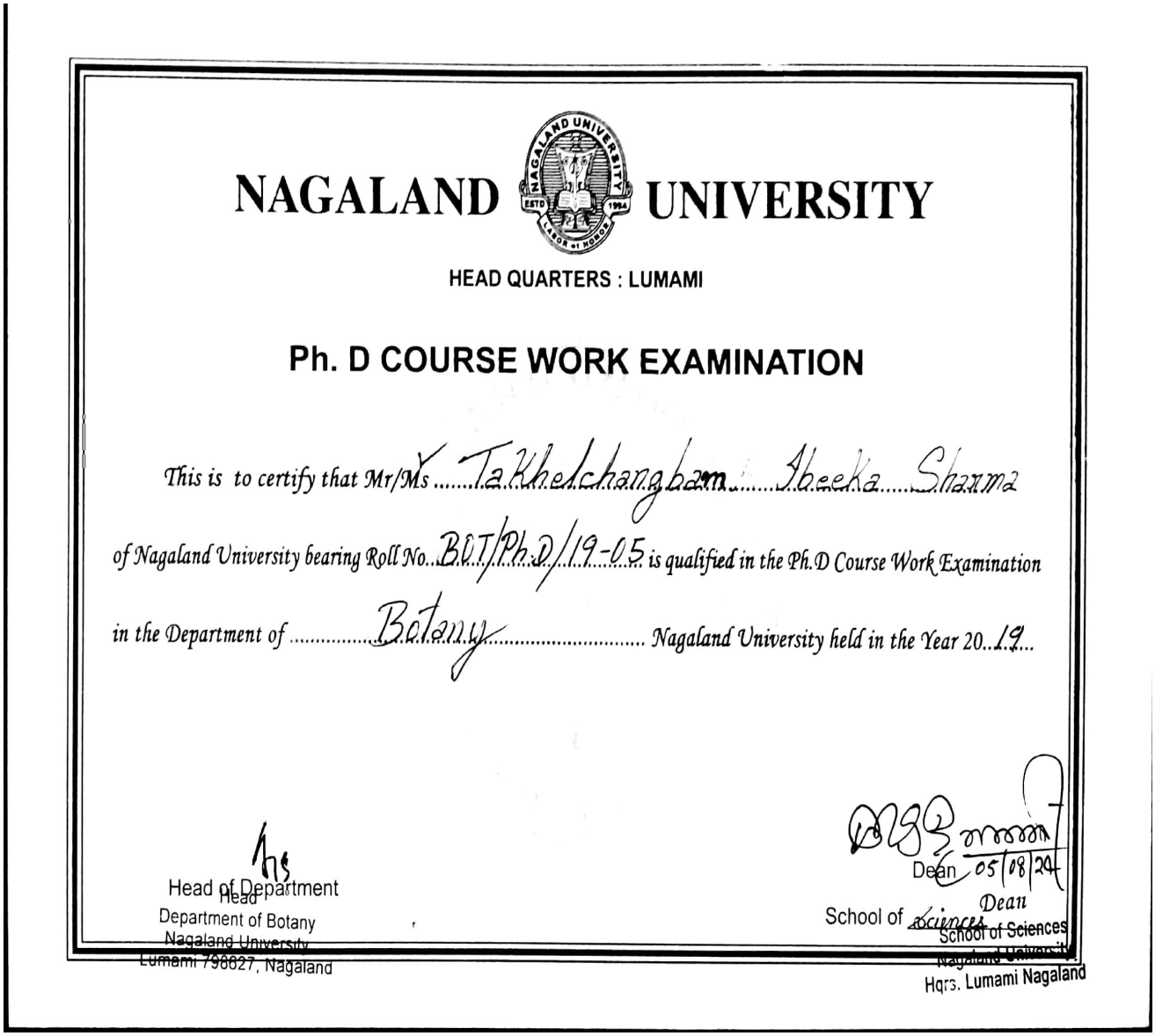

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Abbreviations

Abbreviation	Full Form
%	Percent
-	Negative
+	Positive
°C	Degree Celcius
µg	Microgram
µL	Microliter
α	Alpha
β	Beta
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt
AE	Atropine Equivalents
AGEs	Advanced Glycation End-products
AMPK	Activated Protein Kinase
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
BSA	Bovine Serum Albumin
$CaCl_2$	Calcium Chloride
CO_2	Carbon Dioxide
COI	cytochrome C Oxidase
Conc	Concentration
CTAB	Cetyltrimethylammonium Bromide
DM	Diabetes mellitus
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DNSA	3,5-Dinitrosalicylic Acid
dNTPs	Deoxynucleotide Triphosphates
DPP-4	Dipeptidyl Peptidase 4
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DW	Dry Weight
EtOH	Ethanol
FBS	Fetal Bovine Serum
FRAP	Ferric Reducing Antioxidant Power
G	Gram
GAE	Gallic Acid Equivalent
GC	Guanine-Cytosine
GD	Gestational Diabetes
GE	D-Glucose Equivalent
GLP-1	Glucagon-like Peptide-1
GLUT1	Glucose Transporter 1
GLUT4	Glucose Transporter Type 4
HCl	Hydrochloric Acid
HepG2	Human Hepatoblastoma Cell Line

HPA	Human Pancreatic α-amylase
HPTLC	High Performance Thin Layer Chromatography
hr	Hour
IC_{50}	Half Maximal Inhibitory Concentration
IDF	International Diabetes Federation
IR	Insulin Receptor
ITS	Internal Transcribed Spacer
M	Molar
mM	Millimolar
ml	Milliter
mg	Milligram
matK	Maturase K
MEGA	Molecular Evolutionary Genetics Analysis
MeOH	Methanol
min	Minute
ML	Maximum Likelihood
mtDNA	Mitochondrial Deoxyribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MUSCLE	Multiple Sequence Alignment
N	Normal
Na_2CO_3	Sodium Carbonate
NCCS	National Centre for Cell Sciences
NCBI	National Center for Biotechnology Information
NE	Northeast
nm	Nanometer
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
*p*H	Potential of Hydrogen
pH_2O	Pure Water
pNPG	P-Nitrophenyl-α-D-glucopyranoside
PPHG	Postprandial Hyperglycemia
QE	Quercetin Equivalent
rbcL	Ribulose-bisphosphate carboxylase/oxygenase large subunit
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
ROS	Reactive Oxygen Species
rpm	Revolutions per Minute
rpoC1	Ribonucleic Acid Polymerase C1
RSC	Reducing Sugar Content
SD	Standard Deviation
SE	Standard Error
SGLT2	Sodium Glucose Cotransporter-2
TAE	Tannic Acid Equivalent
TAC	Total Alkaloids Content
TFC	Total Flavonoid Content
TNF	Tumour Necrosis Factor
TPC	Total Phenolic Content

TPTZ	2,4,6-Tri-(2-pyridyl)-5-triazine
TTC	Total Tannin Content
TTRC	Total Triterpenoid Content
TZDs	Thiazolidinediones
UA	Ursolic Acid
UAE	Ursolic Acid Equivalent
v/v	Volume/Volume
VERO	Verda Reno (Green kidney)

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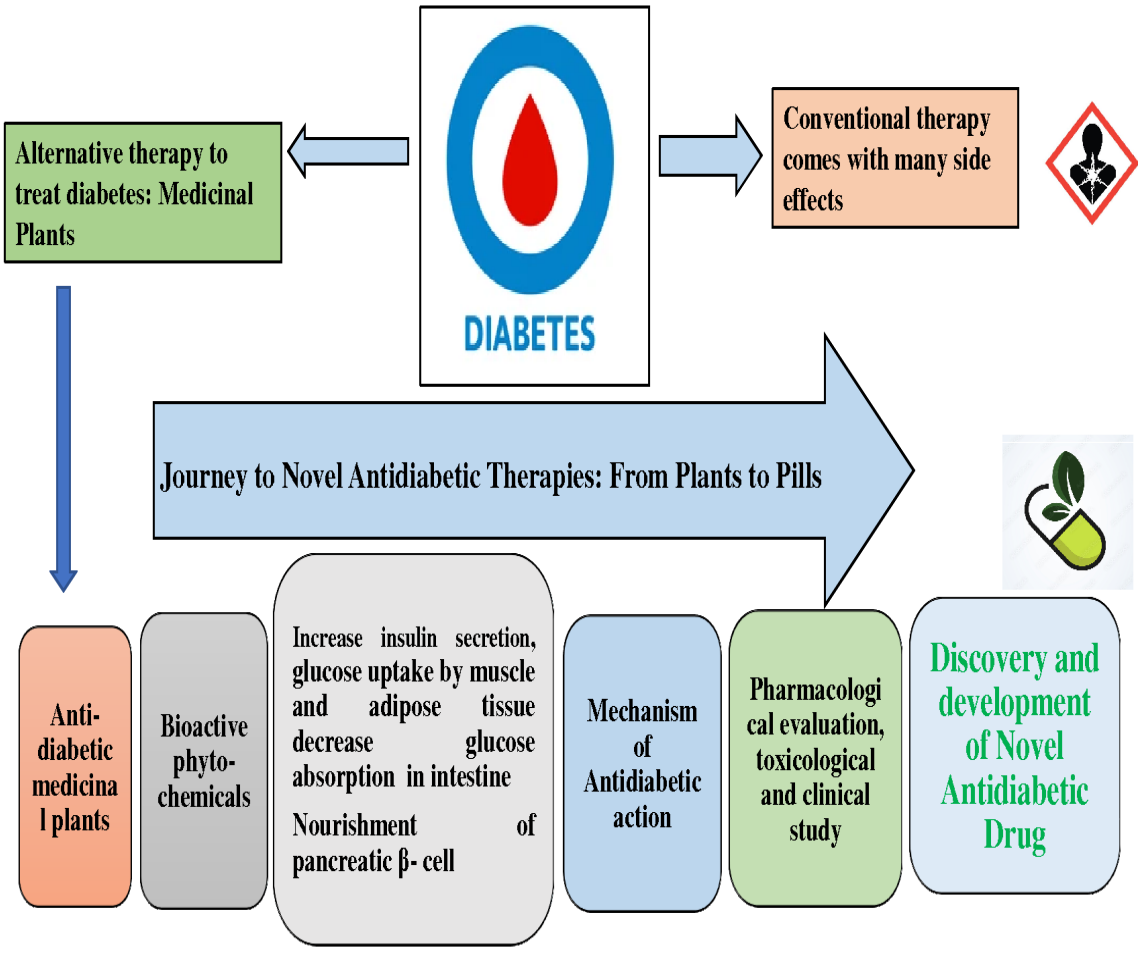
Chapter – 1

Introduction

Graphical Summary



Introduction

Diabetes mellitus (DM) is an incurable illness associated with dysregulation of carbohydrate, protein, and lipid metabolism, leading to insulin secretion anomalies or physiological dysfunctions (Kavishankar, 2011; Lovic et al., 2020). Elevated levels of blood glucose resulting from this dysfunction have the potential to adversely affect multiple physiological systems, specifically impacting blood vessels and nerve function. The chronic consequence of diabetes is the breakdown of essential organ systems like the cardiovascular system, vascular network, ocular structures, renal function, and neural pathways, ultimately resulting in death (Salehi et al., 2019). Diabetes Mellitus (DM) represents a costly and chronic disease prevalent in contemporary society, with its incidence escalating to epidemic proportions globally. This ailment stands out as among the most formidable medical conditions necessitating comprehensive medical attention for the enhanced care of diabetic individuals. The report from the International Diabetes Federation (IDF) in 2021 reveals that diabetes affects 537 million individuals across the globe, with expectations of reaching 783 million by 2045. India has the second largest population affected by diabetes after China, with approximately 77 million people living with the condition (Kangra and Singh, 2023). This number is expected to rise to over 134 million by 2045 (Kansra and Oberoi, 2023). Manifestations of diabetes encompass indicators such as weight reduction, excessive urination, high blood pressure, vision disturbances, extreme thirst, increased appetite, and rapid heart rate (Stratton et al., 2000). The source of this condition is determined by irregularities in the secretion of insulin from the beta cells of the pancreas, resulting in the disruption of glucose concentrations in the bloodstream (Rahman et al., 2021). Thus, the main focus and strategies for diabetes treatment centre on reducing postprandial hyperglycemia.

There are three main types of diabetes. Type 1, or insulin-dependent diabetes mellitus, is marked by a complete lack of insulin production (Daimari et al., 2019). In Type 1 diabetes, high blood sugar levels occur due to the destruction of pancreatic β-cells by the body's T-cells, leading to a loss of insulin. Biomarkers such as islet-targeting autoantibodies, 65 kDa glutamic acid decarboxylase, zinc transporter 8, and insulinoma-associated protein II can be detected before symptoms like excessive urination and thirst appear (Katsarou et al., 2017). In addition to genetic factors, environmental influences play a role in the development of Type 1 diabetes. Exposure to enteroviruses can lead to the destruction of β-cells, and early exposure to cow's milk protein is also linked to the onset of the disease (Akerblom et al., 2002). Type 1 diabetes is more common in children than adults and requires lifelong insulin administration (Atkinson et al., 2014). Although there is no cure, strategies for managing and preventing Type 1 diabetes include targeting T-cells, inducing β-cell tolerance, and β-cell replacement (Gillespie, 2006).

Type 2 diabetes, also known as non-insulin diabetes mellitus, involves impaired insulin secretion, insulin resistance, or both (DeFronzo et al., 2015). Contributing factors include lack of physical exercise, poor diet, obesity, and genetic predisposition (DeFronzo, 2009). The pancreas's β-cells struggle to produce insulin effectively. Patients typically develop prediabetes first, characterized by impaired fasting glucose, glucose tolerance, or elevated haemoglobin HbA1c levels (DeFronzo et al., 2015). Type 2 diabetes significantly increases the risk of microvascular complications like neuropathy and retinopathy, as well as macrovascular complications such as heart attacks, blindness, amputations, and renal failure. It can also lead to severe conditions like chronic liver disease, cancer, and accelerated arthritis (Inzucchi et al., 2012). Practical strategies to combat Type 2 diabetes include lifestyle changes to reduce weight, increasing physical activity, proper diet, and studying the molecular causes of the disease (Dansinger et al.,

2007). The impact on various organs is linked to elevated levels of C-reactive protein, interleukin-6 (IL-6), and tumour necrosis factor (TNF) in the blood (Wang et al., 2013). Treatment options for Type 2 diabetes include metformin, thiazolidinediones, insulin, alpha-glucosidase inhibitors, dipeptidyl peptidase-IV inhibitors, and insulin-releasing glucokinase activators. Other therapies include GLP-1 receptor agonists, SGLT2 inhibitors, and lifestyle interventions such as diet and exercise modifications (Olokoba et al., 2012).

In addition to Type 1 and Type 2 diabetes, some women develop gestational diabetes (GD) during pregnancy. Women with GD have a higher risk of developing diabetes later in life (Buchanan et al., 2005). The GD is often asymptomatic and usually resolves after pregnancy, but it can cause complications such as birth canal lacerations, fetal overgrowth, jaundice, perinatal mortality, cesarean delivery, and shoulder dystocia (Pettitt et al., 1980). Women with a history of GD are at increased risk for kidney, liver, cardiovascular, and retinal diseases, as well as Type 2 diabetes (Daly et al., 2018). To reduce the risk of GD, maintaining a proper diet and physical activity before the fifteenth gestational week is effective (Song et al., 2016). Insulin and metformin are primary treatments for managing GD. After delivery, regular diabetes screening, breastfeeding, and lifestyle interventions should continue (Buchanan et al., 2005).

Diabetes can lead to severe complications such as heart disease, stroke, kidney and nerve damage, blindness, and lower limb amputations, highlighting the need for urgent and effective treatments due to its widespread occurrence and diverse causes (Zakir et al., 2023). Globally, diabetes is a significant cause of morbidity and mortality. India, often called the "Diabetes Capital of the World", has one of the highest diabetes burdens, with over 77 million people affected (David et al., 2023). Type 2 diabetes is far more common in India compared to Type 1, driven by genetic factors, lifestyle choices, and a high-

carbohydrate diet. The country is seeing an alarming increase in young people developing Type 2 diabetes, often linked to obesity and unhealthy eating habits (Unnikrishnan et al., 2017). Diabetes-related complications like diabetic retinopathy and nephropathy are also on the rise. Additionally, India's healthcare system struggles to provide affordable and accessible care to its sizeable diabetic population (Prenissl et al., 2019). Recent statistics show that about 25% of Indian adults over the age of 25 have prediabetes, and the prevalence of diabetes in urban areas is nearly double that of rural areas, further stressing the need for widespread healthcare interventions (Vijayakumar et al., 2019). Several synthetic medications are utilised to manage diabetes, each with its unique set of side effects. Thiazolidinediones (TZDs) employed in the management of diabetes have been associated with the potential adverse effects of weight gain and increased peripheral adiposity. Various investigations and meta-analyses have pointed towards a plausible connection between the utilization of TZDs and elevated susceptibility to ischemic myocardial incidents among individuals with type 2 diabetes. Furthermore, the mechanism of action of TZDs includes the promotion of fluid retention through the augmentation of sodium and fluid reabsorption in the renal system, raising concerns regarding their appropriateness in the treatment of diabetes. Biguanides, such as metformin, are commonly prescribed insulin sensitisers for diabetes. Metformin improves glucose utilisation by acting on insulin receptors and glucose transporters in skeletal muscle and liver cells. However, it can decrease pyruvate dehydrogenase activity, leading to a rare but potentially fatal complication called lactic acidosis, particularly in patients with renal, pulmonary, or cardiac insufficiency or a history of liver disease (Kalsi et al., 2017). Meanwhile, sulfonylureas, which stimulate the pancreas to produce more insulin, can lead to hypoglycemia with symptoms like dizziness and confusion,especially among older peopleand those who frequently skip meals. These drugs are also linked to

increased cardiovascular risk, as they can cause the closure of myocardial K_{ATP} channels while stimulating insulin secretion by closing pancreatic K_{ATP} channels (Aquilante, 2010). Dipeptidyl Peptidase 4 (DPP-4) inhibitors can result in mild gastrointestinal problems, including stomach pain and diarrhoea. Glucagon-like Peptide-1 (GLP-1) agonists are effective at improving blood sugar control and promoting weight loss but can induce nausea and vomiting, particularly when first starting the medication. Lastly, Sodium Glucose Cotransporter-2 (SGLT2) inhibitors can increase the risk of urinary tract and genital fungal infections in some individuals, though they also offer cardiovascular benefits. The choice of diabetes medication should be tailored to an individual's specific needs, and close monitoring, along with lifestyle modifications, is vital to manage and mitigate these side effects effectively, all the while prioritising the benefits of blood sugar control (Sukhikh et al., 2023).

The risks associated with pharmaceutical drugs highlight the importance of alternative medicine, such as ethnomedicinal plants. These natural and minimally processed remedies offer a holistic and gentler approach to healing, reducing adverse reactions and providing a cost-effective option (Arumugam et al., 2013). Moreover, they have historically inspired modern drug development, with numerous pharmaceutical compounds originating from traditional plant-based remedies. This can be observed with the anti-hyperglycemic medication known as metformin. This drug, employed in managing diabetes, can be historically linked to the traditional usage of *Galega officinalis*, a plant utilised for addressing diabetes (Hachkova et al., 2021). Medicinal plants, in a general sense, play a significant role in the management of diabetes due to their various therapeutic properties and the diverse range of bioactive compounds they contain. Medicinal plants offer a natural and holistic approach to managing diabetes, which is often preferred by individuals looking for alternatives to synthetic medications.

These remedies are generally considered safer, with fewer side effects (Banerjee et al., 2020). Medicinal plants are rich sources of secondary metabolites such as flavonoids, alkaloids, polyphenols, and terpenes. These bioactive compounds can help manage diabetes by reducing inflammation and oxidative stress, improving insulin sensitivity, and regulating blood sugar levels (Shanmugam et al., 2021). As complementary therapies, they enhance the effectiveness of conventional treatments and improve overall health outcomes. Many medicinal plants contain compounds that boost insulin sensitivity, helping cells respond better to insulin and regulate glucose metabolism, thus preventing blood sugar spikes. Chronic inflammation, a common issue in diabetes, can be mitigated by the anti-inflammatory properties of these plants, protecting the body from damage (Rahimi et al., 2005). Additionally, medicinal plants are often rich in essential nutrients, contributing to overall health and well-being, which is crucial for individuals with diabetes who may be at risk for other health issues. However, it is essential to use medicinal plants under the guidance of healthcare professionals, especially when combined with synthetic medications. Thus, the identification and isolation of anti-hyperglycemic compounds from plants have become more and more important these days.

The utilisation of medicinal plants for diabetes treatment in India has a long-standing tradition, complementing the availability of modern pharmaceuticals. However, the evaluation of anti-diabetic medicinal plants against contemporary therapeutic standards still needs to be completed (Shukia et al., 2000). Indian Ayurvedic medicines count on various herbal plants for their accessibility, affordability, and minimal side effects. Many of these plants have been integral to the Indian diet (Momin, 1987). Indigenous plants have been employed in diabetes management in India since the 6th Century BC (Valiathan, 1998). India hosts four out of the 36 global biodiversity hotspots:

the Himalayas, Western Ghats, Indo-Myanmar region, and Sunderland. These Indian hotspots, benefiting from diverse climates, provide a habitat for numerous herbal remedies. Among these hotspots, the Himalayan regions extend into northeastern India. With a lower population density compared to other parts of the country, this area harbours undisturbed flora and fauna enriched with medicinal plants (Rama, 2013).

Antidiabetic Plants in Northeast India and Identification

Northeast India comprises Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, and Sikkim. The region, characterized by hilly terrain and limited plains, experiences a predominantly humid climate with intense monsoons and mild winters, fostering rich biodiversity. Northeast India is part of the Indo-Burma hotspot, the world's second-largest hotspot and a sanctuary for the nation's most endangered plant species. It boasts approximately 22 national parks and wildlife sanctuaries, showcasing unique flora and fauna. The region's diverse biodiversity is preserved by ethnic communities with distinct religious customs. Agriculture serves as the primary economic activity, with indigenous groups practicing Jhum cultivation and terrace farming, among others. The distribution of population in the area varies, with 397 individuals per square kilometre in Assam and 17 individuals per square kilometre in Arunachal Pradesh (Dikshit et al., 2014). With 209 tribes, 192 languages, and a blend of races and cultures, northeast India embodies a rich composite culture. Local tribes possess profound knowledge of utilizing biodiversity for sustenance, healthcare, and shelter. Sacred groves serve as reservoirs of biodiversity, harbouring a variety of plant and animal species, some of which may be rare or endangered. They act as genetic reservoirs and play a crucial role in the conservation of regional flora and fauna. The prohibition of tree felling and other destructive practices helps maintain the ecological balance, ensuring the sustainability of these ecosystems (Upadhyay et al., 2019). The

region's cuisine predominantly features natural ingredients and herbs sourced locally, promoting biodiversity conservation and a wholesome lifestyle. Traditional healers have long counted on plant-based solutions to tackle various health problems, imparting their wisdom through generations. Therefore, the comprehensive study of medicinal plants used by local healers in Northeast India holds significant importance for scientists aiming to harness their therapeutic potential and preserve their medicinal properties (Rama, 2013). The various ethnic tribes of NE India have a vast knowledge of identification and traditional ways of using herbal medicinal plants. For adding a new dimension to the management of the disease with phytomedicines, documentation and study of ethnic expertise are critical (Bhutani, 2008). A thorough, comprehensive review by Deb et al. (2023) compiles indigenous knowledge on plant-based treatments for diabetes, highlighting 407 recorded antidiabetic plants along with their details of family, local name, plant part used and ethnomedicinal use in different states of the NE region**.**

Nagaland, a state within Northeastern India, further exemplifies the region's rich biodiversity. It is bordered by Assam to its west, Arunachal Pradesh to the north, Sagaing region of Myanmar to the east and Manipur to the south. Its area is covered by tropical, sub-tropical forests and temperate hills. There are 16 districts in Nagaland *viz*., Chumoukedima, Dimapur, Kiphire, Kohima, Longleng, Mokokchung, Mon, Niuland, Noklak, Peren, Phek, Shamator, Tuensang, Tseminyu, Wokha and Zunheboto. The state is inhibited by 17 significant tribes: Angami, Ao, Chakhesang, Chang, Kachari, Khiamniungan, Konyak, Kuki, Lotha, Phom, Pochury, Rengma, Sangtam, Sumi, Tikhir, Yimchunger, Zeliang. Some other minor tribes include Garo, Mikir, Chirr, Makury, Rongmei and Tilch. The forests of Nagaland receive abundant rainfall (1,800 mm to 2,500 mm annually), which makes the state suitable for a wide variety of flora and fauna (Shankar et al., 2016). Over 80% of the population lives in remote rural areas and

depends on plant-based drugs for ailments of different diseases, including diabetes. In the past, many researchers have published numerous papers on the use of different plants and parts for the treatment of this deadly disease. From various journals on the use of ethnomedicinal plants by various tribes of Nagaland, 47 plants belonging to 29 families are reported to be used, especially as anti-diabetic (Deb and Sharma, 2021). The list of the plants is represented in Table 1.1.

Table 1.1: Ethnomedicinal plants used by the local healers and various ethnic tribes of Nagaland with their local name and part used as antidiabetic medicine

Sl. No.	Scientific Name	Family	Local Name	Habit	Part Used for Anti-diabetic	Method of use
1	*Abroma augustum* (L.) L.f.	Malvaceae	Ulatkambal	Shrub	Fresh leaf	Decoction of fresh leaf
2	*Albizia lebbeck* Linn. Benth	Fabaceae	Moang (Ao tribe)	Tree	Stem and its bark	Dried powder of stem and bark are boiled and extract are drink
3	*Asparagus racemosus* Willd	Asparagaceae	Pongijo (Phom tribe)	Climber	Roots	Decoction of roots
4	*Azadirachta indica* A.Juss	Meliaceae	Neem	Tree	Leaf	Extract of leaf
5	*Bauhinia variegata* L.	Caesalpiaceae	Alphabo(Sumi tribe)	Tree	Roots and bark	Extract of root and bark
6	*Cajanus cajan* (L.) Millsp.	Fabaceae	Chiopi (Zeliang tribe)	Herb	Leaf	Boiled leaf is drink as tea
7	*Catharanthus roseus* (Linn.) G. Don	Apocynaceae	Ampoknaro (Phom tribe), Supienaro (Ao tribe)	Herb	Leaves and flower	Decoction of leaves and flower
8	*Cassia alata* L.	Fabaceae	Dadmari	Shrub	Leaf	Decoction of leaf
9	*Cinnamomum tamala* (Buch-Ham) T.	Lauraceae	Tejpat	Tree	Leaves	Boiled leaves
10	*Cissampelos pareira* Linn.	Menispermaceae	Likhazung (Ao tribe)	Climber	Roots and leaves	Boiled extract of roots and leaves
11	*Clarodendrum colebrookianum* D. Don	Verbenaceae	Oremwa (Ao tribe)	Shrub	Leaves	Leaves are taken by simple boiling

12	*Coccinia indica* W. & A.	Cucurbitaceae	Kundru	Climber	Leaf and fruit	Leaf and fruit is consumed as vegetables
13	*Debregeasia longifolia* (Burm.f.) Wedd	Urticaceae	Natsulawa (Ao tribe)	Shrub	Leaf	Leaf decoction is taken orally
14	*Dicentra scandens* (D.Don) Walp.	Fumariaceae	Phubai (Ao tribe)	Climber	Tubers	Extract of tubers
15	*Dioscorea alata* Linn.	Dioscoreaceae	Achuchu (Sumi tribe)	Climber	Tubers	Extract of tubers
16	*Eclipta prostrata* Roxb.	Asteraceae	Bringaraja	Herb	Leaf	Leaf extract
17	*Emblica officinalis* Gaertn.	Euphorbiaceae	Jakhethi (Lotha tribe), Aonla (Chakhesang tribe)	Tree	Fruit	Fruit extract
18	*Eucalyptus globules* Labill.	Myrtaceae	Eucalyptus (Ao tribe)	Tree	Leaves and flowers	Leaves and flowers extracts
19	*Euphorbia hirta* L.	Euphorbiaceae	Laghudugdhika	Herb	Leaves and flower	Leaves and flower decoction
20	*Gynura crepidioides* Benth.	Asteraceae	Monglibaza (Ao tribe)	Herb	Leaf	Leaf decoction is taken orally
21	*Juniperus racemose* Risso.	Cupressaceae	Vapusa	Tree	Berries	Decoction of berries
22	*Kalanchoe pinnata* (Lam.) Pers.	Crassulaceae	Hohlongkak (Phom)	Herb	Leaves	Leaves decoction
23	*Melothria heterophylla* (Lour.) Cogn.	Cucurbitaceae	Hangkhapaitarere (Tangkhul –Naga tribe)	Climber	Fruits	Fruits is taken as vegetables
24	*Momordica balsamina* L.	Cucurbitaceae	Kora (Chang tribe)	Climber	Leaves and seed, fruits	Leaves as decoction, fruits and seeds as vegetables
25	*Momordica charantia* L.	Cucurbitaceae	Karela	Herb	Fruits	Fruits are taken by frying
26	*Momordica dioica* Roxb. Will	Cucurbitaceae	Bhat karela	Herb	Fruits	Fruits is taken as vegetables
27	*Morus alba* L.	Moraceae	Yong metiong	Tree	Leaf	Leaf is used as tea
28	*Mucuna pruriens* (L.) DC	Fabaceae	Mesener (Ao tribe)	Climbing shrub	Seeds	Seeds are used
29	*Ocimum*	Lamiaceae	Nangparan	Shrub	Leaf	Leaf decoction

	basilicum L.		gtong			
30	*Ocimum tenuiflorum* L.	Lamiaceae	Tulasi	Shrub	Leaf	Leaf decoction
31	*Oroxylum indicum* (Linn.) Benth. ex Kurz.	Bignoniaceae	Kakidziihe (Mao tribe)	Tree	Bark	Decoction of freshly peeled bark, dried peels of bark grounded and mixed with water
32	*Paederia foetida* L.	Rubiaceae	Ajungzu or Sunemli (Ao tribe)	Climbers	Whole plant	Whole plant is pounded into paste and the paste is taken orally
33	*Panax ginseng* C.A. Meyer.	Araliaceae	Tsudirmoz u	Herb	Roots	Dried roots powder is taken orally
34	*Passiflora edulis* Sims.	Passifloraceae	Bel (Angami tribe)	Vine	Leaf	Decoction of leaf
35	*Perilla frutescens* (L.) Britt.	Lamiaceae	Napa –tong (Ao tribe)	Herb	Leaves and inflorescence	Powder of dried leaves and inflorescence are drank with water
36	*Phylogacanthus thytyrsiflorus* Nees.	Acanthaceae	Tuo-mozu (Ao tribe)	Shrub	Leaves.	Tea of powdered dried leaves.
37	*Potentilla fulgens* Wall.	Rosaceae	Kijiichiini (Angami tribe)	Herb	Roots	Roots is tap and then eaten raw or decoction taken
38	*Punica granatum* L.	Puniaceae	Pomegranate, Jarem (Ao tribe)	Shrub	Fruits and seeds	Decoction of fruits and seeds mixed with pure honey is taken orally
39	*Scoparia dulcis* L.	Plantaginaceae	Mithipatta	Herb	Whole plant	Whole plant extract
40	*Solanum nigrum* L.	Solanaceae	Tiitsishe (Chakhesan g tribe)	Herb	Leaves	Leaves is boiled and taken along with extract
41	*Solanum trilobatum* L.	Solanaceae	Longkok (Ao tribe)	Herb	Leaf	Leaf extracts
42	*Solena heterophylla* Lour.	Cucurbitaceae	Bankundri	Climber	Roots	Roots decoction
43	*Syzygium cumini* (L.) Skeels	Myrtaceae	Jamun	Tree	Bark	Bark decoction

44	*Tamarinduss indica* L.	Fabaceae	Imli	Tree	seeds and leaf	Extract of seeds and leaf
45	*Terminalia chebula* Retz.	Combretaceae	Haritaki	Tree	Seeds	Extract of seeds
46	*Tinospora cordifolia* (Thunb.) Miers	Menispermaceae	Guduchii	Herbaceous vine	Stem	Extract of stem
47	*Zanthoxylum armatum* DC.	Rutaceae	Mongmang (Ao tribe), Ganya (Angami)	Shrub	Leaves and fruits	Leaves and fruits are chewed

Albizia lebbeck (L.) Benth., *Catharanthus roseus* (L.) G. Don, *Cissampelos pareira* L., *Clerodendrum colebrookianum* Walp., *Debregeasia longifolia* (Burm. f.) Wedd., *Eucalyptus globules* Labill., *Crassocephalum crepidioides* (Benth.) S. Moore, *Tithonia diversifolia* (Hemsl.) A. Gray, *Urtica dioica* L., *Zanthoxylum rhetsa* (Roxb.) DC. are ten medicinal plants used by the Chungtia tribe for the treatment of diabetes (Malewska, 2014). *Kalanchoe pinnata* is used by the Phom tribe as anti-diabetes (Jamir and Tsurho, 2016). Chang tribe use *Discentra scandens*, *Momordica balsaminas* anti- diabetic (Jamir and Tsurho, 2017). *Asparagus racemosus* Willdenow, *Catharanthus roseus* Linn. is used as an anti-diabetic by the Phom tribe (Imchen and Jamir, 2011). Angami tribe uses *Passiflora edulis* Sims., *Potentilla fulgens* Wall. Medicinal plants as anti-diabetic (Chase and Singh, 2013). *Panax ginseng* C.A. Meyer has also been reported to be used as anti-diabetic in Folk Medicinal Plants of the Nagas in India by Changkija (1999). Shankar et al. (2014) reported *Catharanthus roseus* (L.) G. Don., *Azadirachta indica* A. Juss., *Coccinia indica* W. & A., *Eclipta prostrate* (L.) L., *Momordica dioica* Roxb. Will., *Momordica charantia* L., *Ocimum sanctum* L., *Scoparia dulcis* L., *Syzygium cumini* (L.) Skeels., *Tamarindus indica* L. as anti-diabetic medicinal plants in conservation of some pharmaceutically important medicinal plants from Dimapur district of Nagaland. Local traditional healers and collectors for trading widely used these medicinal plants. Some of the cultivars are also practicing cultivation for some of the medicinal plants (Shankar et

al.,2014). While species like *Abroma augustum* (L.) L.f., *Bauhinia variegata* L., *Cajanas cajan* (L.) Millsp., *Cinnamomum tamala* (Buch-Ham.) T. Nees & C.H. Eberm., *Juniperus racemose* Risso., *Melothria heterophylla* (Lour.) Cogn., *Ocimum tenuiflorum* L., *Tinospora cordifolia* are the antidiabetic medicinal plants found in Kohima, Mokokchung, Tuensang and Zunheboto districts of Nagaland (Shankar et al., 2016). Sumi Naga tribe uses *Bauhinia variegata* Linn., *Dioscorea alata* Linn., and *Passiflora edulis* Sims. as remedies for diabetes patients (Sumi and Shohe, 2018). *Solanum nigrum* and *Emblica officinalis* are anti-diabetic medicinal plants used by the Chakhesang tribe of Nagaland (Bharali et al., 2017). Fruits extract of *Emblica officinalis* Gaertn. is used to treat diabetes by the Lotha tribe (Jamir et al., 2010). *Paederia foetida* L., *Phlogacanthus thtyrsiflorus* Nees., *Perilla frutescens* (L.) Britt., and *Punica granatum* L. are anti-diabetic plants used by the Ao Naga tribe (Jamir, 2012).

Thus, with the rising demand for herbal medicines and supplements, it also faces challenges like misidentification, inadequate cultivation, and long supply chains, which can lead to adulteration of plant ingredients. Ensuring the authenticity and quality of medicinal plants is essential for their safe and effective use despite these obstacles (Bhuyan et al., 2014). The taxonomic identification of plant groups mainly depends on morphological characteristics. However, this method has limitations when distinguishing plants at various developmental stages or identifying processed or fragmented remains (Naim and Mahboob, 2020). Morpho-taxonomic identification requires expertise and experienced taxonomists (Heinrich, 2007), and the wrong medicinal plant identification may lead to serious consequences. Traditional methodologies for species classification are predominantly based on morphological traits, which are susceptible to subjectivity and variability stemming from environmental conditions, developmental phases, and phenotypic flexibility. Precise identification is particularly critical in the realm of herbal

medicine, given that misclassification may result in the utilisation of inappropriate species harbouring potentially inefficacious or harmful properties (Fitzgerald et al., 2020). These challenges can be addressed adequately by utilising the DNA barcode technique for authentication along with morpho-taxonomic characters. Molecular profiling emerges as a more dependable and uniform approach to species differentiation, thus ensuring the accurate selection of plant species for medicinal applications. Besides morpho-taxonomic identification, DNA sequence based molecular characterisation of medicinal plants is crucial to ensure genuine herbal products, as substitutions within plant families can have serious consequences (Akinyemi and Ayodele, 2020). Recognising medicinal plants correctly is essential for their natural use and safety against adulteration. In the domain of processed, semi-processed, or powdered iterations of medicinal plants, the morphological characteristics are frequently indiscernible, thereby presenting a challenge in the differentiation of authentic species from adulterants or substitutes (Naim and Mahboob, 2020). Utilizing molecular identification techniques such as DNA barcoding facilitates the accurate categorization of species, even in these altered forms. Through the process of amplifying and sequencing specific DNA regions like ITS, *rbcL,* and *matK*, a molecular profile can be established for each species (Ismail et al., 2020). This methodology aids in the identification of adulterants and in ensuring the legitimacy of medicinal products. When morphological traits are absent or misleading (sexually dimorphic species), DNA barcodes offer a solution by assigning models to known species (Schindel and Miller, 2005; Ao et al., 2020; Deb and Kamba, 2022; Osman, 2024). Phylogenetic analysis using various molecular markers provides information about the evolutionary relationships between organisms (Patwardhan et al., 2014). Utilising Polymerase Chain Reaction, the dynamic mitochondrial genes containing interspersed highly conserved regions, particularly the 5' end of mtDNA cytochrome C oxidase

subunit I (COI), was identified by Hebert et al. (2003) as the optimal 'DNA barcode' for distinguishing diverse animal species across taxa (Ward et al., 2005). Due to the rapid changes in the structure of the mitochondrial genome of plants, the presence of a universally applicable intergenic spacer at the species level is hindered (Kress et al., 2005). Numerous credible studies involving plastid sequences in plants have led to the identification of several genes suitable as potential barcode markers for plants (Maloukh et al., 2017). Among such barcode markers, ribulose-1, 5-biphosphate carboxylase/oxygenase large subunit (*rbcL*) and *rpo*C1 have been recommended. Due to its conserved nature across most plant lineages, ease of amplification and sequencing, the *rbcL* gene is extensively utilised in plant phylogenetics to gain insights into evolutionary relationships among species and reconstruct phylogenetic trees at various taxonomic levels (Vijayan and Tsou, 2010; Thakur et al., 2016; Deb and Kamba, 2022). Molecular techniques such as DNA fingerprinting, DNA barcoding and Next Generation Sequencing are widely employed in plant classification and discovery. DNA barcoding, in particular, is a fundamental tool that leverages molecular genetics, sequencing technologies, and bioinformatics to classify and discover different plant specimens (Kress, 2017). It uses highly variable short regions of DNA for species identification. Universal DNA barcodes for plants, including key chloroplast gene regions like *rbcL* and *matK*, as well as the *trnH-psbA* intergenic spacer and ribosomal nuclear DNA's internal transcribed spacer (ITS), have been identified to enhance the precision of plant classification and facilitate the generation of extensive species data. Among these, the *matK* and ITS regions are especially recognized and widely used as primary plant barcodes (Ao et al., 2020; Deb and Kamba, 2022; Chac and Thinh, 2023; Osman, 2024). The ITS region is highly variable and evolves faster than other genomic regions, making it suitable for phylogenetic analysis at various taxonomic levels, including species and closely related

taxa (Baldwin et al., 1995). The *matK* gene is highly conserved within plant species, making it suitable for phylogenetic analysis and species identification (Selvaraj et al., 2008).

Over the centuries, the traditional healthcare system has been harnessing medicinal plants for treating various ailments like hypertension, diabetes, digestive problems, liver detoxification, etc. Nagaland is a state in the Northeastern region of India, and the state is a part of a biodiversity hotspot. The ethnic tribes of the state have extraordinary indigenous knowledge of the use of wild plants for the treatment of different ailments in rural areas (Sangtam et al., 2012; Bhuyan et al., 2014; Kichu et al., 2015; Singh et al., 2015; Temsutola et al., 2017; Ozukum et al., 2019; Deb and Sharma, 2021; Deb et al., 2009, 2023). Thus, traditional knowledge and practices of the indigenous communities in Nagaland have long relied on using plant-based remedies for managing different diseases, including diabetes and related conditions.

Antidiabetic Potential Phytocompounds

The emerging field of natural therapy offers promising strategies to mitigate DM. Natural compounds containing active ingredients, such as non-flavonoid polyphenols like tannins and lignans, as well as flavonoids like anthocyanins, quercetin, and rutin etc, have demonstrated potential anti-diabetic properties (Jubaidi et al., 2021). The majority of polyphenols and flavonoids exert their anti-diabetic effects by enhancing glucose control and insulin sensitivity, reducing oxidative stress, lowering levels of inflammatory cytokines, inhibiting the activity of α-amylase and α-glucosidase enzymes, and increasing tyrosine phosphorylation of the insulin receptor (Balbaa et al., 2021).

Numerous vitamins, minerals and secondary metabolites have been studied for their potential contributions to the management of diabetes both in laboratory settings and within living organisms. Secondary metabolites are organic compounds produced by

plants and microorganisms that are not directly involved in the growth and development of the organism but often serve various ecological and physiological functions and contribute to the management of hyperglycemia (Ota and Ulrih, 2017). Some of these secondary metabolites include flavonoids, alkaloids, Tannins, terpenoids, triterpenoids, etc. (Kandar, 2021). Flavonoids are a diverse array of naturally occurring compounds that exhibit a range of phenolic structures and are abundantly found in many fruits, vegetables, and plant-based foods. Flavonoids exhibit promising anti-diabetic effects through various molecular mechanisms. Past research explored the structural requirements for flavonoids with glycogen phosphorylase inhibitory activity, a crucial enzyme involved in glycogen breakdown into glucose in the liver (Kato et al., 2008). Matsuda et al. (2003) delved into the structural necessities for the suppression of Advanced Glycation End-products (AGEs) production, which is linked to persistent hyperglycaemia in diabetes. Increasing hydroxyl groups at specific positions in the flavonoid structure was associated with enhanced inhibition of AGEs. However, methylation or glycosylation of hydroxyl groups at specific locations reduced this inhibitory action. Interestingly, methylation of the hydroxyl group at C3 of the C-ring in flavonols appeared to increase its activity. These findings shed light on the structural determinants that influence the anti-diabetic properties of flavonoids (Shamsudin et al., 2022). Alkaloids such as Berberine are found in various plants, including barberry and goldenseal, while trigonelline is derived from fenugreek seeds. These alkaloids play a significant role in regulating metabolism by influencing key molecules such as AMP-activated protein kinase (AMPK) and glucose transporter 1 (GLUT1), contributing to improved glucose uptake and glycogen synthesis in the liver (Tang et al., 2017). These alkaloids regulate enzymes and AMP-activated protein kinase and enhance hexokinase activity, promoting insulin signalling, glycolysis and glucose utilization, thus

ameliorating carbohydrate metabolism (Muhammad et al., 2021). Tannins are found in various plant foods, including tea, berries, and nuts. Tannins have been associated with decreased feed intake and protein digestibility, but some studies suggest their potential to reduce blood glucose levels and inhibiting α-glucosidase activity involved in starch digestion, highlighting their possible anti-diabetic properties (Ardalani et al., 2021). Plants tannin are natural polyphenols, and they have high antioxidant activity (Ali Asgar, 2013). Triterpenoids like Ursolic acid are found in apple peels, basil, and other plants. Ursolic acid may enhance glucose uptake in skeletal muscles and adipose tissue, improving insulin sensitivity. Under triterpenoids, Oleanolic acid is found in various plants, including olive leaves and basil (Claro-Cala et al., 2022). Triterpenoids showed promising in both in vivo and in vitro anti-diabetic activity by targeting enzymes like α-glucosidase, α-amylase, and protein tyrosine phosphatase. These compounds enhance glucose metabolism, help prevent insulin resistance, and normalize blood glucose and insulin levels in various studies (Ardalani et al., 2021).

Antioxidants play a significant role in the management of diabetes due to their ability to counteract oxidative stress, which is a key factor in the development and progression of diabetes and its complications. Oxidative stress occurs when there is an imbalance between the production of harmful Reactive Oxygen Species (ROS) and the body's ability to neutralise them with antioxidants. In diabetes, elevated blood sugar levels can lead to increased ROS production, causing damage to cells, tissues, and DNA. Antioxidants help mitigate this oxidative stress by neutralizing ROS and reducing cellular damage (Rahimi et al., 2005). Antioxidants, particularly those found in fruits and vegetables, have been shown to protect pancreatic beta cells, which are responsible for producing insulin. Oxidative stress can harm these cells, reducing their ability to secrete insulin effectively, and the antioxidant helps preserve beta cell function, ensuring insulin

production remains adequate. Some antioxidants, such as quercetin and resveratrol, have been linked to improved insulin sensitivity. They enhance the body's ability to respond to insulin, allowing glucose to enter cells more efficiently. This helps maintain blood sugar levels within a healthy range (Khan et al., 2015). Antioxidants can also reduce chronic inflammation by neutralizing pro-inflammatory molecules, which may help improve insulin sensitivity and reduce the risk of diabetes-related complications (Rahimi et al., 2005). Diabetes can lead to damage in blood vessels (diabetic vascular complications) due to oxidative stress. Antioxidants help to maintain vascular health by reducing the impact of oxidative stress on the blood vessel walls, thus preventing complications such as atherosclerosis, nephropathy (kidney damage), retinopathy (eye damage), and neuropathy (nerve damage). Antioxidants improve endothelial function, which is essential for proper blood flow and regulation of blood pressure. Improved endothelial function can reduce the risk of cardiovascular complications in people with diabetes (Dal and Sigrist, 2016). Antioxidants found in a diet rich in fruits, vegetables, and whole grains can help maintain overall health. A balanced diet with a variety of antioxidants supports the immune system and reduces the risk of chronic diseases that can further complicate diabetes management. The ethnic tribes of the state have extraordinary indigenous knowledge of the use of wild plants for the treatment of different ailments in rural areas. Thus, traditional knowledge and practices of the indigenous communities in Nagaland have long relied on using plant-based remedies for managing different diseases, including diabetes and related conditions. It's worth noting that many traditional healing systems, such as Ayurveda and Traditional Chinese Medicine, have a rich history of incorporating medicinal plants into diabetes treatment, contributing to a valuable resource for managing this condition. For instance, Ayurvedic medicine has long recognized the potential of specific herbs like bitter melon and fenugreek seeds in helping to regulate blood sugar

levels (Wang et al., 2013). Phytochemicals derived from medicinal plants like *Bergenia ciliata*, *Mimosa pudica*, and *Phyllanthus emblica* have proven to possess notable antioxidant characteristics and the ability to inhibit enzymes such as α-amylase and α-glucosidase (Sapkota et al., 2022). These characteristics render them potent agents for suppressing digestive enzymes, thus presenting a viable approach to the management of diabetes. These medicinal plants are often abundant in secondary metabolites such as flavonoids, alkaloids, polyphenols, and terpenes, which possess a wide range of properties beneficial for diabetes management. These properties include reducing inflammation and oxidative stress, enhancing insulin sensitivity, and helping to regulate blood sugar levels. Additionally, these compounds serve as complementary therapies alongside prescribed medications, enhancing the effectiveness of conventional treatments and improving overall health outcomes.

The main concern and strategies for treating diabetes lie towards lowering postprandial hyperglycemia. This can be accomplished by targeting the enzymes that can inhibit carbohydrate hydrolyzation in our body, i.e. alpha-amylase and alpha-glucosidase. These two enzymes play a significant role in the digestion of carbohydrates (Nair et al., 2013). They act as primary digestive enzymes and aid in intestine absorption. α-amylase helps in breaking down the long chain of carbohydrates, and α-glucosidase helps convert starch and disaccharides into glucose. Thus, inhibiting the function of these two enzymes leads to slowing down the process of carbohydrate digestion (Reka et al., 2017). Currently, some common α-glucosidase inhibitors, such as Acarbose, Miglitol, etc., are available for treating diabetes, which also comes with various gastrointestinal side effects (Indrianingsih et al., 2015). Plants have been the source of drugs, and various ethnobotanical reports mention the potential of anti-diabetic in numerous plants. Medicinal plants represent abundant reservoirs of secondary metabolites, such as

flavonoids, alkaloids, polyphenols, and terpenes. These bioactive constituents exhibit diverse properties conducive to the management of diabetes, including the reduction of inflammation and oxidative stress, enhancement of insulin sensitivity, and regulation of blood glucose levels (Shanmugam et al., 2021). Comprehensive research has been carried out to study the bioactivity of inhibitors as they have important value in medical management. Various polyphenols that are present in plants can act as insulin by employing glucose and also inhibiting α-amylase, α-glucosidase and oxidative stress (Reddy et al., 2010; Balbaa et al., 2021). Numerous medicinal plants harbour compounds that enhance insulin sensitivity, thereby facilitating cellular responsiveness to insulin and consequently aiding in the regulation of glucose metabolism and prevention of glycemic fluctuations. Medicinal plants frequently abound in vital nutrients, thereby potentially enhancing overall health and well-being, a crucial aspect for individuals with diabetes vulnerable to other health afflictions.

Since diabetic patient have low insulin levels, their α-amylase level also tends to stay low while trying to keep the glucose level under control. Plants use α-amylase inhibitors to disrupt the digestive system of insects, inhibiting their feeding behaviour, and it thus acts as a defence mechanism for various plants (Jayaraj et al., 2013). Therefore, α-amylase inhibitors in plants have the potential to control blood sugar levels. α-glucosidase inhibitors function as competitive antagonists of α-glucosidase enzymes essential for the breakdown of carbohydrates. Within the small intestine, intestinal α-glucosidases breakdown complex carbohydrates into glucose and other monosaccharides (Nair et al., 2013). By inhibiting these enzymatic pathways, it contributes to a reduction in the speed of carbohydrate digestion. Consequently, a lower amount of glucose is absorbed as the carbohydrates remain undigested. For individuals with diabetes, the immediate impact of these enzyme inhibitor medications is a decrease in elevated blood

glucose levels (Ripsin et al., 2009). Hence, it is plausible to employ natural α-amylase and α-glucosidase inhibitors derived from dietary plants as a viable approach in the management of postprandial hyperglycemia while minimizing potential adverse reactions.

Along with studying the various phytochemicals present in antidiabetic medicinal plants and emphasizing the importance of their enzyme actions, it is also essential to analyse the cytotoxicity and cell line studies that play a crucial role in advancing the understanding and treatment of diabetes by providing valuable insights into the disease's pathogenesis and facilitating drug development. Cell lines have significantly advanced research on anti-diabetic treatments, aligning with the principles of the 3Rs (Replace, Refine, Reduce) through the provision of an alternative to animal experimentation (Antony et al., 2019). They facilitate a straightforward assessment of both synthetic and natural compounds regarding their potential in combating diabetes. The practice of cell culturing represents a dependable approach for investigating diseases, cellular harm, and underlying mechanisms, thereby aiding in the assessment of novel therapeutic interventions and the identification of molecular targets for drug development (Beydag-Tasoz et al., 2023). Despite the necessity of specific requirements such as media supplements and growth mediums to sustain them, cell culture remains essential in the realm of anti-diabetic investigations. Diverse cell lines sourced from the liver, bone marrow, and smooth muscles of animals such as rats, mice, and hamsters are utilized in the experimentation of novel medications and toxic substances (Antony et al., 2019). Categorized into normal, transformed, and stem cell lines, these cell cultures are cost-efficient and controllable, yielding pure populations that ensure consistent outcomes. They play a crucial role in the exploration of gene functionality, the creation of artificial tissues, and the production of biological substances. Nevertheless, issues like

contamination and variability within cell lines have the potential to impact findings, while disparities in both genotype and phenotype may result in discrepancies compared to primary cells (Goswami et al., 2010). Various cell lines are utilized in the investigation of anti-diabetic properties, each possessing distinct characteristics and uses. Pancreatic islet cell lines isolated from rats or mice are utilized to explore the mechanisms of insulin secretion. Cell lines that secrete insulin, such as HIT, MIN6, INS-1, RIN, and beta-TC cells, contribute to the study of beta-cell dysfunction and insulin release (Antony et al., 2019; Dash et al., 2023). RIN and INS-1 cell lines derived from rat insulinoma demonstrate similarities to pancreatic beta cells and play a crucial role in elucidating insulin secretion (Karatug and Adıguzel, 2023). Hamster pancreatic beta cells (HIT-T15) and the 3T3 cell line, specifically 3T3-L1 adipocytes, are employed in the examination of insulin signalling, adipogenesis, and glucose metabolism (Fonseca et al., 2023). The L6 cell line is employed as a representation of muscle myogenesis, while mesenchymal stem cell lines differentiate into various cell types, supporting research on anti-diabetic interventions (Cui et al., 2009). Hepatic cell lines, such as HEP G2, originating from human liver cancer cells, are essential for conducting in vitro investigations on liver function and cancer studies. Murine bone marrow-derived BMS2 cell lines contribute to research on multi-potent stromal stem cells, and the BC3H-1 cell line, derived from neoplastic mouse brain tissue, aids in studies related to muscle regulation and differentiation (Qiu et al., 2015). These diverse cell lines provide comprehensive platforms for enhancing our understanding of diabetes and the development of therapeutic strategies.

Assessment methodologies for assessing the anti-diabetic properties encompass various pivotal approaches. One such methodology includes theinvestigation of glucose intake that involves overseeing the absorption of glucose in cells stimulated by insulin

and 2-deoxy-D [3H] glucose, using a scintillation counter to locate 3H-glucose uptake (Antony et al., 2019). Glucose uptake assays are important in analyzing the potential antidiabetic impacts of plant extracts. Several studies have employed diverse approaches to evaluate the activity of glucose uptake. For example, a study on *Abutilon indicum* illustrated a notable enhancement in glucose uptake in 3T3L1 cells following treatment with methanolic leaf extract (Lavanya et al., 2022). Similarly, research on *Duranta repens* by Patil et al. (2022) emphasized an increased glucose uptake in yeast cells attributed to the presence of α-onocerin. Furthermore, the combined ethanolic extract of *Syzygium cumini* and *Psidium guajava* exhibited promising results in glucose uptake in L6 cell lines (Deepa et al., 2018). Moreover, compounds derived from *Salvia africana-lutea*, *Leonotiso cymifolia*, and *Plectranthus madagascariensis* demonstrated elevated glucose uptake in mammalian cells (Etsassala et al., 2020). Additionally, a screening of 41 plant extracts indicated that *Rhus coriaria* and *Pelargonium* sp. extracts stimulated glucose uptake, suggesting their potential as antidiabetic agents (Bashkin et al., 2021). A study by Rajeswari and Sriidevi (2014) in Kaempferol-3-o-α-l-rhamnoside and Apigenin-7-o-β-d-glucuronide from *Cardiospermum halicacabum* leaf extract also showed increased glucose uptake in L-6 cells, indicating potential antidiabetic properties. Kalekar et al. (2013) also conducted glucose uptake assays to evaluate the insulin-sensitizing effects using the 3T3-L1 adipocyte model and concluded the antidiabetic potential of *Phyllanthus emblica*, *Tinospora cordifolia*, and *Curcuma longa*. *Salvia miltiorrhiza* root extract containing salvianolic acid B (SAB) showed enhanced glucose uptake in 3T3-L1 adipocytes, indicating potential antidiabetic properties of the plant (Hu et al., 2014). Schreck and Melzig (2021) studied the methanolic and aqueous extracts from the fruits of *Aronia melanocarpa*, *Cornus officinalis*, *Crataegus pinnatifida*, *Lycium chinense*, and *Vaccinium myrtillus*; the leaves of *Brassica oleracea*, *Juglans regia*, and *Peumus boldus*;

and the roots of *Adenophora triphylla* and observed their effects on intestinal glucose and fructose absorption in $CaCO_3$ cells and the results showed significant and reproducible inhibition of glucose uptake between 40 and 80%. These diverse findings show the importance of glucose uptake assays in identifying plants with antidiabetic effects. Western blot analysis is also another assay in cell line assay, which is employed for molecular investigations involving the identification of proteins through separation, filtration, and centrifugation methodologies (Lafontan, 2008). The study by Okita et al. (2017) showed that modified western blotting can contribute to qualitative or quantitative analyses of diabetes-associated peptides by providing analytical information based on electrophoresis.

Another very important assay that plays a crucial role in the investigation of medicinal plants for diabetes treatment, aiding in the evaluation of the safety and therapeutic effectiveness of plant derivatives, is the Cytotoxicity assay (Kadan et al., 2013). The assessment involves examining how different plant compounds affect cell viability and growth, guaranteeing that the plant extracts utilized in diabetes therapy are both potent and harmless for human use. Research has shown that plant extracts employed in diabetes management can demonstrate cytotoxic properties at varying concentrations, signifying the necessity for precise dosage and administration (Vijayalakshmi and Selvaraj, 2018). They evaluated the cytotoxicity for antidiabetic plants *Sarcostemma brevistigma* using 3T3-L1 cell lines and concluded low cytotoxicity at 1 mg/mL concentration with promising glucose uptake activity. For instance, *Tectona grandis* bark has exhibited neuroprotective effects in high glucose conditions, indicating its potential in addressing diabetes-related nerve complications (Evi et al., 2015). Idris et al. (2022) investigated the total phenolic and flavonoid contents and antioxidant, cytotoxic and antidiabetic properties of *Rhodomyrtus tomentosa* leaf extracts and showed

that the methanol extract had cytotoxicity against MCF-7, HeLa, A549, and B16 cancer cell lines. Also, *Mundulea sericea* bark has presented notable cytotoxicity in cancer cells, implying that while it may have benefits in specific scenarios, its utilization must be closely regulated and supervised (Gangadevi et al., 2020). Madic et al. (2017) evaluated the cytotoxic effect of aqueous extracts of a traditional 'anti-diabetic' herbal preparation, as well as its constituents: *Rubus fruticosus*, *Vaccinium myrtillus*, *Potentilla erecta*, *Geumur banum* and *Phaseolus vulgaris* and showed concentration-dependent root growth inhibition and mitodepressive effects. To assess these impacts, assays like the MTT assay are usually utilized, which gauges cell metabolic function as a measure of cell viability and growth. This method aids in determining the cytotoxic levels of both herbal blends and individual plant extracts, offering crucial insights into their safety profiles. The significance of these discoveries cannot be overstressed. Recognition of cytotoxic effects is crucial for gauging the healing capabilities of anti-diabetic plants, enabling researchers to identify which extracts are reliable and efficient in diabetes management, thus directing further exploration and progress in herbal medicine (Kadan et al., 2013). Additionally, comprehension of the cytotoxicity of these extracts could lead to the recognition of fresh compounds with potential anti-diabetic attributes, ultimately contributing to the formulation of safer, more potent therapies for diabetes. Besides, the utilization of sophisticated strategies like high-throughput screening and bioinformatics can boost the identification and clarification of bioactive substances in plant-derived products. These methodologies facilitate the swift evaluation of numerous samples, offering a more thorough comprehension of their cytotoxic impacts and therapeutic possibilities (Abifarin et al., 2021). Thus,the assessment of cytotoxicity stands as a foundational element in anti-diabetic plant studies, ensuring that plant extracts utilized in diabetes care are secure and efficient, thus opening avenues for the creation of innovative

herbal treatments that can enhance the quality of life for diabetic individuals. Conclusively, to acquire a full comprehension of the therapeutic abilities of anti-diabetic medicinal plants, it is imperative to conduct thorough investigations on their molecular identification, phytochemical composition, enzyme inhibition properties, and cytotoxicity. These examinations are crucial for guaranteeing the safety, effectiveness, and appropriate application of these natural treatments in the management of diabetes.

Thus, the present work is a part of pharmacological studies on some anti-diabetic potential ethnomedicinal plants from Nagaland, India. This study focuses on the molecular authentication of selected ethnomedicinal plants with anti-diabetic potential using three different barcode genes: ITS, *rbcL*, and *matK*. Data from the molecular characterization of DNA sequences in these barcode regions will significantly contribute to existing genetic databases. These genetic sequences will serve as pivotal markers for future research endeavours, playing a crucial role in the validation and verification processes of medicinal flora. By expanding the repository, we are streamlining the identification processes of the scrutinized flora and supporting broader initiatives in preservation, pharmacognosy, and biodiversity exploration. The primary goal of this study was to investigate the properties of 15 anti-diabetic plants *viz*., *Abroma augustum* (L.) L.f., *Bauhinia variegata* L.,*Cajanus cajan* (L.) Millsp., *Catharanthus roseus* (L.) G. Don, *Senna alata* (L.) Roxb., *Clerodendrum colebrookianum* Walp., *Euphorbia hirta* L., *Gynura crepidioides* Benth., *Kalanchoe pinnata* (Lam.) Pers., *Mucuna pruriens* (L.) DC, *Paederia foetida* L., *Passiflora edulis* Sims., *Perilla frutescens* (L.) Britt., *Solanum nigrum* L., and *Solanum trilobatum* L. which are traditionally utilized by different tribes in Nagaland, India. We aimed to analyze the specific phytochemical compounds present in these plants and their antioxidant activity to understand why they have been employed as traditional anti-diabetic remedies. In our journey to study these plants for the

development of anti-diabetic drugs, we need to analyze and quantify the phytocompounds related to anti-diabetic activity and study their mechanisms of action. Following this, we can move forward to toxicological and various clinical trials, ultimately leading to the discovery and development of novel anti-diabetic drugs.

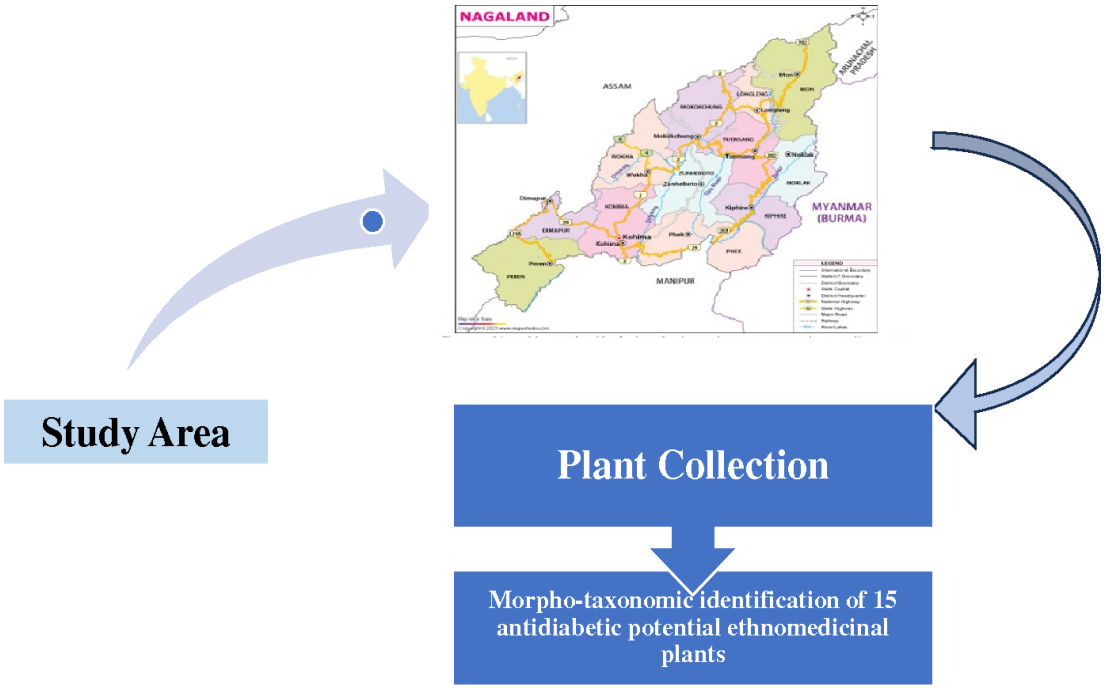
With these points in mind, the present work was undertaken for my Doctoral research with the following objectives:

- Collection of ethnomedicinal plants from Nagaland used as anti-diabetic medicine.
- Molecular characterization of the selected species.
- Phytochemical evaluation of the selected medicinal plants with potential anti-diabetic efficacy.
- *In vitro* evaluation of plant extracts for their α-amylase and α-glucosidase activities and determination of their modes of inhibition.
- Cytotoxicity evaluation and *in vitro* cell line assays of plant extracts.

Chapter – 2

Collection and Morphotaxonomic Identification of Selected Plants

Graphical Summary



Introduction

India is currently witnessing a notable surge in diabetes instances, with approximately 77 million adults diagnosed with diabetes as of 2021, according to the International Diabetes Federation (IDF) report 2021, positioning it as the country with the second-highest diabetic population globally, following China. The diabetes prevalence in India demonstrates significant discrepancies between urban and rural areas, with urban settings typically exhibiting elevated rates (Muralidharan, 2024). The prevalence of diabetes in Nagaland, akin to numerous regions of India, has been on the rise, although there might be constraints on specific and recent data availability. Nonetheless, it is plausible to present a comprehensive overview based on existing information, emphasizing the importance of verifying these statistics with the most recent studies and reports for precision. The precise prevalence figures for Nagaland are less frequently recorded compared to larger states; however, certain surveys and research works offer valuable insights. Kommoju and Reddy (2011) mentioned the necessity for more region-specific investigations in Northeastern states, including Nagaland, owing to lifestyle and genetic elements that could influence diabetes prevalence. A research initiative by the Indian Council of Medical Research (ICMR) carried out in northeastern states disclosed varying prevalence rates. For instance, the ICMR-INDIAB study indicated that diabetes prevalence in neighboring states like Assam and Manipur hovered around 5-7% in urban areas and 2-3% in rural areas (Pradeepa et al., 2015). It can be extrapolated that Nagaland might exhibit similar prevalence rates, warranting localized studies for validation. Surveys on health trends conducted by indigenous health agencies and non-governmental organizations also highlight an upward trajectory in lifestyle-related ailments, including diabetes, albeit specific figures are not consistently recorded or disseminated. The

Ministry of Health and Family Welfare and the National Family Health Survey (NFHS) sporadically encompass data on non-communicable diseases (NCDs), which encompass diabetes documented percentage of diabetics with controlled blood sugar varied from 2.7% (1.6–3.7%) in Nagaland to 11.9% (9.7–14.0%) in Tamil Nadu and was below the national average of 7% in 21 out of 36 states (Maiti et al., 2023). Transformations in lifestyle choices, dietary patterns, and levels of physical activity significantly contribute to the mounting prevalence of diabetes in Nagaland. The process of urbanization and the endorsement of sedentary lifestyles raise considerable concerns regarding the diabetes prevalence scenario (Park et al., 2020). For precise and current prevalence data, it is important to undertake localized investigations concentrating on disparities in prevalence rates between urban and rural regions, the influence of dietary practices and traditional lifestyles, and genetic susceptibilities specific to the native populations of Nagaland. While detailed and contemporary statistics regarding the prevalence of diabetes in Nagaland may be scarce, the available information indicates a similar upward trend as observed in other Northeastern states.

The increasing prevalence of diabetes, exacerbated by urbanization and sedentary lifestyles, stressing the pressing necessity to investigate efficient and enduring management approaches. One potential method is rooted in the application of ancestral medicinal wisdom handed down through generations. It is important to record the antidiabetic plants of Nagaland to safeguard traditional knowledge, validate medicinal attributes via scientific investigation, and advocate for the sustainable utilization and preservation of these botanical species. This record not only assists in safeguarding ecological variety but also fosters economic growth within local communities by facilitating the production of herbal remedies and medications. Furthermore, it offers a valuable healthcare asset for controlling diabetes using locally accessible treatments,

particularly in regions with restricted conventional medical services. Moreover, it functions as an educational instrument for students, scholars, and healthcare professionals, stimulating further research and advancements in ethnobotany, pharmacology, and traditional medicine.

The preservation and utilization of traditional knowledge by local healers shows the significant role of indigenous wisdom in the realm of healthcare. The recording and assimilation of this ancestral medicinal knowledge, especially in relation to antidiabetic plants, correspond with the customs of traditional healers in India who have historically utilized medicinal plants for diverse therapeutic purposes (Priyadharshana et al., 2022). The involvement of traditional healers in India in the application of medicinal plants for healthcare is pivotal, as evidenced by numerous research studies. These healers possess unique indigenous knowledge concerning medicinal flora, with research demonstrating the importance of traditional medicinal plants in addressing various health conditions. The traditional healers, who often serve as custodians of herbal medicine knowledge, have transmitted this wisdom through generations, thereby enriching the extensive reservoir of traditional medicine in India (Radha, 2022). The utilization of therapeutic plants in traditional healing practices is deeply entrenched in Indian culture, with roughly 3000 plant species recognized for their medicinal properties in systems such as Ayurveda, Homeopathy, Unani, and Siddha, among others (Sahu et al., 2013). The alignment of medicinal plants with the human physiological system honed over centuries, signifies the importance of harmonizing traditional healing methods with contemporary medicine to enhance healthcare outcomes in India (Anamika et al., 2023).

Nagaland, with its rich biodiversity and varied topography, is home to a plethora of medicinal plants that have been utilized by indigenous communities for centuries. The forests of the region are abundant with a wide array of flora, many of which are believed

to possess notable therapeutic properties. *Aconitum ferox* (Monk's Hood) and *Asparagus racemosus* (Shatavari) are among the prominent plants known for their analgesic, anti-inflammatory, rejuvenating, and fertility-enhancing attributes. *Dioscorea bulbifera* (Air Potato) holds significance as a traditional remedy for conditions such as fever and dysentery. Furthermore, *Rauvolfia serpentina* (Indian Snakeroot), recognized for its efficacy in managing hypertension, is prevalent in the area. Noteworthy is the utilization of *Momordica charantia* (Bitter Melon) for its established antidiabetic properties in regulating blood glucose levels, providing a natural approach to addressing diabetes. The transmission of knowledge regarding these plants spans across generations, constituting an essential component of the Naga culture and traditional medicinal practices. To safeguard this knowledge and to meet the increasing demand for medicinal plants, there is a need to explore medicinal plants with antidiabetic properties.

Materials and Methods

Survey Area

Nagaland, located in the northeastern region of India, is characterized by undulating terrain and verdant valleys. It shares borders with Arunachal Pradesh to the north, Assam to the west, Manipur to the south, and Myanmar to the east, encompassing an area of around 16,579 square kilometres (Figure 2.1). The topography is predominantly mountainous, featuring the Naga Hills reaching heights of up to 3,840 meters. Mount Saramati, the tallest summit in Nagaland, rises to 3,826 meters and is positioned along the Myanmar border. The state is intersected by a multitude of rivers and streams, with notable ones including the Doyang and Dikhu. Dense forests, covering approximately one-sixth of the state's land area, harbour a diverse range of plant and animal species. The climate in Nagaland varies from sub-tropical to temperate, characterized by copious

monsoon rainfall between May and September, which enhances the lush vegetation and agricultural output.

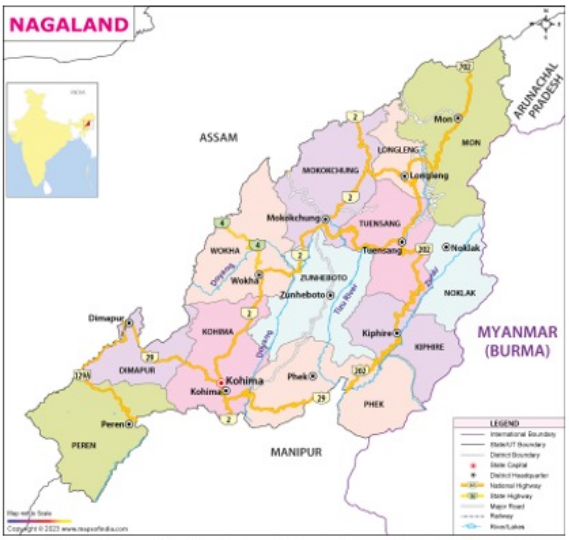


Figure 2A: Map of Nagaland showing the survey and sampling area. (https://images.app.goo.gl/DVHMP9RFT5RAZAXX6)

Figure 2.1: Map of Nagaland

Plant Collection and Identification

Fifteen ethnomedicinal plant specimens were gathered from diverse locations within Nagaland during their respective blooming periods in the year 2021. The process of identifying the plant samples involved a thorough examination of their vegetative, morphological, and reproductive attributes. Identification of the selected plant species was carried out in consultation with botanical experts and available botanical references, such as the "Checklist of Flora of Nagaland, 2017". Each plant specimen was assigned a unique herbarium accession number. Furthermore, The Plant List was consulted to authenticate the botanical nomenclature.

Results

The study on ethnomedicinal plants with anti-diabetic properties used by tribes of Nagaland, India, revealed significant findings regarding the traditional remedies employed by the local tribes. A systematic review of the literature identified a total of 47 plants from 29 families utilized as anti-diabetic agents among different tribes in Nagaland (Deb and Sharma, 2021; Shanker et al., 2016; Jamir et al., 2010; Sumi and Shohe, 2018). The research highlighted the popularity of herbal remedies in the state due to the perceived lower or nonexistent side effects associated with plant-based drugs. It was observed that herbs, particularly the leaf part of the plant, are predominantly used by the tribes in Nagaland. Among the identified families, Cucurbitaceae, Fabaceae, and Lamiaceae were the most commonly occurring with anti-diabetic properties. These families have been extensively utilized by the tribes for their medicinal benefits in managing diabetes. Each plant is identified by its local name, habitat type (including shrubs, trees, climbers, and herbs), parts used (such as leaves, roots, stems, and fruits), and specific methods of preparation and consumption (including decoctions, extracts, and teas). In districts like Kohima, Mokokchung, Tuensang, and Zunheboto, species such as *Abroma augusta* (L.) L.f., *Bauhinia variegata* L., *Cajanus cajan* (L.) Millsp., *Cinnamomum tamala* (Buch-Ham.) T. Nees & C.H. Eberm., *Juniperus racemose* Risso., *Melothria heterophylla* (Lour.) Cogn., *Ocimum tenuiflorum* L., and *Tinospora cordifolia, Dioscorea alata* Linn., and *Passiflora edulis* Sims. are used (Shankar et al., 2016). *Albizia lebbeck* Linn. Benth., *Catharanthus roseus* (Linn.) G. Don, *Cissampelos pareira* Linn., *Clerodendrum colebrookianum* D. Don, *Debregeasia longifolia* (Burm. f.) Wedd., *Eucalyptus globules* Labill., *Gynura crepidioides* Benth., *Tithonia diversifolia* (Hemsl.) A. Gray, *Urtica dioica* L., *Zanthoxylum rhetsa* (Roxb.) DC. represent a selection of ten medicinal plants that have been utilized by the Chungtia tribe for addressing diabetes. *Kalanchoe pinnata* is employed by the Phom tribe for its anti-diabetic properties (Malewska, 2014). The Chang tribe incorporates *Discentra scandens* and *Momordica*

balsamina in their anti-diabetic treatments (Jamir and Tsurho, 2017). *Asparagus racemosus* Willdenow and *Catharanthus roseus* Linnaeus are recognized by the Phom tribe for their anti-diabetic attributes (Imchen and Jamir, 2011). The Angami tribe specifically utilizes *Passiflora edulis* Sims. and *Potentilla fulgens* Wall. among other medicinal plants for their anti-diabetic effects (Chase and Singh, 2013). Additionally, *Panax ginseng* C.A. Meyer is documented to have anti-diabetic properties in the context of Folk Medicinal Plants of the Nagas in India, as observed by Changkija. Shankar et al. (2016) have documented the usage of *Catharanthus roseus* (L.) G. Don., *Azadirachta indica* A. Juss., *Coccinia indica* W. & A., *Eclipta prostrate* Roxb., *Momordica dioica* Roxb. Will., *Momordica charantia* L., *Ocimum sanctum* L., *Scoparia dulcis* L., *Syzygium cumini* (L.) Skeels., and *Tamarindus indica* L. as medicinal plants with anti-diabetic properties, within the framework of conserving various pharmaceutically important medicinal plants from Dimapur district of Nagaland. Among all the antidiabetic plants recorded from Nagaland, 15 were selected due to their popularity and widespread use among various tribes (Table 2.1). This chapter focuses on the morphological identification of these selected plant samples, while future studies will explore their molecular characterization, phytochemical analysis, enzyme inhibition, and cytotoxicity.

Table 2.1: Showing the distribution of 15 anti-diabetic ethnomedicinal plants used by the local healers and various ethnic tribes of Nagaland with its local name and part used as medicine along with their GPS coordinates from collection sites.

S.No.	Herbarium / Accession No	Sample Name	Family	Local name in Nagaland	Method of use	Latitude (N)	Longitude (E)	Elevation (m AMSL)
1	NU/BOT/IS-CRD/0006	*Abroma augustum* (L.) L.f.	Malvaceae	Ulatkambal	Fresh leaves decoction	26°13'47.31″	94°28'19.91″	998.8
2	NU/BOT/IS-CRD/0004	*Bauhinia variegata* L.	Fabaceae	Alphabo (Sumi tribe)	Extract of root and bark	26°13'30.80″	94°28'28.69″	1010
3	NU/BOT/IS-CRD/0007	*Cajanus cajan* (L.) Millsp	Fabaceae	Chiopi (Zeliang tribe)	Boiled leaf is drink as tea	26°13'1.73″	94°28'30.90″	952.5
4	NU/BOT/IS-CRD/0008	*Catharanthus roseus* (L.) G. Don	Apocynaceae	Ampoknaro (Phom tribe), Supienaro (Ao tribe)	Decoction of leaves and flower	26°13'0.08″	94°28'31.86″	957.3
5	NU/BOT/IS-CRD/0009	*Senna alata* (L.) Roxb.	Fabaceae	Dadmari	Decoction of leaves	26°13'52.78″	94°28'20.58″	980
6	NU/BOT/IS-CRD/0001	*Clerodendrum colebrookianum* Walp.	Lamiaceae	Oremwa (Ao tribe)	Leaves are taken by simple boiling	26°13'30.14″	94°28'32.33″	984.1
7	NU/BOT/IS-CRD/0002	*Euphorbia hirta* L.	Euphorbiaceae	Laghudugdhika	Leaves and flower decoction	26°12'59.8″	94°28'34.2″	954.5
8	NU/BOT/IS CRD/0010	*Gynura crepidioides* (Benth.) Moore.	Asteraceae	Monglibaza (Ao tribe)	Leaves decoction is taken orally	26°12'36.28″	94°28'31.73″	1037
9	NU/BOT/IS-CRD/0005	*Kalanchoe pinnata* (Lam.) Pers.	Crassulaceae	Hohlongkak (Phom tribe)	Leaves decoction	26°13'53.09″	94°28'21.07″	980.1
10	NU/BOT/IS-CRD/0003	*Mucuna pruriens* (L.) DC.	Fabaceae	Mesener (Ao tribe)	Seeds are used	26°12'35.35″	94°28'36.09″	1037.9
11	NU/BOT/IS-CRD/0011	*Paederia foetida* L.	Rubiaceae	Ajungzu or sunemli (Ao tribe)	Paste of whole plant taken orally	26°13'31.29″	94°28'24.53″	1022
12	NU/BOT/IS-	*Passiflora edulis*	Passifloraceae	Bel (Angami	Leaves decoction	26°13'49.55″	94°28'23.29″	981.1

	CRD/0012	Sims.		tribe)				
13	NU/BOT/IS-CRD/0013	*Perilla frutescens* (L.) Britt.	Lamiaceae	Napa –tong (Ao tribe)	Dried leaves and inflorescence powder taken with water	26°13'49.30″	94°28'23.59″	980.6
14	NU/BOT/IS-CRD/0014	*Solanum nigrum* L.	Solanaceae	Tiitsishe (Chakhesang tribe)	Boil leaves consumed	26°13'31.83″	94°28'22.83″	1028
15	NU/BOT/IS-CRD/0015	*Solanum trilobatum* L.	Solanaceae	Longkok (Ao tribe)	Leaf extracts	26°13'8.57″	94°28'40.98″	916.7

The morphological descriptions of the species are given below:

1. *Abroma augustum* (L.) L.f. (Synm. *Abroma augusta* (L.) L.f.) (Figure 2.2 a)

Family: Malvaceae

Local name: Ulatkambal

Part used: Fresh leaf

Herbarium / Accession No: NU/BOT/IS-CRD/0006

It is a shrub with a height of 3-5 m. The stems and leaves are covered with soft, bristly hairs. Leaves are ovate in shape with 20-30 cm long, and 10-15 cm broad. The flowers are purple in colour. Petals are five in number, and they fall off soon.

Method of use: Decoction of fresh leaf.

2. *Bauhinia variegata* L. (Figure 2.2 b)

Family: Fabaceae

Local name: Alphabo (Sumi tribe)

Part used: Fresh bark and roots

Herbarium / Accession No:NU/BOT/IS-CRD/0004

It's a flowering medium-sized tree of 10-12 m in height. The leaf has two lobes and a 15-20 cm obcordate shape. The flower is bright pink and white in colour, around 8-10cm in diameter, and has five petals. The fruit is in the seedpods with several fruits inside it.

Method of use: Extract of root and bark.

3. *Catharanthus roseus* (L.) G. Don (Figure 2.2 c)

Family: Apocynaceae

Local name: Ampoknaro (Phom tribe), Supienaro (Ao tribe)

Part used: Fresh Leaves and Flower

Herbarium / Accession No: NU/BOT/IS-CRD/0008

It's a perennial herb of 90 cm in height. Stem are erect, light green in colour and have flexible branches. Leaves are petiolate and opposite and 2-4cm wide and 3-9cm long. The flowers range from white with a yellow or red centre to dark pink with a darker red centre, with a basal tube 2.5–3 cm long and a corolla 2–5 cm diameter with five petal-like lobes. The fruit is a pair of follicles 2–4 cm long and 3 mm wide.

Method of use: Decoction of leaves and flowers.

4. *Senna alata* (L.) Roxb. (Synm. *Cassia alata* (L.) Roxb.) (Figure 2.2 d)

Family: Fabaceae

Local name: Dadmari

Part used: Fresh leaves

Herbarium / Accession No: NU/BOT/IS-CRD/0009

It is a shrub of around 3 m tall, stems are marked with leaf scars and has persistent stipules. Leaves are 50-80 cm long and leave close in dark. Leaves have 9-10 leaflets, oblong, asymmetrical rounded base and apiculate at apex. It has yellow inflorescence, and the fruit pod is around 25 cm long and dark brown in colour. The pod contains 50-60 flattened, triangular seeds.

Method of use: Decoction of leaf.

5. *Euphorbia hirta* L. (Figure 2.2 e)

Family: Euphorbiaceae

Local name: Laghudugdhika

Part used: Fresh leaves and flower

Herbarium / Accession No: NU/BOT/IS-CRD/0002

It is a herb of about 40-50 cm, and the stem is round and red in colour. It has white hair and produces white latex. Leaves occur in opposite pairs on the stem. Leaves are simple, hairy and have a dentate margin. The flowers are found in axillary cyme at each

leaf node. They lack petals and are generally on a stalk. The fruit is a capsule with three valves and produces tiny, oblong, four-sided red seeds.

Method of use: Leaves and flower decoction.

6. *Clarodendrum colebrookianum* Walp. (Figure 2.2 f)

Family: Lamiaceae

Local name: Oremwa (Ao tribe)

Part used: Fresh leaves

Herbarium / Accession No: NU/BOT/IS-CRD/0001

It's a shrub 2-3 m in height, branches are 4 angled when they are young. Leaves are simple and opposite, heart-shaped with pointed tips. Flowers are white and borne in 4-6-branched corymbose cymes at the end of branches. Fruit is a drupe with 4 1-seeded pyrenes, sometimes separating into 2 2-loculated or 4 1-locular mericarps.

Method of use: Leaves are taken by simple boiling.

7.*Gynura crepidioides* (Benth.) Moore (Synm. *Crassocephalum crepidioides* (Benth.) Moore.) (Figure 2.2 g)

Family: Asteraceae

Local name: Monglibaza (Ao tribe)

Part used: Fresh Leaf

Herbarium / Accession No: NU/BOT/IS-CRD/0010

It is a shrub of around 100 cm. Stem with rounded or fluted cross-section, solid, hairy. The stem, rather solid, is furrowed and covered with short and thick bristles in its upper part. The branches are covered with a dense pubescence. The leaves are simple, alternate, and very variable in shape. The lamina is generally oval or elliptical, 6 to 18 cm long and 2 to 5 cm wide, having 0 to 4 pinnated lobes more or less deep towards the base. The apex is acute to acuminate. The flowers are 9 to 11 mm long and 0.5 mm wide,

yellow in colour, dark orange-red at the tip, and rarely completely yellow. The anthers are purple. The style is long bifid.

Method of use: Leaf decoction is taken orally.

8. *Passiflora edulis* Sims. (Figure 2.2 h)

Family: Passifloraceae

Local name: Bel (Angami tribe)

Part used: Leaf

Herbarium / Accession No: NU/BOT/IS-CRD/0012

A vine with dense branches, leaves are usually 3-5 lobed to halfway, sub-orbicular-ovate, and the base of the leaves is cordate. Flowers are about 4 cm across, axillary, often solitary. Petals are slightly shorter than the calyx lobes. The fruit is berry, sub-globose, approximately 4 cm across.

Method of use: Decoction of leaf.

9. *Paederia foetida* L. (Figure 2.2 i)

Family: Rubiaceae

Local name: Ajungzu or Sunemli (Ao tribe)

Part used: Whole plant

Herbarium / Accession No: NU/BOT/IS-CRD/0011

A slender climber. Leaves opposite, elliptic-ovate, smell unpleasant. They grow 2–11 cm long with conspicuous stipules. Flowers are greyish-purple, and petals join to form a tube (corolla), with usually five spreading lobes. Fruit ellipsoids & are reddish; each fruit has two seeds that are black, roundish, and often dotted with white, needle-shaped crystals.

Method of use: The whole plant is pounded into paste, and the paste is taken orally.

10. *Kalanchoe pinnata* (Lam.) Pers. (Figure 2.2 j)

Family: Crassulaceae

Local name: Hohlongkak (Phom)

Part used: Fresh: Leaf

Herbarium / Accession No: NU/BOT/IS-CRD/0005

It is succulent and around 1m tall with a fleshy cylindrical stem and reddish in colour. The leaves are thick, fleshy, 10-30 cm long, with three to five pairs of fleshy limb lobes. The leaves produced bulbils. The fruits are follicles of 10–15 mm, which are found in the persistent calyx and corolla.

Method of use: Leaf decoction.

11. *Solanum trilobatum* L. (Figure 2.2 k)

Family: Solanaceae

Local name: Longkok or likok (Ao tribe)

Part used: Leaf

Herbarium / Accession No: NU/BOT/IS-CRD/0015

It is a slender prickly scrambling shrub, and its leaves are rounded-ovate in outline, obtusely 3-5-lobed, 2-7 cm long, 1-4 cm wide, with a few prickles along the petiole and midrib. Inflorescences extra-axillary, peduncle short, 3-9-flowered; pedicels 1-2 cm long, widely divergent. Fruits are globose around 15 mm in diameter, and seeds are 3 mm in diameter.

Method of use: Leaf extracts.

12. *Perilla frutescens* (L.) Britt. (Figure 2.2 l)

Family: Lamiaceae

Local name: Napatong (Ao tribe)

Part used: Leaves and inflorescence

Herbarium / Accession No: NU/BOT/IS-CRD/0013

It is an annual herb with hairy stalk. The leaves are opposite, 7–12 cm long and 5–8 cm wide, with a broad oval shape, pointy ends, serrated margins, and long leafstalks. The flowers bloom on racemes at the end of branches. Perilla seeds can be soft or hard, being white, grey, brown, and dark brown in colour and globular in shape.

Method of use: Powder of dried leaves and inflorescence are drunk with water.

13. *Solanum nigrum* L. (Figure 2.2 m)

Family: Solanaceae

Local name: Tiitsishe (Chakhesang tribe)

Part used: Leaves

Herbarium / Accession No: NU/BOT/IS-CRD/0014

A bushy or herbaceous plant growing up to 1.25 m tall. Stems are sparsely hairy and are rough in texture. The alternately arranged leaves (2-13 cm long and 1-8 cm wide) are borne on stalks 5-30 mm long. The small star-shaped flowers are borne in several-flowered clusters in the leaf forks near the tips of the branches. The flowers have five white or purple-tinged petals that are fused together at the base and a yellow central cone. The rounded fruit turns from green to dull black or purplish-black in colour when mature, and it contains numerous small seeds.

Method of use: Leaves are boiled and taken along with the extract.

14. *Cajanus cajan* (L.) Millsp (Figure 2.2 n)

Family: Fabaceae

Local name: Chiopi (Zeliang tribe)

Part used: Fresh leaf

Herbarium / Accession No: NU/BOT/IS-CRD/0007

It is a leguminous shrub of 2-3 m in height. The leaves are alternate and trifoliate, and the leaflets are oblong and lanceolate 5-10 cm long and 2-4 cm wide. The flowers are yellow in colour with purple streaks.The fruit is a flat, straight and pubescent pod, 5-9 cm long and 12-13 mm wide. It has 4-9 seeds and can be round to lens-shaped.

Method of use: Boiled leaf is drunk as tea.

15. *Mucuna pruriens* (L.) DC. (Figure 2.2 o)

Family: Fabaceae

Local name: Mesener (Ao tribe)

Part used: Fresh Seeds

Herbarium / Accession No: NU/BOT/IS-CRD/0003

It's a climbing shrub with long vines that reach up to more than 15 m. The plants are covered with fuzzy hairs. The leaves are tri-pinnate, ovate, or rhomboid-shaped. The flowers are arranged in axillary arrayed panicles, 15 to 32 cm long, and each has two to many flowers. The accompanying leaves are about 12.5 cm long. It has white, lavender or purple flowers. Its pods are about 10-20 cm long and are covered with loose white to creamish hairs that cause severe itching if they come in contact with skin. The seeds are round or flattened, uniform, ellipsoid, 1.0 to 1.9 cm long, 0.8 to 1.3 cm wide and 4 to 6.5 cm thick.

Method of use: Seeds are used.

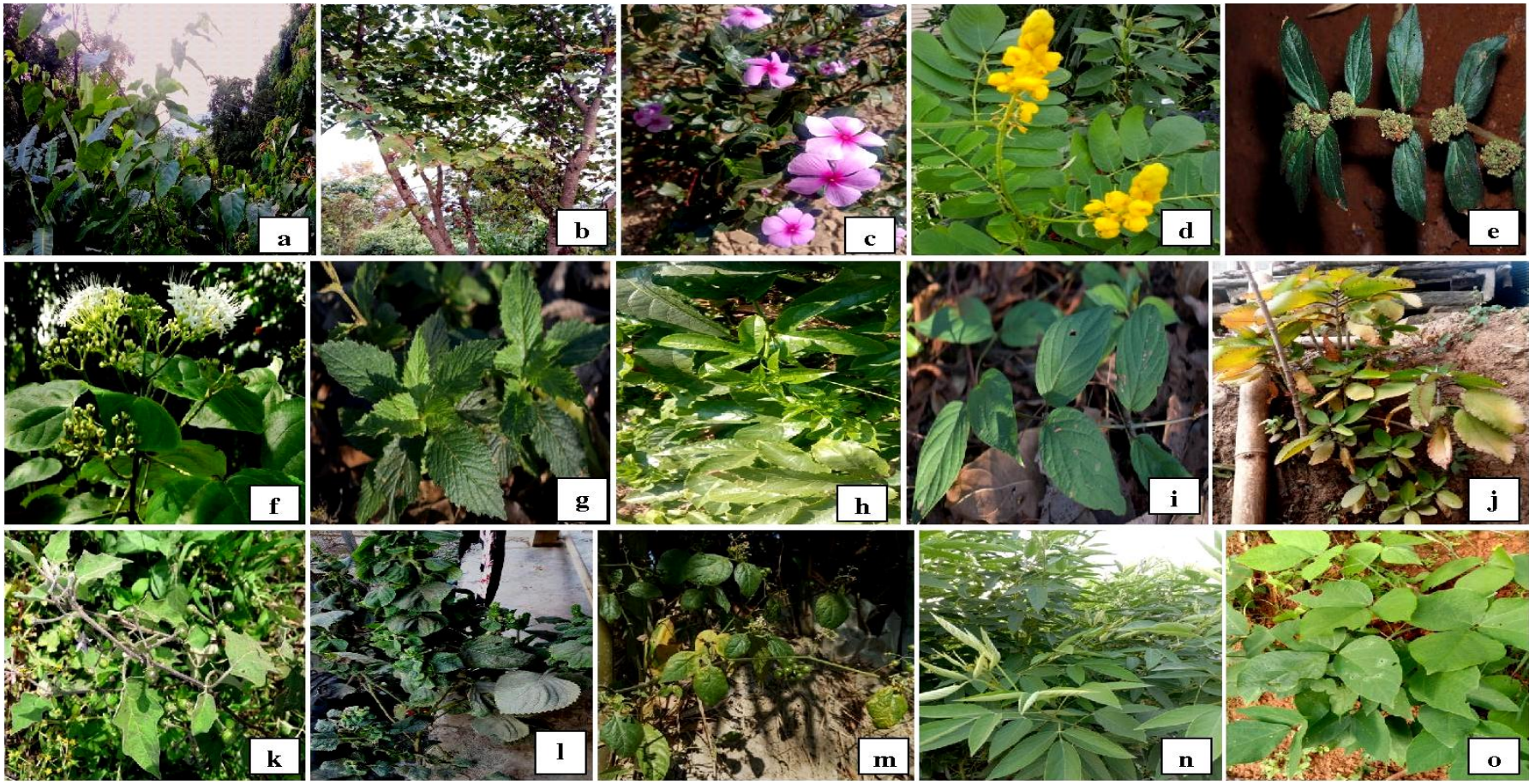


Figure 2.2: Selected anti-diabetic potential ethnomedicinal plants of Nagaland. a. *Abroma augustum*, b. *Bauhinia variegata*, c. *Catharanthus roseus*, d. *Senna alata,* e. *Euphorbia hirta*, f. *Clerodendrum colebrookianum*, g. *Gynura crepidioides,* h. *Passiflora edulis*, i. *Paederia foetida*, j. *Kalanchoe pinnata*, k. *Solanum trilobatum*, l. *Perilla frutescens*, m. *Solanum nigrum*, n. *Cajanus cajan*, o. *Mucuna pruriens*.

Discussion

The transmission of knowledge regarding the utilization of traditional medicinal plants from older generations to younger ones is currently at risk of extinction (Shanker et al., 2016). Thus, it is crucial to compile and document the ethnomedicinal uses before the irreversible disappearance of this valuable information. The use of different plant components, such as leaves, bark, roots, and entire plants, has been observed. One common treatment method involves preparing plant parts as decoctions using water. Additionally, some plants are consumed either raw or after cooking. Numerous studies have emphasized the ethnomedicinal significance and phytochemical analysis of diverse antidiabetic plant species across different regions globally. Even so, there is a deficiency of information about the molecular characterization and phytochemical analysis of native antidiabetic plant genera uncovered in Nagaland. Therefore, this study serves as a foundational platform for evaluating the biochemical properties and elucidating the potential mechanisms of anti-diabetic action exhibited by the aforementioned native plant species in Nagaland. The selection of plant parts is based on the empirical knowledge of local communities, necessitating scientific validation. Multiple tests have revealed anti-hyperglycemic effects in animal models induced with STZ, with particular plants displaying notable inhibition of alpha-glucosidase and alpha-amylase were also observed. *Abroma augustum* (L.) L.f. has been researched for its antidiabetic properties, with various phytochemicals, including taraxerol, flavonoids, phenolic compounds, abromine, and sterols, contributing to its antidiabetic effects (Khanra et al., 2015; Goswami et al., 2023). Studies have shown that *Abroma augustum* leaf extract displays substantial antidiabetic activity by lowering fasting blood glucose levels and enhancing serum biochemical markers in diabetic rats (Khanra et al., 2015). Moreover, the aqueous extract of *Abroma augustum* leaves has demonstrated the potential to mitigate biochemical

irregularities and histomorphological changes in alloxan-induced diabetic rabbits, indicating its efficacy as an antidiabetic agent (Hussain et al., 2013). These findings shows the promising role of *Abroma augustum* in diabetes management through its diverse phytochemical composition and positive effects on glucose metabolism and associated complications. *Bauhinia variegata* is rich in various phytochemicals such as kaempferol, hesperidin, and rutoside, which contribute to its antidiabetic properties (Kaushik et al., 2023; Kumari et al., 2023). These compounds have demonstrated hypoglycemic effects, some resembling insulin's function, thereby aiding in diabetes management. Additionally, the traditional use of *Bauhinia variegata* extracts in Ayurveda and other traditional medicine systems for their therapeutic benefits, including antidiabetic effects, bringing out its potential as a natural remedy for diabetes (Charu et al., 2022). Pigeon pea (*Cajanus cajan*) holds promise for both nutritional and medicinal applications, warranting further investigation into its bioactive properties (Dolly and Vinod, 2023). Research on *Cajanus cajan* has shown that its root extracts possess hypoglycemic and antihyperlipidemic effects through mechanisms such as inhibiting carbohydrate-hydrolyzing enzymes and enhancing methylglyoxal-trapping effects on advanced glycation end products formation (Shu et al., 2022). *Catharanthus roseus* (L.) G. Don contains antidiabetic alkaloids, including vindoline, vindolidine, vindolicine, and vindolinine, showcasing potential for managing diabetes and possessing antioxidant properties (Tiong et al., 2013). *Senna alata* (L.) Roxb is rich in phytoconstituents with antidiabetic properties, such as phenol, flavonoid, tannin, alkaloids, rutin, kaempferol, rhein, and luteolin (Thilak et al., 2023). Additionally, *Senna auriculata* (L.) Roxb, a related plant, is reported to have alkaloids, flavonoids, tannins, and other bioactive compounds with antidiabetic properties (Nille et al., 2021). *Clerodendrum colebrookianum* leaves have antidiabetic properties, and further studies are needed to

understand the mechanism and develop new drugs (Sarma, 2022). *Euphorbia hirta* is known for its medicinal properties, containing polyphenols, flavonoids, steroids, tannins, and alkaloids that exhibit antidiabetic effects (Ghosh et al., 2019; Chandel et al., 2023). Similarly, *Crassocephalum crepidioides*, a species of the Asteraceae family, is reported to have antidiabetic properties due to the presence of secondary metabolites like alkaloids, flavonoids, and phenolic compounds (Pierre et al., 2022). Both plants have been traditionally used in various regions for their pharmacological activities, including antidiabetic effects, making them valuable resources in the management of diabetes. *Kalanchoe pinnata* is also known to contain phytoconstituents such as alkaloids, flavonoids, and phenolic compounds that contribute to its antidiabetic activity (Quazi et al., 2011; George et al., 2018; Singh et al., 2021). On the other hand, *Mucuna pruriens*, also recognized for its antidiabetic effects, contains bioactive compounds like alkaloids, flavonoids, and phenolic compounds that play a role in managing diabetes (Oyinloye et al., 2023). Sumathy (2017) did a comparative study between *Solanum nigrum* and *Solanum trilobatum* and explored the antidiabetic potential of these plant's leaves along with phytochemical, antioxidant, and antimicrobial activities.Phytochemical analysis of *Solanum trilobatum* revealed chlorophyll, carotenoids, sugars, proteins, amino acids, and minerals, indicating potential antidiabetic compounds in the plant (Ahmed et al., 2016). Data relating to acute and chronic toxicity are in high demand to develop safe plant-based supplements and drugs against diabetes. There is a chance of using improper portions of the plants; thus, screening and evaluating biochemical constituents are essential.

Conserving medicinal plants presents numerous challenges due to a combination of ecological, economic, and social factors. Habitat destruction, driven by urbanization, agriculture, and deforestation, poses a significant threat to the natural environments where these plants thrive (Adla et al., 2022). Overharvesting, spurred by high demand for

traditional medicine and pharmaceutical use, further depletes wild populations. Inadequate regulation and enforcement exacerbate this issue, as unsustainable practices often go unchecked. Climate change also plays a critical role in altering ecosystems and affecting the growth and survival of these plants (Hounsou et al., 2024). Additionally, a lack of comprehensive scientific research on many medicinal species impedes efforts to develop effective conservation strategies. The erosion of indigenous knowledge, crucial for identifying and utilizing medicinal plants, compounds the problem, as does limited public awareness about the importance of plant conservation (Wani et al., 2021). Addressing these challenges requires a multifaceted approach, integrating habitat protection, sustainable harvesting practices, legal frameworks, scientific research, and community engagement. Sustainable harvesting of medicinal plants can be achieved through a variety of methods that balance the needs of human consumption with the preservation of plant populations. One effective approach is the implementation of regulated harvesting practices, which involve setting quotas and establishing seasons to prevent overharvesting (Marcelino et al., 2023). Harvesting techniques should be designed to minimize damage to the plant and its environment, such as selective harvesting, where only certain parts of the plant are collected, leaving the rest to regenerate. Cultivation of medicinal plants in controlled environments, such as botanical gardens or farms, can also alleviate pressure on wild populations (Schippmann et al., 2002). Additionally, community-based management, where local communities are empowered to oversee and manage harvesting activities, can ensure that traditional knowledge is integrated with conservation efforts, promoting both ecological and cultural sustainability.

Conservation strategies for medicinal plants must be comprehensive and multidisciplinary. In situ conservation, which involves protecting plants in their natural

habitats, is crucial for maintaining the ecological interactions and genetic diversity of these species. This can be supported by establishing protected areas, such as national parks and nature reserves, and by restoring degraded habitats. Ex-situ conservation, including the creation of seed banks and living collections in botanical gardens, provides a backup in case wild populations are threatened (Bhaskar, 2024). Research and monitoring are essential components, enabling the identification of at-risk species and the development of effective conservation plans. Public education and awareness campaigns can foster a sense of stewardship and encourage sustainable practices. Collaborations between governments, non-governmental organizations, scientists, and local communities are vital to ensure the long-term conservation of medicinal plants (Shukla, 2023).

Conclusions and Summary

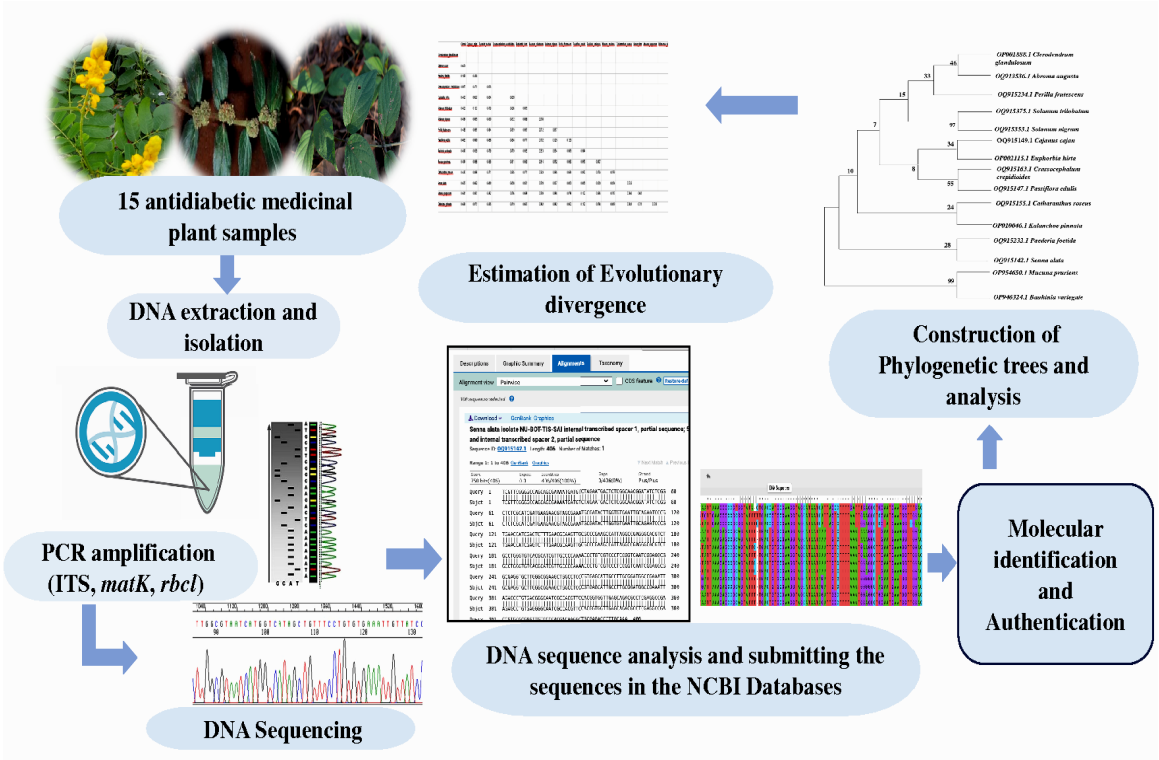
This study provides evidence supporting the traditional use of medicinal plants and emphasizes the importance of documenting ethnobotanical knowledge to ensure it is passed down through generations. The current research highlights that herbs, especially the leaves, are predominantly used by the tribes of Nagaland for their antidiabetic properties. Families such as Cucurbitaceae, Fabaceae, and Lamiaceae are commonly found to have antidiabetic effects. Many bioactive compounds isolated from these plants have demonstrated hypoglycemic effects, proving effective for diabetes treatment, though numerous bioactive agents remain uncharacterized. To confirm the safety and effectiveness of these plant species, it is crucial to conduct phytochemical analyses and establish their chemical profiles and antidiabetic mechanisms. This effort will also raise awareness among younger generations about the importance of preserving these valuable plants, which are at risk due to deforestation. Additionally, the toxicity of these plants needs to be elucidated to ensure their safe use. Given the increasing demand for drugs, it is essential to utilize medicinal plants and promote the mass multiplication of various

species. The mentioned plants can be developed into functional foods to help prevent diabetes and serve as nutritional supplements for diabetic patients. Detailed evaluation of the bioactive compounds and their mechanisms of action is a priority for future research. This will not only support traditional knowledge but also scientifically validate and enhance the current use of medicinal plants.

Chapter – 3

Molecular Characterization of the Investigated Species

Graphical Summary



Introduction

Diabetes Mellitus is a prevalent condition arising from disruptions in carbohydrate, protein, and lipid metabolism, along with irregularities in insulin secretion or its physiological impacts (Ojo et al., 2023). The various forms of diabetes arise because of insulin resistance or because of lifestyle preferences such as obesity, sedentary behaviour, and genetic predisposition and their complications include heart ailments, strokes, renal and nerve impairment, as well as ocular and podiatric issues (Yun and Ko, 2021). These ramifications emphasize the pressing requirement for efficient therapies owing to the extensive prevalence and varied etiologies of diabetes (Harding et al., 2019). Present treatments encompass insulin therapy, pharmacotherapy, and dietary regulation, with blood sugar-lowering drugs operating through diverse mechanisms. Nevertheless, these drugs may elicit adverse effects like gastrointestinal disturbances, weight fluctuations, and hepatic issues. Given the hazards linked with pharmaceutical agents, complementary medicine, particularly the utilization of ethnomedicinal flora, has garnered attention. These plants provide a holistic and less refined approach to recovery, diminishing the probability of unfavourable reactions and proving more economical (Bilal et al., 2018).

The demand for herbal medicines and supplements is increasing, yet challenges such as misidentification, lack of proper cultivation practices, and extended supply chains contribute to the adulteration of plant ingredients. Ensuring the authenticity and quality of medicinal plants is crucial for their safe and effective use (Wang et al., 2023). Traditionally, the taxonomic identification of plant species has been based primarily on morphological characteristics (Ilham et al., 2022). However, this method is limited by various environmental factors that can influence plant morphology (Quain et al., 2018). Morphological identification often cannot differentiate between species, as exemplified by *Colletotrichum* spp. (Savian et al., 2023). Additionally, morphological markers are

constrained by individual phenotypic variations (Bekele and Bekele, 2014). In the case of mangrove plants, morphological identification is challenging due to their similarities with associated species (Vyas et al., 2014). Similarly, identifying Ramie plants (*Boehmeria nivea* L. Gaud) based on morphology is difficult because of variability in distinguishing characteristics such as leaf colour (Mayerni et al., 2020).

Morpho-taxonomic identification requires specialized knowledge and skilled taxonomists (Heinrich, 2007), as the misidentification of medicinal plants can lead to serious repercussions. Conventional methods of species classification heavily depend on morphological characteristics, which are subject to subjectivity and variability due to various factors such as environmental conditions, developmental stages, and phenotypic plasticity. Precision in identification is particularly critical in the field of herbal medicine, where misidentifying species can result in the utilization of inappropriate plants with potentially ineffective or harmful attributes. These obstacles can be effectively tackled by incorporating DNA sequence-based barcodes for verification in conjunction with morpho-taxonomic traits. Techniques in molecular characterization, such as RAPD and AFLP, provide more precise and distinctive identification, ensuring the accurate selection of plant species for medicinal purposes. Alongside morpho-taxonomic identification, the molecular characterization of medicinal plants based on DNA sequences is imperative to ensure the authenticity of herbal products, as substitutions within plant families can have significant consequences. The use of microsatellite markers for molecular characterization is crucial for precise clonal identification due to genetic variations. Molecular markers surpass the constraints of traditional methods by leveraging DNA polymorphisms, thereby improving the accuracy of genetic diversity evaluation in programs aimed at crop enhancement. The accurate identification of medicinal plants is essential for their safe and natural utilization, guarding against adulteration. Techniques like AFLP and SSR markers play a pivotal role in distinguishing cultivars accurately and

resolving synonymy (Rotondi et al., 2003). Moreover, molecular data complements conventional methods by resolving uncertainties in the phylogeny of seed plants, enhancing precision, and reconciling conflicting hypotheses (Mathews, 2009).

Molecular characterization is of paramount importance in the identification of medicinal plants, contributing to authentication, quality evaluation, and analysis of genetic diversity. The utilization of DNA barcoding, employing markers like Internal Transcribed Spacer (ITS), RAPD, and ISSR, has proven to be effective in species identification and evaluation of genetic similarity in various plant species (Dastagir et al., 2023; Pukhrambam et al., 2023). This methodology enriches our comprehension of the medicinal attributes of plants such as *Polygonum posumbu* and *Hedychium coronarium*, allowing for species and hybrid differentiation, aiding in conservation endeavours, and the exploration of novel genes (Parida et al., 2017; Ao et al, 2020; Deb and Kamba, 2022; Pukhrambam et al., 2023). Furthermore, molecular markers play a role in detecting adulteration, ensuring the genuineness of herbal medicines, and enhancing the quality of medicinal plants through Marker Assisted Selection (MAS) for the improvement of active ingredients (Shaik et al., 2022). The application of molecular identification methodologies such as DNA barcoding facilitates precise species classification, even when presented in modified forms. By amplifying and sequencing specific DNA regions such as ITS, *rbcL*, and *matK*, a molecular blueprint is established for each species, assisting in the identification of contaminants and guaranteeing the legitimacy of medicinal products. In cases where morphological characteristics are lacking or deceptive, DNA barcodes provide a solution by linking models to recognized species (Schindel and Miller, 2005; Ao et al., 2020; Deb and Kamba, 2022). The analysis of phylogenetics utilizing diverse molecular markers furnishes insights into the evolutionary connections between organisms (Patwardhan et al., 2014). A small segment of the

mitochondrial *cytochrome c oxidase 1* (COI) gene sequence is effectively used as a universal barcode in animals. However, this gene is not suitable for plants because the rate of nucleotide substitution is very low in the plant mitochondrial genome (Mower et al., 2007). To address this issue, plastid DNA sequences have been used to establish DNA barcodes for plants. The Plant Working Group of the Consortium for the Barcode of Life (CBOL Plant Working Group 2009) evaluated seven plastid barcode loci such as *psbK–psbI*, *atpF–atpH*, *trnH–psbA* spacers, and the *matK, rbcL, rpoB*, and *rpoC1* genes. They recommended *rbcL* and *matK* as standard DNA barcode regions and additionally suggested ITS and *trnH–psbA* as supplementary barcode loci.

Studies have indicated the efficacy of these primers in the identification of plant species present in herbal commodities, showcasing high detection efficiencies in gDNA plant pools and biomass mock controls through the utilization of *rbcL* and ITS2 metabarcodes (Travadi et al., 2023). Notably, ribulose-1, 5-biphosphate carboxylase/oxygenase large subunit (*rbcL*) and *rpoC1* are widely accepted in this context. The *rbcL* gene is extensively utilized in plant phylogenetics due to its conserved nature across various plant lineages, ease of amplification, and sequencing capabilities, offering insights into evolutionary relationships and assisting in the development of phylogenetic trees at different taxonomic levels (Vijayan and Tsou, 2010; Thakur et al., 2016; Deb and Kamba, 2022). Numerous investigations on the DNA barcoding of medicinal plants have highlighted the efficacy of ITS2 and *matK* regions. For example, these regions can differentiate *Rauvolfia serpentina* (L.) Benth. Ex Kurz, whose root extracts are utilized as an antihypertensive medication, from other species within the genus (Eurlings et al., 2013). They also verify *Eurycoma longifolia* Jack, whose plant extracts (especially roots) are employed for the treatment of cough and possess anticancer and aphrodisiac properties. Furthermore, *matK* is recognized for offering optimal identification for Philippine ethnomedicinal Apocynaceae (Mohammed et al., 2017;

Cabelinet al., 2016; Ao et al., 2020; Deb and Kamba, 2022). The ITS region, characterized by its significant variability and rapid evolution in comparison to other genomic regions, is well-suited for phylogenetic investigation at diverse taxonomic levels, encompassing species and closely related taxa (Baldwin et al., 1995). The *matK* gene, acknowledged for its high conservation within plant species, also proves to be suitable for both phylogenetic analysis and species identification (Selvaraj et al., 2008).

The present work is a part of pharmacological studies on some anti-diabetic potential ethnomedicinal plants from Nagaland, India. The focus of this part of the work was on molecular authentication of the selected ethnomedicinal anti-diabetic potential plants using three established barcode genes: ITS, *rbcL* and *matK*. The data from molecular characterization of DNA sequences in the ITS, *rbcL*, and *matK* barcode regions will contribute significantly to the existing genetic databases. These genetic sequences are poised to act as pivotal markers for forthcoming research endeavours, playing a crucial role in the validation and verification processes of medicinal flora. Through the expansion of the repository, we are not only streamlining the identification processes of the scrutinized flora but also bolstering broader initiatives in the realms of preservation, pharmacognosy, and the exploration of biodiversity.

Material and Methods

Genomic DNA isolation, amplification and sequencing

Fresh tender leaves were collected from various parts of Nagaland and utilized for DNA extraction following the appropriate adjustments of CTAB genomic DNA extraction protocols outlined by Doyle and Doyle (1987) and Kamba and Deb (2018) (Annexure I). The quality of the isolated DNA samples was assessed on agarose gel (0.9%, w/v) and quantified using a Thermo Scientific Nanodrop Spectrophotometer. Evaluation of the amplification efficiency of each potential DNA barcode was conducted through PCR

using universal primers and standardized conditions. The ITS region amplification utilized ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) primers as described by Doyle and Doyle (1987). The *rbcL* region amplification employed *rbcL-1f-F* (ATGTCACCACAAACAGAGACTAAAGC) and *rbcL-724-R* (TCGCATGTACCTGCAGTA) primers following the methodology of Vijayan and Tsou (2010). Additionally, for the amplification of the *matK* region, *matK*-3FKIM-r (CGTACAGTACTTTTGTGTTTACGAG) and *matK*-1RKIM-F (ACCCAGTCCATCTGGAAATCTTGGTTC) primers were utilized according to Selvaraj et al. (2008). A total of 15 candidate sample species genomic DNA was used as the template for the PCR reaction mixture, comprising specific components in precise quantities.

The PCR thermal cycling conditions involved an initial denaturation step at 94 °C for 3 minutes, followed by 30 cycles at 94 °C for 45 sec, 57 °C for 45 sec during annealing, and 72 °C for 1 min for extension, with a final extension step at 72°C for 10 minutes, consistently applied to all primers. The amplification reaction was carried out using the Thermo Scientific Arktik Thermal Cycler. Subsequent confirmation of the amplicons was achieved through gel electrophoresis on 1.5% (w/v) agarose gels, stained with 0.8 µg/ml ethidium bromide, and visualized using a Chemi-Doc gel documentation system (Bio-Rad). Sequencing of the amplified products was performed at 1st Base Laboratories, Singapore, and Chromous Biotech Pvt. Ltd., Bangalore, India. The sequences obtained underwent quality assessment using BioEdit sequence alignment Editor Software 7.0.9.0.

Sequence alignment and analysis

The nucleotide sequences of the plant species from ITS, *rbcL*, and *matK* regions were subsequently deposited in the National Centre for Biotechnology Information (NCBI) GenBank. Comparative analysis of the ITS, *rbcL*, and *matK* DNA sequences against entries in the GenBank was conducted using the Basic Local Alignment Search Tool (BLAST). Multiple sequence alignment was carried out using the MUSCLE tool within the MEGA-11 software. Subsequently, the nucleotide sequences of the identified candidate species from ITS, *rbcL*, and *matK* regions were submitted to NCBI GenBank, and corresponding accession numbers were obtained (**Table 3.1).** Phylogenetic trees were constructed utilizing MEGA-11 software (Tamura et al., 2021) employing statistical models such as Tamura-Nei, Tamura 3-parameter, and General Time Reversible methods commonly utilized in Maximum Likelihood (ML) analysis for evolutionary relationship estimation based on DNA sequence data. Inter-specific distances were calculated using the Kimura 2-parameter method in MEGA 11 to assess inter-specific variation. A consensus tree was generated in each form of phylogenetic analysis by performing 1000 bootstrap replicates. The resulting consensus tree was considered a depiction of the evolutionary history of the analysed taxa.

Results

The barcode loci ITS, *rbcL*, and *matK* from the nuclear and chloroplast genomes were used for the molecular characterization of 15 ethnomedicinal anti-diabetic plants from Nagaland. All the targeted regions were successfully amplified for the investigated species, generating sequences with lengths ranging from 249 to 1399 base pairs (Annexure II). These sequences were then submitted to the NCBI GenBank, and accession numbers were obtained (**Table 3.1).** Species resolution for the loci ITS, *rbcL*, and *matK* was calculated using BLAST analysis, phylogenetic tree method and genetic distances (**Table 3.2, 3.3, 3.4**).

BLAST analysis

During the investigation, 9 out of the 15 species showed the highest BLAST hit of 100% identity for the ITS region and the remaining 5 species exhibited slightly lower similarity specifically, *Mucuna pruriens* (99.42%), *Kalanchoe pinnata* (96.80%), *Gynura crepidiodes* (99.38%), *Euphoria hirta* (98.98%), and *Catharanthus roseus* (97.95%) identity for ITS region. *Clerodendrum colebrookianum* resulted in an ambiguous identification, and its BLAST hit with the nearest species is 80.19% (*Clerodendrum bracteatum*); however, with *matK* and *rbcL* markers, the plant sample was identified as *Clerodendrum colebrookianum*. For the *rbcL* region, 11 out of 15 species displayed the highest blast hit of 100% identity using the BLAST algorithm, while *Passiflora edulis, Paederia foetida, Gynura crepidiodes* and *Clerodendrum colebrookianum* exhibited 99.83, 99.30, 99.45 and 98.88% identity respectively. But for the *matK* region, 13 out of 15 species exhibited a 100% BLAST hit, indicating significant homology with the target sequences. This suggested the *matK* region's potential hypervariability. Notably, *Senna alata* and *Clerodendrum colebrookianum* showed 99.88 and 98.77% similarity, respectively (**Table 3.1**).

Table 3.1: BLASTn statistics of different barcode marker genes (ITS1, *rbcL, matK*) sequence analysis of 15 selected anti-diabetic plants

Sl. No.	Selected plant species	Sequence statistics with subject sequences									
		Barcode marker studied	GenBank Acc. No.	Max score	Total score	Base pair length	Query Cover (%)	E value	Identity (%)	Closest similar species	GenBank Accession No. of closest species
1	*Abroma augustum*	ITS1	OQ913536	569	569	384	80	1e-157	100	*Abroma augustum*	MF348969
		rbcL	OQ916148	1142	1142	621	99	0.0	100		KR528698
		matK	OQ916152	1362	1362	740	99	0.0	100		MF349845
2	*Bauhinia variegata*	ITS1	OP946324	542	542	358	81	2e-149	100	*Bauhinia variegata*	MZ314018
		rbcL	OQ916147	992	992	540	99	0.0	100		MH549747
		matK	OQ916151	1528	1528	830	99	0.0	100		MT176420
3	*Cajanus cajan*	ITS1	OQ915149	1338	1338	730	99	0.0	100	*Cajanus cajan*	MG991099
		rbcL	OQ913364	2582	2582	1398	100	0.0	100		KU729878
		matK	OP966870	1624	1624	879	100	0.0	100		MH392013
4	*Catharanthus roseus*	ITS1	OQ915155	1098	1098	636	99	0.0	97.95	*Catharanthus roseus*	KC952025
		rbcL	OQ913366	1264	1264	684	100	0.0	100		NC021423
		matK	OP966869	1585	1585	858	100	0.0	100		JN228930
5	*Senna alata*	ITS1	OQ915142	745	745	406	99	0.0	100	*Senna alata*	MH050233
		rbcL	OQ913370	1157	1157	626	100	0.0	100		LC385919
		matK	OQ913371	1565	1565	867	98	0.0	99.88		EU362042
6	*Clerodendrum*	ITS1	OP001888	464	464	651	96	1e-125	80.19	*Clerodendrum*	KX228227

	colebrookianum									*breacteatum**	
		rbcL	OP020130	1273	1273	724	98	0.0	98.88	*Clerodendrum colebrookianum*	NC_069069
		matK	OP035401	1445	1445	818	99	0.0	98.77		NC_069069
7	*Euphorbia hirta*	ITS1	OP002115	1230	1230	703	97	0.0	98.98	*Euphorbia hirta*	MH768144
		rbcL	OQ913367	981	981	531	100	0.0	100		MG889511
		matK	OQ913374	1386	1386	750	100	0.0	100		MG889539
8	*Gynura crepidioides*	ITS1	OQ915163	1171	1171	648	99	0.0	99.38	*Crassocephalum crepidiodes*	MH050154
		rbcL	OQ913368	1310	1310	728	99	0.0	99.45		NC057993
		matK	OQ658381	1061	1283	694	100	0.0	100		MF159418
9	*Kalanchoe pinnata*	ITS1	OP010046	416	416	249	100	1e-111	96.80	*Kalanchoe* sp.	MW297180
		rbcL	OQ913365	922	922	499	100	0.0	100		OP711354
		matK	OQ913373	1288	1288	697	100	0.0	100		GU135118
10	*Mucuna pruriens*	ITS1	OP954650	628	703	386	100	2e-175	99.42	*Mucuna pruriens*	LC494602
		rbcL	OQ916146	817	817	442	100	0.0	100		MK244684
		matK	OQ916150	917	917	499	100	0.0	100		KX721059
11	*Paederia foetida*	ITS1	OQ915232	1120	1120	609	99	0.0	100	*Paederia foetida*	MH710857
		rbcL	OQ913369	1299	1299	726	99	0.0	99.30		NC062416
		matK	OQ913372	2326	2326	1259	100	0.0	100		AY358409
12	*Passiflora edulis*	ITS1	OQ915147	761	761	415	99	0.0	100	*Passiflora edulis*	MN852305

		rbcL	OQ916149	1107	1107	602	100	0.0	99.83	MF807938
		matK	OQ921094	1328	1328	725	99	0.0	100	MF807938
13	*Perilla frutescens*	ITS1	OQ915234	1351	1351	737	99	0.0	100	*Perilla frutescens* MH710687
		rbcL	OQ921088	955	955	520	99	0.0	100	KX397889
		matK	OQ921093	1474	1474	801	99	0.0	100	KT220692
14	*Solanum nigrum*	ITS1	OQ915353	1297	1297	708	99	0.0	100	*Solanum nigrum* MW018361
		rbcL	OQ921089	1022	1022	556	99	0.0	100	MH168549
		matK	OQ921092	1640	1640	891	99	0.0	100	MH265199
15	*Solanum trilobatum*	ITS1	OQ915375	1099	1099	598	99	0.0	100	*Solanum trilobatum* MH283725
		rbcL	OQ921090	1304	1304	708	99	0.0	100	NC039602
		matK	OQ921091	1389	1389	758	99	0.0	100	ON623024

*Indicates ambiguous identification.

Phylogenetic tree analysis

To study the phylogeny of the selected 15 anti-diabetic medicinal plants, the Tamura-Nei, Tamura 3-parameter, and General Time Reversible models of the Maximum Likelihood method were employed. The phylogenetic tree was constructed with 1000 bootstrap replicates. The phylogenetic tree from ITS sequences was constructed using the Tamura-Nei model of Maximum Likelihood, revealing two major clades (Figure 3.1). *Bauhinia variegata* and *Mucuna pruriens* formed a clade with a bootstrap value of 99, while, *Cajanus cajan* and *Euphorbia hirta* were separated from *Gynura crepidiodes* and *Passiflora edulis*, forming subclades with low bootstrap values.

In phylogeny with *matK* sequences, two major clades and three main subclasses were observed (Figure 3.2). *Senna alata, Gynura crepidiodes,* and *Abroma augusta* formed a clade with a bootstrap value of 100, while the rest comprised a separate clade that was further subdivided into respective subclades. Genera *Clerodendrum, Perilla, Solanum, Catharanthus*, and *Paederia* formed a clade with a bootstrap value of 72, distinguishing them from the other genera, while *Cajanus* and *Mucuna* formed a distinct cluster with a bootstrap value of 100. But, genera *Kalanchoe, Passiflora, Euphorbia, Bauhinia, Cajanus*, and *Mucuna* formed a clade with a bootstrap value of 47.

The phylogenetic tree generated from *rbcL* using the Maximum Likelihood Tamura 3-parameter model resolved into two major clades (Figure 3.3). The genera *Clerodendrum, Paederia*, and *Gynura* formed a clade with a bootstrap value of 100. The genera *Solanum* constituted a subclade with a bootstrap value of 86. The genera *Perilla, Catharanthus, Kalanchoe, Abroma, Passiflora, Euphorbia, Bauhinia, Senna, Mucuna* and *Cajanus* formed different clades with lower bootstrap values. A subclade with a bootstrap value of 88 included *Cajanus, Mucuna, Senna, and Bauhinia.*

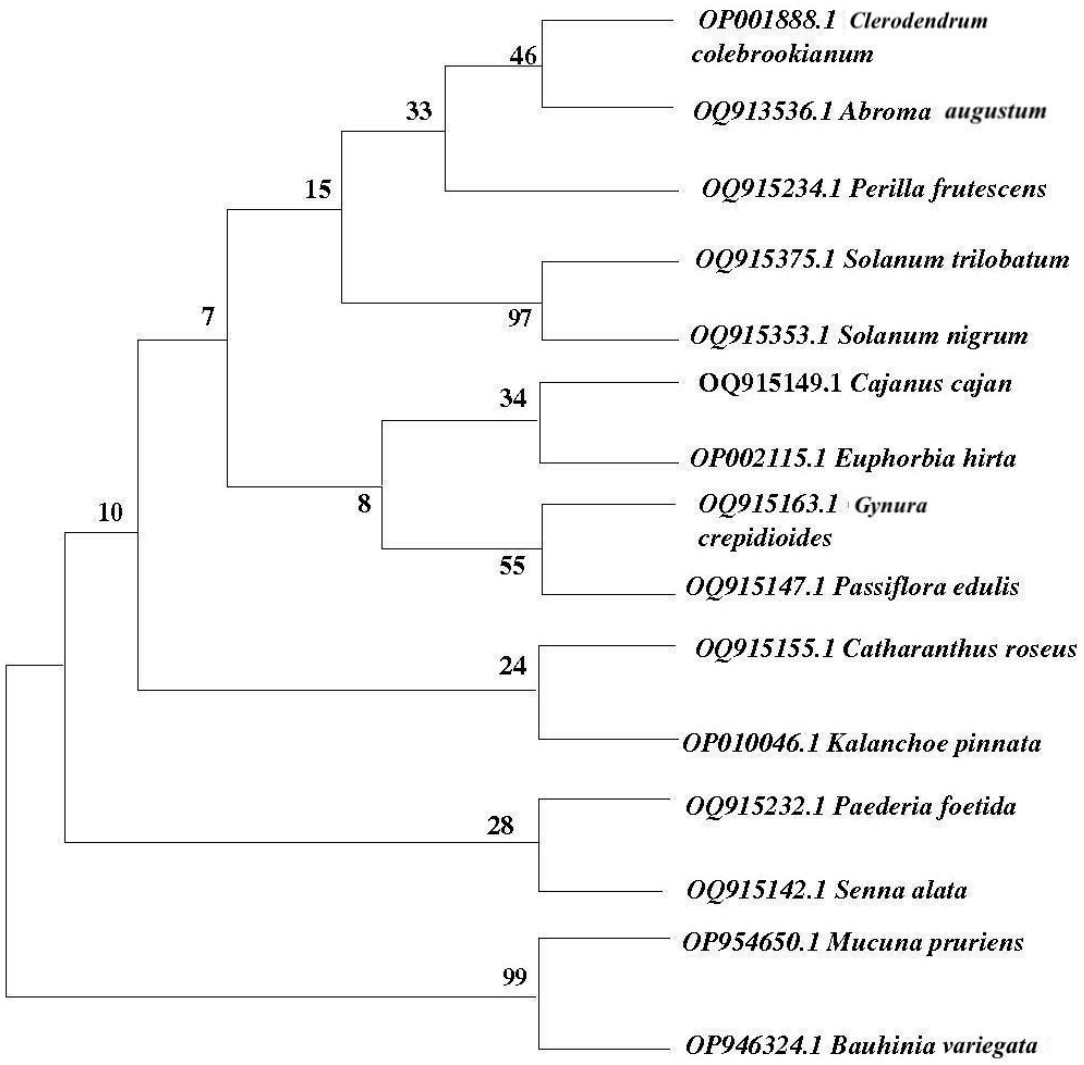


Figure 3.1: Phylogenetic tree based on ITS sequences using the Tamura- Nei model of Maximum likelihood method.

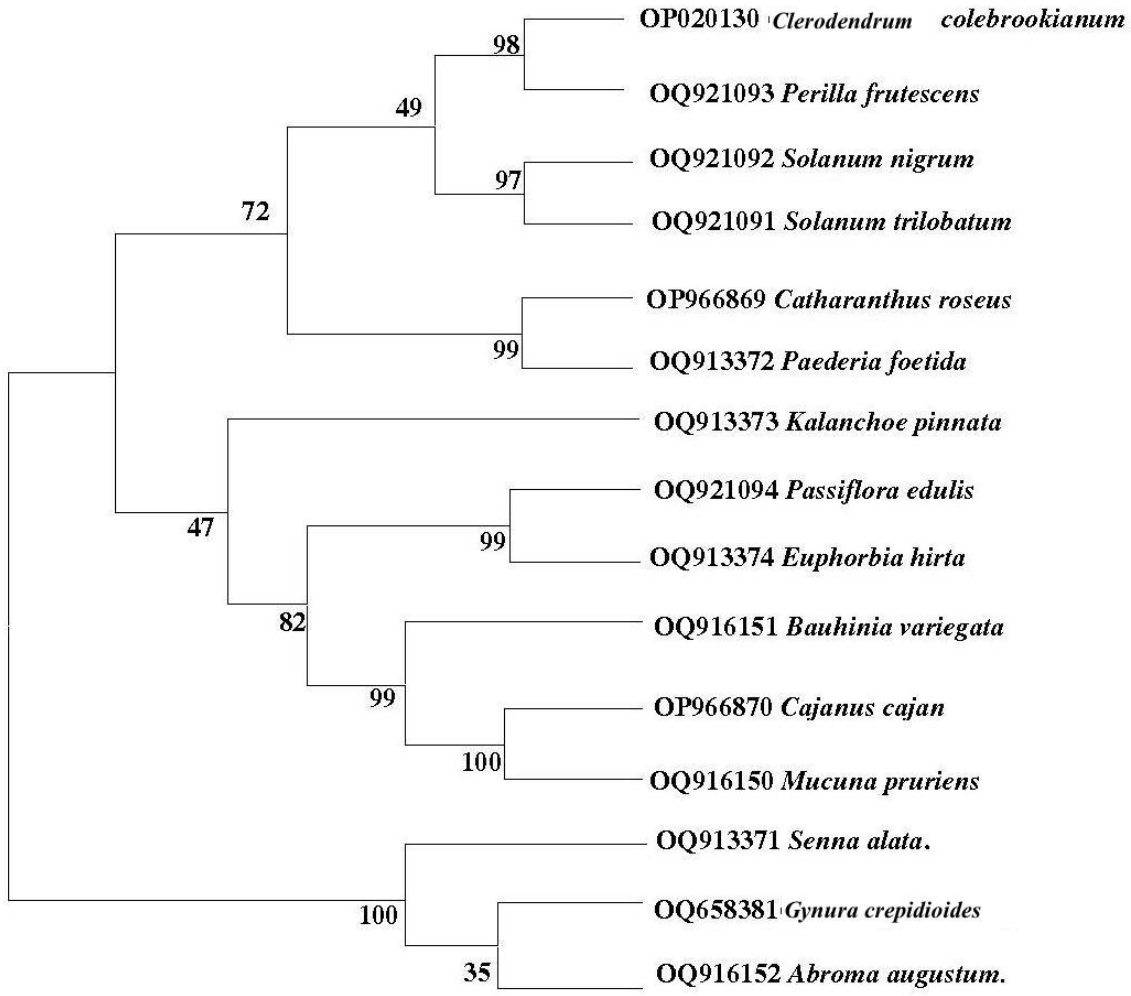


Figure 3.2: Phylogenetic tree generated using *matK* sequences using the General time reversible model of Maximum likelihood method.

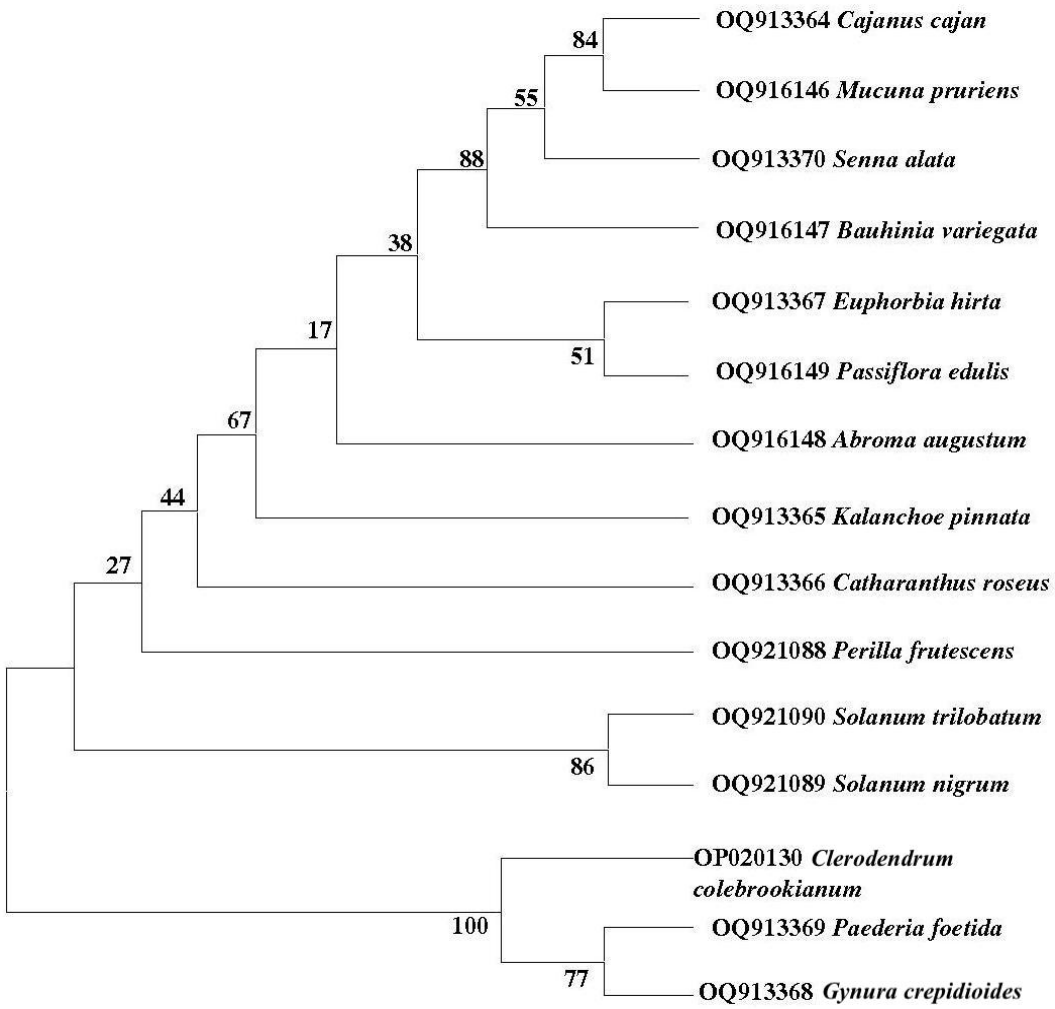


Figure 3.3: Phylogenetic tree generated using *rbcL* sequences using the Tamura-3 parameter model of Maximum likelihood method.

Table 3.2: Estimates of evolutionary divergence between sequences (ITS)

	Clerodendrum colebrookianum	***Cajanus cajan***	***Catharanthus roseus***	***Senna alata***	***Paederia foetida***	***Gynura crepidioides***	***Passiflora edulis***	***Perilla frutescens***	***Solanum trilobatum***	***Solanum nigrum***	***Abroma augustum***	***Kalanchoe pinnata***	***Bauhinia variegata***	***Euphorbia hirta***	***Mucuna pruriens***
Clerodendrum colebrookianum															
Cajanus cajan	0.449														
Catharanthus roseus	0.405	0.369													
Senna alata	0.397	0.321	0.296												
Paederiafoetida	0.397	0.329	0.297	0.278											
Gynura crepidioides	0.457	0.386	0.369	0.337	0.362										
Passiflora edulis	0.383	0.290	0.269	0.259	0.270	0.293									
Perilla frutescens	0.358	0.327	0.328	0.299	0.271	0.385	0.278								
Solanum trilobatum	0.369	0.294	0.308	0.256	0.291	0.354	0.237	0.260							
Solanum nigrum	0.375	0.311	0.302	0.263	0.261	0.353	0.254	0.271	0.120						
Abroma augustum	0.370	0.330	0.326	0.346	0.352	0.365	0.266	0.272	0.297	0.289					
Kalanchoe pinnata	0.496	0.462	0.436	0.437	0.423	0.473	0.452	0.424	0.419	0.439	0.497				
Bauhinia variegata	0.554	0.493	0.492	0.530	0.492	0.547	0.486	0.495	0.459	0.486	0.532	0.594			
Euphorbia hirta	0.442	0.330	0.355	0.308	0.328	0.382	0.276	0.336	0.301	0.311	0.313	0.473	0.512		
Mucuna pruriens	0.555	0.465	0.451	0.474	0.459	0.508	0.464	0.485	0.450	0.470	0.521	0.588	0.507	0.503	

Genetic distance

Interspecific variations among the species were calculated by preparing a distance matrix by aligning the sequences of all the species.

ITS pairwise genetic distance

When data was analyzed for pair-wise genetic distance, it was found that *Clerodendrum colebrookianum* had a similarity score of 0.449 with *Cajanus cajan*, indicating a moderate level of similarity. *Catharanthus roseus* had a similarity score of 0.369 with *Cajanus cajan*, suggesting a relatively lower similarity than other species. *Kalanchoe pinnata* had relatively high similarity scores with multiple species, such as *Abroma augustum* (0.497) and *Mucuna pruriens* (0.555), suggesting closer relationships. *Solanum nigrum* was also found to have a similarity score of 0.120 with *Solanum trilobatum*, indicating a relatively lower similarity between these two species. *Euphorbia hirta* had a moderate similarity score (0.442) with *Clerodendrum colebrookianum* and relatively higher scores with some other species like *Bauhinia variegata* (0.512) (Table 3.2).

***matK* pairwise genetic distance**

Catharanthus roseus and *Clerodendrum colebrookianum* exhibited a low similarity score of 0.1954. *Gynura crepidioides* had a relatively high similarity score with *Clerodendrum colebrookianum* (0.5435), *Catharanthus roseus* (0.5292), *Passiflora edulis* (0.5268) and *Perilla frutescens* (0.5495) suggested closer relationships. *Solanum trilobatum* showed low similarity with *Clerodendrum colebrookianum* (0.1695), *Catharanthus roseus* (0.1550), *Passiflora edulis* (0.2368) and *Perilla frutescens* (0.1600). *Senna alata* had a high similarity score with *Clerodendrum colebrookianum* (0.5285), *Catharanthus roseus* (0.5170), *Passiflora edulis* (0.5317), *Perilla frutescens* (0.5326), *Solanum nigrum* (0.5147), *Cajanus cajan* (0.5376), *Mucuna pruriens* (0.5349), *Bauhinia variegata* (0.5212), *Euphorbia hirta* (0.5275), *Kalanchoe pinnata* (0.5287) and *Paederia foetida* (0.5319) showed their close relationship (Table 3.3).

Table 3.3: Estimates of evolutionary divergence between sequences (*matK*)

	Clerodendrum colebrookianum	***Catharanthus roseus***	***Passiflora edulis***	***Perilla frutescens***	***Gynura crepidioides***	***Solanum nigrum***	***Solanum trilobatum***	***Abroma augustum***	***Cajanus cajan***	***Bauhinia variegata***	***Euphorbia hirta***	***Kalanchoe pinnata***	***Paederia foetida***	***Mucuna pruriens***	***Senna alata***
Clerodendrum colebrookianum															
Catharanthus roseus	0.1954														
Passiflora edulis	0.2462	0.2303													
Perilla frutescens	0.1089	0.1725	0.2469												
Gynura crepidioides	0.5435	0.5292	0.5268	0.5495											
Solanum nigrum	0.1768	0.1466	0.2304	0.1641	0.5250										
Solanum trilobatum	0.1695	0.1550	0.2368	0.1600	0.5254	0.0257									
Abroma augustum	0.5167	0.5039	0.5188	0.5264	0.2234	0.5008	0.5015								
Cajanus cajan	0.2688	0.2362	0.2345	0.2532	0.5350	0.2283	0.2309	0.5291							
Bauhinia variegata	0.2302	0.2160	0.2044	0.2222	0.5312	0.1939	0.1937	0.5163	0.1596						
Euphorbia hirta	0.2426	0.2298	0.1683	0.2362	0.5423	0.2272	0.2227	0.5250	0.2513	0.2011					
Kalanchoe pinnata	0.2551	0.2219	0.2583	0.2522	0.5548	0.2258	0.2258	0.5290	0.2478	0.2113	0.2518				
Paederia foetida	0.2308	0.1568	0.2441	0.2123	0.5601	0.1867	0.1907	0.5316	0.2535	0.2451	0.2487	0.2594			
Mucuna pruriens	0.2757	0.2485	0.2204	0.2786	0.5474	0.2445	0.2445	0.5289	0.0762	0.1463	0.2525	0.2637	0.2665		
Senna alata	0.5285	0.5170	0.5317	0.5326	0.2222	0.5147	0.5085	0.1992	0.5376	0.5212	0.5275	0.5287	0.5319	0.5349	

Table 3.4: Estimates of evolutionary divergence between sequences (*rbcL*)

	Clerodendrum colebrookianum	***Cajanus cajan***	***Paederia foetida.***	***Gynura crepidioides***	***Euphorbia hirta***	***Solanum trilobatum***	***Solanum nigrum***	***Perilla frutescens***	***Passiflora edulis***	***Bauhinia variegata***	***Mucuna pruriens***	***Catharanthus roseus***	***Senna alata***	***Abroma augustum***	***Kalanchoe pinnata***
Clerodendrum colebrookianum															
Cajanus cajan	0.4654														
Paederia foetida.	0.1066	0.4894													
Gynura crepidioides	0.0975	0.4761	0.0956												
Euphorbia hirta	0.4455	0.0667	0.4614	0.4659											
Solanum trilobatum	0.4606	0.1307	0.4799	0.4686	0.0875										
Solanum nigrum	0.4384	0.0815	0.4570	0.4512	0.0896	0.0108									
Perilla frutescens	0.4435	0.0981	0.4644	0.4519	0.0951	0.0712	0.0617								
Passiflora edulis	0.4663	0.0979	0.4888	0.4804	0.0771	0.1102	0.1205	0.1250							
Bauhinia variegata	0.4547	0.0506	0.4799	0.4779	0.0583	0.0833	0.0874	0.0918	0.0944						
Mucuna pruriens.	0.4344	0.0385	0.4535	0.4511	0.0590	0.0814	0.0792	0.0853	0.0973	0.0407					
Catharanthus roseus	0.4480	0.0868	0.4771	0.4605	0.0771	0.0629	0.0665	0.0654	0.0972	0.0706	0.0769				
Senna alata	0.4537	0.0402	0.4849	0.4688	0.0521	0.0799	0.0827	0.0808	0.0868	0.0260	0.0294	0.0735			
Abroma augustum	0.4641	0.0821	0.4862	0.4706	0.0688	0.0789	0.0888	0.0788	0.1002	0.0665	0.0747	0.0660	0.0601		
Kalanchoe pinnata.	0.4654	0.0701	0.4805	0.4719	0.0617	0.0862	0.0862	0.0902	0.1202	0.0768	0.0819	0.0882	0.0721	0.0782	

***rbcL* pairwise genetic distance**

Clerodendrum colebrookianum and *Cajanus cajan* exhibited a moderate level of similarity score of 0.4654, while a similarity score of 0.1066 was found between *Paederia foetida* and *Clerodendrum colebrookianum* indicated a relatively high score with multiple species such as *Clerodendrum colebrookianum* (0.4663), *Paederia foetida* (0.4888) and *Gynura crepidiodes* (0.4804). Further, it was found that *Passiflora edulis* and *Perilla frutescens* exhibited a similarity score of 0.1250 between the species. But, *Kalanchoe pinnata* had a moderate similarity score with *Clerodendrum colebrookianum* (0.4654), *Paederia foetida* (0.4805) and *Gynura crepidiodes* (0.4719) (Table 3.4).

Table 3.5: Mean GC content of the studied antidiabetic plants

Sequenced region	Mean GC content (%)
ITS	59.67
rbcL	42.80
matK	32.6

Additionally, the Guanine-Cytosine (GC%) content, an essential characteristic of DNA sequences, was observed to vary among the studied regions (**Table 3.5**). These variations in GC content could have reflected differences in nucleotide composition and functional constraints among the genomes of the studied species. Moreover, GC% could have influenced the stability of DNA secondary structures and the efficiency of PCR amplification.

Discussion

An expeditious and precise approach to species authentication is crucial to guarantee the secure utilisation of herbal drugs manufactured from ethnomedicinal plants. Nonetheless, conventional morphological and chemical identification methods prove intricate. Various molecular techniques have been employed to verify the authenticity of medicinal plants, utilising DNA regions found in the chloroplast and nucleus (Hebert

et al., 2003). In a study by Chen et al. (2010), an evaluation was conducted on seven potential DNA barcodes (*psbA-trnH*, *rpoC1, ycf5, matK, rbcL*, ITS and ITS2) derived from various medicinal plant species. The findings of this study hold significant implications for both traditional medicine practitioners and the healthcare community. Combining traditional knowledge with modern scientific approaches can bridge the gap in between ancient healing practices and contemporary healthcare systems. The findings of the present investigation will contribute to the scientific validation and preservation of Nagaland's medicinal plant heritage, ultimately benefiting the local communities and facilitating the integration of indigenous medicines into mainstream healthcare frameworks. DNA barcoding finds extensive application in distinguishing between species, preserving endangered species, and identifying traditional medicine, among other uses (Kress et al., 2005). The results of the molecular authentication were compared with the morphological identifications, and a high degree of concordance was observed between the two methods. This strengthens the accurate authentication of the plant species using molecular markers. The successful amplification and characterisation of genetic markers, as demonstrated in the present study, align with the growing trend in herbal medicine plant research towards molecular identification, which has been highlighted in previous works by Liu et al. (2012), Ao et al. (2020), and Deb and Kamba (2022). This approach ensures the accurate identification of plant species for traditional remedies. Further, it was found that there were significant variations in sequence lengths (ranging from 249 to 1399 base pairs), attributed to the genetic diversity within the studied plants, including the presence of introns, variable regions, and gene duplications. The successful amplification and sequencing of the barcode loci ITS*, rbcL*, and *matK* from these ethnomedicinal anti-diabetic plants, as highlighted in the present study, aligned with the recognition of these regions as significant and valuable genetic markers

for DNA barcoding purposes, as also emphasised by Heubl (2010). The study by Zhang and Jiang (2020) reaffirmed the importance of using multiple markers in DNA barcoding. This approach helps overcome the limitations of relying on a single barcode's genetic variation and improves the accuracy of species identification. It not only aids in the accurate identification and characterisation of these plants but also provides valuable genetic data that can be used for further research, conservation efforts, and the preservation of traditional medicinal knowledge. The ITS sequence has found extensive application in phylogenetic analyses across diverse groups of plant species, including but not limited to *Calycadenia* (Asteraceae), *Paeonia* (Ranunculaceae), *Antennaria* (Asteraceae), the subtribe *Helianthinae* (Asteraceae), as well as various *Dendrobium* species within the Orchidaceae family (Deb and Kamba, 2022). During the BLAST analysis, the unidentified barcode sequence of the individual was queried using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). This search aimed to locate a highly similar sequence within a reference database comprising accurately identified species' barcodes. Several species, including *Abroma augustum, Bauhinia variegata, Cajanus cajan, Senna alata, Paederia foetida, Passiflora edulis, Perilla frutescens, Solanum nigrum*, and *Solanum trilobatum*, showed high BLAST hit identities for the ITS and *rbcL* sequences. Some species did not achieve 100% identity, but the high levels of similarity in the majority of cases indicate that these regions are effective for species identification. The fact that *Clerodendrum colebrookianum* had ambiguous identification in the ITS region (with an 80.19% identity to the nearest species) raises questions about its genetic uniqueness or the presence of genetic variation within this species. The potential sources of species ambiguities could arise from multiple factors, including variations in the names assigned to the same plants across different ethnic groups, suboptimal post-harvest preservation conditions leading to DNA degradation, or

inadvertent inclusion of mixed plant material (Naim and Mahboob, 2020). However, a significant finding worth noting is the consistently high genetic similarity, reaching up to 98%, observed in the *rbcL* and *matK* regions of *Clerodendrum colebrookianum*. This genetic similarity reinforces the reliability of utilising these specific DNA barcode markers for accurate molecular identification despite potential challenges introduced by variations in nomenclature and preservation conditions. Such results emphasise the importance of selecting appropriate genetic markers for precise species identification in studies involving ethnomedicinal plants.

Phylogenetic methods rooted in tree-based analyses have found extensive application within DNA barcode research. These approaches serve to accurately link a species with its corresponding taxonomic category. Nonetheless, a universally applicable barcode method for plants has yet to be established, prompting researchers to employ diverse gene markers to enhance the efficacy of species identification (Naim and Mahboob, 2020). In the phylogenetic analysis, the study employed various models of the Maximum Likelihood method, backed by 1000 bootstrap replicates, to ensure the robustness of the results. The phylogenetic trees generated from these markers provided valuable insights. In the ITS tree, two major clades emerged, with Genera *Bauhinia* and *Mucuna* forming a closely related clade, while Genera *Cajanus* and *Euphorbia* displayed a more complex branching pattern. The *matK* based analysis revealed a well-supported clade comprising *Senna alata*, *Gynura* and *Abroma*, indicating their close genetic affinity, while *Clerodendrum, Perilla, Solanum, Catharanthus*, and *Paederia* formed a distinct clade. In the *rbcL* tree, *Clerodendrum, Paederia*, and *Gynura* clustered together robustly, and *Solanum* formed a subclade. Slight differences in clustering patterns of 15 plants can also be observed depending on the barcode markers applied. Since each marker targets distinct regions of the genome, which can vary in their mutation rates, evolutionary

histories, and susceptibilities to selection pressures and recombination, clustering patterns are affected (Aghayeva et al., 2021). Different barcode markers, such as chloroplast *rpoB, rbcL, matK* and nuclear ITS regions, exhibited varying levels of genetic variation and resolution capabilities, impacting the clustering outcomes. For instance, the nuclear ITS marker showed high species-level assignment success rates in orchids, indicating its effectiveness in capturing genetic diversity and phylogeographic signals (Gill et al., 2019). The phylogenetic findings provide a deeper understanding of the genetic relationships among these medicinal plants, aiding in their classification and offering insights into their evolutionary history and potential for traditional medicinal uses.

Other dominant markers like SCoT, iPBS, ISSR, etc., can be employed to study the genetic relationship between plants. These markers are useful for assessing genetic diversity, population structure, and phylogenetic relationships due to their high polymorphism and ability to target different genomic regions. But, markers like ITS, *rbcL*, *matK,* etc. can be used for species authenticity as used in the present investigation because of their universality, the well-conserved region across a wide range of plant species. There are also extensive databases like GenBank and the Barcode of Life Data Systems (BOLD) that contain reference sequences for barcode regions. This facilitates the identification and comparison of the studied plants with existing data (Meiklejohn et al., 2019).

The GC% (guanine-cytosine content) of the DNA sequences was analysed, revealing variations among the studied species. These differences in GC% reflect variations in nucleotide composition and functional constraints on the genomes of the plants. The GC% can also affect DNA stability and PCR amplification efficiency (Mamedov et al., 2008). Pair-wise genetic distances were computed for the ITS, *rbcL*, and *matK* regions. The ITS region showed varying degrees of similarity between species,

with some species showing moderate similarity while others had relatively lower or higher similarity scores. In various studies by Ro and McPheron (1997), Kushwaha and Joshi (2023), and Steinitz et al. (2005), the ITS region allowed clear molecular distinction between different species, aiding in phylogenetic analyses and evolutionary relationship assessments. Similar trends were observed in the *matK* and *rbcL* regions, with some species showing close relationships and others displaying more distant relationships. Furthermore, the molecular barcodes produced for each of the specimens within this current study will prove invaluable for forthcoming research endeavours and conservation initiatives focused on these extensively employed medicinal plants with anti-diabetic properties among the area's indigenous communities. Understanding the phylogenetic relationships and genetic diversity of these medicinal plants is essential to facilitate accurate identification, responsible cultivation, sustainable utilization, and the preservation of the available resources.

Summary and Conclusions

Present study findings indicate that ITS1 and ITS4 for the ITS region, *rbcL-1f-F* and *rbcL*-724-R for the *rbcL* region and *matK*-3FKIM-r and *matK*-1RKIM-F for the *matK* region, serve as not only standard phylogenetic markers but also valuable DNA barcodes. The study successfully amplified the barcode loci ITS, *rbcL*, and *matK* of 15 ethnomedicinal anti-diabetic plants native to Nagaland. These sequences, ranging in length from 249 to 1399 base pairs, were deposited in the NCBI GenBank, ensuring their accessibility for future research and contributing to the preservation of traditional medicinal knowledge. The phylogenetic analysis using multiple models and bootstrap replicates provided insights into the evolutionary relationships among these plants. The trees revealed well-supported clades and subclades, indicating close genetic relationships within certain genera and highlighting potential evolutionary complexities in others.

Notably, the genetic similarity observed in the *rbcL* and *matK* regions of *Clerodendrum colebrookianum*, despite variations in the ITS region, stresses the reliability of using these DNA barcode markers for molecular identification. This finding adds credibility to the study's results and emphasises the importance of selecting appropriate genetic markers for accurate species identification. Overall, this research contributes to our understanding of the genetic diversity and evolutionary history of ethnomedicinal plants in Nagaland. It provides a solid foundation for further taxonomic and conservation efforts and elucidates the significance of molecular tools in preserving traditional medicinal knowledge and supporting biodiversity research.

Chapter –4

Analysis of Antidiabetic Potential Phytochemicals of the Selected Ethnomedicinal Plants

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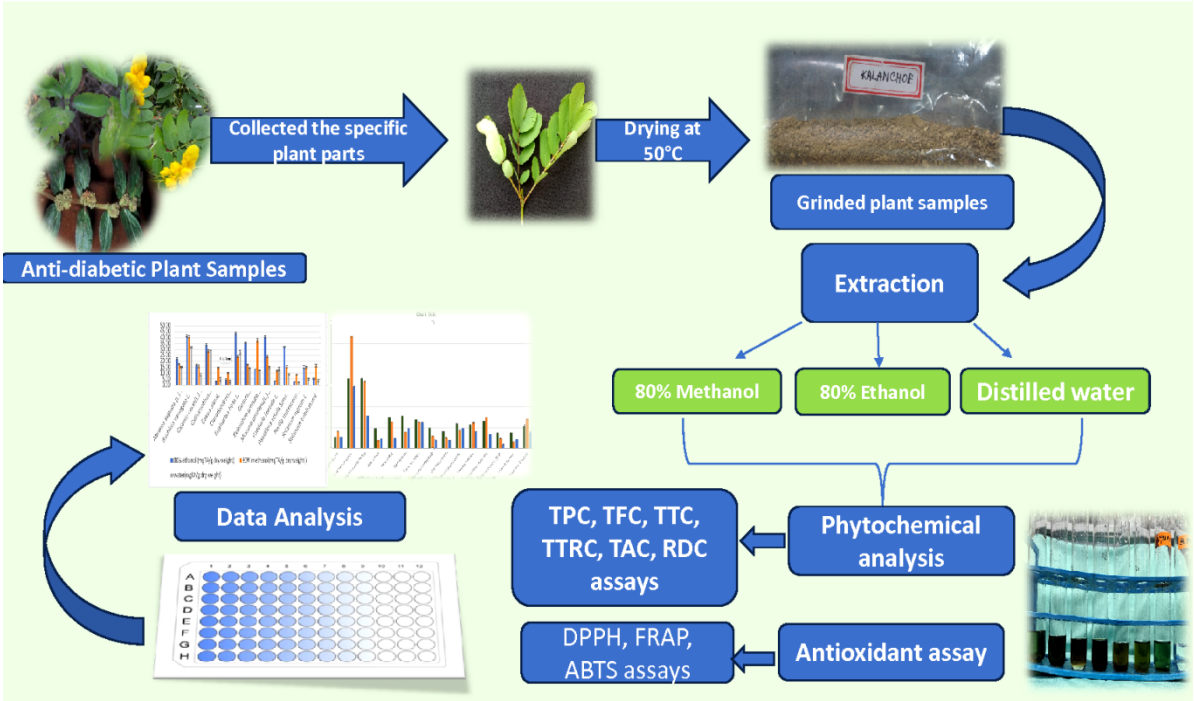
Graphical Summary



Introduction

Diabetes is a very serious condition that necessitates a comprehensive and multifaceted therapeutic approach. Patients suffering from diabetes exhibit either insufficient insulin production or cellular resistance to it. The management of post-prandial hyperglycemia involves the use of digestive treatments containing glucosidase inhibitors like acarbose, miglitol, and voglibose (Sen et al., 2023; Sharma and Deb, 2024). These inhibitors function by impeding the breakdown of carbohydrates, consequently diminishing the cell's capacity to uptake glucose. Metformin and glibenclamideare commonly prescribed for the treatment of diabetes. Despite the availability of such treatments, they are accompanied by notable limitations, including high expenses and potential adverse effects like hypoglycemia, weight gain, gastrointestinal complications, and hepatotoxicity (Sukhikh et al., 2023). In light of these challenges, ongoing investigations are being conducted to formulate efficacious anti-diabetic and antioxidant therapies, particularly in light of the impact of oxidative stress on the progression of diabetes. Historically, herbal medicines have played a significant role in the management of diabetes due to the presence of a diverse array of bioactive compounds with anti-diabetic properties. Individuals seeking alternatives to synthetic medications often favour herbal remedies as they provide a natural and comprehensive approach to diabetes management. Such remedies are generally perceived as safer, with relatively fewer side effects (Banerjee et al., 2020). Although more than 400 traditional plant remedies for diabetes have been documented, only a small proportion have undergone systematic or clinical scrutiny to determine their effectiveness. Several plant extracts have exhibited hypoglycemic effects in both human and animal models of Type 2

diabetes. Nonetheless, the widespread integration of herbal therapies into modern medicine is impeded by a dearth of thorough scientific and clinical substantiation. Further clinical investigations are imperative, along with the establishment of straightforward bioassays for biological standardization, pharmacological and toxicological assessments, and extensive utilization of animal models to assess toxicity and safety (Zanzabil et al., 2023). Identifying the active compounds in these plant extracts is also crucial. Medicinal plants containing phytochemicals like flavonoids, prophenyl phenols, alkaloids etc. show potential in managing diabetes by improving insulin sensitivity and addressing complications (Malode et al., 2021). The relationship between diabetes mellitus and the preventive roles of various phytochemicals on diabetes and the major challenging issue in diabetes management is the obstruction of various complications that remain the main cause of diabetes-related mortality. Phytomedicine also plays a crucial role in managing diabetes by offering anti-hyperglycemic, anti-inflammatory, and apoptotic properties, aiding in developing alternative or supportive therapies (Durazzo et al., 2021). Additionally, medicinal plants are often rich in essential nutrients, contributing to overall health and well-being, particularly for individuals with diabetes who may be at risk for other health issues. It is crucial to use medicinal plants under the guidance of healthcare professionals, especially when combined with synthetic drugs, to ensure safe and effective diabetes management.

Plant-derived phytochemicals like steroids, alkaloids, phenolic compounds, lycopene, genistein, quercetin and glycosides have shown efficacy in treating diabetes, offering potential as alternative medicines with fewer side effects compared to conventional drugs (Awuchi, 2022; Baile et al., 2023). These phytoconstituents are beneficial for managing diabetes by enhancing insulin release or inhibiting glucose

absorption. They treat diabetic nephropathy by targeting oxidative stress, inflammation, and other pathways, offering potential therapeutic leads for managing diabetes-related kidney complications (Mitra et al., 2024).

Various vitamins, minerals, and phyto-secondary metabolites have been investigated for their potential roles in diabetes management, both *in vitro* and *in vivo*. Plant-derived phenolic compounds, such as polyphenols, have shown promising potential as antidiabetic agents due to their ability to reduce oxidative stress and hyperglycemia and prevent diabetic complications like retinopathy, nephropathy, and neuropathy (Akpoveso et al., 2023). These phenolic compounds, including curcumin, catechin, flavonoids, tannins, resveratrol, and berberine, exhibit antidiabetic effects through various mechanisms such as inhibiting carbohydrate metabolism enzymes, improving pancreatic β-cell function, and enhancing antioxidant capacity (Deka et al., 2022). Studies on *Veronica biloba* and *Schoenoplectus triqueter* have demonstrated their strong inhibition of angiotensin-I converting enzyme (ACE) and Type-II diabetes enzymes like α-glucosidase and α-amylase, indicating their potential as antihypertensive and antidiabetic agents rich in phenolic compounds (Hassan et al., 2022). Flavonoids, essential low molecular weight phenolic compounds found abundantly in plants, offer various potential benefits as antidiabetic agents. This compound offers benefits in managing type 2 diabetes mellitus by exerting anti-oxidative, anti-inflammatory, glucose and lipid metabolism regulation effects, potentially leading to novel hypoglycemic medications (Yi et al., 2023).Venugopala et al. (2022) highlighted the different types of flavonoids as potent antidiabetic agents along with their structure-activity relationship (SAR) studies, which targeted various biological pathways like xanthine oxidase, SGLT-II, α-glucosidase, PPAR-γ, DPP-4, and glycogen phosphorylase. Flavonoids from plants, like quercetin in *Bauhinia strychnifolia* and *Spatholobussu berectus*, exhibit high antioxidant

and antiglycative properties, making them potential agents for combating oxidative stress, diabetic complications, inhibiting enzymes, enhancing glucose uptake, and potentially offering alternative treatments for blood sugar control (Praparatana et al., 2022; Wong et al., 2022). Flavonoids protect pancreatic beta cells from degradation and increase both insulin secretion and sensitivity. They help maintain glucose homeostasis and facilitate glucose absorption through glucose transporter 2 (GLUT-2) (Ridho, 2023). Alkaloids exhibit antidiabetic properties through multiple mechanisms. They inhibit aldose reductase (AR) and protein tyrosine phosphatase 1B (PTP1B), activate AMP-activated protein kinase (AMPK), and inhibit dipeptidyl peptidase-4 (DDP-4) and advanced glycation end products (AGEs). These compounds also enhance glucose absorption, promote the regeneration of pancreatic beta cells, and modulate the activity of glucose transporter 4 (GLUT-4), glycogen synthase kinase 3 (GSK-3), sterol regulatory element-binding protein 1 (SREB-1), acetyl-CoA carboxylase (ACC), peroxisome proliferator-activated receptors (PPARs), and glucokinase (Ridho, 2023). Plant tannins are high molecular weight natural polyphenolic compounds, categorized into hydrolyzable and condensed tannins (Ali, 2013). They exhibit significant antioxidant activity, which can enhance the body's antioxidant status and protect against degenerative diseases. Tannins are found in various plant foods, including tea, berries, and nuts. Some studies suggest their potential to reduce blood glucose levels and inhibit α-glucosidase activity involved in starch digestion, indicating their possible anti-diabetic properties (Ardalani et al., 2021). Total triterpenoids from medicinal plants have shown promising potential as antidiabetic agents. Research has highlighted the effectiveness of triterpenoids such as arjunolic acid, asiatic acid, and cyclocaric acid B in increasing glucose uptake and exhibiting antidiabetic activity (Liu et al., 2018). Additionally, compounds like hederagenin, 3-epiakebonoic acid, and arjunolic acid have demonstrated strong inhibition

of α-glucosidase activity, with the ability to stimulate glucose uptake in insulin-resistant cells (Bian et al., 2021). Furthermore, pentacyclic triterpenoids like oleanolic acid, ursolicacid, and betulinic acid from various medicinal plants have shown multiple biological activities affecting glucose absorption, insulin secretion, and diabetic complications, offering a promising approach for diabetes management (Alqahtani et al., 2013). The study by Fikriyah et al. (2020) isolated two triterpenoid compounds from *Anisophyllea disticha* with potential antidiabetic activity, indicating the presence of triterpenoids in medicinal plants as antidiabetic agents. Tetracyclic triterpenoids in medicinal plants like *Panax ginseng*, *Momordica charantia*, etc., exhibit antidiabetic properties, but the total triterpenoid content specific to this function was not specified (Hamid et al., 2015).

Antioxidants play a crucial role in managing diabetes by reducing oxidative stress, preventing lipid peroxidation, and supporting antioxidant enzymes, potentially slowing or preventing diabetes progression (Kanwugu et al., 2022; Tuell et al., 2023). Oxidative stress and reactive oxygen species play a role in the initiation and progression of type 2 diabetes. Natural antioxidant products such as vitamin E, vitamin C, beta-carotene, selenium, and manganesehelp combat type 2 diabetes (Tuell et al., 2023). Free radicals, including reactive oxygen species (ROS) and reactive nitrogen species derived from oxygen and nitrogen, are highly reactive molecules produced in various cellular locations, and their increased generation is associated with hyperglycemia (Opara, 2002). Oxidative stress is crucial in developing various diseases, including cancer, cardiovascular disease, diabetes, ageing, liver, and lung diseases. It results from an imbalance between radical production and radical scavenging systems. Various studies have indicated that the overproduction of free radicals and a deficiency in antioxidant protection are involved in the pathogenesis of diabetes. The mechanisms behind the prooxidant-antioxidant

imbalance in diabetes mellitus include glucose auto-oxidation, increased formation of advanced glycation end products (AGEs), the polyol pathway, the hexosamine pathway, and the mitochondrial respiratory chain (Singh et al., 2009). Antioxidants like astaxanthin, are found mostly in marine organisms and have been shown to protect β-cells, neurons, as well as several organs, including the eyes, kidney, liver, etc. Thus, it plays a crucial role in managing diabetes by protecting cells and organs and improving glucose metabolism, offering potential benefits in diabetes treatment and complications (Kanwugu et al., 2022). Antioxidants like vitamins C and E, minerals, and flavonoids help manage diabetes by combating oxidative stress, maintaining cellular balance, and supporting normal cell signalling mechanisms (Saini et al., 2023; Tuell et al., 2023). In individuals diagnosed with type 2 diabetes mellitus and deemed high-risk, the implementation of intensive interventions incorporating a variety of pharmaceutical combinations and adjustments in lifestyle has exhibited favourable outcomes in terms of vascular complications and diminished mortality rates stemming from cardiovascular disease and other etiologies. The management of diabetes is significantly influenced by the role of antioxidants, which serve to reduce complications such as endothelial dysfunction, cardiomyopathy, retinopathy, nephropathy, and neuropathy, consequently mitigating the likelihood of developing diabetes and its associated complications (Zatalia and Sanusi, 2013).

Numerous traditional medicinal practices, such as Ayurveda and Traditional Chinese Medicine, have historically incorporated medicinal herbs like bitter melon and fenugreek seeds for the purpose of managing diabetes, leveraging their rich concentration of beneficial compounds such as flavonoids, alkaloids, polyphenols, and terpenes. Within Nagaland, recognized as a region of high biodiversity in the Northeastern part of India, local indigenous communities possess substantial expertise in utilizing indigenous flora

to address various health conditions, including diabetes. The present study constitutes a segment of pharmacological investigations on select ethnomedicinal plants with potential anti-diabetic properties indigenous to Nagaland, India. A key objective of this research endeavour was to explore the phytochemical constituents with anti-diabetic potential in 15 ethnomedicinal plants traditionally utilized by various tribes in Nagaland, India. Emphasis was placed on the scrutiny of distinct anti-diabetic phytochemical compounds present in these plants and their antioxidative capabilities, aiming to elucidate the rationale behind their historical use as traditional anti-diabetic remedies. Additionally, the current investigation delved into evaluating the efficacy of different solvents, notably ethanol, methanol, and water, in isolating diverse phytochemical compounds from these plants. Acquiring insights into the effects of various solvents could offer valuable knowledge for prospective studies and the formulation of efficacious anti-diabetic therapies derived from these natural reservoirs.

Materials and Methods

Plant Extracts Preparation

The specific plant parts to be used for analysis were collected during the flowering seasons of all the plants under study, where the secondary metabolites are known to be present at the peak concentrations as per traditional knowledge. The collected samples were washed with tap water and then dried in a hot air oven at 50°C till the consistent weight of the samples was ensured. The dried plant parts were then ground separately into fine powder. For the preparation of the different extracts, 50 mg of each of the powdered plant parts was mixed with 10 ml each of methanol (80%, v/v) (MeOH), ethanol (80%, v/v) (EtOH) and pure water (pH_2O) separately. The mixtures were then incubated in a water bath for 2h at 60°C followed by centrifugation at 10000 rpm for 10 min. Post centrifugation, the supernatants were filtered using Whatman's filter paper No.

1 and the extracts were stored at 4ºC till used for analysis purposes. The ThermoScientific UV-Visible Spectrophotophometer (Thermo Scientific Evolution 201 Series) and Thermo Scientific Multiskan Spectrophotometer were used to measure absorbance for quantification of phytochemicals and antioxidant activity, respectively.

Phytochemical Analysis

Quantification of total phenol content (TPC)

For the quantification of TPC, the modified Folin-Ciocalteu method (Genwali et al., 2013) was used. For this purpose, 200 µL each of the plant extracts of the three solvents were mixed with 2.8 ml pH_2O, 2 ml 7% (w/v) sodium carbonate solution and 0.5 ml 10% (v/v) Folin-Ciocalteu reagent and incubated at room temperature in the dark for 90 min. Post incubation, the absorbance was read at 765 nm. The TPC was quantified and expressed as milligrams of Gallic acid equivalent per gram (mgGAE/gm) of the sample based on dry weight basis. All the experiments were performed in triplicates for each solvent.

Quantification of total flavonoid content (TFC)

To quantify the TFC, the protocol of Tan (Tan et al., 2018) was followed. To a 200 µL plant extracts, 0.15 ml 0.5M sodium nitrate and 0.15 ml 0.3M aluminium chloride were added. The final volume was adjusted to 4.0 ml using 30% methanol (v/v) and allowed to settle the mixture for 5 min. Subsequently, 1 ml of 1M sodium hydroxide (NaOH) was added to the reaction mixture, and the absorbance was read at 415 nm. The resulting absorbance values were utilised to determine the concentration of TFC with reference to a Quercetin standard, expressed as milligrams of Quercetin equivalent per gram (mgQE/gm) of the sample on a dry weight basis.

Quantification of total tannin content (TTC)

The Folin-Dennis method (Bhattacharyya et al., 2014) was used for the estimation of TTC. Reaction mixtures were prepared by mixing 200 µL each of the plant extracts with 3.8 ml pH_2O, 0.5 ml each of both Folin-Dennis reagents and 0.5 ml 20% sodium carbonate solution and mixed thoroughly, and absorbance was read at 775 nm. The TTC was calculated based on a calibration curve using Tannic acid as a reference standard and expressed as milligrams of tannic acid equivalent per gram (mgTAE/gm) of the sample on a dry weight basis.

Quantification of total triterpenoid content (TTRC)

To quantify the TTRC, a standard curve was prepared using Ursolic acid as a reference (Ke et al., 2014). For this purpose, 40 µL of each plant extract was mixed with 800 µL sulfuric acid and 400 µL 5% Vanillin-Glacial Acetic Acid reagent. The reaction mixtures were then incubated at 60°C for 15 min, allowing for chemical reactions followed by mixed 5 ml of glacial acetic acid to the mixture, ensuring complete homogenisation. The absorbance was read at 545 nm, and a standard curve was generated using Ursolic acid, with known concentrations linked to absorbance values at 545 nm. By employing this curve, the TTRC in the samples were determined and expressed as milligrams of Ursolic acid equivalent per gram (mgUAE/gm) of the sample.

Quantification of total alkaloid content (TAC)

To determine the TAC, the procedure outlined by Patel et al. (2015) with minor adjustments was followed. Initially, the raw sample was subjected to extraction using three different solvents: 80% methanol, 80% ethanol, and pH_2O in, a Soxhlet apparatus. The resulting extracts were then condensed and subsequently evaporated to obtain dry residues for further analysis. These residues were subsequently dissolved by adding 2N hydrochloric acid (HCl). In the separating funnel, 1ml of the test solution was transferred,

and to this, 5 ml of 0.2 M phosphate buffer (pH 4.7) and 10-4 M bromocresol green were added. Variable volumes of chloroform (1, 2, 3, and 4 ml) were added into the mixtures and vigorously shaken to allow the complexes to form and collected in 10ml volumetric flask. The final volume was adjusted to 10 ml with chloroform. Subsequently, the absorbance of the complex was read at 470 nm. The TAC was quantified in terms of milligrams of Atropine equivalents per gram (mgAE/gm) of the sample on a dry weight basis.

Quantification of reducing sugar content (RSC)

The RSC was determined using the 3,5-dinitrosalicylic acid (DNSA) method (Khatri and Chhetri, 2020). To prepare the DNSA reagent, 1.5 g of 3,5-dinitro salicylic acid was dissolved in 30 ml of 2M NaOH and 40 g of sodium-potassium tartaric acid in 100 ml of distilled water. These solutions were mixed to create the DNSA reagent, and the volume was adjusted to 150 ml with pH_2O. In the experiment, 600 µL of each plant extract was mixed with 2.4 ml pH_2O and 1mL of DNSA reagent. The tubes were sealed with cotton and then placed in boiling water for 5 min. After cooling, the absorbance of the reactions was measured at 540 nm. The RSC was determined from the calibration curve of standard D-glucose, and the results were expressed as milligrams of D-glucose equivalent per gram (mgGE/gm) dry extract weight.

Calculation for TPC, TFC, TTC, TTRC, TAC, RSC

(TPC, TFC, TTC, TTRC, TAC, RSC) $=\frac{(C\times V\times D)}{W}$,

Where:

C = Concentration of the compound in the sample (mg/mL), **V** = Volume of the extract used (mL), **D** = Dilution factor (if applicable), W = weight of the sample (g).

Quantification of antioxidant activity

2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay

Assessment of the radical scavenging activity of the sample extracts was done using DPPH free radical scavenging assay (Brand-William et al., 1995). Different concentrations of the sample extracts were mixed with 3 ml of DPPH reagent (0.1mM dissolved in 80% methanol). The final volume was adjusted to 4 ml using 80% methanol, and the mixture was incubated for 30 min in the dark. Post-dark incubation, the absorbance of the solution was read at 517 nm. The percentage inhibition of DPPH free radicals was determined using the following formula:

$$\textbf{Free radical inhibition } \% = \frac{control\ absorbance \times Sample\ absorbance}{control\ absorbance} \textbf{x 100}$$

Ferric reducing antioxidant power (FRAP) assay

The assessment of antioxidant activity was also executed using the FRAP assay relies on the capacity of antioxidants to convert ferric (Fe_3^+) ions into ferrous (Fe_2^+) ions in the presence of 2,4,6-tri-(2-pyridyl)-5-triazine (TPTZ) (Rakholiya et al., 2015). To prepare the FRAP reagent, a solution was created by combining freshly prepared 10mM TPTZ, 20 mM ferric chloride, and 300 mM sodium acetate buffer (pH 3.6) in a 10:1:1 ratio. This reagent was then placed in a 37°C water bath. In the test, sample extracts were mixed with 3ml of the FRAP reagent in a test tube, and the final volume was adjusted to 4 ml using pH_2O. After 30 min of incubation in the dark at 37°C, the absorbance read at 593 nm. A standard curve was established using ferrous sulphate, and based on the sample's absorbance, the FRAP units were calculated, expressed in mM of $Fe2^+$ per gram of dry sample.

2, 2-Azino-(3-ethyl) benzothiazoline-6-sulfonic acid diammonium salt (ABTS) radical cation scavenging assay

The ABTS assay was done following a modified version of the Babbar et al. (2011) method to evaluate the antioxidant capacity of the sample extracts. An ABTS stable stock solution was prepared by incubating a 7 mM/L aqueous ABTS solution and a 2.45 mM/L potassium persulfate solution in a dark room at room temperature for 16 h. The absorbance of this solution was adjusted to 0.70±0.02 absorbance units (AU) at 734 nm by diluting it with 80% ethanol prior to use. In the test, an appropriate volume of the sample extracts was mixed with 3 ml of the ABTS working solution, with methanol serving as the control. After a 30 min incubation period in darkness, the absorbance was measured at 734 nm to assess the antioxidant capacity.

Statistical Analysis

All the experiments were executed in triplicate and repeated thrice, and deviations were calculated as the standard error of the mean (SEM). In addition, One-Way Analysis of Variance Analysis (ANOVA) and the Least Significant Test were done at a 95% confidence level ($p \leq 0.05$) using Microsoft Excel software.

Results

In the present study, for the extraction of crude phytochemicals, three different solvents were used. In general, 80% ethanol extract was found to be a better solvent, followed by 80% methanol and pure water, except for the TFC, where 80% methanol extract was found to be better than the other two. For TTC, though EtOH was found to be a better solvent, but other two solvents were found to be competitive.

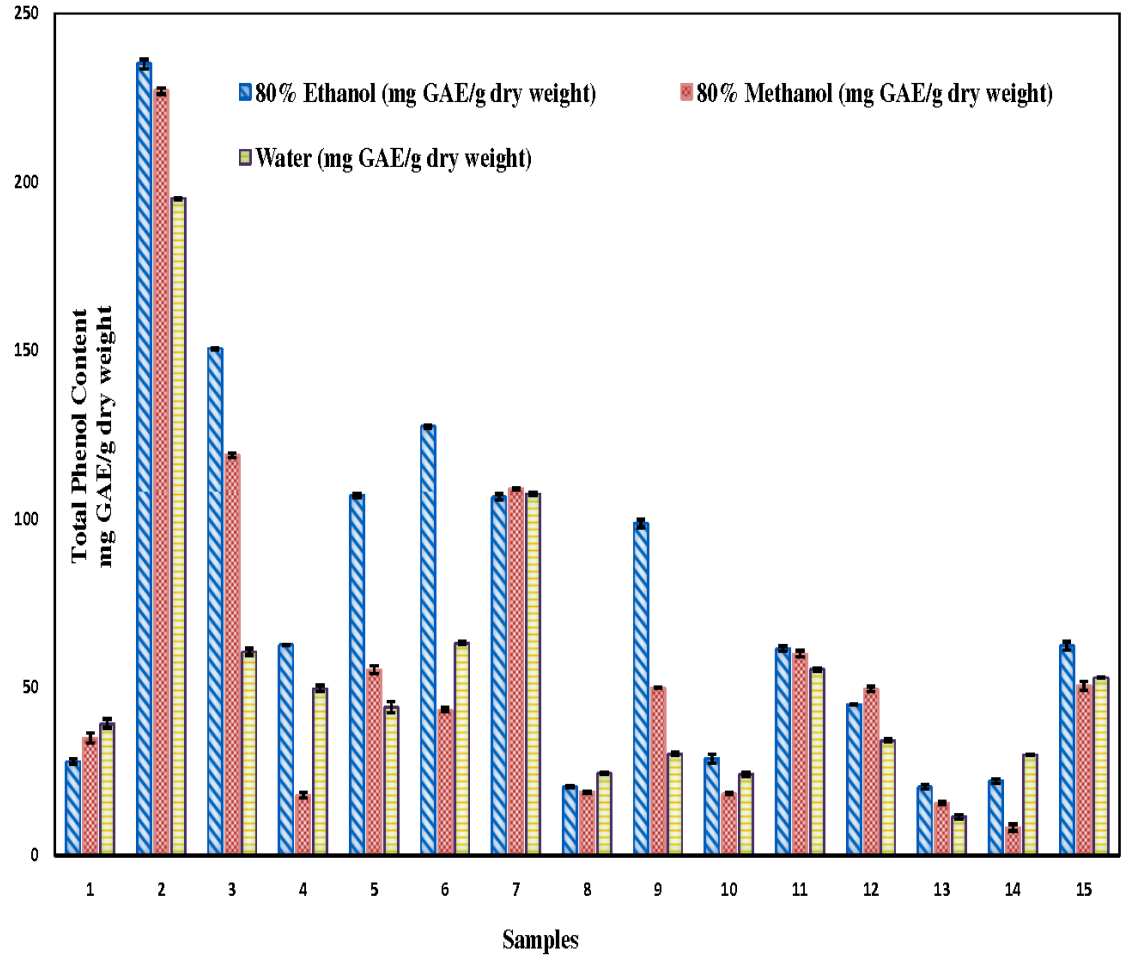


Figure 4.1: Total phenolic contents of 15 medicinal plants in three different solvents used for treating diabetes. 1. *Abroma augustum,* 2. *Bauhinia variegata,* 3. *Cajanus cajan,* 4. *Catharanthus roseus,* 5. *Senna alata,* 6. *Clerodendrum colebrookianum,* 7. *Euphorbia hirta,* 8. *Gynura crepidioides,* 9. *Kalanchoe pinnata,* 10. *Mucuna pruriens,* 11. *Paederia foetida,* 12. *Passiflora edulis,*13. *Perilla frutescens,* 14. *Solanum nigrum,* 15. *Solanum trilobatum*.

Total phenolic content

Figure 4.1 illustrates the TPC of various plant species under investigation. It is worth noting that all the assessed plant species contain good phenolic compounds. The highest TPC concentration was observed in the 80% EtOH extract of *Bauhinia variegata* (235.02 mgGAE/gm), and the lowest was recorded from the leaves of *Perilla frutescens*; 20.36 mgGAE/gm in EtOH extract and 11.52 mgGAE/gm in pH_2O extract. In 80% MeOH extract, *Solanum nigrum* leaves extract showed the lowest TPC (8.33 mgGAE/gm). In general, of the 15 plant extracts evaluated using three different solvents, 80%EtOH extract was found to be a better solvent for analysis of TPC. In *Abroma augustum*, *Gynura crepidioides*, *Mucuna pruriens*, *Passiflora edulis*, *Perilla frutescens,* and *Solanum nigrum* leaves, the TPC was ≤50 mgGAE/g in 80% EtOH extracts (Table 4.1).

Table 4.1: Quantification of anti-diabetic potential phytochemicals from 15 ethnomedicinal plants using different solvent extracts

Anti-diabetic potential plants	Total Phenol Content			Total Flavonoid Content			Total Tannin Content			Total alkaloid Content			Total Triterpinoid Content			Total Reducing Sugar Content		
	80% EtOH (mgGAE/g)	80% MeOH (mgGAE/g)	Pure Water (mgGAE/g)	80% EtOH (mg QE/g)	80% MeOH (mg QE/g)	Pure Water (mgQE/g)	80% EtOH (mgTA/g)	80% MeOH (mgTA/g)	Pure Water (mgTA/g)	80% EtOH (mg AE/g)	80% MeOH(mg AE/g)	Pure Water (mgAE/g)	80% EtOH (mgUAE/g)	80% MeOH (mgUAE)	Pure Water (mgUAE)	80% EtOH (mg E/g)	80% MeOH (mgGE/g	Pure Water (mgGE/g)
Abroma augustum	27.97 ±0.7^{h}	34.96 ±1.5^{h}	39.22 ±1.3^{f}	26.78 ±0.7^{h}	40.93 ±0.7^{e}	26.80 ±0.6^{g}	19.418 ±1.07^{g}	11.327 ±0.02^{h}	31.800 ±1.4^{e}	22.37 ±0.88^{e}	17.58 ±0.78^{e}	15.02 ±0.34^{d}	32.465 ±1.88^{j}	30.436 ±0.98^{h}	14.131 ±0.74^{f}	219.677 ±1.0^{b}	132.233 ±1.3^{b}	92.667 ±0.6^{e}
Bauhinia variegata	235.02 ±1.4^{a}	227.04 ±0.9^{a}	195.02 ±0.3^{a}	167.20 ±1.4^{a}	266.87 ±0.7^{a}	148.23 ±0.7^{a}	163.290 ±1.8^{a}	142.018 ±1.9^{a}	148.830 ±1.9^{a}	41.29 ±0.72^{a}	40.55 ±0.66^{a}	31.76 ±0.58^{a}	134.871 ±0.80^{b}	134.871 ±0.76^{b}	134.871 ±0.88^{b}	588.878 ±0.6^{a}	477.977 ±1.0^{a}	402.111 ±1.1^{a}
Cajanus cajan	150.52 ±0.2^{b}	118.82 ±0.7^{b}	60.54 ±1.0^{c}	166.64 ±1.1^{a}	160.06 ±0.8^{b}	78.57 ±0.9^{b}	67.012 ±0.8^{c}	64.279 ±0.4^{c}	46.436^{c} ±0.7	16.71 ± 0.84^{f}	15.63 ± 1.25^{f}	8.48 ± 0.88^{f}	46.995 ±0.94^{i}	49.491 ±0.25^{f}	24.476 ±0.78^{e}	119.866 ±0.3^{e}	70.111 ±0.8^{c}	94.122 ±0.7^{e}
Catharanthus roseus	62.61 ±0.1^{f}	17.93 ±0.8^{i}	49.57 ±0.9^{e}	47.40 ±0.6^{f}	18.77 ±0.5^{h}	23.80 ±0.8^{g}	40.945 ±0.8^{d}	27.873 ±1.1^{f}	40.558 ±0.9^{d}	34.13 ±0.52^{c}	28.88 ±1.26^{c}	29.22 ±0.19^{b}	49.901 ±0.72^{h}	53.95 ±0.66^{e}	30.3 ±0.59^{d}	105.988 ±1.6^{f}	45.977 ±1.6^{e}	108.700 ±0.4^{d}
Senna alata	106.90 ±0.5^{d}	55.22 ±1.1^{e}	43.98 ±1.7^{f}	73.60 ±0.5^{c}	63.74 ±0.4^{d}	24.52 ±0.9^{g}	29.593 ±2.3^{f}	20.206 ±0.4	25.461 ±1.5^{f}	3.00 ±0.14^{h}	14.58 ±0.39^{f}	4.82 ±1.23^{g}	56.205 ±0.84^{f}	47.904 ±0.89^{f}	23.339 ±0.63^{e}	102.178 ±1.1^{f}	59.011 ±1.0^{d}	87.233 ±1.4^{f}
Clerodendrum colebrookianum	127.34 ±0.4^{c}	43.29 ±0.6^{g}	63.16 ±0.5^{c}	78.25 ±1.3^{b}	40.38 ±0.6^{e}	47.24 ±0.7^{d}	42.739 ±1.3^{d}	49.697 ±1.1^{d}	18.660 ±0.6^{g}	4.63 ±0.382	10.00 ± 0.52^{g}	2.89 ± 0.95^{h}	118.803 ±0.82^{c}	99.3 ±0.32^{c}	72.911 ±0.93^{c}	88.300 ±1.3^{g}	38.711 ±0.8^{f}	33.400 ±1.8^{i}
Euphorbia hirta	106.48 ±0.9^{d}	108.73 ±0.4^{c}	107.41 ±0.5^{b}	69.75 ±0.9^{d}	62.07 ±0.7^{d}	61.89 ±0.6^{c}	86.563 ±0.8^{b}	83.327 ±0.6^{b}	99.436 ±2.1^{b}	43.58 ±1.15^{a}	24.05 ± 0.89^{d}	27.45 ± 1.83^{c}	28.969 ±1.05^{l}	17.675 ±0.79^{i}	9.594 ±1.03^{g}	152.389 ±1.7^{d}	72.300 ±1.3^{c}	95.111 ±1.5^{e}
Gynura crepidioides	20.39 ±0.3^{i}	18.89 ±0.3^{i}	24.39 ±0.3^{i}	49.22 ±0.9^{f}	29.40 ±0.6^{f}	20.28 ±0.5^{h}	10.266 ±0.5^{i}	10.588 ±0.3^{h}	16.891 ±1.7^{g}	35.58 ±0.48^{c}	17.05 ±0.30^{e}	14.23 ±0.37^{d}	81.058 ±078^{d}	67.279 ±0.93^{d}	29.911 ±0.87^{d}	67.711 ±1.2^{i}	47.388 ±0.8^{e}	30.544 ±0.8^{i}
Kalanchoe pinnata	98.59 ±1.2^{e}	49.77 ±0.1^{f}	30.19 ±0.5^{h}	40.68 ±0.8^{g}	25.76 ±0.1^{g}	19.52 ±0.2^{h}	23.212 ±1.6^{g}	15.855 ±0.9^{g}	24.497 ±0.7^{f}	13.42 ± 0.11^{g}	37.54 ±1.39^{b}	12.24 ±0.44^{e}	59.136 ±0.51^{e}	42.921 ±1.90^{g}	24.687 ±0.74^{e}	83.378 ±0.7^{h}	43.155 ±1.1^{e}	68.900 ±1.7^{g}
Mucuna pruriens	28.78 ±1.3^{h}	18.37 ±0.3^{i}	24.20 ±0.6^{i}	58.11 ±1.3^{e}	43.33 ±0.7^{e}	47.60 ±0.9^{d}	16.745 ±0.6^{h}	17.594 ±2.6^{g}	14.224 ±1.1^{g}	40.71 ±0.92^{b}	23.96 ±1.11^{d}	14.51 ±0.82^{d}	19.426 ±1.92^{m}	17.367 ±1.31^{i}	8.137 ±0.52g	66.956 ±1.2^{i}	33.900 ±1.4^{g}	40.200 ±0.2^{h}
Paederia foetida	61.41 ±0.7^{f}	59.91 ±0.9^{d}	55.27 ±0.5^{d}	56.56 ±1.8^{e}	60.98 ±1.5^{d}	40.83 ±0.5^{e}	45.381 ±0.3^{d}	32.485 ±0.4^{e}	26.430 ±0.6^{f}	3.42 ±0.23^{h}	12.04 ±0.82^{f}	13.33 ±1.73^{e}	51.573 ±0.73^{g}	41.314 ±0.32^{g}	23.078 ±0.73^{e}	153.856 ±1.6^{d}	53.511 ±0.4^{d}	128.333 ±1.8^{c}
Passiflora edulis	44.83 ±0.1^{g}	49.50 ±0.7^{f}	34.14 ±0.5^{g}	66.19 ±0.2^{d}	73.84 ± 0.5^{c}	35.23 ±0.8^{f}	35.606 ±1.4^{e}	26.655 ±1.2^{f}	26.509 ±0.6^{f}	32.00 ±0.26^{d}	15.50 ±0.80^{e}	8.89 ±0.62^{f}	55.966 ±0.86^{f}	42.767 ±0.70^{g}	23.716 ±1.12^{e}	173.800 ±1.6^{c}	73.900 ±1.8^{c}	137.689 ±1.6^{b}

Solanum nigrum	22.11 ±0.6^{i}	8.33 ±1.0^{j}	29.94 ±0.1^{h}	37.29 ±0.1^{g}	24.32 ±0.7^{g}	10.25 ±0.4^{i}	8.703 ±0.5i	2.891 ±0.8^{j}	3.491 ±0.5^{i}	2.63 ±0.07^{h}	8.84 ±0.25^{g}	2.35 ±0.42^{h}	36.819 ±0.17k	31.488 ±0.85^{h}	16.277 ±0.72^{f}	66.200 ±1.9^{i}	32.800 ±0.4^{g}	27.522 ±0.9^{j}
Solanum trilobatum	62.28 ±1.2^{f}	50.38 ±1.4^{f}	52.88 ±0.1^{d}	37.43 ±1.4^{g}	16.03 ±1.3^{h}	21.87 ±0.2^{h}	14.836 ±0.2^{h}	7.527^{i} ±0.3	19.279 ±0.7^{h}	15.08 ±1.39^{g}	14.58 ±0.67^{e}	4.74 ±0.73^{h}	227.415 ±0.39^{a}	219.906 ±0.72^{a}	180.604 ±0.43^{a}	92.533 ±0.9^{g}	47.733 ±0.9^{e}	41.567 ±1.2^{h}

Note: EtOH: 80% EtOH, MeOH: 80% MeOH; Water: Pure water; Data are expressed as Mean ± SE, n=3. * *p*-value has been calculated using one-way ANOVA, and values with the same superscript letters in the column do not differ significantly ($p \leq 0.05$).

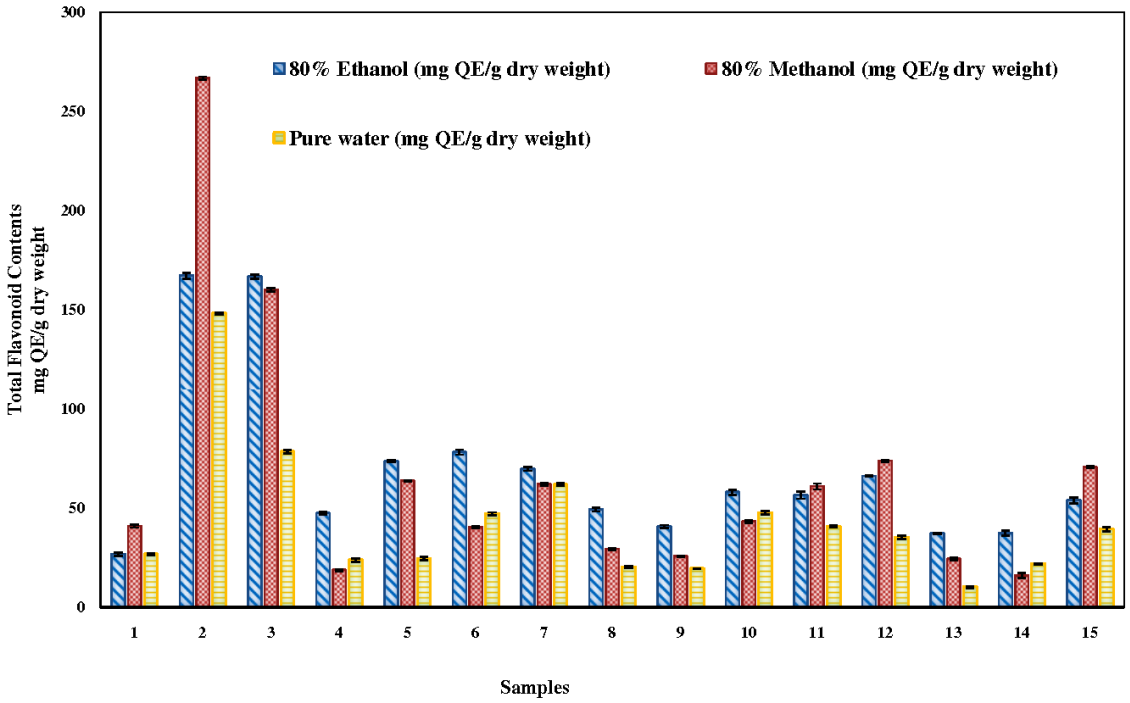


Figure 4.2: Total flavonoid contents of 15 medicinal plants in three different solvents used for treating diabetes. 1. *Abroma augustum,* 2. *Bauhinia variegata,* 3. *Cajanus cajan,* 4. *Catharanthus roseus,* 5. *Senna alata,* 6. *Clerodendrum colebrookianum,* 7. *Euphorbia hirta,* 8. *Gynura crepidioides,* 9. *Kalanchoe pinnata,* 10. *Mucuna pruriens,* 11. *Paederia foetida,* 12. *Passiflora eduli,* 13. *Perilla frutescens,* 14. *Solanum nigrum,* 15. *Solanum trilobatum*.

Total flavonoid content

The depicted Figure 4.2 provides an overview of the presence of flavonoids in the various plant species under investigation. Notably, the MeOH extract was found to be a better choice for the extraction of TFC, and water was the poorest. In all the extraction solvents, *Bauhinia variegata* stem extract was consistently observed to possess the highest TPC with values of 167.20 mgQE/gm in EtOH, 266.87 mgQE/gm in MeOH, and 148.23 mgQE/gm in water extracts. In contrast, plant species such as *Abroma augustum*, *Catharanthus roseus, Gynura crepidioides, Kalanchoe pinnata, Perilla frutescens*, and

Solanum nigrum leaves extracts, when compared to all the three solvent extractions, exhibited TPC values was ≤50mgQE/gm(Table 4.1).

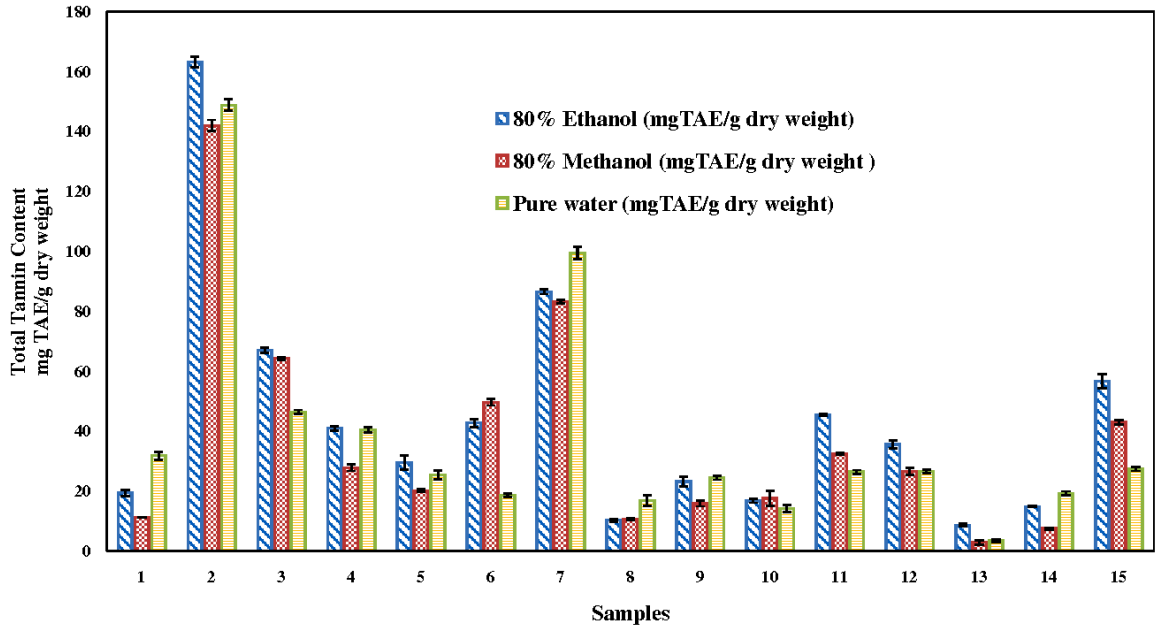


Figure 4.3: Total Tannin Contents per dry weight of 15 medicinal plants in three different solvents used for treating diabetes. 1. *Abroma augustum,* 2. *Bauhinia variegata,* 3. *Cajanus cajan,* 4. *Catharanthus roseus,* 5. *Senna alata,* 6. *Clerodendrum colebrookianum,* 7. *Euphorbia hirta,* 8. *Gynura crepidioides,* 9. *Kalanchoe pinnata,* 10. *Mucuna pruriens,* 11. *Paederia foetida,* 12. *Passiflora edulis,* 13. *Perilla frutescens,* 14. *Solanum nigrum,* 15. *Solanum trilobatum.*

Total tannin content

The TTC of all the 15 studied species in all three solvents are presented in Figure 4.3. Remarkably, *Bauhinia variegata* leaves extract consistently displayed the highest TTC in all three solvents, with measurements of 163.290, 142.018 and 148.830 mg/gm in EtOH, MeOH and water extracts respectively. Conversely, *Perilla frutescens* leaves exhibited the lowest TTC in all three solvent extracts, with values of 8.703, 2.891, and 3.491 mg TAE/gm in EtOH, MeOH and water extracts, respectively. Besides *Bauhinia*

variegata, only *Cajanus cajan* leaves and *Euphorbia hirta* whole plant part extracts exhibited TTC values exceeding 50 mgTAE/gm (Table 4.1).

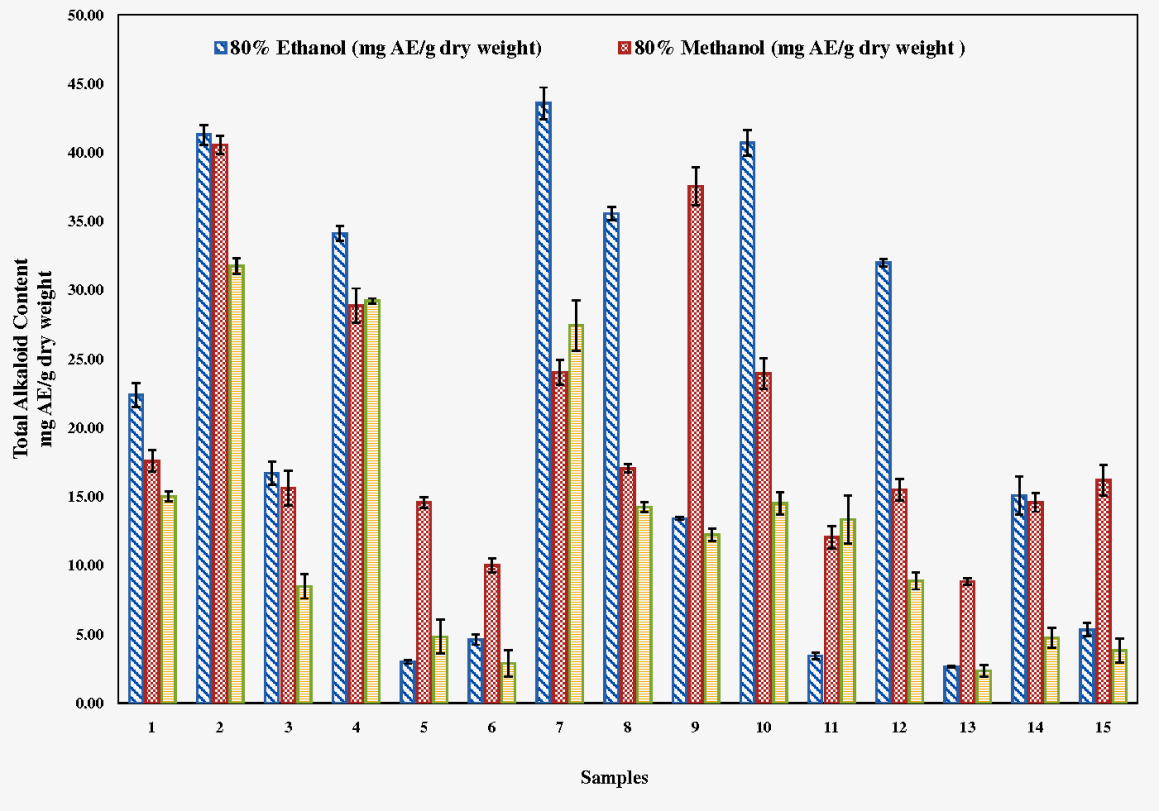


Figure 4.4: Total Alkaloid Contents per dry weight of 15 medicinal plants in three different solvents used for treating diabetes. 1. *Abroma augustum,* 2. *Bauhinia variegata,* 3. *Cajanus cajan,* 4. *Catharanthus roseus,* 5. *Senna alata,* 6. *Clerodendrum colebrookianum,* 7. *Euphorbia hirta,* 8. *Gynura crepidioides,* 9. *Kalanchoe pinnata,* 10. *Mucuna pruriens,* 11. *Paederia foetida,* 12. *Passiflora edulis,* 13. *Perilla frutescens,* 14. *Solanum nigrum,* 15. *Solanum trilobatum*.

Total alkaloid content

Of the 15 species studied in the present investigation, top 7 species where comparatively higher TAC values were recorded in *Bauhinia variegata, Catharanthus roseus, Euphorbia hirta, Gynura crepidioides, Mucuna pruriens, Passiflora edulis, Abroma augustum* in all the extraction solvents (Figure 4.4). However, the values varied significantly in different solvents. In EtOH solvent highest TAC was recorded in

Euphorbia hirta (43.58 mgAE/gm) followed by *Bauhinia variegata* (41.29 mgAE/gm)>*Mucuna pruriens* (40.71 mgAE/gm)>*Gynura crepidioides* (35.58 mgAE/gm)>*Catharanthus roseus* (34.13 mgAE/gm)>*Passiflora edulis* (32.00 mgAE/gm)>*Abroma augustum* (22.38 mgAE/gm) and least was in *Perilla frutescens* (2.63mgAE/gm). While, in MeOH extract, values were *Euphorbia hirta* (24.04 mgAE/gm), *Bauhinia variegata* (40.54 mgAE/gm), *Mucuna pruriens* (23.96 mgAE/gm), *Gynura crepidioides* (17.04 mgAE/gm), *Catharanthus roseus* (28.88 mgAE/gm), *Passiflora edulis* (15.50mgAE/gm) and in *Perilla frutescens* (8.83mgAE/gm) (Table 4.1). When comparing all three solvents, it was found that *Bauhinia variegata* contained the highest TAC among the 15 species studied. The remaining species, such as *Senna alata*, *Cajanus cajan*, *Clerodendrum colebrookianum, Paederia foetida,* and *Solanum trilobatum* leaf extracts detected less than 20 mgAE/gm TAC in all the solvents.

Total triterpenoid content

The experiment involved the extraction of plant samples using three distinct solvents: 80% EtOH, 80% MeOH, and pH2O. Figure 4.5 shows that among these solvents, the 80% EtOH extraction method exhibited the highest efficacy in terms of TTRC. Specifically, the extract from *Solanum nigrum*, regardless of the solvent used, consistently demonstrated the highest TTRC values. In the case of 80% EtOH, the TTRC value for *Solanum nigrum* was 227.41 mgUA/gm, while for 80% MeOH, it was 219.906 mgUA/gm, and for pH2O, it measured 180.604 mgUA/gm. Similarly, the extract from *Bauhinia variegata* also consistently exhibited relatively high TTRC values across all three solvents. In the case of 80% EtOH, the TTRC value for *Bauhinia variegata* was 134.871 mgUA/gm. For 80% MeOH, it was 134.871 mgUA/gm, and for pH2O, it was 134.871 mgUA/gm. Conversely, *Mucuna pruriens* displayed the lowest TTRC values in all three solvents. The TTRC value for *Mucuna pruriens* was 19.426 mgUA/gm when

extracted with 80% EtOH, 17.367 mgUA/gm with 80% MeOH, and 8.137 mgUA/gm with pH2O, respectively(Table 4.1). These findings indicate that the choice of solvent significantly influences the extraction efficiency of TTRC, with 80% EtOH proving to be the most effective for extracting TTRC from *Solanum nigrum*, while *Bauhinia variegata* and *Mucuna pruriens* also displayed varying TTRC values in response to different solvents.

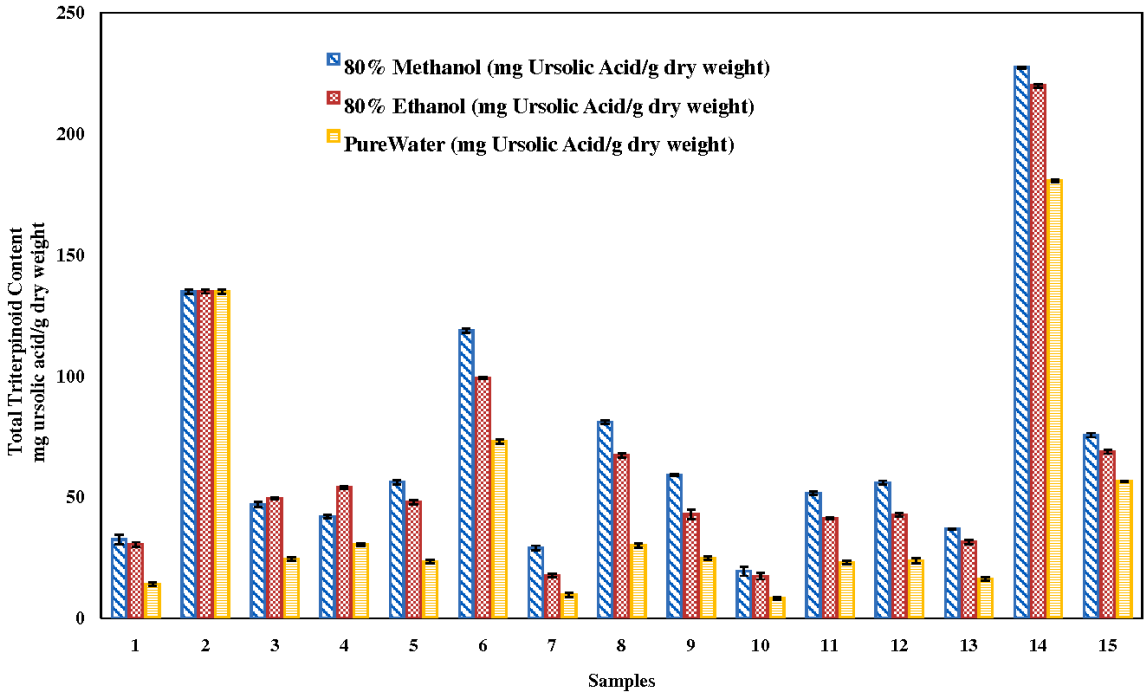


Figure 4.5: Total Triterpenoid Contents per dry weight of 15 medicinal plants in three different solvents used for treating diabetes. 1. *Abroma augustum,* 2. *Bauhinia variegata,* 3. *Cajanus cajan,* 4. *Catharanthus roseus,* 5. *Senna alata,* 6. *Clerodendrum colebrookianum,* 7. *Euphorbia hirta,* 8. *Gynura crepidioides,* 9. *Kalanchoe pinnata,* 10. *Mucuna pruriens,* 11. *Paederia foetida,* 12. *Passiflora edulis,* 13. *Perilla frutescens,* 14. *Solanum nigrum,* 15. *Solanum trilobatum*.

Total reducing sugar content

The analysis of RSC results is presented in Figure 4.6. Data reveals the noteworthy variations across the three different solvent extracts in different plants studied. *Perilla frutescens* consistently exhibited the lowest RSC, with recorded values of 66.200 mgGE/gm with 80% EtOH, 32.800 mgGE/gm with 80% MeOH, and 27.522 mgGE/gm with pH_2O.Conversely, *Bauhinia variegata* was found to contain the highest RSC among all the plant samples studied (588.878 mgGE/gm in 80% EtOH, 477.977 mgGE/gm in 80% MeOH, and 402.111 mgGE/gm in pH_2O) (Table 4.1). Furthermore, *Gynura crepidioides, Kalanchoe pinnata, Mucuna pruriens, Solanum nigrum,* and *Solanum trilobatum* leaves extracts exhibited RSC values ≤100 mgGE/gm of the sample. These findings underscore the substantial disparities in RSC among the plant samples and emphasise the significance of solvent choice in the extraction process.

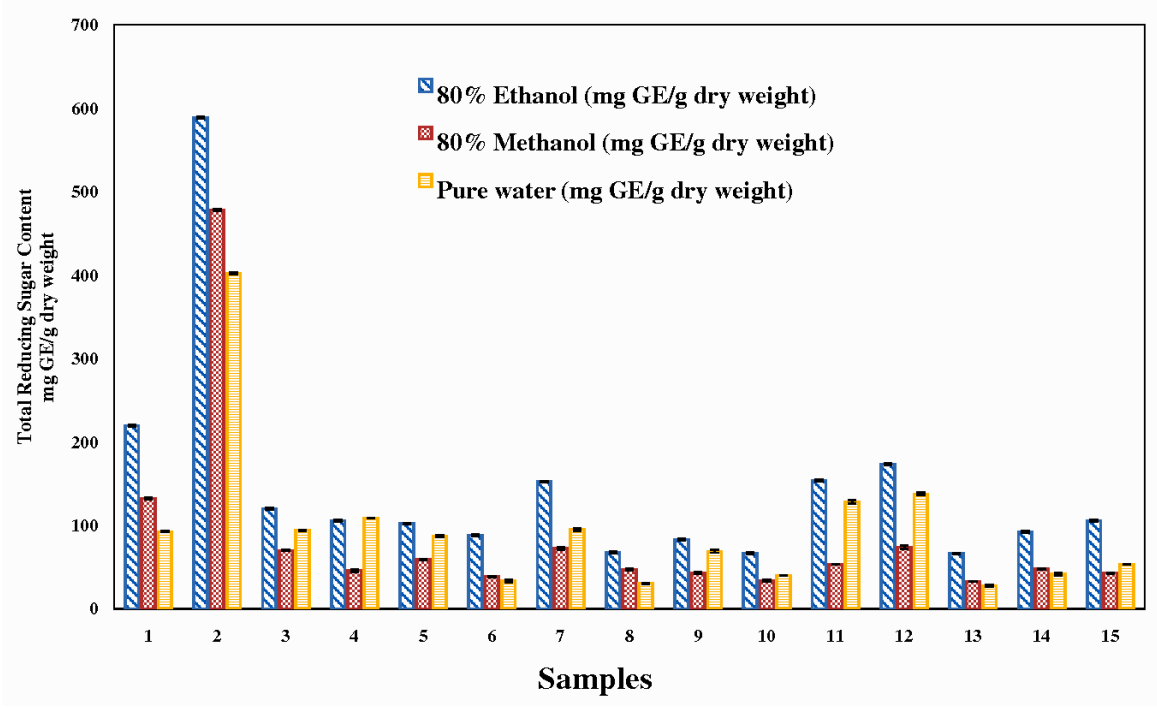


Figure 4.6: TRS content per dry weight of 15 medicinal plants in three different solvents used for treating diabetes. 1. *Abroma augustum,* 2. *Bauhinia variegata,* 3. *Cajanus cajan,* 4. *Catharanthus roseus,* 5. *Cassia alata,* 6. *Clerodendrum colebrookianum,* 7. *Euphorbia*

hirta, 8. *Gynura crepidioides,* 9. *Kalanchoe pinnata,* 10. *Mucuna pruriens,* 11. *Paederia foetida,* 12. *Passiflora edulis,* 13. *Perilla frutescens,* 14. *Solanum nigrum,* 15. *Solanum trilobatum*.

Antioxidant activity evaluation

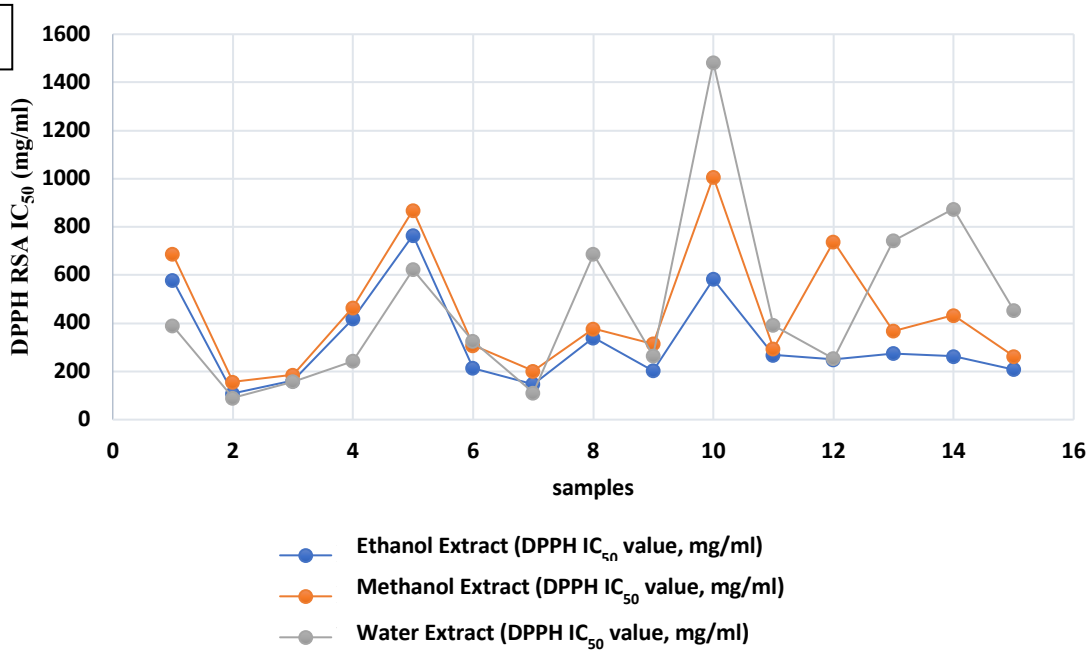
The antioxidant potential of all 15 species was investigated using three different extracts and three different techniques, and the findings are presented in Table 4.2. The IC_{50} values are indicative of the DPPH radical scavenging activity for 80% EtOH, 80% MeOH, and pH_2O extracts from various plant samples, with Trolox serving as the positive control. The results reflect the radical scavenging potential of the plant extractsin Figure 4.6. Notably, all plant extracts exhibited robust radical scavenging activity, showcasing their antioxidant capabilities. Trolox, employed as a benchmark, displayed an IC_{50} value of 42.48 mg/ml. Of particular significance, *Bauhinia variegata* demonstrated the most potent antioxidant activity, as evidenced by its lowest IC_{50} values. Specifically, the IC_{50} values were 109.02 mg/ml for 80% EtOH, 156.98 mg/ml for MeOH, and 90.60 mg/ml for pH_2O. Lower IC_{50} values are indicative of higher antioxidant activity. On the other hand, the 80% EtOH extract from *Senna alata* exhibited notably high IC_{50} values of 765.43 mg/ml, while the 80% MeOH extract had an IC_{50} value of 868.41 mg/ml. This implies that the antioxidant activity of *Senna alata* is relatively lower in comparison to other plant samples. Furthermore, the deionised water extracts from *Mucuna pruriens, Senna alata*, and *Solanum nigrum* displayed elevated IC_{50} values, indicating less efficient radical scavenging activity, with values of 1483.15 mg/ml, 623.68 mg/ml, and 874.28 mg/ml, respectively. It is notable that the ethanol extracts consistently yielded the lowest IC_{50} values for most of the plant samples, suggesting that ethanol is the preferred solvent for antioxidant extraction from the investigated plant samples. This emphasizes the

Table 4.2: Comparative analysis of antioxidant activities of 15 anti-diabetic potential ethnomedicinal plants through different analytical techniques and different extraction processes

Anti-diabetic potential plants	80% EtOH extract			80% MeOH extract			Pure Water extract		
	DPPH RSA IC_{50} value (mg/ml)*	FRAP (mM Fe^{2+}/g)	ABTS IC_{50} values* (mg/ml)*	DPPH RSA IC_{50} value (mg/ml)*	FRAP (mM Fe^{2+}/g)	ABTS IC_{50} values (mg/ml)*	DPPH RSA IC_{50} value (mg/ml)*	FRAP (mM Fe^{2+}/g)	ABTS IC_{50} values (mg/ml)*
Abroma augustum	578.75±1.09 ^{b}	11.132±1.69^{g}	7.537± 0.038^{d}	686.87±1.13^{d}	11.697±0.31^{j}	8.35±0.162^{d}	389.83±1.87^{g}	23.124±0.80^{h}	9.78±0.069^{e}
Bauhinia variegata	109.02±1.83^{j}	66.781±1.48^{b}	2.19± 0.072^{f}	156.98±1.26^{n}	68.739±0.38^{b}	2.77±0.082^{g}	90.6±1.54^{n}	71.227±1.02^{a}	5.34±0.082^{h}
Cajanus cajan	162.17±0.95^{h}	73.617±0.51^{a}	2.87± 0.058^{f}	186.27±1.04^{m}	71.600±0.35^{a}	4.53±0.0628^{f}	158.03±1.49^{l}	60.724±2.00^{c}	6.35±0.059^{g}
Catharanthus roseus	418.09±1.23^{c}	40.279±1.33^{e}	4.68±0.098^{e}	465.161±1.63^{e}	52.161±1.48^{f}	6.88±0.106^{e}	243.37±0.82^{k}	67.485±2.48^{b}	8.72±0.104^{e}
Senna alata	765.43±0.08^{a}	15.134±2.06^{g}	5.657±0.052^{d}	868.41±0.94^{b}	14.973±3.09^{i}	6.533±0.031^{e}	623.68±0.49^{e}	7.501±0.98^{k}	8.03±0.067^{e}
Clerodendrum colebrookianum	214.28±1.14^{g}	65.876±2.34^{b}	4.74±0.081^{e}	308.1±0.73^{h}	69.990±1.17^{b}	6.11± 0.066^{e}	325.84±0.63^{h}	43.063±0.37^{e}	7.26±0.114^{f}
Euphorbia hirta	147.3±1.07^{i}	75.205±1.11^{a}	2.85±0.088^{f}	201.75±1.49^{l}	74.384±1.32^{a}	2.87±0.092^{g}	111.8±0.43^{m}	72.583±2.24^{a}	9.38±0.038^{e}
Gynura crepidioides	339.74±1.85^{d}	8.717±0.15^{h}	8.66±0.097^{c}	377.69±0.68^{g}	14.507±0.74^{i}	17.11±0.306^{b}	687.08±1.52^{d}	22.800±1.98^{i}	19.58±0.153^{d}
Kalanchoe pinnata	202.63±1.09^{g}	26.986±0.11^{f}	8.24± 0.096^{c}	314.51±1.27^{i}	17.685±0.22^{h}	11.23±0.059^{c}	265.44±1.62^{i}	39.825±0.12^{f}	9.66±0.004^{e}
Mucuna pruriens	582.59±0.69^{b}	9.014±0.47^{h}	14.38± 0.19^{b}	1007.68±1.55^{a}	14.905±0.54	18.28±0.37^{b}	1483.15±1.82^{a}	30.775±0.34^{h}	25.18±0.495^{b}
Paederia foetida	268.3±1.82^{e}	52.217±0.28^{d}	5.36±0.041^{e}	295.39±1.81^{j}	57.795±1.10^{d}	7.49± 0.052^{d}	392.25±0.94^{g}	39.892±0.98^{f}	22.82±0.112^{c}
Passiflora edulis	249.54±0.39^{f}	48.473±0.90^{d}	5.97± 0.085^{d}	738.61±1.74^{c}	38.776±1.52^{g}	7.2±0.063^{d}	254.04±1.67^{j}	36.841±0.76^{g}	9.84±0.347^{e}
Perilla frutescens	275.44±1.58^{e}	1.711±0.09^{i}	20.48±0.069^{a}	367.83±0.52^{g}	2.794±0.10^{l}	33.84±1.203^{a}	742.76±1.49^{c}	9.260±0.75^{i}	37.21±0.568^{a}
Solanum nigrum	263.56±0.89^{e}	1.645±0.27^{i}	15.27±0.041^{b}	433.177±1.66^{f}	4.466±0.19^{k}	17.78±0.434^{b}	874.28±1.84^{b}	7.394±0.42^{k}	18.73±0.077^{d}
Solanum trilobatum	207.92±1.62^{g}	59.310±0.87^{c}	6.99± 0.083^{d}	262.84±0.82^{k}	66.671±0.78^{c}	4.88±0.032^{f}	454.68±0.76^{f}	46.382±0.40^{d}	7.59±0.063^{f}

Note: RSA: Radical scavenging activity; Data represents the mean of three replicates Standard error from the mean. * p-value has been calculated using One-way ANOVA, and values with the same superscript letters in the column do not differ significantly ($p \leq 0.05$).

i

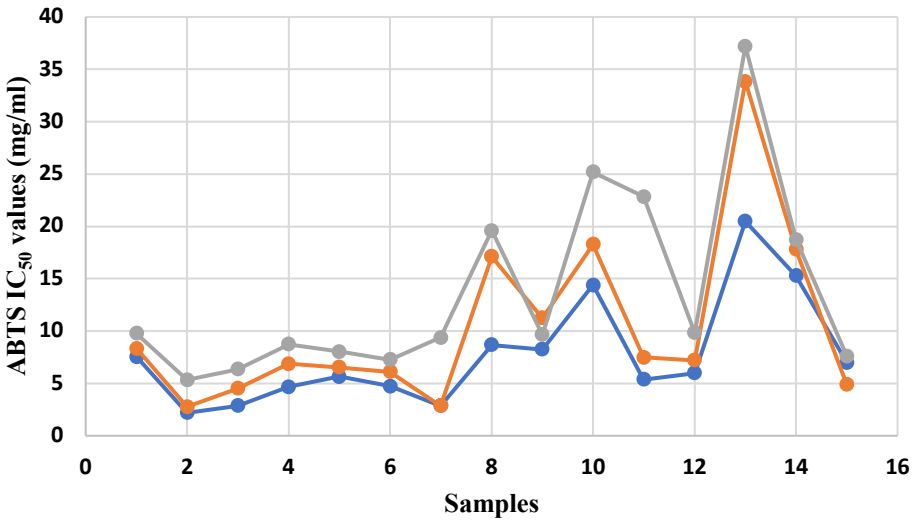


Ethanol Extract (DPPH IC_{50} value, mg/ml)

Methanol Extract (DPPH IC_{50} value, mg/ml)

Water Extract (DPPH IC_{50} value, mg/ml)

ii



Ethanol Extract (ABTS IC_{50} value, mg/ml)

Methanol Extract (DPPH IC_{50} value, mg/ml)

Water Extract (DPPH IC_{50} value, mg/ml)

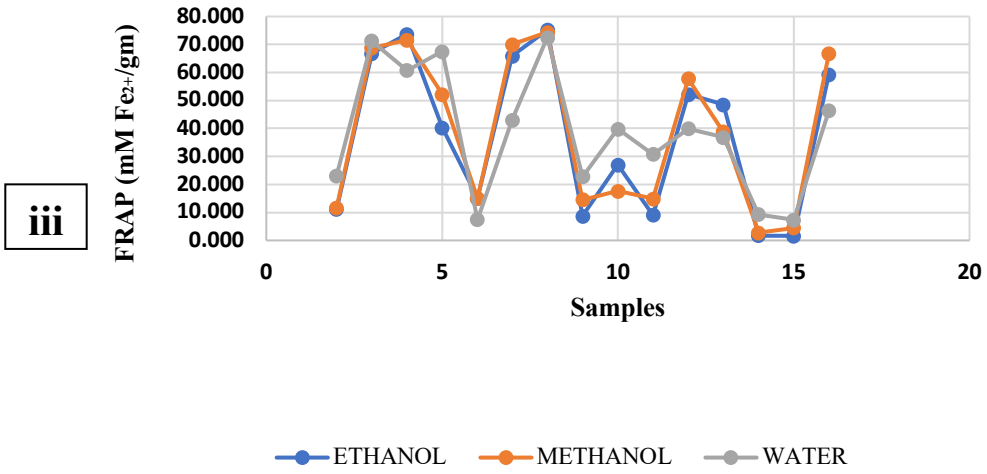


Figure 4.7: Comparative analysis of EtOH extracts, MeOH extracts and Pure water extracts of 15 antidiabetic plant samples in **i)** DPPH free radical scavenging assay, **ii)** ABTS free cation radical scavenging assay, **iii)** FRAP assay.

significance of solvent selection in optimising the extraction of antioxidants with high radical scavenging potential.

The data presented in Table 4.2 illustrates the Ferric-Reducing Power (FRAP) of aqueous extracts using 80% EtOH, 80% MeOH and pH_2Oextracts. The FRAP assay assesses the reducing power of antioxidants present in the samples, and the values obtained reflect their ability to reduce ferric ions. Importantly, all examined plant extracts exhibited a dose-dependent reducing activity. Regarding the 80% EtOH extracts, the FRAP values follow the order of *Euphorbia hirta>Cajanus cajan>Bauhinia variegata>Clerodendrum colebrookianum>Solanum trilobatum>Paederia foetida*. Among the three solvent extracts, *Euphorbia hirta* displayed the highest FRAP value and consequently exhibited the most potent reducing power. Specifically, the values were 75.205±1.11 $mMFe_2^+$/gm for 80% EtOH, 74.384±1.32$mMFe_2^+$/gm for 80% MeOH, and 72.583±2.24 $mMFe_2^+$/gm for pH_2O. Conversely, *Perilla frutescens* and *Solanum nigrum* exhibited the lowest FRAP values, signifying the least potent reducing power among the

plant samples. The values were 1.711±0.09 $mMFe_2^+$/gm in 80% EtOH, 2.794±0.10 $mMFe_2^+$/gm in 80% MeOH and 9.260±0.75 $mMFe_2^{+/}$gm in pH_2O for *Perilla frutescens* extract. For *Solanum nigrum,* the value is respectively 1.645±0.27 $mMFe_2^{+/}$gm, 4.466±0.19 $mMFe_2^{+/}$gm, 7.394±0.42 $mMFe_2^{+/}$gm in 80% EtOH, 80% MeOH and pH_2O. These findings provide valuable insights into the reducing power of various plant extracts and the impact of different solvents on their antioxidant potential. *Euphorbia hirta* emerges as a particularly strong candidate for its robust reducing power, while *Perilla frutescens* and *Solanum nigrum* demonstrate comparatively lower reducing power in this context.

Results of IC_{50} values of ABTS scavenging activity are presented in Table 4.2 for all three extracts and plants under study with Trolox as a positive control. Trolox exhibited IC_{50} values of 7.33 mg/ml. Among the plant samples, *Bauhinia variegata* displayed the most potent antioxidant activity (lowest IC_{50} values: 2.19 mg/ml for the 80% EtOH, 2.77 mg/ml for the MeOH, and 5.34 mg/ml for pH_2O extract. In contrast, the EtOH extract from *Perilla frutescens* exhibited notably high IC_{50} values (20.48 mg/ml), followed by MeOH extract (33.84 mg/ml) and pH_2O extract (37.21 mg/ml). *Perilla frutescens* demonstrated the lowest antioxidant value compared to the other investigated plant species. *Cajanus cajan*, *Catharanthus roseus*, and *Euphorbia hirta* also displayed comparatively high antioxidant activity compared to the IC_{50} values of the standard Trolox. The EtOH extract consistently showed low IC_{50} values, indicating it is a more effective solvent in the extraction process and is associated with high antioxidant activity.

Discussion

The ethnomedicinal plants investigated for anti-diabetic potential phytochemicals in the present study are being traditionally utilised by different tribes in Nagaland, India, for treating and or managing diabetes (Deb and Sharma, 2021). The present study shows high concentrations of polyphenol and flavonoid contents in *Bauhinia variegata, Cajanus cajan, Clerodendrum colebrookianum, Euphorbia hirta, Senna alata, Passiflora edulis*, and *Solanum trilobatum*. The high concentration of these compounds in these plants shows their potency of using them as anti-diabetic drugs. The plant extracts exhibited notable ferric-reducing ability, and their substantial antioxidant activity is evident through the DPPH assay and ABTS radical cation scavenging assay. The extract's concentration-dependent reductive capability suggests that higher concentrations may boost antioxidant activity. This is particularly relevant in diabetes complications, where oxidative stress plays a role, and antioxidants are explored as potential treatments (Reaven et al., 1995).

Polyphenols and flavonoids, prominent constituents found in plants renowned for their antioxidant properties, may contribute to the healing characteristics of plant extracts through their redox features. Various studies (Krings and Berger, 2001; Ali et al., 2008) have described the inherent antioxidant attributes in medicinal plants characterised by an abundance of phenolic compounds. Natural antioxidants derived from plants predominantly exist in diverse forms, encompassing flavonoids, phenolic acids, and similar constituents. Various research has shown the relationship of polyphenols and their compounds with a range of therapeutic potentials, including antioxidative, antidiabetic, anticancer, and antimicrobial effects (Han et al., 2007; Mohammed et al., 2016). Previous studies have indicated robust connections between the levels of polyphenols and the antioxidant capabilities in numerous medicinal plant species (Gorinstein et al., 2004). It is important to acknowledge that the phytochemical and antioxidant profiles varied across

plant species, indicating that not all plants possess the same bioactive compounds or antioxidant capacity. According to Balbaa et al. (2021), it was noted that the level of polyphenols in plant extracts has a significant influence on their antioxidant potential. In the present study, the stem bark of *Bauhinia variegata* consistently stood out as a rich source of phytochemicals, exhibiting remarkable antioxidant activity and showing its potential for medicinal applications. The abundant presence of flavonoids and other phenolics in *Bauhinia variegata* within our investigation was expressly linked to its demonstrated antioxidant and antidiabetic effects. A study by Kamal et al. (2022) shows the potential of *Bauhinia variegata* in various therapeutic areas because of its high phytochemical and antioxidant content. In the MeOH extract of *Bauhinia variegata* (Negi et al., 2012) reported a total phenol content of 88.71 µg/mg extract GAE, a total flavonoid content of total tannin content of 96.71 µg/mg extract TAE. The difference in the antioxidant activity results of the plant samples between the current investigation and prior studies could arise from differences in the extraction solvent/technique, plant parts used, elevation, soil factors, and climatic conditions (Mpofu et al., 2006). Mahitha et al. (2015) reported that the leaf extracts of *Cajanus cajan* contain significant amounts of phenolic and flavonoid content and their contribution to the antioxidant activity. In the study of the plant extract of *Euphorbia hirta*, a significant amount of phenolic and flavonoid content and antioxidant potential was also observed (Akporhuarho et al., 2022; Asgar et al., 2023).

In the present study, *Perilla frutescens* consistently demonstrated lower phytochemical content and antioxidant activity, aligning with findings by Dimita et al. (2022), who reported similarly low total phenolic content (up to ~110 mgGAE/gm DW). Also the low phenolic and flavonoid content in *Solanum nigrum* in the present study aligns with the study of Veerapagu et al. (2018) where the MeOH extract of *Solanum*

nigrum fruits has a total phenol content (TPC) of 4.57±0.57 mgGAE/gm and a total flavonoid content (TFC) of 3.61 ± 0.07 mgQAE/gm, indicating that it has a moderate amount of phenolic and flavonoid content. Notably, studies, including Razgonova et al. (2022), highlight variations linked to growth stage and geographical origin, with differences observed in total phenolic content among regions like Japan, Korea and China. This variability emphasises the importance of meticulous plant and extraction method selection for targeted bioactive compounds or antioxidant potential in addressing specific health conditions.

Both reducing and non-reducing sugars contribute significantly to central metabolic pathways (growth, development, metabolism, stress response, and disease resistance), fostering the production of secondary metabolites that augment the medicinal properties of plants (Khatri and Chhetri, 2002). In the investigated plant species, the noteworthy presence of substantial polyphenols, antioxidants, total triterpenoid and flavonoid content accentuate their potential bioactive properties. The presence of bioactive substances in medicinal plants has the ability to selectively influence metabolic pathways related to diabetes, including glucose uptake in peripheral tissues and controlling of blood glucose levels (Gupta et al., 2023). Akdad et al. (2023) have mentioned that 14 biomolecules including epicatechin, catechin, epigallocatechin 3-gallate, quercetin, quercetin 3-glucoside, berberine, rutin, linoleic acid, oleanolic acid, oleic acid, chlorogenic acid, gallic acid, hesperidin, and corosolic acid have promising activity against diabetes and its complications in clinical studies. Various phytocompounds extracted from different plant species have been validated scientifically for their anti-diabetic potential and for managing the complications as a result of diabetes (Khan et al., 2019). Intriguingly, in our study, alongside these beneficial compounds, a significant elevation in total reducing sugar content was observed. Several factors contribute to this phenomenon, which

reflects the dynamic of various biological processes within the plants. It is plausible that the high reducing sugar levels are a consequence of active metabolic pathways influenced by optimal nutrient availability in the soil. Environmental conditions, such as temperature and humidity, also play a role where stress responses potentially trigger sugar production (Khatri and Chhetri, 2020). Additionally, genetic variations among plant species or varieties could contribute to differing sugar accumulation patterns. Further investigations, including targeted biochemical analyses and comparative studies, are warranted to elucidate the specific mechanisms underpinning the observed high total reducing sugar content. This intriguing finding not only expands our understanding of the biochemical composition of these plants but also raises compelling questions regarding the intricate regulatory networks governing their metabolic responses.

The preferential efficacy of 80% ethanol (EtOH) in extracting a diverse spectrum of phytochemicals and antioxidants establishes it as the solvent of choice. This observation aligns with the findings of a study conducted by Mohammed et al. (2016), Atun et al. (2022), Sethumadhavan and Natchimuthu (2022) on distinct plant species. The variations observed in each assay underscored the importance of a multi-faceted approach in evaluating plant extracts for their medicinal potential. The study opens avenues for further investigations into the specific compounds responsible for the observed bioactivities, facilitating the development of targeted herbal remedies for diabetes treatment.

Significant antioxidant activity that is observed in *Catharanthus roseus, Abroma augustum*, *Clerodendrum colebrookianum* is in accordance with various studies where these species show rich pharmaceutical potential, antimicrobial, antigenotoxic, and antimutagenic properties (Miah et al., 2020; Sarma, 2022; Sharma et al., 2022). Nevertheless, certain plant extracts demonstrated unexpectedly elevated IC_{50} values in the

DPPH assay, thereby signifying diminished antioxidant efficacy. The factors that may contribute to these elevated IC_{50} values could encompass reduced concentrations of bioactive antioxidant constituents, solubility challenges, suboptimal extraction methodologies, as well as the influence of geographical and environmental variables on the concentrations of secondary metabolites (Mpofu et al., 2006). To rectify these challenges, it is imperative that additional research is conducted. Subsequent investigations should prioritize the optimization of the extraction processes to guarantee the comprehensive recovery of antioxidant compounds. Furthermore, it would be advantageous to examine the effects of geographical and environmental variables on the antioxidant characteristics of these botanical specimens. Standardizing collection protocols and incorporating a broader array of samples from diverse locations could yield a more thorough comprehension of the fluctuations in antioxidant activity. Enhanced assay methodologies or alternative assays ought to be contemplated to corroborate and validate the findings.

Summary and Conclusions

The findings of the present investigation provide scientific support for the folkloric use of specific herbs in Nagaland as potential anti-diabetic agents. The results disclose important antidiabetic potential biochemical in the studied plant species, marking the first scientific report on the biochemical contents of these plants from Nagaland, India. Various studies have also confirmed that the presence of these phytocompounds contributes to their antidiabetic potential. Among the 15 medicinal plants scrutinised, seven of them exhibited noteworthy levels of the studied phytochemicals. The high concentrations of polyphenols and flavonoids in species such as *Bauhinia variegata*, *Cajanus cajan*, *Clerodendrum colebrookianum*, *Euphorbia hirta*, *Senna alata*, *Passiflora edulis*, and *Solanum trilobatum* were linked to their significant

antioxidant and potential antidiabetic activities. Notably, *Bauhinia variegata* emerged as a potent source of phytochemicals with strong medicinal applications. On the contrary, species like *Perilla frutescens* and *Solanum nigrum* exhibited lower phytochemical content and antioxidant potential, which varied according to factors such as plant growth stage and geographical origin.Therefore, the extracts of these plant species, can be used as a very good source of antidiabetic drugs. The preferential use of 80% ethanol for extracting phytochemicals proved effective, supporting its use in future studies for maximizing yield and activity. However, some plant extracts showed lower antioxidant activity as indicated by higher IC_{50} values, suggesting the need for optimization of the extraction process and further studies on the impact of environmental factors on secondary metabolite levels. The traditional practice of using these plant species is recommended and future work to isolate and characterise the active antidiabetic compounds elucidating the mechanism of its action and followed by clinical trials to determine its antidiabetic efficacy need to be carried out.

Chapter – 5

In Vitro α-Amylase and α-Glucosidase Inhibition Assessment by Different Extracts and Determining Their Mode of Inhibition

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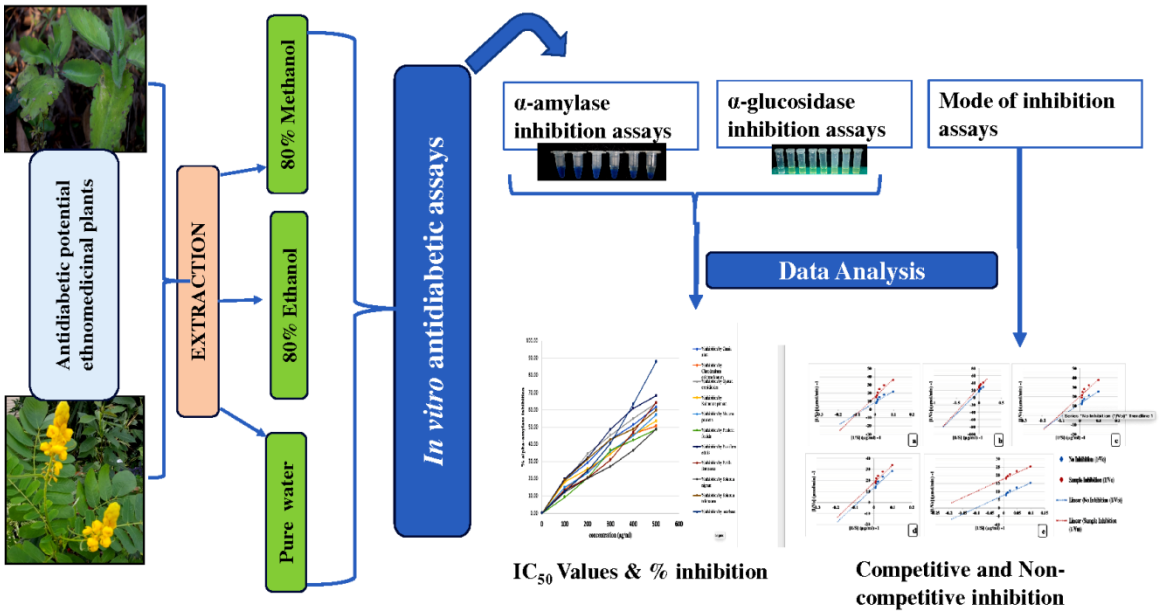
Graphical Summary



Introduction

Diabetes encompasses a group of metabolic conditions characterized by hyperglycemia, where there is an increase in blood sugar levels due to either insufficient insulin secretion by the pancreas or the inadequate response of the body's cells to the produced insulin (Jadon et al., 2024). A critical therapeutic approach in diabetes management involves the substantial reduction of postprandial hyperglycemia. An exceedingly successful technique to complete this task is by effectively blocking carbohydrate-hydrolyzing enzymes, specifically α-amylase and α-glucosidase (Bui et al., 2024). The pancreatic α-amylase is key in the process of digestion by kickstarting the breakdown of starch into smaller oligosaccharides like maltose, maltotriose, and various α-(1-6) and α-(1-4) oligoglucans. These oligosaccharides are later converted into glucose by α-glucosidases, which are then absorbed into the bloodstream. The rapid breakdown of dietary starch leads to increased postprandial hyperglycemia (PPHG). Research findings indicate that the existence of human pancreatic α-amylase (HPA) in the small intestine is associated with increased postprandial glucose levels, highlighting its control as a crucial element in the treatment of type-2 diabetes (Matsui et al., 1996; Kim et al., 2005). Inhibitors of pancreatic α-amylase decelerate carbohydrate breakdown, consequently lessening the pace of glucose absorption and decreasing postprandial blood glucose levels. Glucosidase blockers, obtained from different origins, like plants and microorganisms, have been extensively researched. In the 1970s, it was revealed that inhibiting intestinal disaccharidases and pancreatic α-amylase could impact carbohydrate absorption, offering a therapeutic strategy for managing non-insulin-dependent diabetes mellitus (Kajaria et al., 2013). Several compounds, including flavonoids, quinazolinones, and phytochemical constituents sourced from plants like *Plectranthus ecklonii*, have exhibited inhibitory effects on α-amylase and α-glucosidase, positioning them as potential

contenders for diabetes treatment (Modh and Patel, 2022; Etsassala et al., 2022). These inhibitors delay the absorption of glucose, thereby reducing postprandial glucose levels and the likelihood of long-term diabetes complications. Hence, diverse herbal extracts with potent inhibitory properties against both enzymes play a significant role in diabetes management.

Traditional medicines like the Siddha formulation, such as 'Gandhakasarkkarai', have demonstrated significant anti-diabetic activity by inhibiting these enzymes, highlighting the diverse sources of compounds that can aid in the treatment of diabetes while potentially minimizing side effects associated with conventional medications (Iyswarya et al., 2022). Plants have been the source of drugs, and various ethnobotanical reports mention the potential of anti-diabetic in numerous plants. Various studies have focused on screening compounds for their inhibitory effects on α-amylase and α-glucosidase, aiming to develop new treatments for type 2 diabetes. Research has identified natural flavonoids like Galangin, Maackiain, and Corylin (Bui et al., 2024), as well as synthetic compounds such as quinazolinones (Modh and Patel, 2022), and extracts from medicinal plants like *Cycas* sp. and *Curcuma longa* (Tulin et al., 2024) and herbal extracts with potent inhibitory activity for both enzymes (Mony, 2023). Herbal medicines serve as abundant reservoirs of secondary compounds, such as flavonoids, alkaloids, polyphenols, and terpenes. These active components display a wide array of properties beneficial in the management of diabetes, such as inflammation reduction, oxidative stress mitigation, enhancement of insulin sensitivity, and regulation of blood glucose levels (Roy et al., 2022). Extensive investigation has shown the bioactive nature of these agents, emphasizing their considerable significance in medical treatment. Notably, polyphenols have the capacity to imitate the actions of insulin by fostering glucose absorption and hindering α-amylase, α-glucosidase, and oxidative stress (Cisneros-

Yupanqui et al., 2023). Moreover, various medicinal plants encompass compounds that enhance insulin sensitivity, thereby facilitating cellular receptivity to insulin and assisting in the regulation of glucose metabolism, consequently averting fluctuations in glycemic levels. Additionally, medicinal plants frequently contain essential nutrients that can improve overall health and well-being, an indispensable factor for individuals with diabetes, who face heightened susceptibility to other health issues. This holistic approach involving the utilization of medicinal plants not only addresses the regulation of blood sugar but also promotes broader health advantages.

Since diabetic patients typically have low insulin levels, their α-amylase levels also tend to remain low as they work to regulate glucose levels. In plants, α-amylase inhibitors serve as a defence mechanism against insects by disrupting their digestive systems and inhibiting their feeding behaviour (Jayaraj et al., 2013). This natural defence mechanism suggests that α-amylase inhibitors in plants could potentially help control blood sugar levels in humans. α-glucosidase inhibitors, on the other hand, act as competitive antagonists against α-glucosidase enzymes, crucial for breaking down complex carbohydrates into glucose and other monosaccharides within the small intestine (Nair et al., 2013). By inhibiting these enzymatic pathways, they slow down carbohydrate digestion, reducing the amount of glucose absorbed as undigested carbohydrates remain. For individuals with diabetes, this inhibition leads to a significant reduction in elevated blood glucose levels (Ripsin et al., 2009). Therefore, utilizing natural α-amylase and α-glucosidase inhibitors from dietary plants represents a promising approach to managing postprandial hyperglycemia, potentially minimizing adverse reactions commonly associated with other treatments.

The main objective of this current investigation was to examine the inhibitory effects of α-amylase and α-glucosidase by the selected ethnomedicinal plants from

Nagaland using *in vitro* assays. Subsequent to these analyses, the outcomes can be utilized to substantiate the therapeutic properties of the bioactive compounds acquired from the plants. Moreover, the botanical specimens have the potential to serve as valuable natural reservoirs for the development of sustainable anti-diabetic medications stemming from natural sources.

Materials and Methods

Preparation of Plant Extracts

The collected specimens were washed with tap water and subsequently dried in a hot air oven set at 50°C until attaining a stable weight. Subsequently, the dried plant samples were individually ground into a fine particulate form. To generate various extracts, 50mg of each powdered plant component was combined with 10 ml of methanol (80%, v/v) (MeOH), ethanol (80%, v/v) (EtOH), and pure water (pH_2O) separately. These amalgams were subjected to 2 h incubation in a water bath at 60°C, succeeded by centrifugation at 10000 rpm for 10 min. Following centrifugation, the resulting supernatants were filtrated using Whatman's filter paper No. 1 and the extracts were stored at 4°C until required for analytical procedures.

Chemical Used

Acarbose (from Sigma Aldrich Chemical Pvt. Ltd.), Porcine Pancreatic Amylase (4 units/ml) (Sigma Aldrich), Starch Azure, 0.5M Tris HCl buffer, 0.01M Calcium chloride, 50% Acetic acid, 2% Dimethyl sulfoxide (DMSO), 3 mM p-nitrophenyl α-D-glucopyranoside (p-NPG), α-glucosidase (Maltase) ex. Yeast (2 units/ml) (from SRL), 100 mM Phosphate buffer (pH-7), 0.2 M Sodium Carbonate.

α-Amylase Inhibition Assay

The experiment was conducted in accordance with standard protocol with minor adjustments (Hansawasdi et al., 2000). A quantity of 2 mg of starch azure was dispersed

in 0.2 mL of 0.5M Tris–HCl buffer (pH 6.9) supplemented with 0.01M CaCl2, constituting the substrate solution. Subsequently, the tubes containing the substrate solution were subjected to boiling for 5 min, followed by a pre-incubation period at 37°C for another 5 min. The EtOH extracts of various plant samples were dissolved in 2% DMSO to yield concentrations of 100, 200, 300, 400, and 500µg/mL. Then, 0.2 mL of the plant extract at the specified concentration was introduced into the tube containing the substrate solution. Furthermore, 0.1 mL of porcine pancreatic amylase mix in Tris–HCl buffer (2 units/mL) was combined with the plant sample extract and substrate solution. The enzymatic reaction ensued at 37°C for a duration of 10 min. Termination of the reaction was achieved by the addition of 0.5 mL of 50% acetic acid to each tube. Subsequent centrifugation of the reaction mixture was carried out at 3000 rpm for 5 min at 4°C. The absorbance of the resultant supernatant was quantified at 595 nm in the UV-Vis spectrophotometer (Thermo Scientific Evolution 201 Series). The same methodology was replicated for the evaluation of α-amylase inhibitory effects of other plant extracts (MeOH and pH_2O). Acarbose, a recognized α-amylase inhibitor, was employed as a standard drug. The experimental procedures were repeated thrice. The % inhibition was calculated using the formula:

$$\text{Percent Inhibition} = \frac{absorbance\ 595\ (Control) - absorbance\ 595\ (extract)}{absorbance\ 595\ (control)} \times 100$$

The α-amylase inhibitory activities were expressed as a percentage of inhibition (IC_{50}) determined through regression analysis based on a graph illustrating scavenging activity in relation to concentration.

α-Glucosidase Inhibition Assay

The α-glucosidase was solubilised in a phosphate buffer with a concentration of 100 mM and a pH of 6.8, subsequently employed as the enzymatic extract. P-Nitrophenyl-α-

D-glucopyranoside (pNPG) was used as the substrate. The EtOH, MeOH and pH2O extracts of various plant samples were dissolved in DMSO to yield concentrations of 100, 200, 300, 400, and 500 µg/mL. 10 µL of various concentrations of plant samples were treated with p-NPG (250 µL, 3 mM) and phosphate buffer solution (490 µL, 100 mM, pH 7). The solution was first incubated at 37°C for 5 min. Then, 250 µL of α-glucosidase enzyme (2 units/mL) was added, and the reaction continued for 15 min. The reaction was halted by the addition of 1 ml 0.2 M Na_2CO_3. Acarbose served as the standard α-Glucosidase inhibitor. The mixtures were measured at 400 nm using a Thermo Scientific Multiskan Spectrophotometer. The experimental procedures were replicated three times for each test (Indrianingsih et al., 2015). The % inhibition was calculated with the formula:

$$\text{Inhibition}\% = \frac{absorbance\ 400\ (Control) - absorbance\ 400\ (extract)}{absorbance\ 595\ (control)} \times 100$$

The IC_{50} values were ascertained through analysis of plots depicting the percentage of inhibition against the logarithm of inhibitor concentration and were derived utilizing non-linear regression techniques based on the average inhibitory data.

Determining the Mode of Inhibition

For determining the mode of inhibition of α-Amylase and α-Glucosidase, α-Amylase and α-Glucosidase assay was achieved by varying the substrate concentration and using IC_{50} of sample extract as concentration and as the source of inhibitor. A Lineweaver-Burk plot was constructed to identify their mode of inhibition (Indrianingsih et al., 2015).

Statistical Analyses

All values were expressed as Mean±SE. Statistical difference and linear regression analysis were performed using Microsoft Excel software.

Results

The comparison of α-amylase and α-glucosidase inhibitory activities of selected indigenous ethnobotanical plant species from Nagaland, India, is presented in Table 5.1. Percentage Inhibition of α- amylase and α-Glucosidase by 80% EtOH, 80% MeOH and pure water extract at a concentration of 500 µg/ml is presented in Table 5.2. The analysis of inhibitory effects was implemented using three solvent extracts: EtOH, MeOH, and pure water. The percentage inhibition of α-amylase enzyme by EtOH, MeOH, and pure water is also presented in Figure 5.1 (a, b, c). *Bauhinia variegata* methanol extract exhibited significant inhibitory activity on α-amylase with an IC_{50} value of 286.41 µg/ml and percentage inhibition of 65.63%, signifying the most potent extract among the others, followed by *Cajanus cajan* (IC_{50}: 288.00 µg/ml and 79.29 % inhibition), *Passiflora edulis* (IC_{50}: 362.72 µg/ml and 65.04% inhibition). While, in EtOH and pH_2O extracts, *Bauhinia variegata, Cajanus cajan, Abroma augustum* and *Euphorbia hirta* demonstrated notable good inhibitory effects IC_{50} value ranging from 314.68 µg/ml to 394.52 µg/ml. The MeOH and pH_2O extracts of *Solanum nigrum* show consistently low inhibition with IC_{50} values of 499.88 µg/ml and 468.55 µg/ml with an inhibition of 43.39% and 55.73%, respectively. For water extract also, the α-amylase inhibition was found to be the highest in *Bauhinia variegata* with its IC_{50} value of 364.27 µg/ml, and percentage inhibition was 65.63%.

The percentage inhibition of α-glucosidase enzyme by EtOH, MeOH and pH_2O are presented in Figure 5.2 (a, b, c). The MeOH and pH_2O extracts of *Gynura crepidioides* exhibited significant α-glucosidase inhibitory activity with its IC_{50} value of 328.67 µg/ml and inhibition of 68.66% for MeOH extract and 403.43 µg/ml and 61.55% inhibition for pure water extract. EtOH extract of *Passiflora edulis* and *Bauhinia variegata* was found to have 75.24% and 68.19% inhibition and significantly high α-glucosidase inhibition

with an IC_{50} value of 335.37 µg/ml and 335.139 µg/ml each. *Solanum nigrum* showed a considerably low value in inhibition against α-glucosidase, with IC_{50} values ranging from 477.33 to 529.31 µg/ml. The percentage inhibition for *Solanum nigrum* was 45.67% in pH_2O extract, 48.90% in EtOH extract, and 50.99% in MeOH extract. The standard drug, i.e. Acarbose, showed an IC_{50} of 54.408 µg/ml for α-amylase inhibition and 45.433 µg/ml for α-glucosidase inhibition.

Table 5.1: α-amylase and α-glucosidase inhibitory activity (IC_{50}, μg/ml) of some anti-diabetic potential ethnomedicinal plant species of Nagaland

Investigated Plants	α-amylase inhibitory activity (IC_{50}, μg/ml)			α-glucosidase inhibition (IC_{50} μg/ml)		
	80% EtOH extract (±SE)*	80% MeOH extract (±SE)*	Pure water extract (±SE)*	80% EtOH extract (±SE)*	80% MeOH extract (±SE)*	Pure water extract (±SE)*
Abroma augustum	357.57± 10.09^{d}	490.30 ± 14.73^{n}	394.99 ±08.76^{d}	393.12±09.63h	389.51±17.67^{g}	422.35±13.62^{g}
Bauhinia variegata	314.68± 08.74 ^{a}	286.41 ± 16.65 ^{a}	364.27± 14.01^{a}	335.37±12.82^{a}	332.72±13.89^{b}	371.86±13.81^{a}
Cajanus cajan	333.61± 12.63 ^{b}	288.00 ± 10.77 ^{a}	375.50± 10.84^{b}	381.32±13.51^{e}	365.11±10.55^{d}	403.65±11.85^{c}
Catharanthus roseus	376.56± 13.82^{i}	415.73 ± 09.03^{h}	410.00± 09.75^{e}	381.13± 14.33^{e}	395.07±11.41^{i}	420.47±13.62^{e}
Senna alata	427.56± 09.21^{o}	381.26 ± 11.06^{f}	434.94±13.34^{h}	383.55±12.07^{g}	390.45± 15.49^{h}	431.01± 12.11^{i}
Clerodendrum colebrookianum	385.35± 11.38^{j}	418.15 ± 13.66^{i}	451.85± 10.66^{m}	461.87±16.72m	449.11± 18.55^{k}	486.15± 26.71^{m}
Euphorbia hirta	358.81± 14.66 ^{d}	352.56 ± 07.13^{c}	394.52± 12.8^{d}	347.73±14.38^{c}	376.97±12.46^{e}	397.72±16.43^{b}
Gynura crepidioides	374.11± 07.89^{g}	373.66 ± 13.03 ^{e}	431.18± 09.63^{g}	360.72±11.32^{d}	328.67± 13.11^{a}	403.43± 14.84^{c}
Kalanchoe pinnata	359.45± 11.03^{f}	417.07 ± 15.2^{i}	436.25± 10.89^{i}	449.47±15.48l	412.53± 12.44^{j}	474.74± 15.12^{k}
Mucuna pruriens	373.26± 12.52^{g}	407.50 ± 12.48^{g}	424.11± 11.47^{f}	436.21±10.36k	383.17± 17.63^{f}	426.30± 14.65^{h}
Paederia foetida	411.50± 10.22^{n}	461.90 ± 13.94^{l}	435.93± 09.64^{i}	482.38±23.65n	503.95± 23.82^{n}	468.09± 17.58^{j}
Passiflora edulis	332.20± 08.74^{b}	362.72 ± 09.11^{d}	435.64± 07.54^{i}	335.139±12.44^{a}	344.04± 11.38^{c}	420.02± 11.54^{e}
Perilla frutescens	401.60± 13.72^{l}	471.32 ±13.62m	442.65± 16.22^{l}	417.45±15.71j	518.69± 21.23^{o}	474.03± 17.13^{k}

Solanum nigrum	392.35± 10.63^{k}	499.88 ± 14.73o	468.55±12.45^{n}	529.31±18.03^{o}	477.33± 12.55^{l}	519.49± 21.66^{o}
Solanum trilobatum	405.81± 11.38^{m}	421.04 ± 08.94^{k}	467.76± 14.83^{n}	392.97±11.57^{h}	474.32± 17.76^{l}	502.33± 16.39^{n}
Acarbose	54.40816327±4.21			45.4330544±2.66		

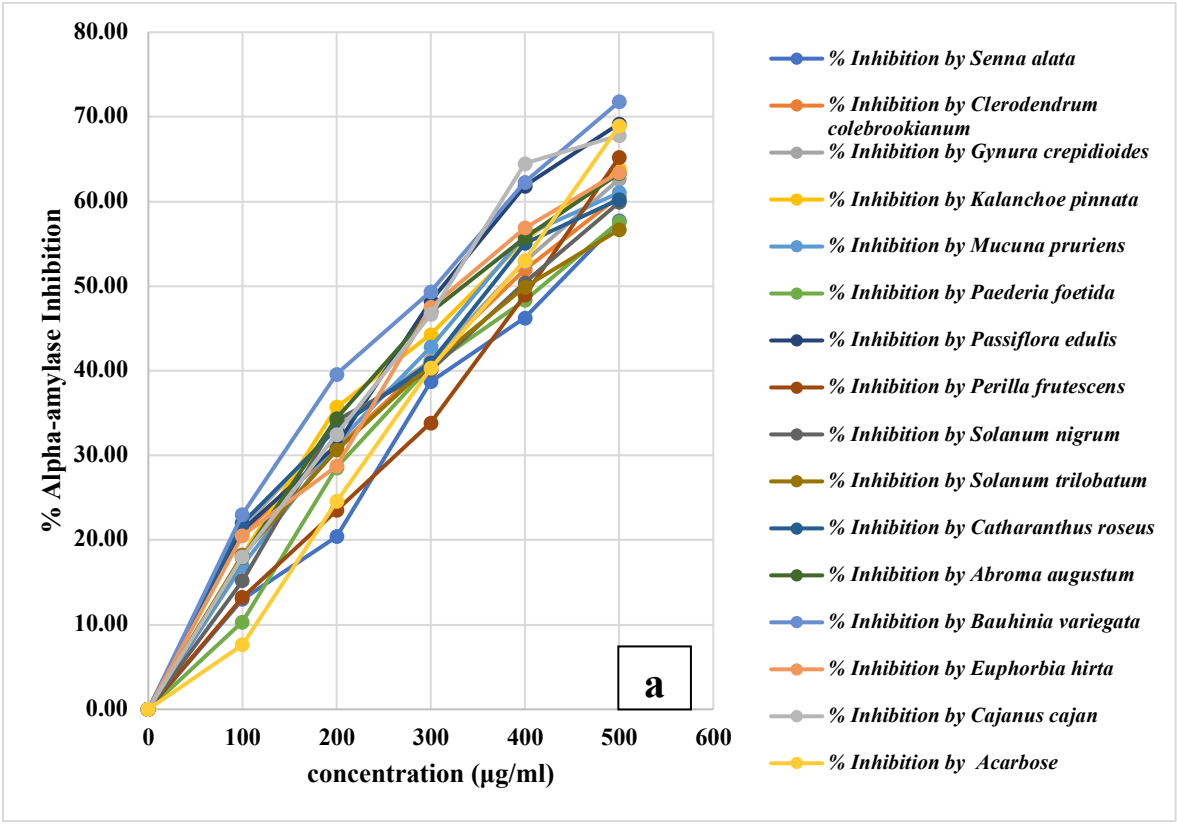
Note: Data represents the mean of three replicates ± Standard error from the mean. * *p*-value has been calculated using one-way ANOVA, and values with the same letters in the column do not differ significantly ($p \leq 0.05$).

Table 5.2: Percent inhibition of α- amylase and α- glucosidase by 80% EtOH, 80% MeOH and pure water extracts at a concentration of 500 µg/ml

Samples	Percentage Inhibition of α- Amylase at 500µg/ml			Percentage Inhibition of α- Glucosidase at 500µg/ml		
	80%EtOH Extract	80% MeOH Extract	Pure Water Extract	80%EtOH Extract	80% MeOH Extract	Pure Water Extract
Senna alata	57.71± 0.417^{e}	61.35± 1.332^{d}	53.33± 1.842^{e}	61.71 ±1.704^{d}	61.04± 1.416^{c}	56.98± 0.721^{c}
Clerodendrum colebrookianum	60.63± 1.233^{c}	63.31± 2.424^{d}	53.33± 1.048^{e}	50.63± 2.330^{g}	59.78± 0.371^{d}	50.12± 0.583^{d}
Gynura crepidioides	62.64± 0.926^{c}	61.17 ± 1.106^{d}	59.24± 0.707^{c}	63.73± 0.835^{c}	68.66± 0.529^{b}	61.55± 1.211^{b}
Kalanchoe pinnata	63.87± 0.502^{c}	62.80± 0.482^{d}	57.74± 1.622^{d}	53.72± 0.439^{f}	63.01± 1.457^{c}	54.30± 0.688^{c}
Mucuna pruriens	61.04± 0.412^{c}	66.30± 0.401^{c}	55.63± 0.223^{d}	57.04± 0.442^{e}	67.29± 0.634^{b}	54.45± 1.772^{c}
Paederia foetida	57.60± 0.732^{e}	59.84± 0.692^{e}	53.44± 0.446^{e}	48.60± 0.104^{g}	55.49± 0.411^{e}	50.99± 0.594^{d}
Passiflora edulis	69.19± 0.284^{b}	65.04± 0.250^{c}	60.94± 0.863^{c}	68.19± 0.285^{b}	67.95± 0.842^{b}	61.02± 0.325^{b}
Perilla frutescens	65.21± 0.372^{b}	50.16± 1.241^{f}	58.13± 0.274^{d}	64.21± 0.762^{c}	45.10± 1.655^{g}	55.66± 0.249^{c}
Solanum nigrum	59.90± 0.117^{d}	48.39± 2.490^{f}	55.73± 0.692^{d}	48.90± 1.086^{g}	50.99± 0.463^{f}	45.67± 1.407^{e}
Solanum trilobatum	56.67± 1.348^{e}	59.32± 0.108^{e}	51.46± 1.556	59.67± 0.633^{d}	54.82± 1.572^{e}	48.56± 0.738^{e}
Catharanthus roseus	60.26± 1.224^{d}	66.28± 0.540^{c}	56.24± 0.518^{d}	68.98± 0.744^{b}	60.18± 0.924^{d}	54.34± 0.226^{c}
Abroma augustum	63.33± 0.382^{c}	54.69± 0.273	57.92± 0.392^{d}	60.19± 0.392^{d}	60.55± 0.627^{d}	54.22± 0.463^{c}

Bauhinia variegata	71.77± 1.104[a]	88.65± 0.317[a]	65.63± 0.941[a]	75.24± 0.153[a]	69.46± 0.489[a]	65.01± 0.382[a]
Euphorbia hirta	63.44± 0.645[c]	65.13± 1.217[c]	58.23± 1.329[d]	67.32± 1.831[b]	61.34± 2.541[c]	57.99± 1.518[c]
Cajanus cajan	67.81± 0.386[b]	79.29± 0.933[b]	62.81± 1.660[b]	60.72± 0.649[d]	64.23± 0.806[c]	59.63± 0.672[b]
Acarbose	**68.93± 0.251**			**87.97± 0.837**		

Note: Data represents the mean of three replicates ± Standard error from the mean. * *p*-value has been calculated using one-way ANOVA, and values with the same letters in the column do not differ significantly ($p \leq 0.05$).


80.00
70.00
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40.00
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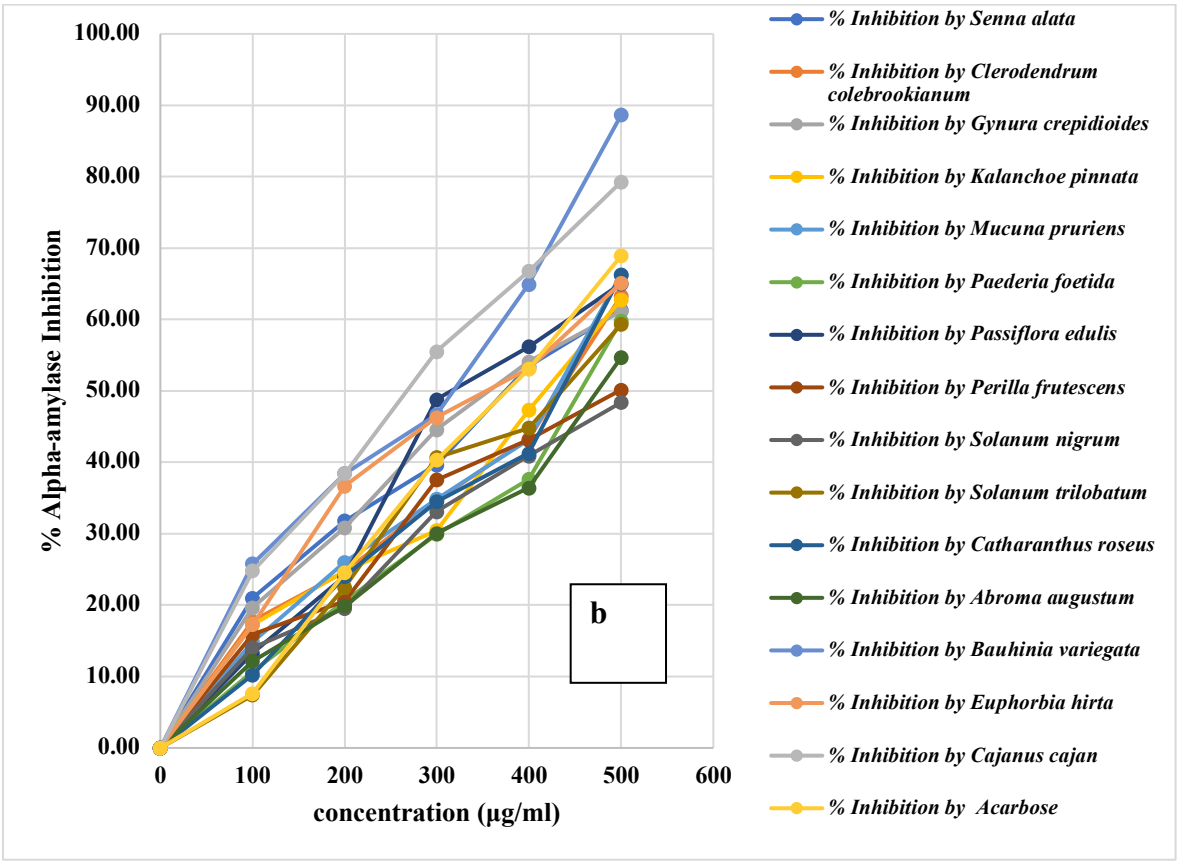
% Alpha-amylase Inhibition
0
100
200
300
400
500
600
concentration (µg/ml)
a
% Inhibition by Senna alata
% Inhibition by Clerodendrum colebrookianum
% Inhibition by Gynura crepidioides
% Inhibition by Kalanchoe pinnata
% Inhibition by Mucuna pruriens
% Inhibition by Paederia foetida
% Inhibition by Passiflora edulis
% Inhibition by Perilla frutescens
% Inhibition by Solanum nigrum
% Inhibition by Solanum trilobatum
% Inhibition by Catharanthus roseus
% Inhibition by Abroma augustum
% Inhibition by Bauhinia variegata
% Inhibition by Euphorbia hirta
% Inhibition by Cajanus cajan
% Inhibition by Acarbose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concentration (µg/ml)
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% Inhibition by Senna alata
% Inhibition by Clerodendrum colebrookianum
% Inhibition by Gynura crepidioides
% Inhibition by Kalanchoe pinnata
% Inhibition by Mucuna pruriens
% Inhibition by Paederia foetida
% Inhibition by Passiflora edulis
% Inhibition by Perilla frutescens
% Inhibition by Solanum nigrum
% Inhibition by Solanum trilobatum
% Inhibition by Catharanthus roseus
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% Inhibition by Euphorbia hirta
% Inhibition by Cajanus cajan
% Inhibition by Acarbose

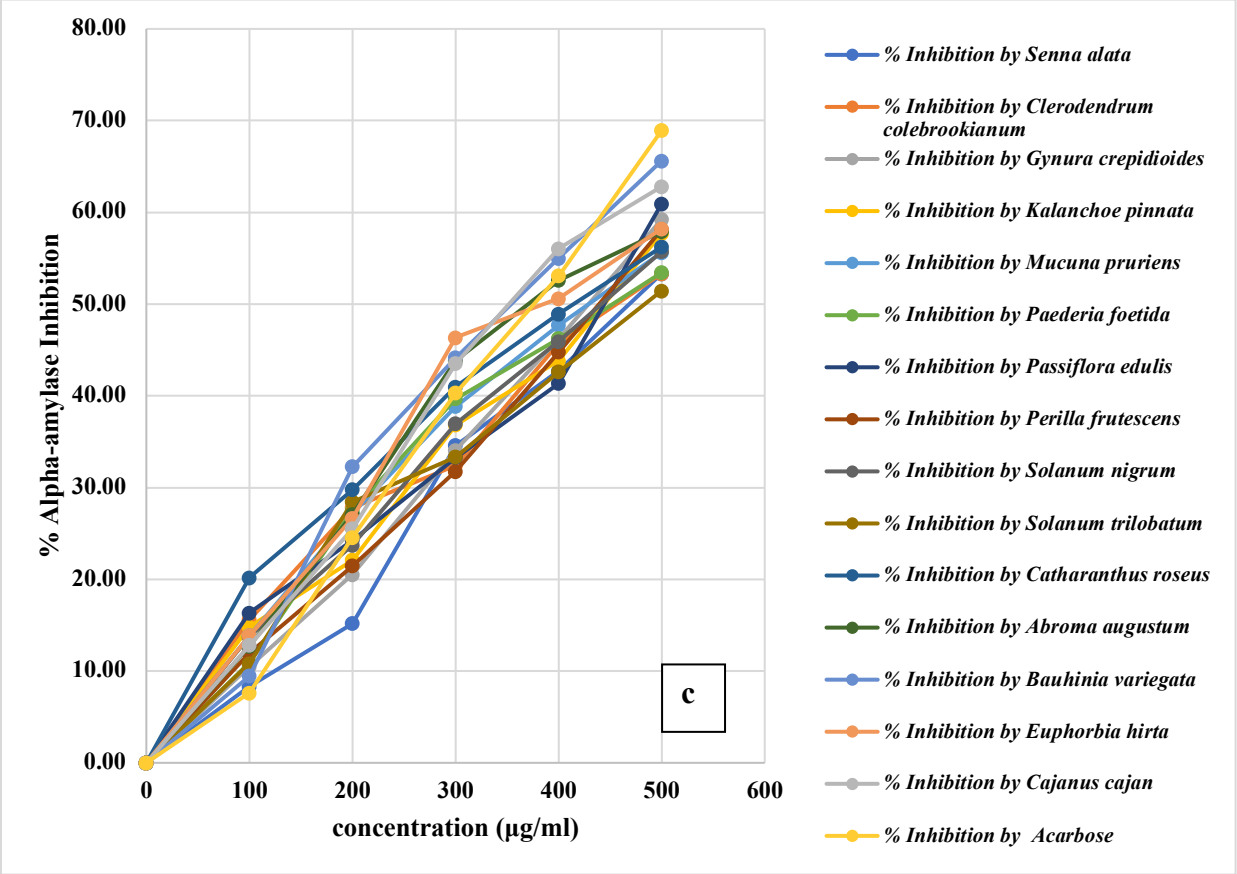
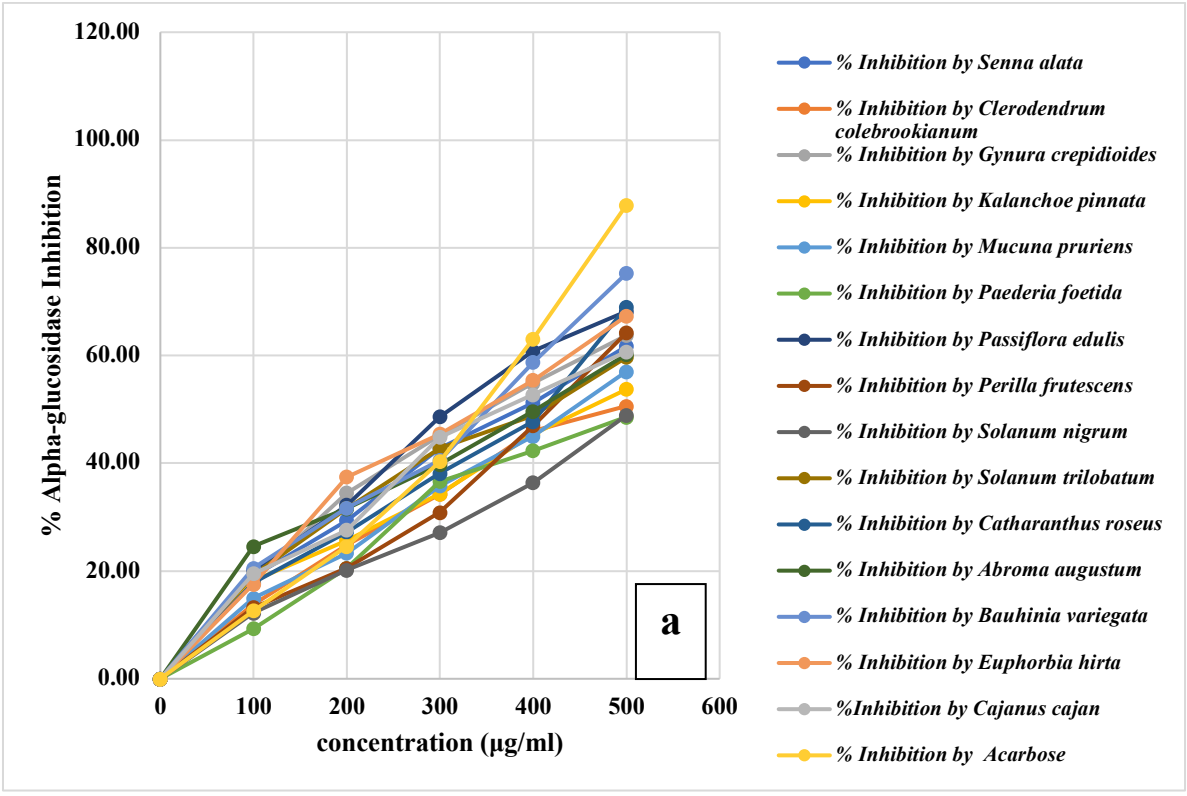


Figure 5.1: Percent inhibition of α-amylase by different extracts. a.80% ethanol extract, b. 80% methanol extract, and c. Pure water extract.


120.00
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% Alpha-glucosidase Inhibition
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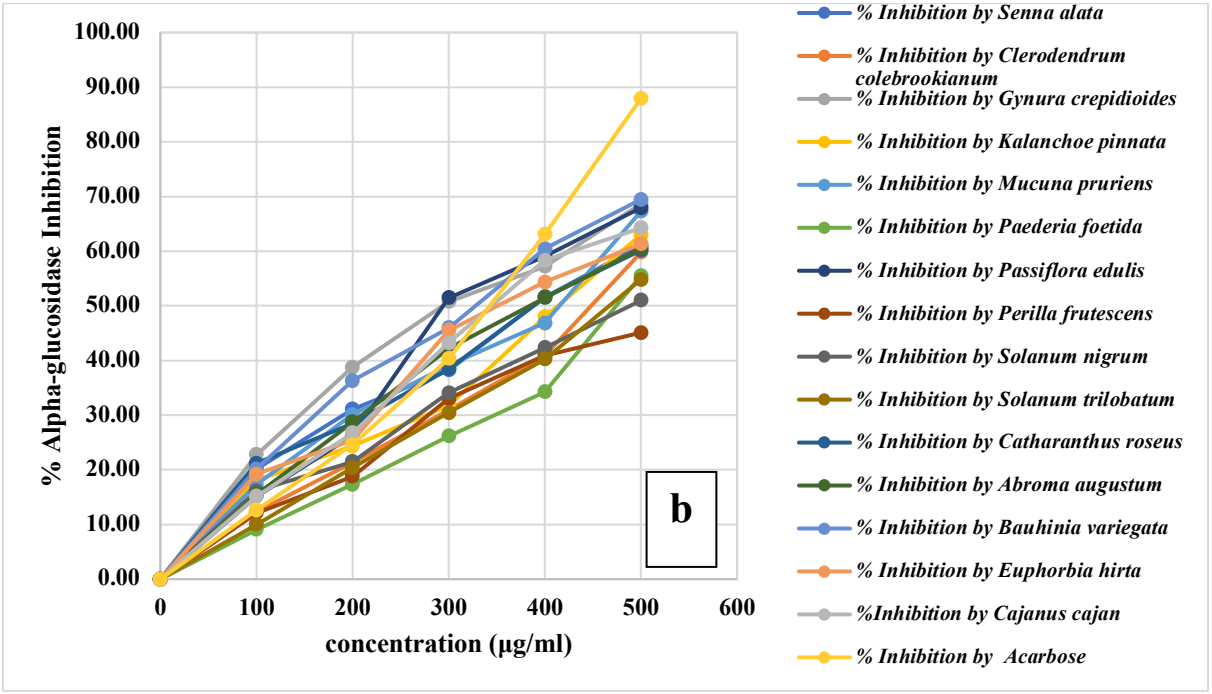
concentration (µg/ml)
a
% Inhibition by Senna alata
% Inhibition by Clerodendrum colebrookianum
% Inhibition by Gynura crepidioides
% Inhibition by Kalanchoe pinnata
% Inhibition by Mucuna pruriens
% Inhibition by Paederia foetida
% Inhibition by Passiflora edulis
% Inhibition by Perilla frutescens
% Inhibition by Solanum nigrum
% Inhibition by Solanum trilobatum
% Inhibition by Catharanthus roseus
% Inhibition by Abroma augustum
% Inhibition by Bauhinia variegata
% Inhibition by Euphorbia hirta
%Inhibition by Cajanus cajan
% Inhibition by Acarbose


100.00
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% Alpha-glucosidase Inhibition
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200
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concentration (µg/ml)
b
% Inhibition by Senna alata
% Inhibition by Clerodendrum colebrookianum
% Inhibition by Gynura crepidioides
% Inhibition by Kalanchoe pinnata
% Inhibition by Mucuna pruriens
% Inhibition by Paederia foetida
% Inhibition by Passiflora edulis
% Inhibition by Perilla frutescens
% Inhibition by Solanum nigrum
% Inhibition by Solanum trilobatum
% Inhibition by Catharanthus roseus
% Inhibition by Abroma augustum
% Inhibition by Bauhinia variegata
% Inhibition by Euphorbia hirta
%Inhibition by Cajanus cajan
% Inhibition by Acarbose

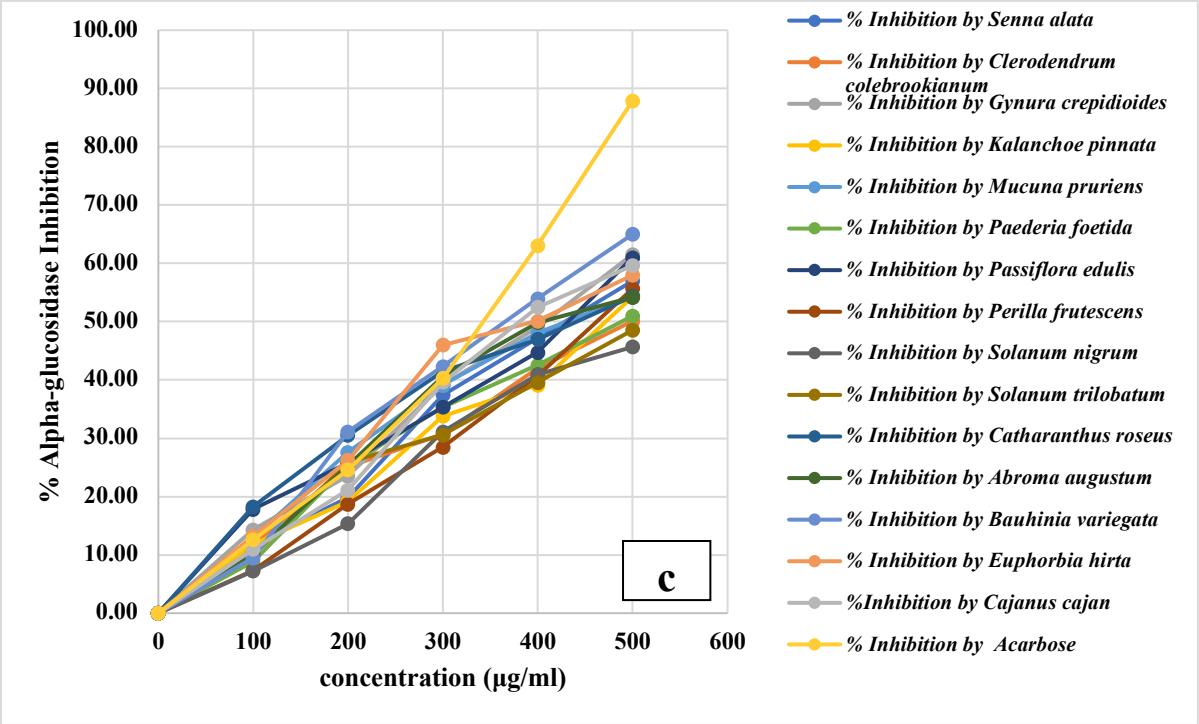
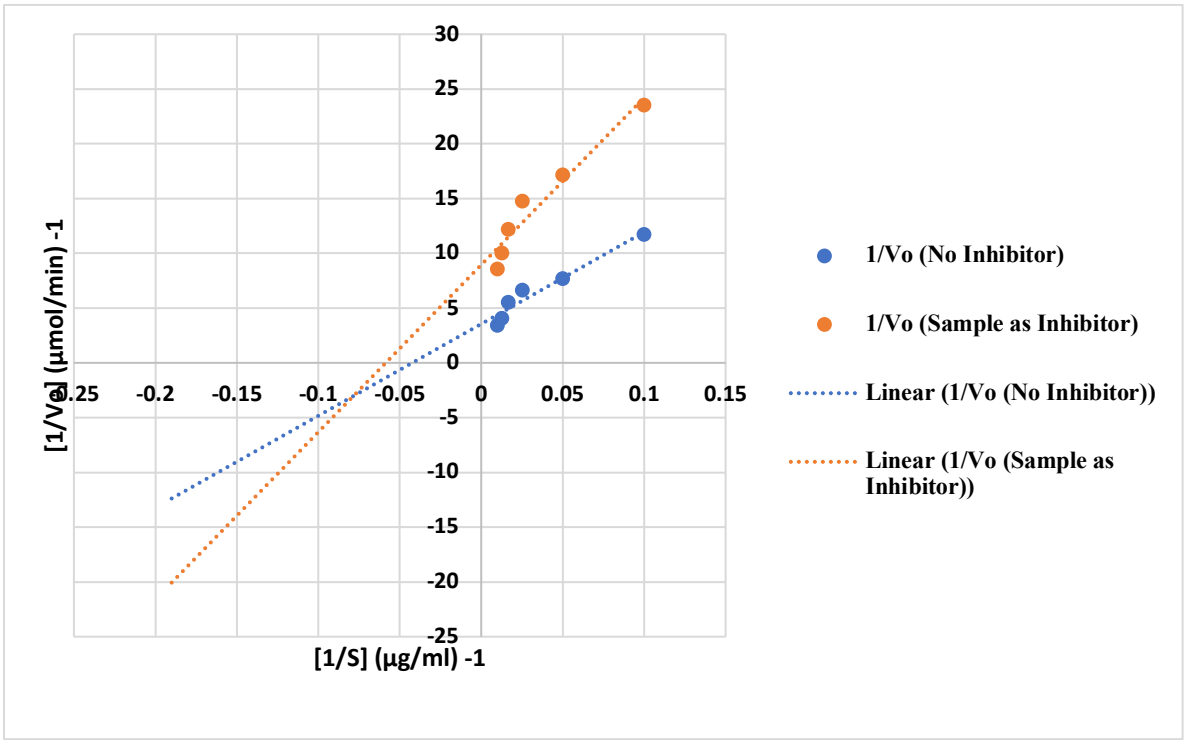


Figure 5.2: Percent inhibition of α-glucosidase by different extracts. a. 80% ethanol extract, b. 80% methanol extract, and c. Pure water extract.

For the present study, Acarbose was used as a standard drug for reference. The inhibition percentage of Acarbose was 68.93% for α-amylase enzyme and 87.97% for α-glucosidase enzyme when taking into account the data and conducting a comparison of the inhibitory impact of the three extracts (EtOH, MeOH and pH_2O) on α-amylase and α-glucosidase enzymes. The resulting data reveals that EtOH extract was a better extraction as higher enzyme inhibitions were registered in all the species studied. The results also show significant variations in inhibitory potency among the plant species and extracts analyzed.



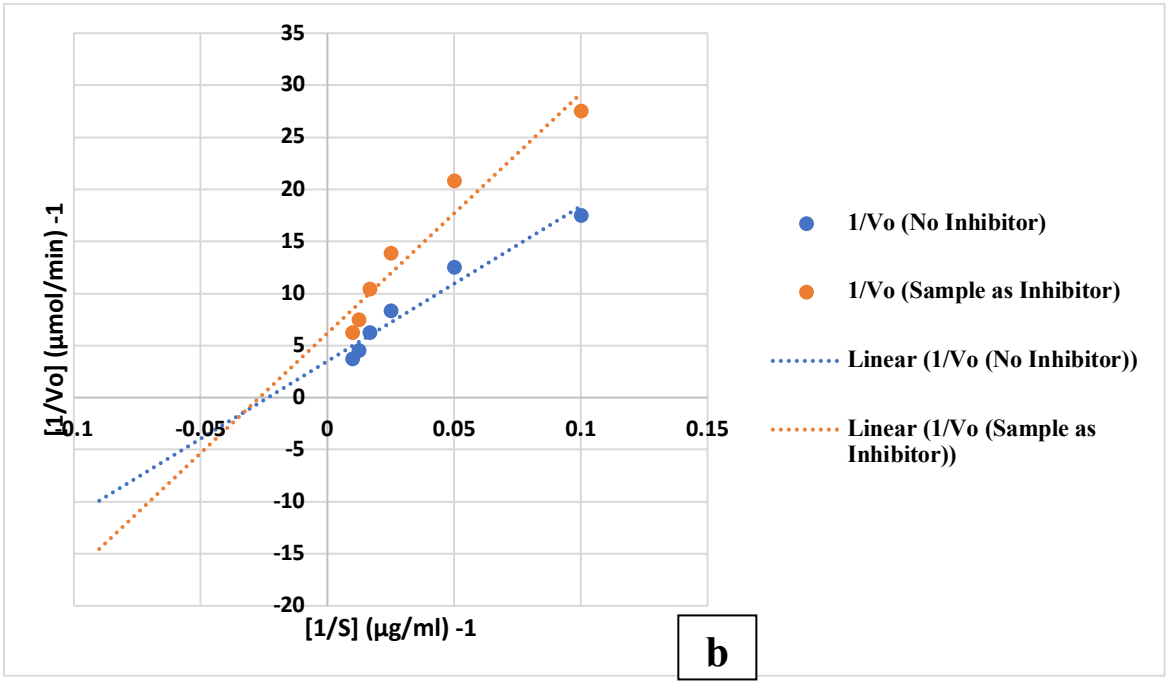


Figure 5.3: Mode of competitive inhibitions by *Bauhinia variagata* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.

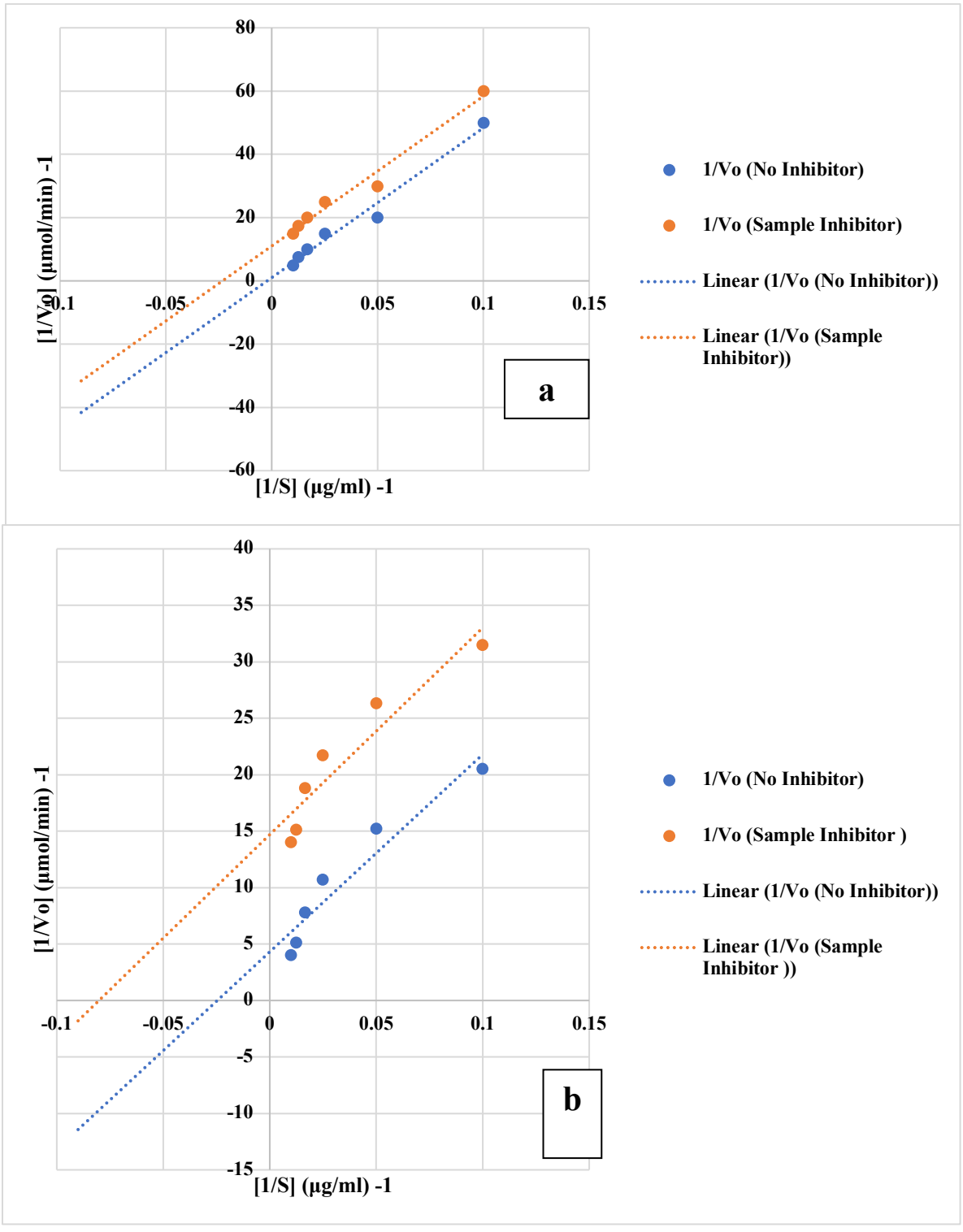
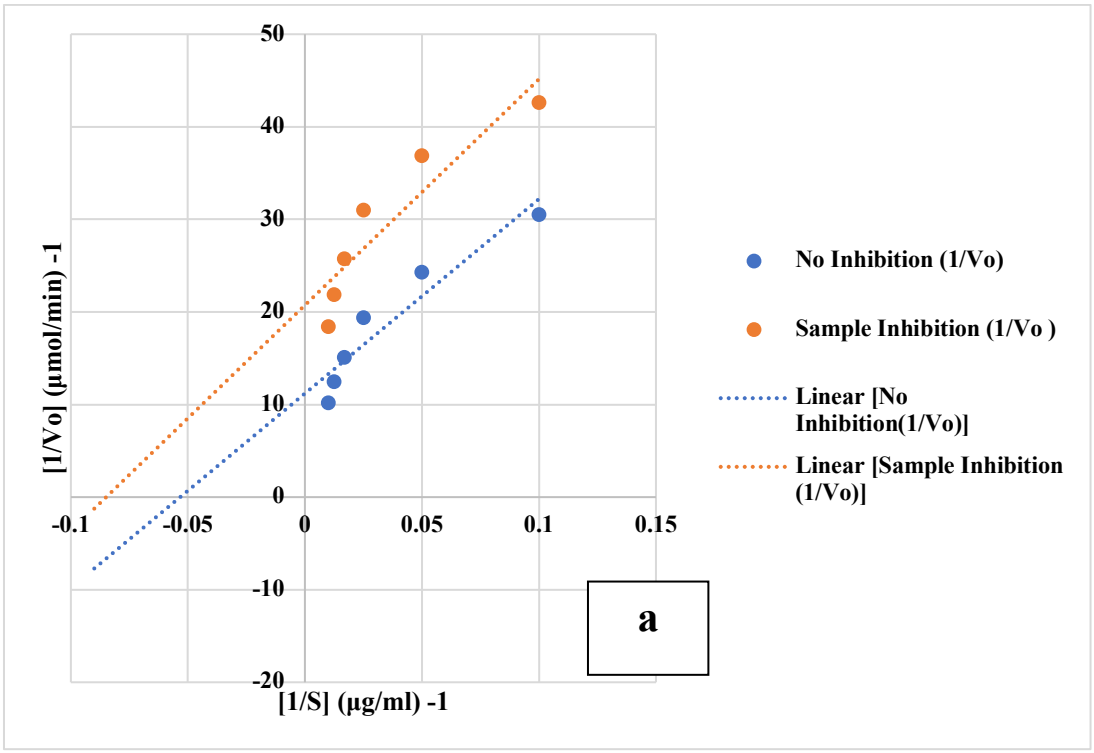


Figure 5.4: Mode of non-competitive inhibitions by *Euphorbia hirta* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.



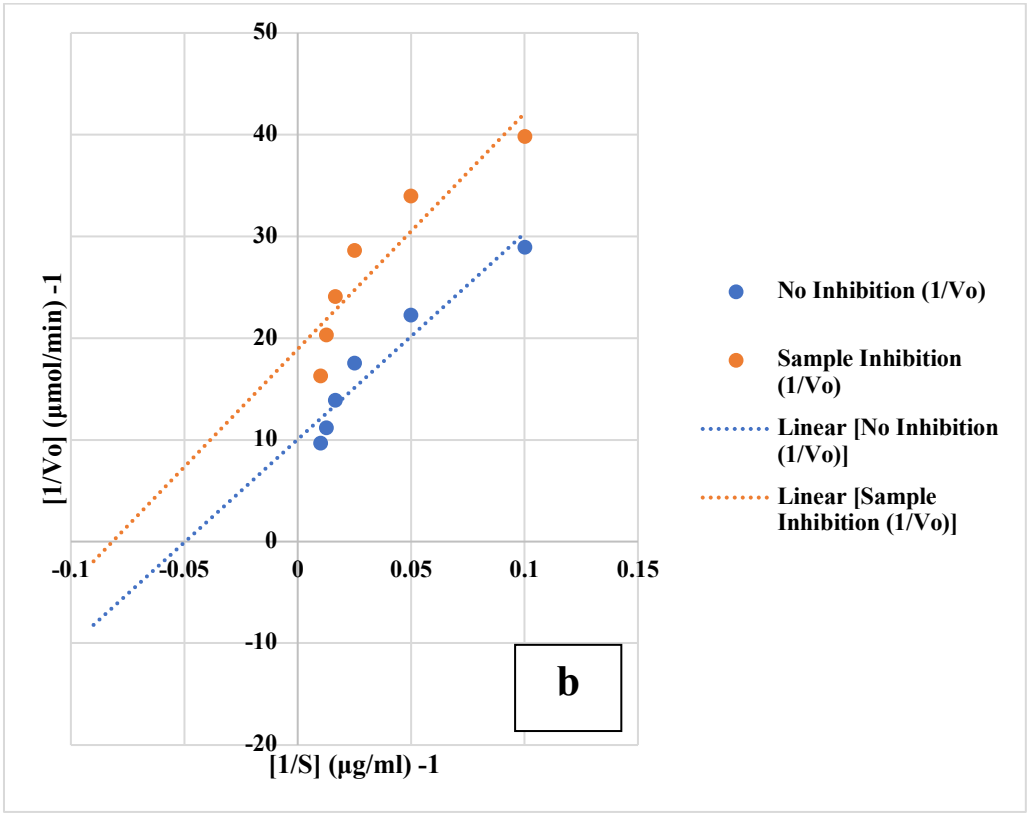
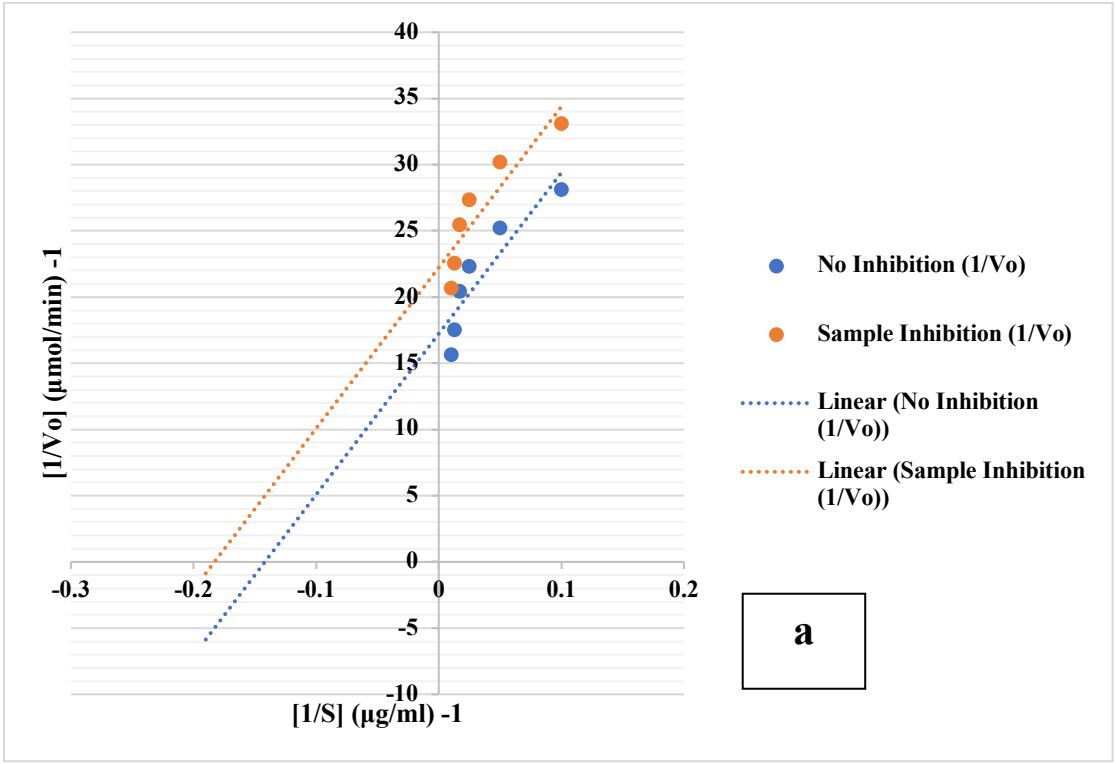


Figure 5.5: Mode of non-competitive inhibitions by *Passiflora edulis* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.



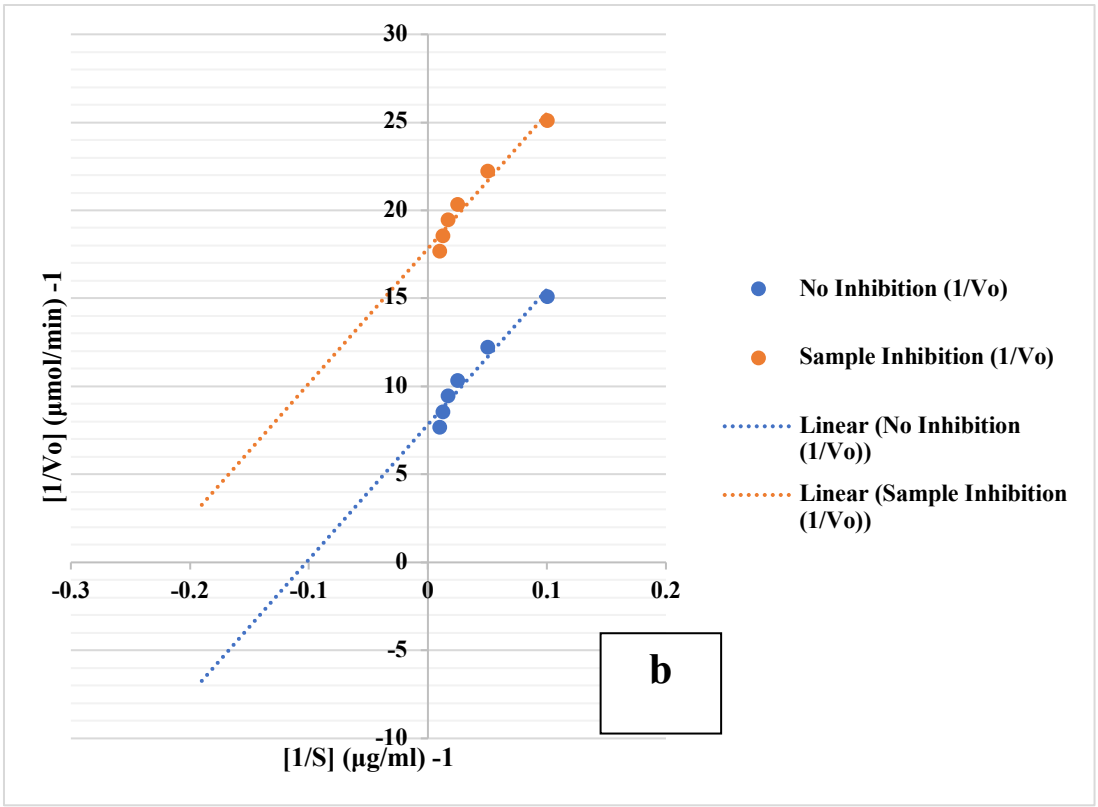


Figure 5.6: Mode of non-competitive inhibitions by *Clerodendrum colebrookianum* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.

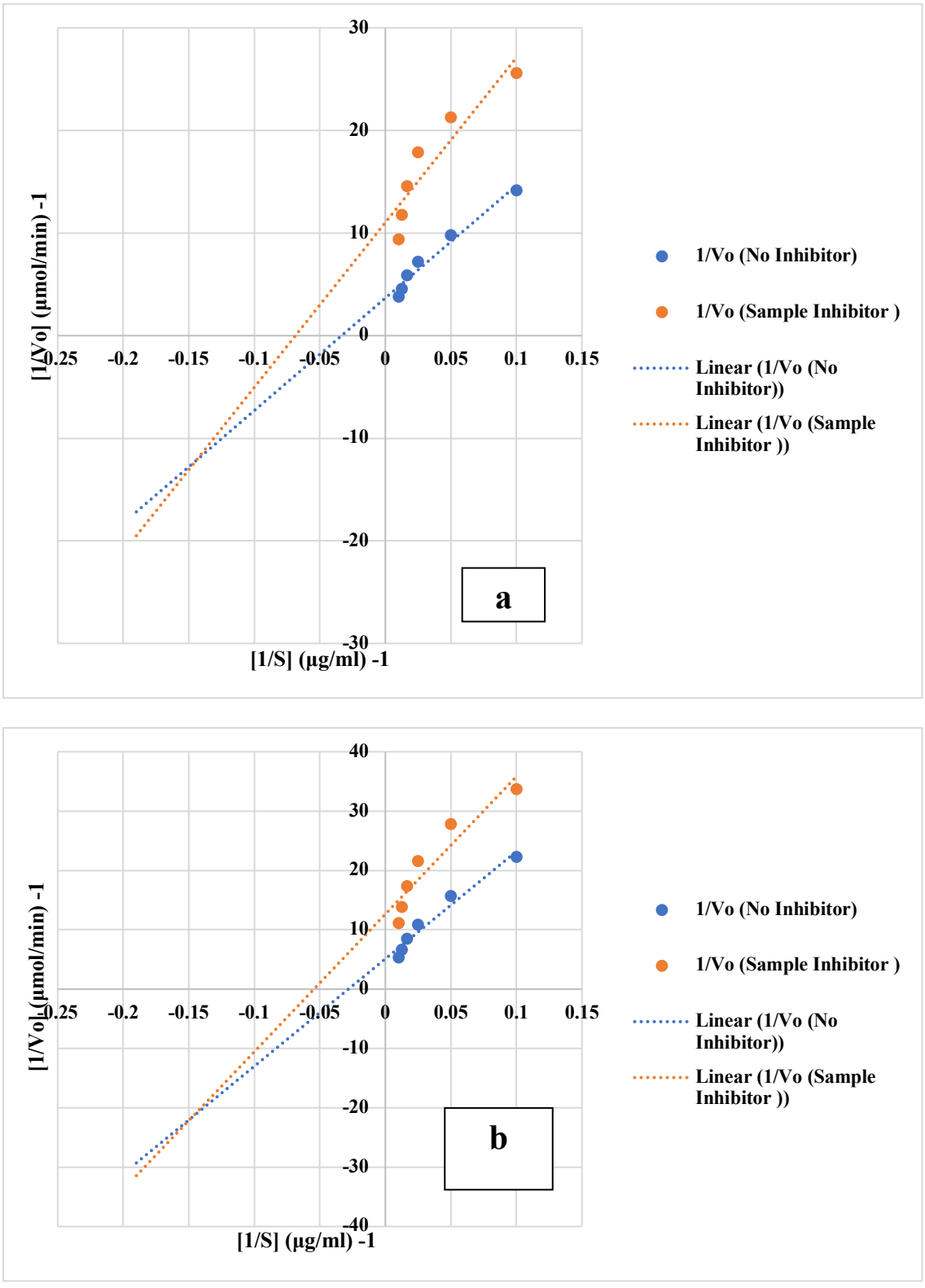
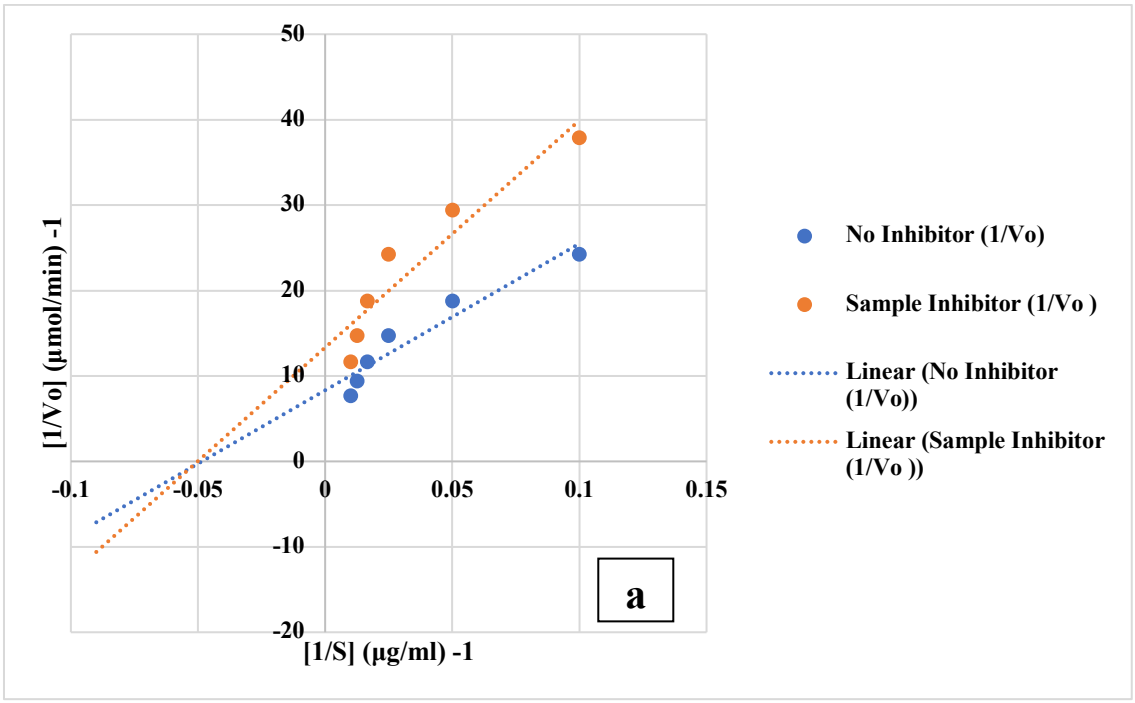


Figure 5.7: Mode of competitive inhibitions by *Catharanthus roseus* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.



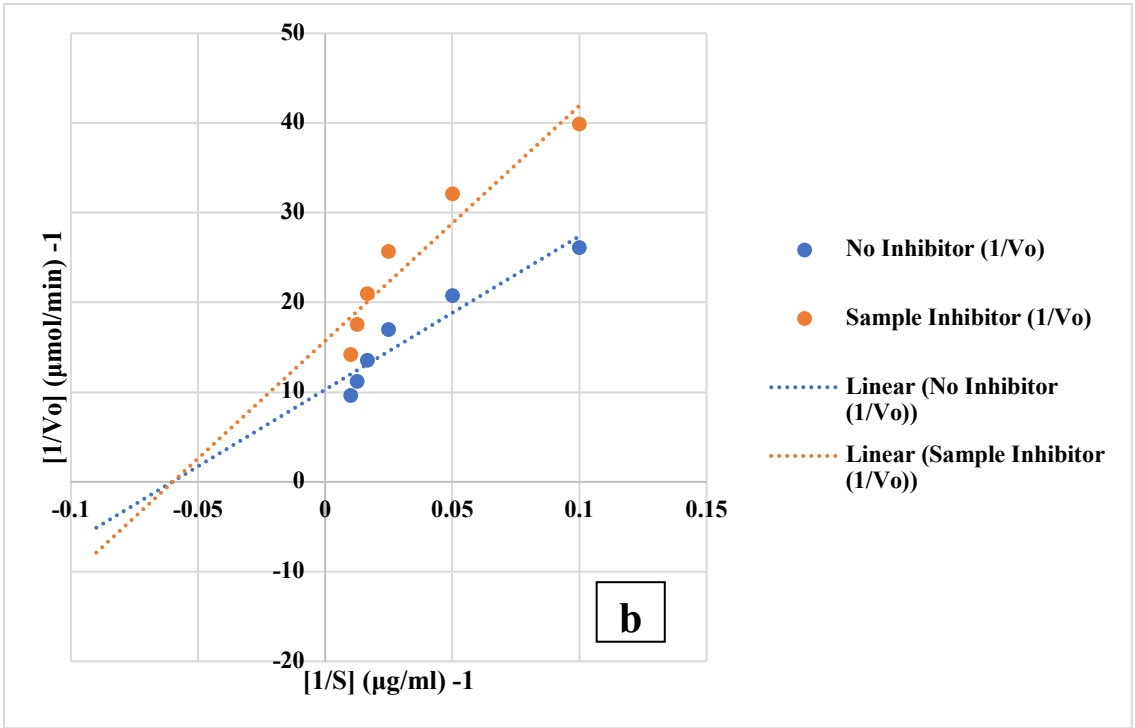
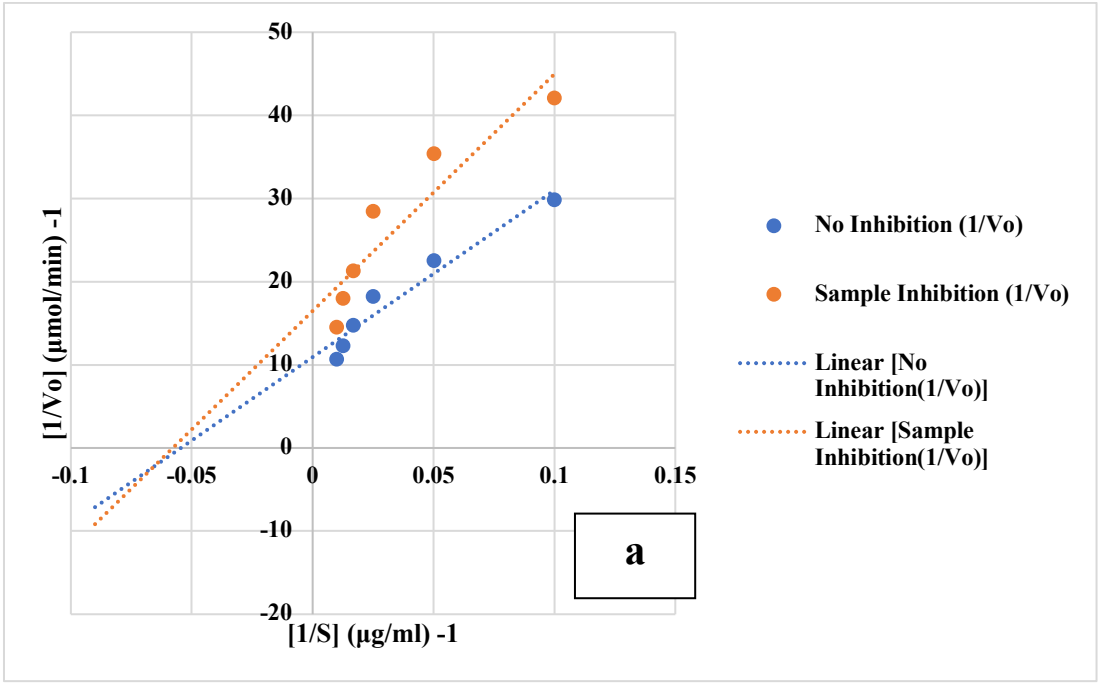


Figure 5.8: Mode of competitive inhibitions by *Abroma augustum* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.



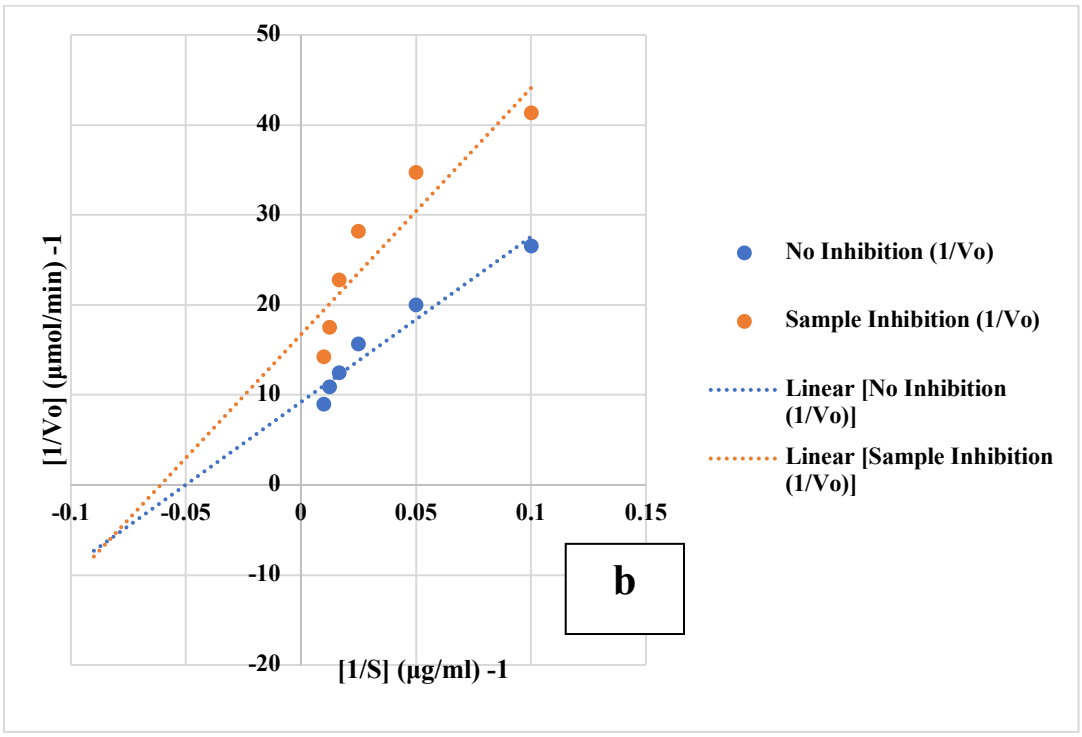
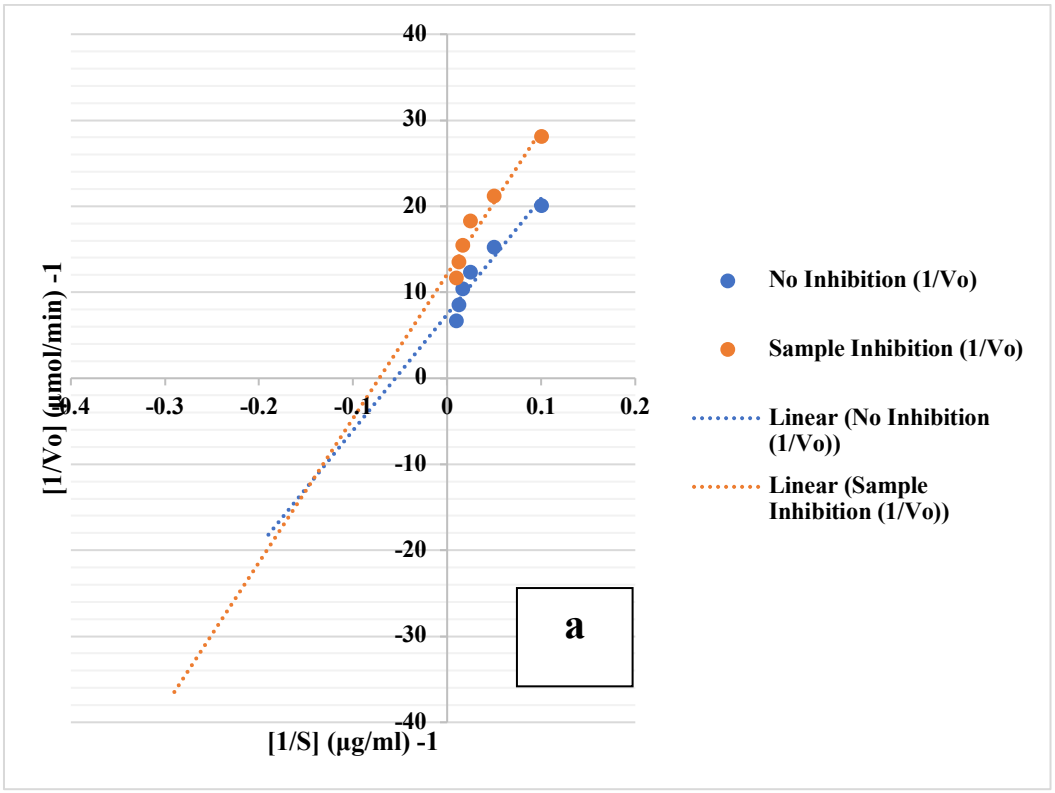


Figure 5.9: Mode of competitive inhibitions by *Gynura crepidioides* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.



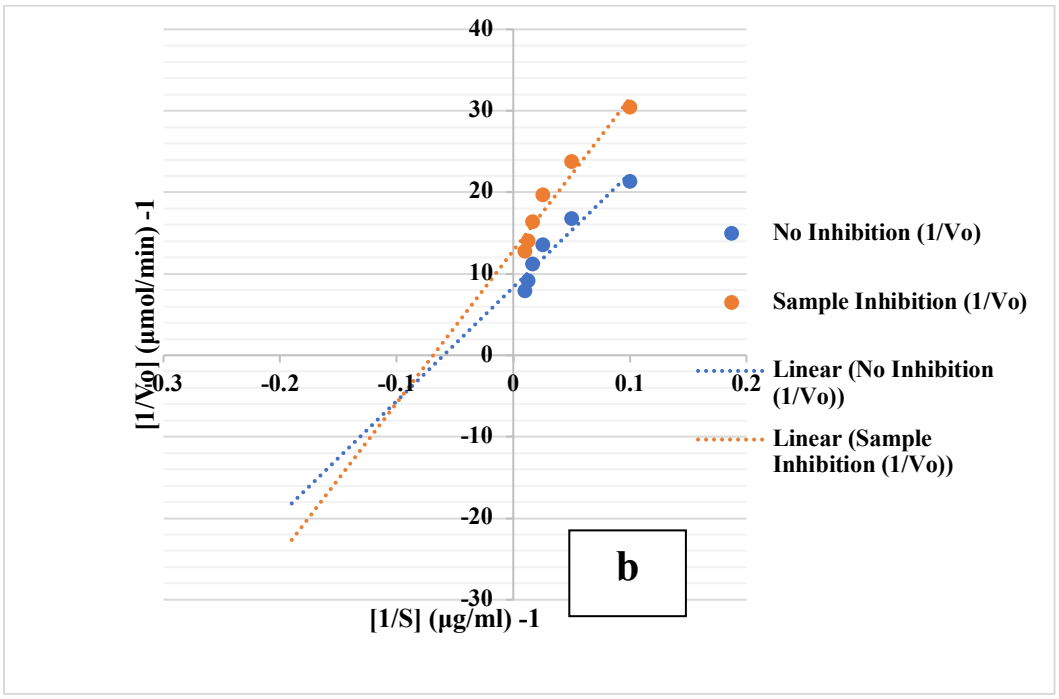
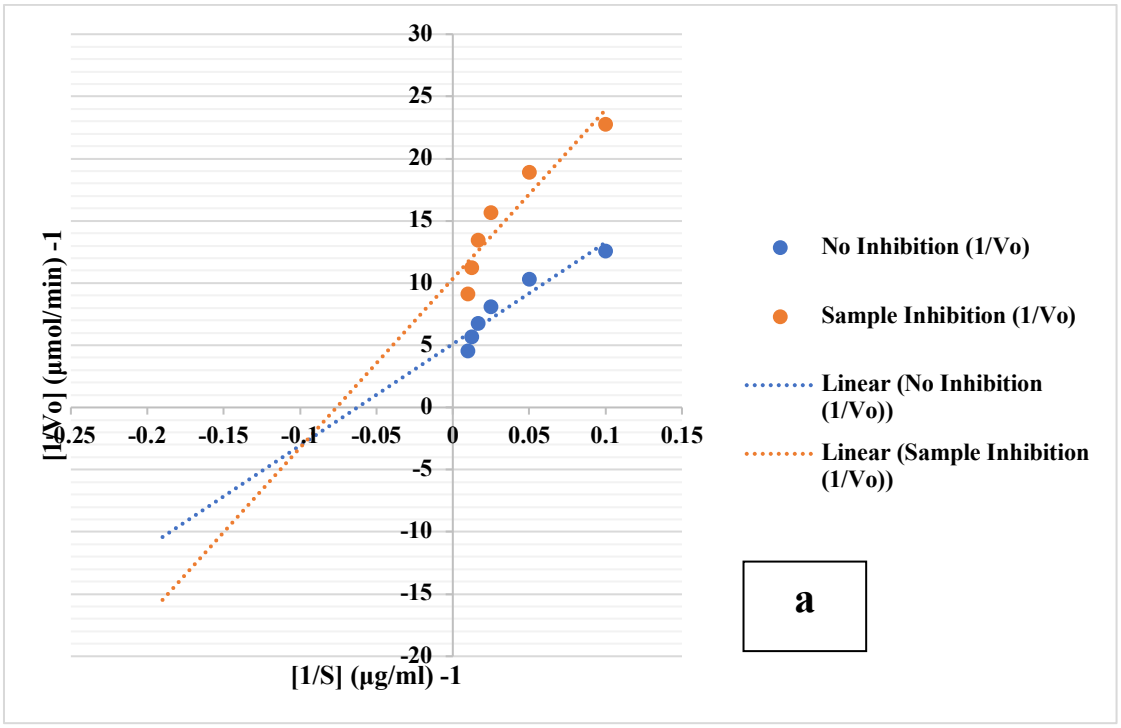


Figure 5.10: Mode of competitive inhibitions by *Cajanus cajan* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.



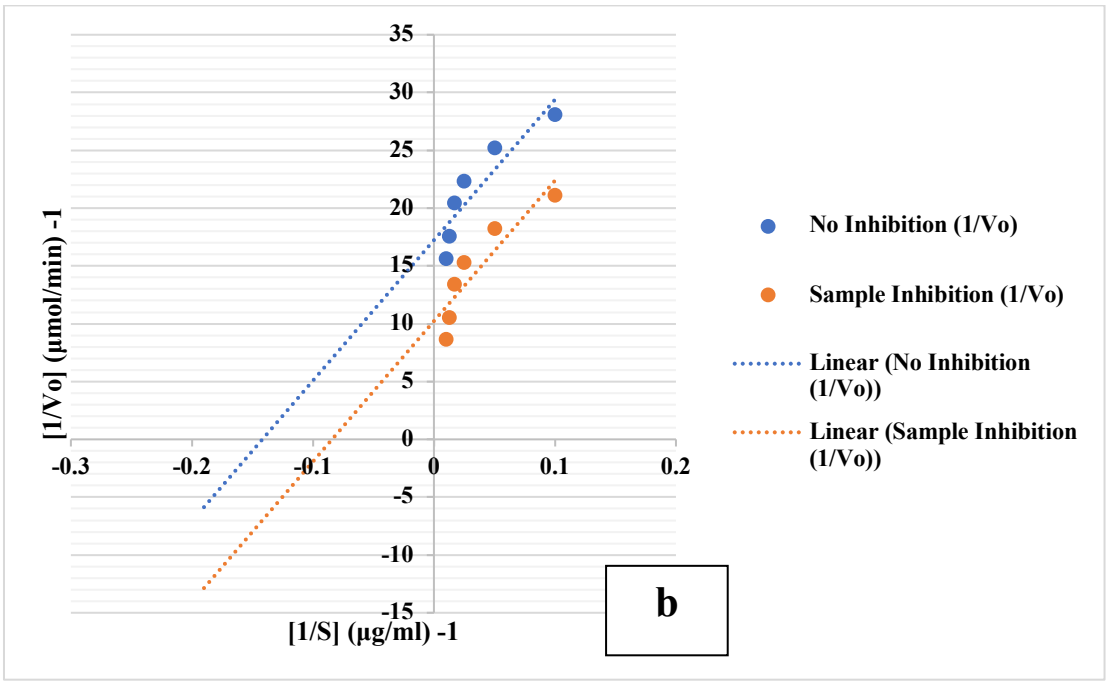
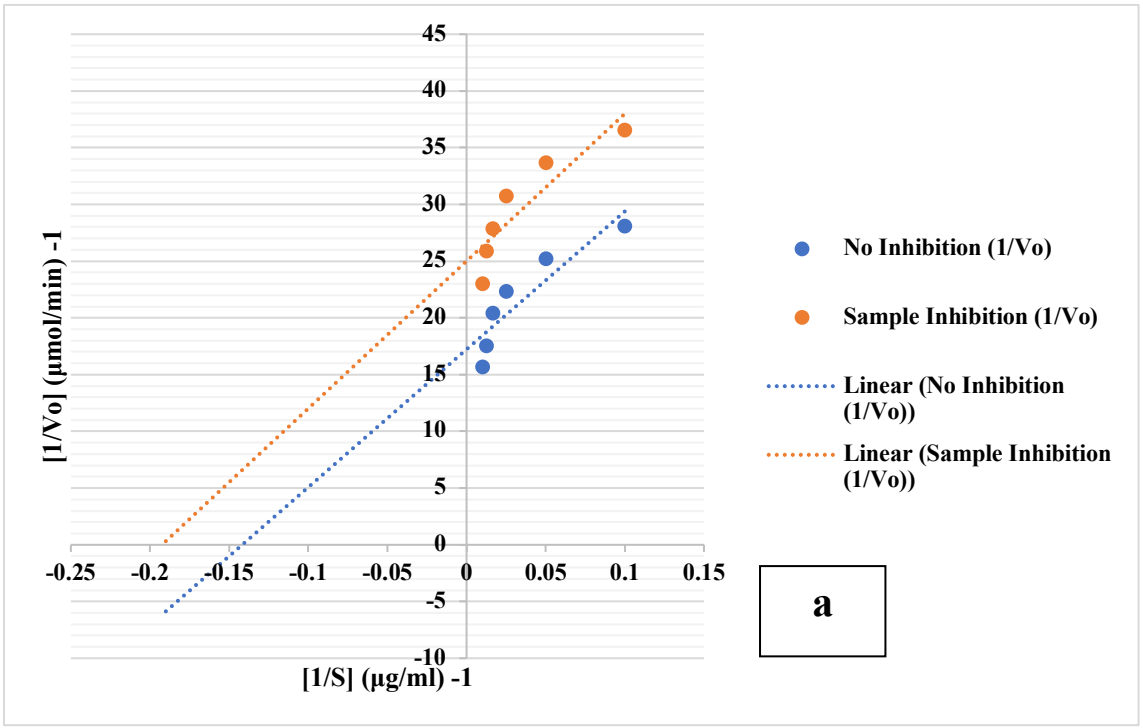


Figure 5.11: Mode of competitive and non-competitive inhibitions by *Senna alata* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.



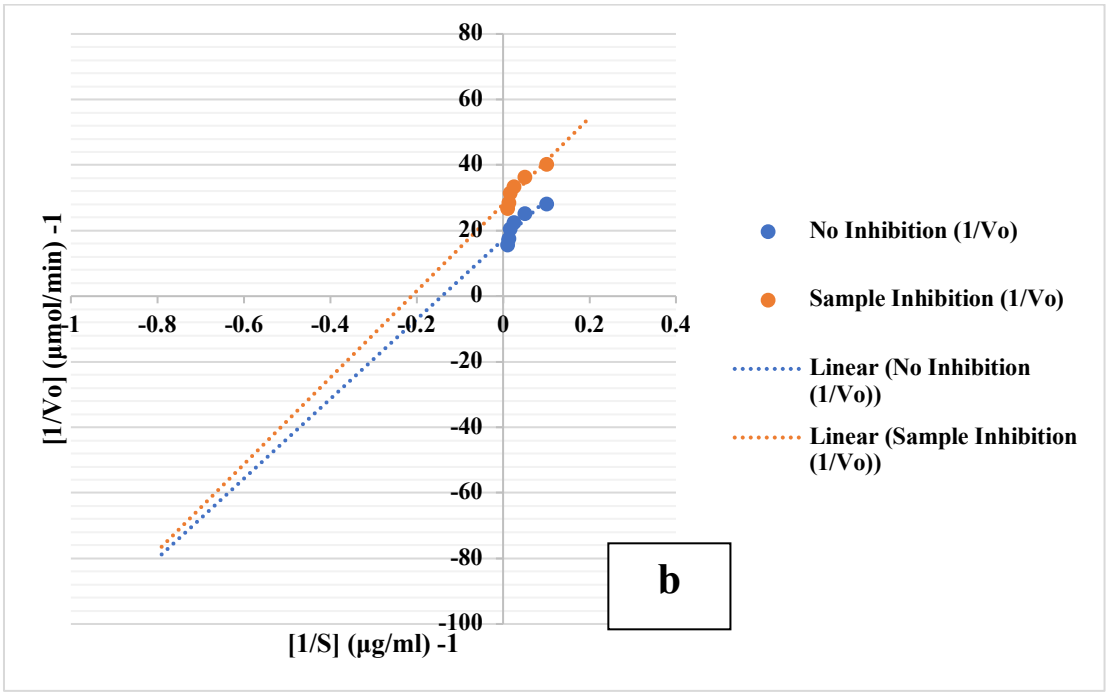


Figure 5.12: Mode of non-competitive and competitive inhibitions by *Perilla frutescens* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.

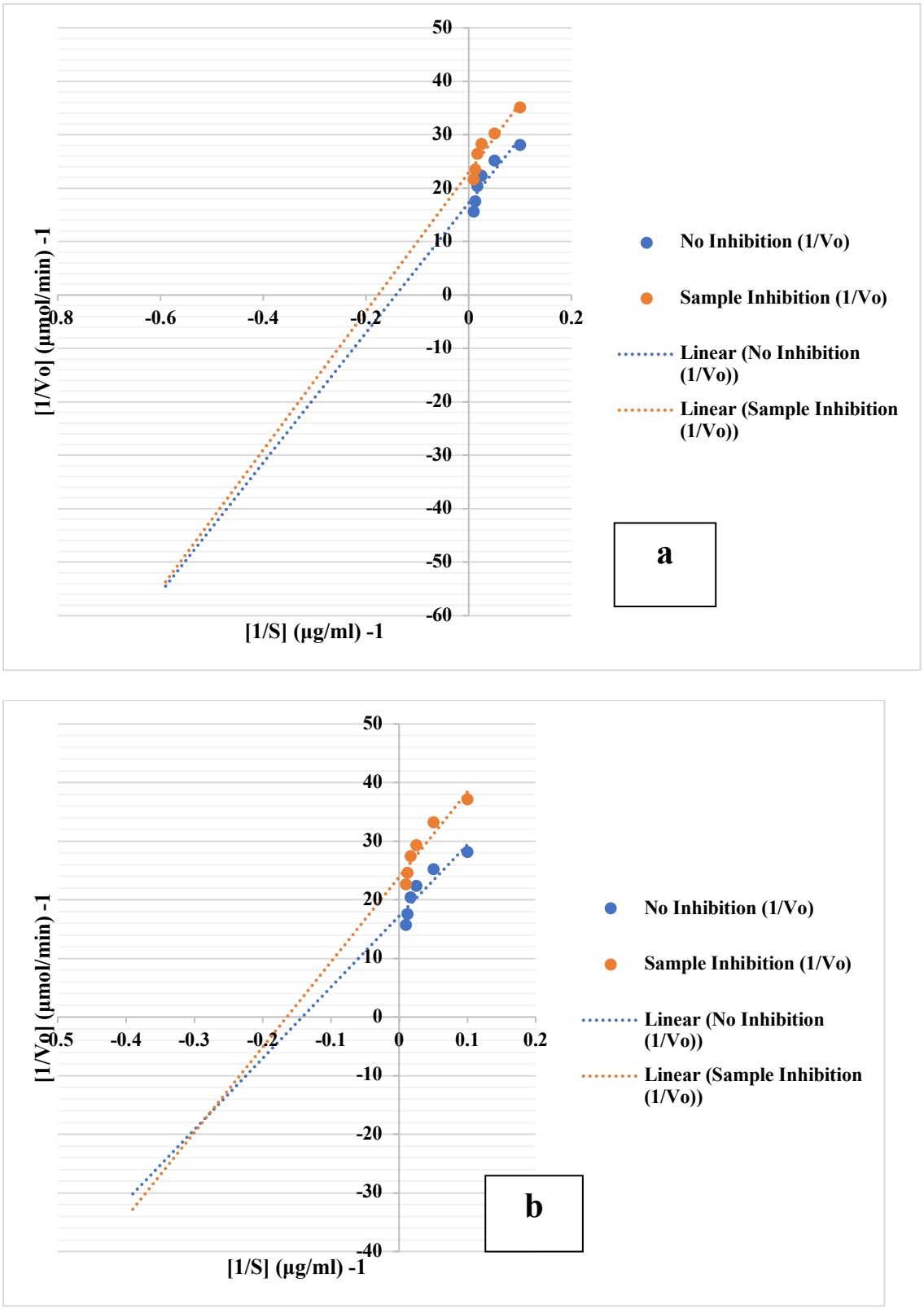
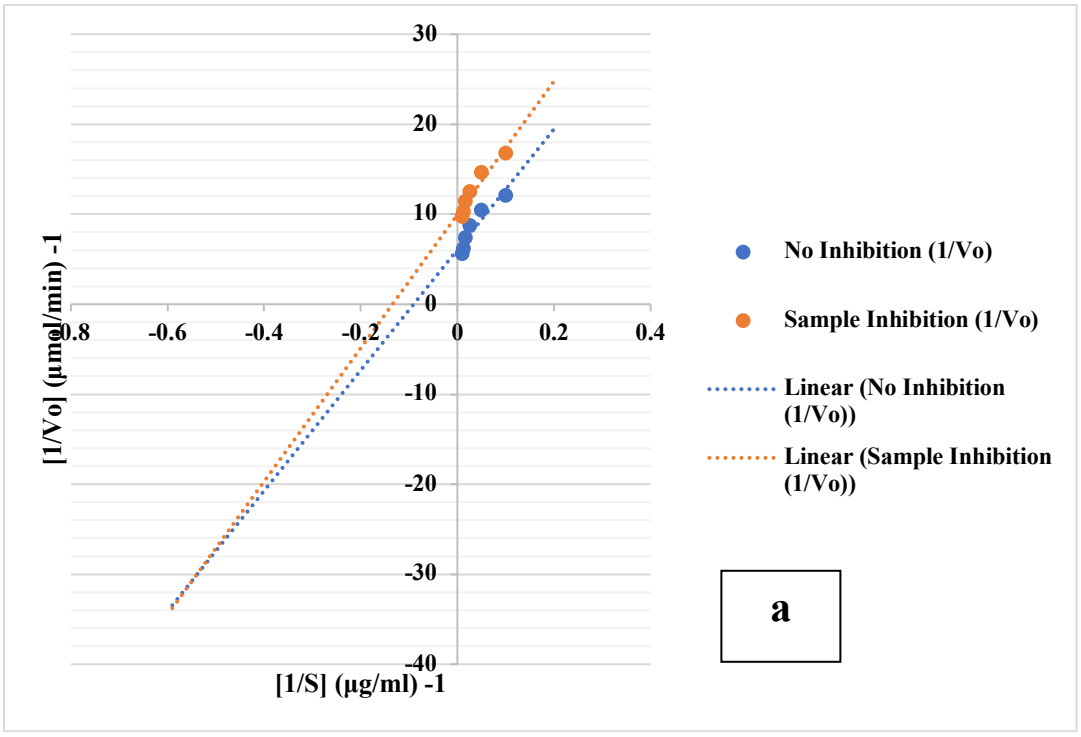


Figure 5.13: Mode of competitive inhibitions by *Kalanchoe pinnata* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.



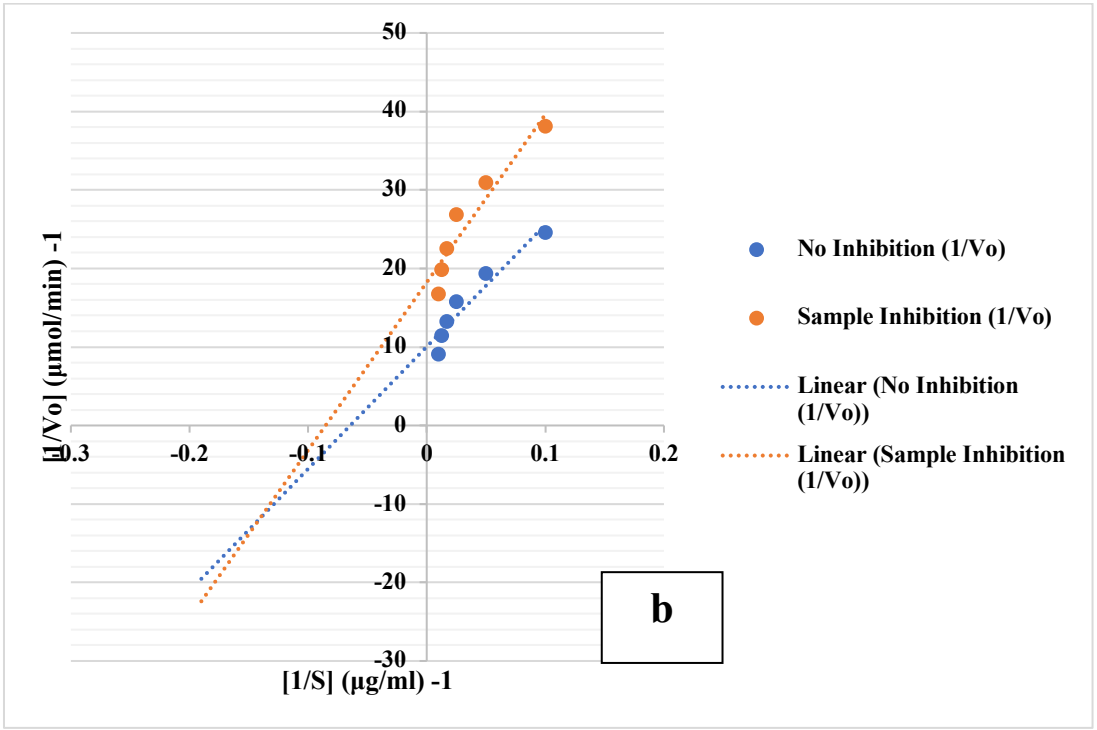


Figure 5.14: Mode of competitive inhibitions by *Mucuna pruriens* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.

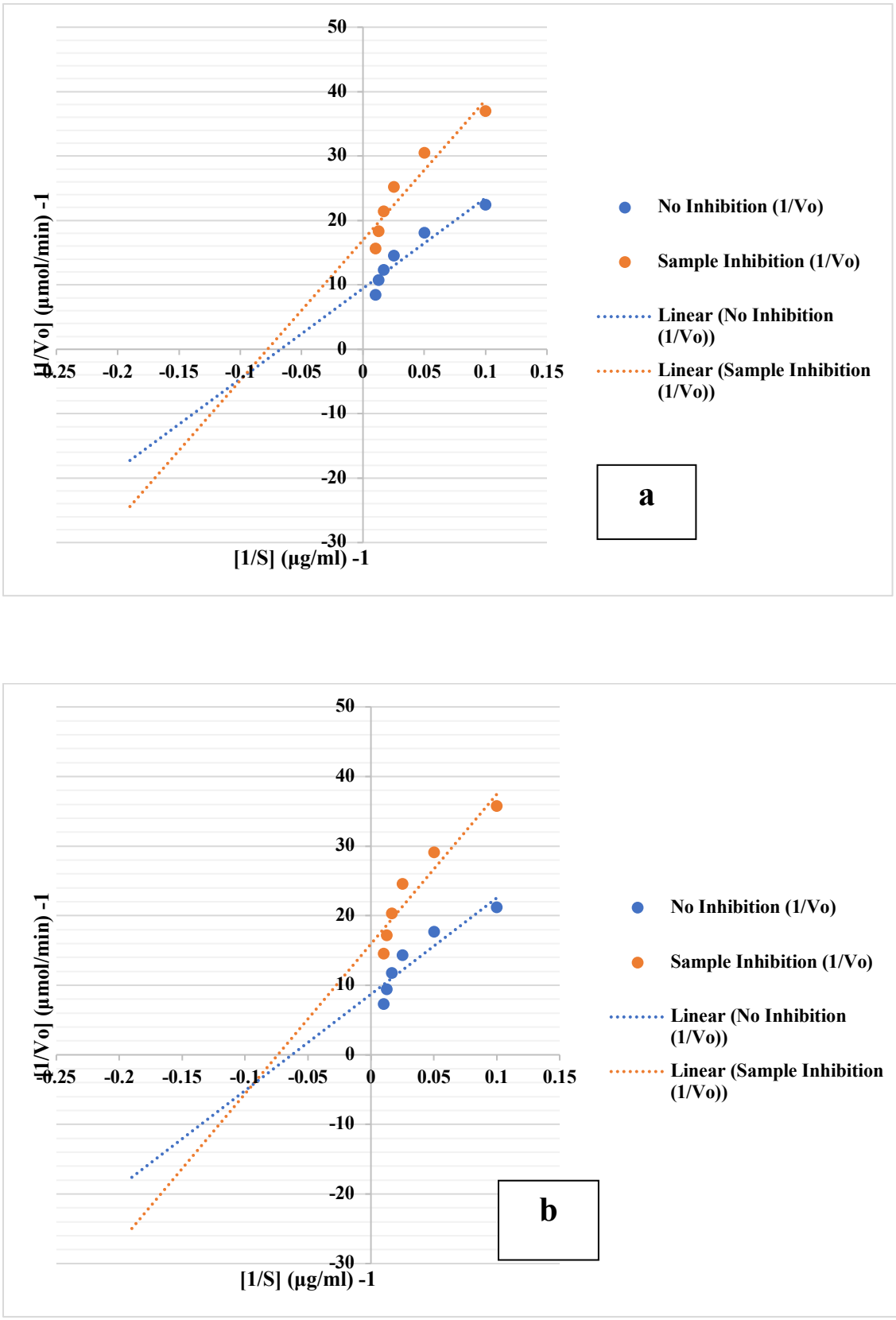
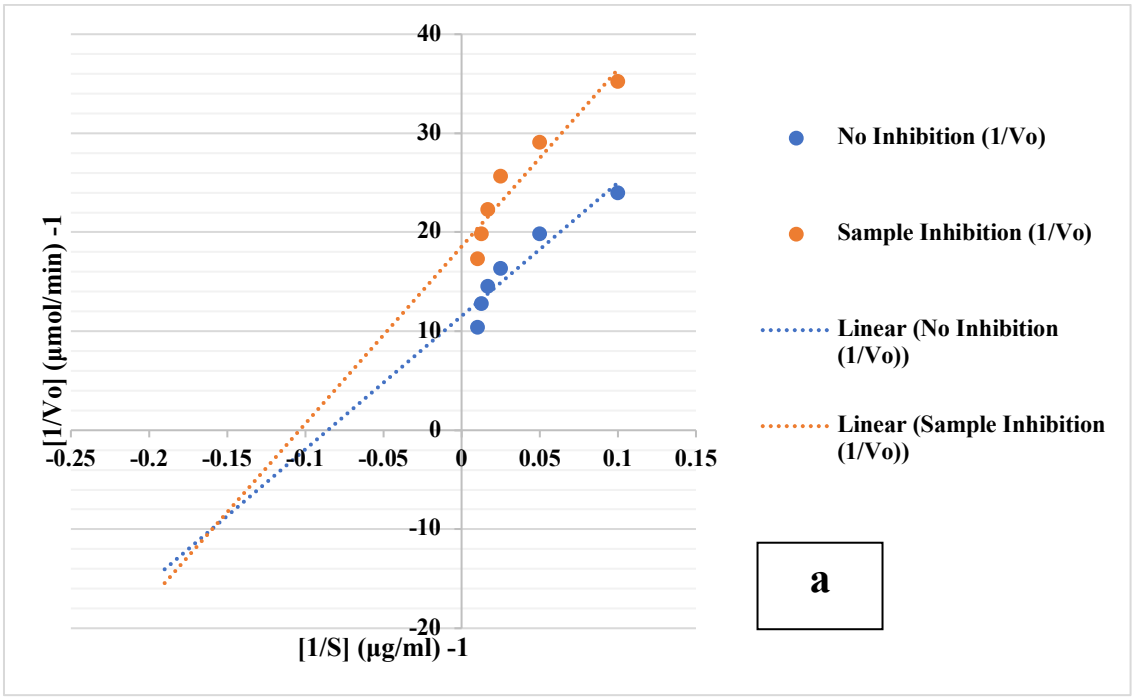


Figure 5.15: Mode of competitive inhibitions by *Paederia foetida* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.



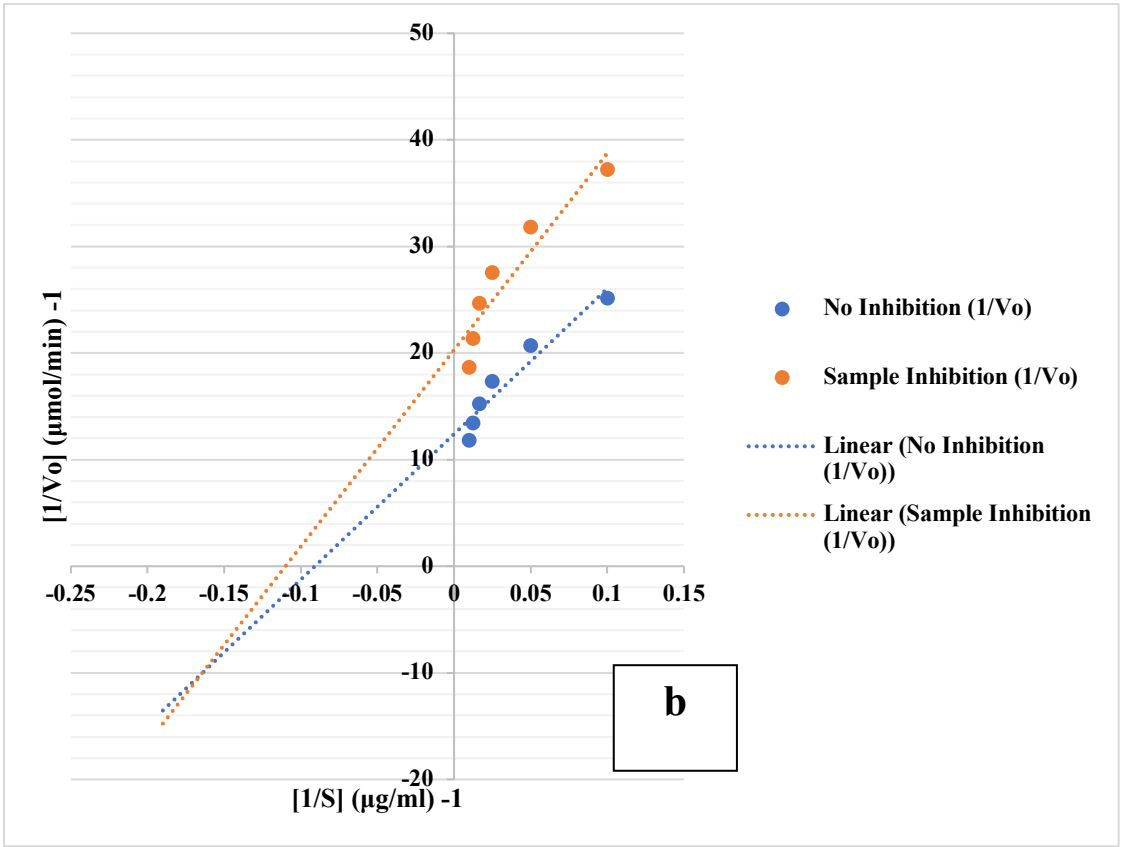


Figure 5.16: Mode of competitive inhibitions by *Solanum nigrum* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.

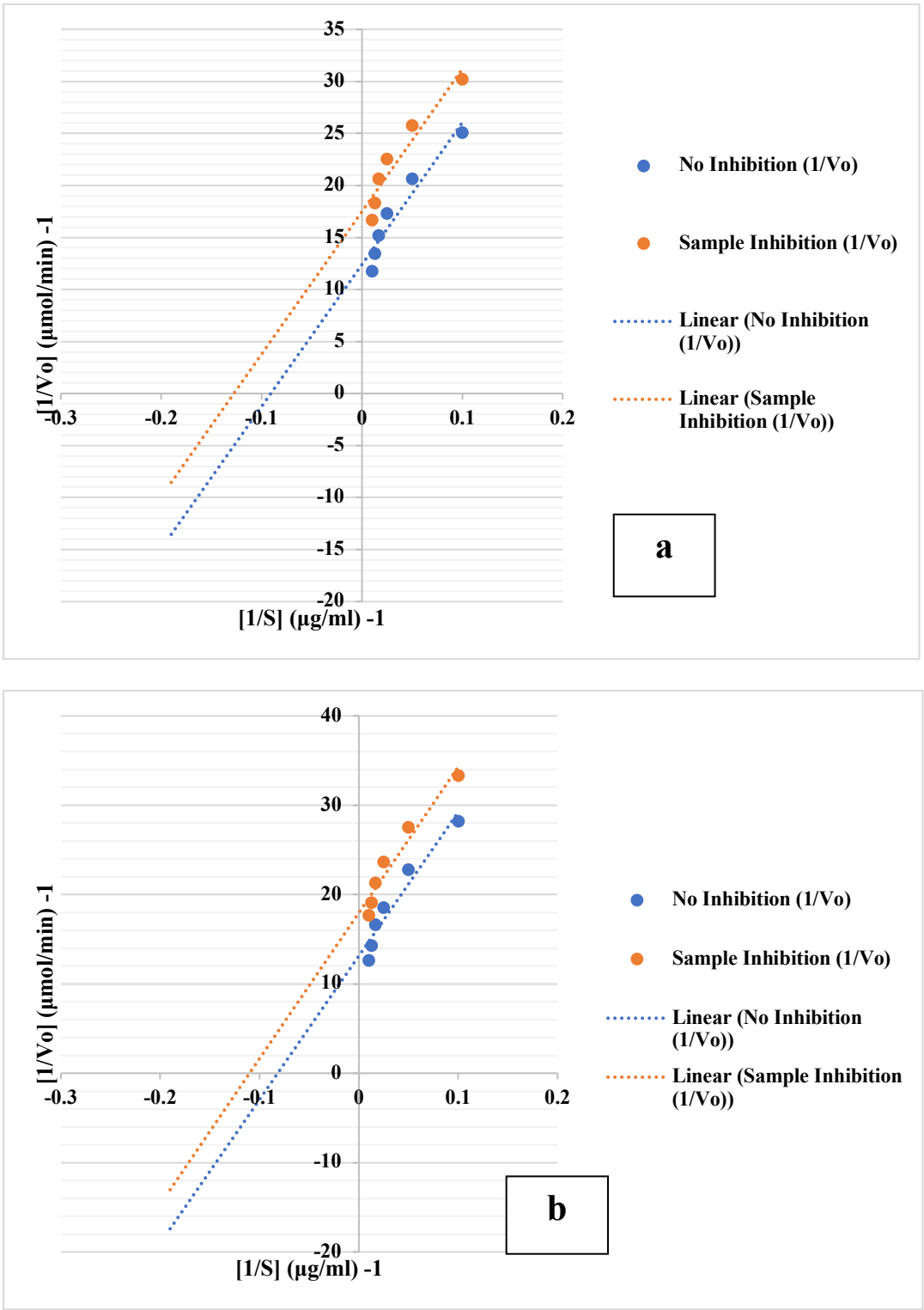


Figure 5.17: Mode of non-competitive inhibitions by *Solanum trilobatum* extracts on enzyme activities. **a.** On α-amylase enzyme activity, **b**. α-glucosidase enzyme activity.

The inhibitory mechanisms of both α-amylase and α-glucosidase enzymes were investigated utilizing ethanol extracts derived from selected ethnomedicinal plant samples through the utilization of Lineweaver-Burk plots. The regression analysis of Lineweaver-Burk plots indicates a competitive mode of inhibition for *Bauhinia variegata*, illustrated in Figure 5.3 (a, b) and a non-competitive mode of inhibition for *Euphorbia hirta*, *Passiflora edulis, Clerodendrum colebrookianum* depicted in Figure 5.4 (a, b); 5.5 (a, b); 5.6 (a, b). *Catharanthus roseus, Abroma augustum, Gynura crepidioides, Cajanus cajan* exhibited a competitive mode of inhibition in both α-glucosidase and α-amylase inhibition, as displayed in Figure 5.7 (a, b); 5.8 (a,b), 5.9 (a,b); 5.10 (a, b). Ethanol extract of *Senna alata* shows competitive mode of inhibition withα-amylase enzyme and non-competitive mode of inhibition with α-glucosidase enzymes and is depicted in Figure 5.11 (a, b). A non-competitive and competitive inhibition is observed in α-amylase and α-glucosidase enzymes, respectively, for *Perilla frutescents* (Figure 5.12 a, b). The ethanol extracts of *Kalanchoe pinnata*, *Mucuna pruriens*, *Paederia foetida*, and *Solanum nigrum* showcased a competitive mode of inhibition in both enzyme inhibition assays and is shown in Figure 5.13 (a, b); Figure 5.14 (a, b); Figure 5.15 (a, b); and Figure 5.16 (a, b). In the regression analysis of Lineweaver-Burk plots, *Solanum trilobatum* demonstrated a non-competitive mode of inhibition, as illustrated in Figure 5.17 (a, b) for both α-amylase and α-glucosidase enzymes.

Discussion

Numerous herbal extracts have been documented for their anti-diabetic properties and are employed in various practices to manage diabetes. The utilization of herbal extracts has been observed both directly and indirectly in the formulation of numerous medicines. The present study observed the inhibitory effect of various extracts from selected ethnomedicinal plants on porcine pancreatic α-amylase and α-glucosidase

enzymes. The plant extract has notable inhibition ability in both the enzyme inhibition assays. α-amylase acts as a catalyst and is required in the breakdown of carbohydrates; it also facilitates the conversion of polysaccharides such as starch into simple oligosaccharides like maltose. The hindrance of α-amylase results in a deceleration of subsequent biochemical reactions that are responsible for generating and absorbing glucose within the gastrointestinal tract. Consequently, these enzymes are the main subjects for controlling elevated glucose levels post-meal in individuals afflicted with diabetes. α-glucosidase inhibitors are also regarded as a novel prospect in the combat against diabetes. A vast array of over 400 distinct compounds, comprising 61 terpenes, 37 alkaloids, 49 quinines, 37 phenols, 73 phenylpropanoids, 103 flavonoids, 8 steroids, and 43 various other compounds, exhibiting glucosidase inhibitory properties, has been identified from diverse categories of plants (Yin et al., 2014).

Bauhinia variegata, Abroma augustum, Euphorbia hirta, Passiflora edulis, *Gynura crepidioides*, and *Kalanchoe pinnata* exhibited significant inhibition toward α-amylase and α-glucosidase enzymes. A study by Dos Santos et al. (2022) also studied the α-amylase inhibitory effect of *Passiflora edulis*, and its seed extract showed alpha-amylase inhibition with an IC_{50} of 32.1±2.7µg/mL, indicating potential anti-diabetic properties warranting further investigation for managing diabetes. Research on *Bauhinia variegata* demonstrated that methanolic extracts of leaves and bark exhibited high phenolic and flavonoid contents (Kamal et al., 2022), while *Bauhinia variegata* flower extract-synthesized silver nanoparticles showed potent alpha-amylase enzyme inhibition with an IC_{50} value of 38 µg/ml (Johnson et al., 2018). Various other studies have also demonstrated that *Cajanus cajan* seeds possess significant inhibitory activities against these enzymes, indicating their role in managing diabetes (Mony, 2023; Sharma et al., 2023). Additionally, a pair of new lignan conformers, one new flavonoid glycoside, as

well as nineteen known compounds were purified from the twigs and leaves of *Cajanus cajan*. These compounds showed α-glucosidase inhibitory activities (Lei et al., 2022). Malathi et al. (2010) investigated the α-amylase and α-glucosidase inhibitory activities of alcoholic extracts from the flower and leaf of *Catharanthus roseus* to evaluate their anti-diabetic potential. The study revealed significant enzyme inhibition by both extracts, with the leaf extract showing the highest inhibitory action at 10 mg/ml (IC_{50}), while the flower extract had an IC_{50} at 12.5 mg/ml. Singh et al. (2001) have also demonstrated that the aqueous extract of *Catharanthus roseus* leaves showed significant α-amylase and α-glucosidase inhibitory activity in vitro. The study suggested that the inhibitory effects could be attributed to the presence of various phytochemicals such as flavonoids, alkaloids, and phenolic compounds. Extract of *Gynura crepidioides* showed efficient protection activity of pancreatic β- cells from cell death in the INS-1 cell line and showed inhibition of alpha-amylase and reduction of oxidative stress, indicating its potential anti-diabetic activity (Bahar et al., 2017). Adedayo et al. (2015) studied the effect of treating hot water on the radicals 1,1-diphenyl-2 picrylhydrazyl and hydroxyl scavenging, Fe^{2+}-induced lipid peroxidation and α-amylase and α-glucosidase inhibitory abilities of *Gynura crepidioides* leaf using *in vitro* models were evaluated and they also conclude that raw leaves had higher α-amylase and α-glucosidase inhibitory abilities. Efizal et al. (2007) also demonstrated that methanol extract and ethyl acetate fraction from *Kalanchoe pinnata* Pers. leaves have inhibitory activity against α-glucosidase enzyme and their IC_{50} in between 72.8 to 982.6 µg/mL. The generated data from this present study shows IC_{50} values from 359.45 to 436.25 µg/mL for α-amylase inhibition and 412.53 to 474.74 µg/mL for α-glucosidase inhibition. The variations in IC_{50} values may be because of several factors, including differences in extraction methods, solvent polarity, plant material source, and assay conditions. The different solvents and differences in

fractionation methods can influence the composition and concentration of bioactive compounds extracted, thus affecting the inhibitory potency against the α-glucosidase enzyme.

Bharadwaj et al. (2018) studied *Mucuna pruriens*, and they found that alpha-amylase inhibitors in it showed inhibition against human salivary α-amylase, suggesting potential in managing nutritional disorders like diabetes and obesity. Various *in vitro* physicochemical, phytocompound, antimicrobial and anti-diabetic studies on methanol extract of *Mucuna pruriens* showed significant α-amylase and α-glucosidase inhibition, and it indicates potential antidiabetic agent in traditional medicine (Kusuma et al., 2016). This also aligns with our observed data, where both 80% ethanol and 80% methanol extracts of *Mucuna pruriens* displayed significant inhibitory activity against α-amylase and α-glucosidase enzymes. A study conducted by Dendup et al. (2014) explored three novel isoflavanones along with thirteen previously identified compounds that were extracted from the root extracts of *Mucuna pruriens*. These compounds were studied, and they exhibited properties as inhibitors of α-glucosidase enzymes. The study also highlighted the influence of change in season on the synthesis of bioactive metabolites in *M. pruriens* and supported the idea of the plant's promising role as a potential anti-diabetic agent. They also observed significant inhibitory effects of the plant on α-amylase and α-glucosidase enzymes. In another study by Kazeem et al. (2015), the *Senna alata* extract using acetone displayed the highest inhibitory activity against α-amylase (IC_{50}=6.41 mg/mL), while extract using hexane exhibited the highest inhibitory effect on α-glucosidase (IC_{50}=0.85 mg/mL). In our study, the 80% ethanol extract exhibited the highest and effective inhibitory activity against both α-amylase and α-glucosidase for *Senna alata*. This suggests that different solvent systems can indeed yield extracts with varying bioactive profiles, emphasizing the importance of solvent selection in extraction

procedures for targeting specific bioactivities. Research conducted by Tan et al. (2019) also offers valuable input into the potential anti-diabetic compounds that were discovered in the twigs of *Paederia foetida*. This suggests that the plant exhibits therapeutic properties for diabetes management, which is consistent with our own findings that demonstrate IC_{50} inhibition in *Paederia foetida* leaves ranging from 411.50 to 503.95 µg/mL. Additionally, the root extract of *Paederia foetida* contains an enzyme with amylolytic activity, identified as β-amylase, which is stable under various pH and temperature conditions (Sottirattanapan et al., 2017). With alignment to our study, Choi et al. (2023) mentioned that 75% EtOH extract from *Perilla* seed meal had higher phenol content (105.58 mg GAE/g DW) and flavonoid contents and exhibited better antioxidant as well as better inhibition against α-glucosidase and α-amylase. Wang et al. (2021) also presented that ethyl acetate extract of *Perilla frutescens* showed potential α-glucosidase inhibition activity with an IC_{50} value of 190.03 µg/mL, indicating its potential as a natural anti-diabetic agent. The MeOH extract of *Perilla frutescens* leaves also exhibited α-amylase inhibition, indicating potential anti-diabetic properties, and HPTLC revealed 17 polyvalent phytoconstituents in the MeOH extract (Dixit et al., 2022). The current data also correlates with Wang et al. (2020) findings that *Perilla frutescens* extract from leaves showed increased IC_{50} values for α-glucosidase inhibition after digestion, indicating reduced activity post-digestion. This shows the importance of considering the stability and availability of bioactive phytocompounds in natural extracts. Increased suppression of carbohydrate metabolism and enzyme activities in α-amylase and α-glucosidase while employing ethanol extracts may also be the result of active solubilisation of bioactive compounds in ethanol.

The Lineweaver-Burk plot regression analysis revealed distinct modes of inhibition for each extract. For *Euphorbia hirta, Passiflora edulis, Clerodendrum colebrookianum,*

Perilla frutescens and *Senna alata's* α-glucosidase enzyme inhibition, *Solanum trilobatum*, a non-competitive mode of inhibition was observed. This implies that the inhibitor, i.e., the extract, binds to the enzyme at a site other than its active site, altering its conformation and enzyme-substrate complex formation without impacting substrate binding. This finding suggests a subtle interaction between the extract components and the enzyme, possibly involving allosteric regulation or conformational changes. The Lineweaver-Burk plot regression analysis for *Bauhinia variegata*, *Catharanthus roseus*, *Abroma augustum*, *Cajanus cajan*, *Gynura crepidioides*, *Senna alata's* α-amylase, *Kalanchoe pinnata*, *Mucuna pruriens*, *Paederia foetida* and *Solanum nigrum* revealed a competitive mode of inhibition. In this case, the inhibitor, i.e., extract, competes with its substrate for binding to its active site of the enzyme. The competitive nature of this inhibition suggests that the extract components share structural similarities with the substrate, effectively blocking its access to the enzyme's active site (Johnson et al., 2018). Plant extracts have shown promising inhibitory effects on key enzymes involved in diabetes management through competitive and non-competitive modes of inhibition. A study on *Piper betle* leaf extracts exhibited competitive inhibition of α-amylase and α-glucosidase, showcasing their ability to reduce blood glucose levels in diabetic mice (Mahmud et al., 2023). Moreover, the methanolic extract of *Cornus capitata* displayed competitive inhibition of α-glucosidase, emphasizing its potential as a treatment for postprandial hyperglycemia (Bhatia et al., 2019). Additionally, extracts of *Launaea taraxacifolia* and *Strychnos spinosa* leaves showed significant inhibitory effects on α-glucosidase in an uncompetitive mode, further supporting their role in managing diabetes (Adinortey et al., 2018). Ashwini et al. (2022) have studied and compared the herbal formulation and extracts of *Trigonella foenum graecum* and *Coriandrum sativum* seeds/fruits and the concoction showed significant α-amylase and α-glucosidase

inhibitory activity. The extracts exhibited competitive inhibition of α-amylase and non-competitive inhibition of α-glucosidase, showing potential for antidiabetic effects through enzyme inhibition. The plant extracts from *Stillingia lineata, Faujasiopsis flexuosa, Erythroxylum laurifolium, Elaeodendron orientale*, and *Antidesmam adagascariensis* were studied and they exhibited uncompetitive and mixed type of inhibition on α-amylase and α-glucosidase enzymes, indicating both competitive and non-competitive modes of inhibition (Picot et al 2014). The aqueous methanolic extract of *Ailanthus altissima* bark has also exhibited a competitive mode of enzyme inhibition against alpha-glucosidase, showing significant anti-diabetic activity in vitro (Ulhasan et al., 2023). No significant papers studying the mode of enzyme inhibition relating to our studied plant samples were found. The collective findings highlight the various mechanisms by which plant extracts can efficiently suppress crucial enzymes associated with the management of diabetes.

The ability to inhibit α-glucosidase and α-amylase by plant extracts from selected anti-diabetic/anti-hyperglycemic plants of the present research suggests that further anti-diabetic activities of these plants and additional mechanisms beyond α-glucosidase inhibition and α-amylase inhibition need to be studied to move forward with potential drug development.

Summary and Conclusions

These findings underscore the significant potential of ethnobotanical flora from Nagaland in the development of natural products possessing anti-diabetic properties. Upon investigating the inhibitory potential on α-amylase within extracts derived from assorted anti-diabetic flora, all extracts indicated a substantial degree of inhibition for α-amylase and α-glucosidase that is consistent with the reference compound Acarbose. It was noted that all species analyzed displayed anti-diabetic activity in vitro. *Bauhinia variegata, Abroma augustum, Euphorbia hirta, Passiflora edulis, Gynura crepidioides*,

and *Kalanchoe pinnata* demonstrated notable enzyme inhibition activities, suggesting their potential as anti-diabetic agents. The findings revealed competitive and non-competitive modes of inhibition, indicating different mechanisms of action among the plant extracts. Variations in IC_{50} values can be attributed to factors such as extraction methods, solvent polarity, and environmental conditions. Furthermore, it was noted that the inhibitory effects were markedly higher in ethanol and methanol extracts while exhibiting slightly reduced activity in pure water extracts. The plant materials presumably harbor natural compounds capable of inhibiting enzymatic activity; thus, further research is warranted to identify these bioactive compounds through comprehensive structural analysis and characterization methodologies. To properly assess these plant specimens as potential anti-diabetic and anti-hyperglycemic agents within living organisms, it is important to investigate them more deeply, while also discovering new inhibitors of α-amylase and α-glucosidase and clarifying their modes of action.

Chapter–6

Cytotoxicity Evaluation and *In Vitro* Cell Line Assays of Selected Plant Extracts

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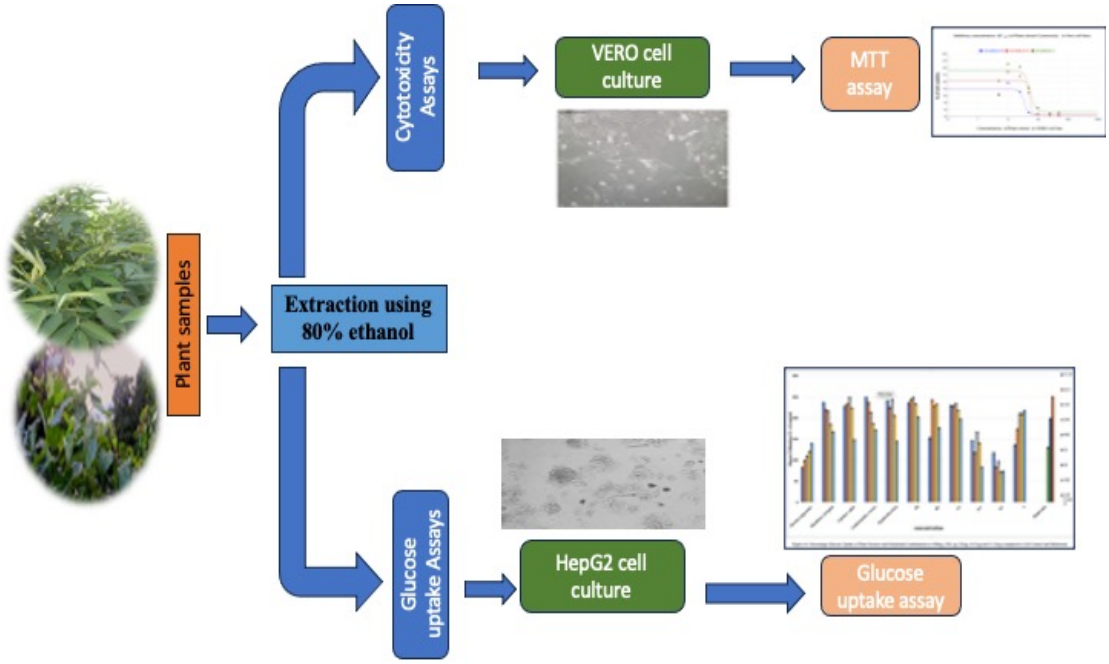
Graphical Summary



Introduction

Diabetes mellitus has emerged as one of the most prevalent chronic diseases in contemporary society, largely due to changes in lifestyle and the increasing prevalence of metabolic disorders (Antar et al., 2023). In the pursuit of innovative antidiabetic pharmacological agents, a significant number of researchers are exploring natural sources for potential remedies. Ethnobotanical methodologies, which are deeply entrenched in indigenous traditions, have yielded crucial insights into identifying flora possessing medicinal properties (Mekonnen et al., 2022).

The inhabitants of Nagaland, located in the North Eastern region of India, have historically depended on traditional herbal interventions for managing diabetes. Various tribes of Nagaland use several medicinal plants to treat diabetes such asChungtia tribe uses species like *Albizia lebbeck*, *Catharanthus roseus*, and *Zanthoxylum rhetsa* (Malewska, 2014; Deb and Sharma, 2021); while, the Phom tribe uses *Kalanchoe pinnata* and *Asparagus racemosus* (Jamir and Tsurho, 2016). Additionally, *Momordica balsamina* and *Discentra scandens* are known for their antidiabetic use among the Chang tribe (Jamir and Tsurho, 2017). Several other tribes, including the Angami and Chakhesang, also rely on various local plants like *Passiflora edulis* and *Solanum nigrum* for diabetes management (Chase and Singh, 2013; Bharali et al., 2017). This rich ethnobotanical knowledge not only highlights the cultural significance of these plants but also paves the way for scientific exploration into their potential therapeutic benefit.

Importance of Cell Line Studies in Cytotoxicity and Glucose Uptake

In modern pharmacological research, cell line studies play an essential role in bridging the gap between basic biochemical assays and clinical trials. These *in vitro* models allow researchers to gain insights into how plant extracts or bioactive compounds

function at the cellular level, providing vital preliminary data on both safety and efficacy before proceeding to animal or human studies.

One of the most critical aspects of assessing plant extracts for medicinal use, particularly in the context of antidiabetic treatments, is their potential cytotoxicity. Cytotoxicity assays are designed to evaluate whether a compound exerts harmful effects on healthy, non-cancerous cells (Ranjitka et al., 2021). This is crucial because even though a plant extract may show promise in inhibiting enzymes like α-amylase and α-glucosidase, it must also be confirmed as non-toxic to human cells at therapeutic doses. If an extract displays cytotoxic effects, it can limit its potential for safe use as a treatment. Thus, cytotoxicity studies serve as a safeguard, identifying any adverse effects early in the research process (Hussain et al., 2022). The MTT assay is a widely utilized method for assessing cytotoxicity. This colorimetric assay measures the metabolic activity of cells by converting yellow tetrazolium salt (MTT) into purple formazan crystals through mitochondrial enzymes. The amount of formazan produced is directly proportional to the number of viable cells, and this is quantified spectrophotometrically. By measuring cell viability, the MTT assay provides a reliable method for determining the concentration at which a plant extract becomes toxic to cells (Ghasemi et al., 2021). This helps in establishing a therapeutic window i.e. the range of concentrations where the plant extract is effective without being harmful.

For antidiabetic research, cytotoxicity studies are particularly significant. Diabetes treatments must be safe for long-term use, often requiring frequent or sustained administration (Kadan et al., 2013). If an extract exhibits cytotoxic effects, even at low levels, it could pose risks to patients, especially those with comorbidities or compromised immune systems. Therefore, evaluating the safety of plant extracts through cytotoxicity

assays is a foundational step before considering them as viable antidiabetic agents (Ala et al., 2013).

Equally important to safety is the efficacy of these plant extracts in managing blood glucose levels. Diabetes, particularly type 2 diabetes mellitus, is characterized by insulin resistance and impaired glucose uptake in peripheral tissues, including the liver, skeletal muscle, and adipose tissue (Antar et al., 2023). Glucose uptake assays are designed to assess how effectively a plant extract can promote glucose absorption at the cellular level. This is vital for determining whether the extract can influence glucose metabolism and, thus, mitigate hyperglycemia, which is a key feature of diabetes (Petersen and Shulman, 2018).

HepG2 cells, a human liver cancer cell line, are commonly used to study glucose uptake because they mimic many metabolic characteristics of human liver cells, including insulin sensitivity and glucose processing (Zang et al., 2004). The liver is a central organ in glucose homeostasis, regulating blood sugar levels by either storing glucose as glycogen or releasing it into the bloodstream. In type 2 diabetes, hepatic glucose uptake is often impaired, contributing to elevated blood sugar levels (Jiang et al., 2020). Therefore, HepG2 cells serve as a valuable *in vitro* model to study how plant extracts may improve glucose metabolism by enhancing cellular glucose absorption or modulating insulin sensitivity (Sefried et al., 2018). The glucose uptake assay typically involves exposing HepG2 cells to varying concentrations of plant extracts, after which the amount of glucose absorbed by the cells is measured. This can be done through enzymatic methods or colorimetric assays, where a decrease in glucose concentration in the culture medium indicates enhanced glucose uptake by the cells (Yang et al., 2010). These studies are crucial for identifying plant extracts that can stimulate glucose transporters like GLUT4, which facilitate glucose entry into cells, or activate other pathways involved in glucose

metabolism (Wang et al., 2011). Enhancing glucose uptake in liver cells is a promising mechanism for controlling hyperglycemia and improving insulin sensitivity, making these assays a key component in evaluating the antidiabetic potential of plant-based therapies (Aladejana et al., 2020).

Overall, cell line studies of cytotoxicity and glucose uptake are indispensable for the preclinical evaluation of plant extracts in antidiabetic research. Cytotoxicity assays ensure that these extracts do not harm healthy cells, while glucose uptake assays provide direct evidence of their ability to modulate glucose metabolism at the cellular level. Together, these studies offer a comprehensive assessment of the safety and efficacy of medicinal plants, bringing us one step closer to identifying effective, plant-based treatments for diabetes.

Rationale for Plant Selection

In this study, the selection of plant species was guided by a combination of ethnobotanical significance, preliminary phytochemical screenings, and enzyme inhibition assays targeting α-amylase and α-glucosidase. Out of the 15 medicinal plants initially studied, five were selected for further investigation based on their potent antidiabetic activity, favourable phytochemical profiles, and historical use in traditional medicine. These species: *Abroma augustum, Bauhinia variegata, Cajanus cajan, Catharanthus roseus*, and *Euphorbia hirta* were prioritized for *in vitro* cytotoxicity and glucose uptake studies for several reasons. Each of these plants has been traditionally used by indigenous tribes in Nagaland for managing diabetes or related metabolic disorders (Deb and Sharma, 2021). This long-standing usage offers a strong cultural and empirical foundation, suggesting that these plants have been effective in the management of hyperglycemia for generations. In addition, the phytochemical profiles of these plants revealed high concentrations of bioactive compounds, such as flavonoids, alkaloids,

phenolic acids, and terpenoids, which have shown potential in managing diabetes by modulating insulin sensitivity, enhancing glucose uptake, and inhibiting enzymes like α-amylase and α-glucosidase (Rahman et al., 2022). The enzyme inhibition assays conducted in earlier chapters further validated the anti-hyperglycemic potential of these species. The selected five species demonstrated the most potent enzyme inhibitory activity, indicating their effectiveness in reducing postprandial glucose levels, which is a key therapeutic target in diabetes management. This strong enzymatic inhibition aligns with the traditional use of these plants and offers a biochemical rationale for their further testing at the cellular level. By narrowing down to these five species, the study focuses on plants with both traditional backing and scientific validation from initial screening, ensuring that the most promising candidates undergo in-depth evaluation.

In addition to studying these plants individually, this research also explores the potential of polyherbal formulations. The decision to test combinations of plants was driven by the growing interest in the synergistic effects that polyherbal treatments can offer. Polyherbal formulations are commonly utilized in traditional medicine to enhance therapeutic efficacy and minimize toxicity (Parasuraman et al., 2014). By combining multiple plants, the bioactive compounds from different species may interact synergistically, producing enhanced therapeutic outcomes that target multiple biological pathways simultaneously (Vaou et al., 2022). This approach can lead to a more potent antidiabetic effect compared to using individual plants alone. Moreover, polyherbal formulations hold the potential to reduce the toxicity associated with individual extracts, thereby improving the overall safety profile of the treatment, which is critical for long-term use in chronic conditions like diabetes. Given that diabetes is a multifactorial disease, involving dysregulation of glucose metabolism, insulin resistance, oxidative stress, and inflammation, employing a polyherbal approach allows for a more holistic

strategy in addressing these complexities (Guan et al., 2024). The selected plants each offer distinct mechanisms of action, whether through enzyme inhibition, enhancement of glucose uptake, or antioxidant effects, thus providing a comprehensive strategy for diabetes management.

Thus, the objective of this study is to comprehensively evaluate the antidiabetic potential of selected medicinal plants from Nagaland, focusing specifically on *Abroma augustum, Bauhinia variegata, Cajanus cajan, Catharanthus roseus*, and *Euphorbia hirta*. The study seeks to assess the cytotoxicity of the individual plant extracts and their polyherbal formulations. By employing the MTT assay across various human cell lines, including VERO cells, the research aims to determine the safety profiles of these plant extracts. Furthermore, the study intends to investigate the glucose uptake capacity of these medicinal plants using HepG2 cells, a widely recognized human liver cancer cell line that closely resembles human hepatocytes. The overarching goal of this study is to contribute to the scientific understanding of the medicinal flora of Nagaland and its potential role in diabetes management. By substantiating the antidiabetic attributes of these selected plants through rigorous *in vitro* evaluations, the research aspires to pave the way for future investigations aimed at developing effective, plant-based therapeutic agents. Ultimately, the results derived from this investigation may yield significant contributions to the development of secure and effective polyherbal interventions for diabetes, leveraging the extensive ethnobotanical expertise and customary practices of the indigenous communities in Nagaland. This aim encapsulates the aspiration to close the gap between traditional medicinal systems and current scientific exploration, signifying the importance of integrating indigenous wisdom with modern research methodologies.

Materials and Methods

Plant Material

The plant material for this study was selected from five ethnomedicinal species known for their traditional use in diabetes management. The chosen species were ***Abroma augustum* (L.) L. f.**, ***Bauhinia variegata* L.**, ***Cajanus cajan* (L.) Millsp.**, ***Catharanthus roseus* (L.) G. Don**, and ***Euphorbia hirta* L.** These plants were collected from various regions of Nagaland, India, based on their established antidiabetic potential in local traditional medicine. Healthy plant parts, leaves and stems, were harvested, washed thoroughly with tap water, oven-dried, and ground into fine powder using a mechanical grinder. The dried plant material was stored in airtight containers at room temperature until further use. The rationale for selecting these five species stemmed from prior phytochemical analyses and enzyme inhibition assays conducted on fifteen antidiabetic plants, where these species exhibited the most potent α-amylase and α-glucosidase inhibition activities.

Polyherbal Formulations

To investigate the potential synergistic effects of combining different plant extracts, equal amounts of powdered plant material from the selected species were combined to create the following polyherbal formulations (1:1 ratio):

1. Formulation AB: *Abroma augustum* + *Bauhinia variegata*

2. Formulation BC: *Bauhinia variegata* + *Cajanus cajan*

3. Formulation CC: *Cajanus cajan* + *Catharanthus roseus*

4. Formulation EA: *Euphorbia hirta* + *Abroma augustum*

5. Formulation EC: *Euphorbia hirta* + *Cajanus cajan*

6. Formulation A: Combination of all five plant species

These polyherbal formulations along with individual plant extracts, were subjected to extraction and further tested for cytotoxicity and glucose uptake to explore their antidiabetic potential and safety.

Plant Extracts Preparation

The specific plant parts to be used for analysis were collected during the flowering seasons of all the plants under study, where the secondary metabolites are known to be present at the peak concentrations as per traditional knowledge. The collected samples were washed with tap water and then dried in a hot air oven at 50°C till the consistent weight of the samples was ensured. The dried plant parts were then ground separately into fine powder. For the preparation of the extract, 50 mg of powdered plant material from each plant species was mixed with 10 ml of 80% ethanol (v/v). This ethanol concentration was used uniformly for all individual plant extracts and polyherbal formulations. The mixtures were then incubated in a water bath for 1h at 60°C followed by centrifugation at 10000 rpm for 10 min. Post centrifugation, the supernatants were filtered using Whatman's filter paper No. 1 and the extract was stored at 4ºC till used for analysis purposes.

Cytotoxicity Assay

Cell culture

In the present investigation, the VERO cell line was used to assess the cytotoxicity of the plant extracts. VERO cells, derived from the kidney of the African green monkey, are commonly employed in cytotoxicity assessments due to their non-tumorigenic nature and relevance for predicting human cell responses (Ammerman et al., 2008). These cells are suitable for evaluating the potential toxic effects of plant extracts on normal, healthy cells, which is essential for ensuring the safety of therapeutic candidates.

MTT colorimetric assay

To evaluate the cytotoxic effects of the plant extracts, the MTT colorimetric assay was employed. This assay measures cell viability by assessing mitochondrial activity, as the mitochondrial enzymes reduce the MTT reagent (a yellow tetrazolium salt) to purple formazan crystals. The amount of formazan produced is directly proportional to the number of viable cells, allowing the quantification of cytotoxic effects. Following 24 h of cell culture, the VERO cells were exposed to various concentrations of the plant extracts (5 µg, 10 µg, 25 µg, 50 µg, 100 µg, 250 µg and 500 µg per well). After an incubation period of 18-20 h, the bioactivity of the extracts was assessed using the MTT assay. In this method, 100µL of MTT reagent was added to each well, and the cells were incubated for 4 h in a CO_2 incubator. After incubation, the supernatant was discarded, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The absorbance of each well was measured at 570nm using a microplate reader, with higher absorbance values indicating greater cell viability. This assay was performed in triplicate to ensure the reliability of the results. The IC_{50} values (the concentration of extract required to inhibit 50% of cell viability) were calculated using Microsoft Excel, providing a quantitative measure of the cytotoxic effects of each extract.

The Effect of Plant Extracts on Glucose Uptake Using HepG2 Cells

Cell culture

The human hepatoma cell line (HepG2) was procured from the National Centre for Cell Sciences (NCCS), Pune. The HepG2 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified incubator at 37°C with 5% CO_2. The cells at 80–90% confluence were split and then used for further experiments.

Cell viability assay

Inhibition of cell growth was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay for different extracts with different concentrations (5–500 µg/mL) (Akinrinde et al., 2018). The MTT (0.5 mg/mL) was added to the appropriate wells of each plate, which were then incubated at 37°C. HepG2 cells (5*104 cells/mL) were seeded in 96 well plates and incubated at 37°C for 24 h to grow. Further, cells were treated with different concentrations of the test compound for 24 h. After the treatment, 10 µl MTT dye (5 mg/ml) was added to each well and incubated for 3 h at 37°C to obtain coloured formazan products. At the end of incubation, the medium was aspirated and 100 µg/mL of DMSO was added to solubilize the formazan crystals formed in the cells. The absorbance of the wells was read at 540 nm and percent viability was calculated.

Glucose uptake assay

HepG2 cells that were seeded in culture plates at 5*105 cells/mL were allowed to adhere and grow at 37°C for 24 h in an incubator supplemented with 5% CO_2. Before assaying, cells were pre-incubated at 37°C for 48 h with different concentrations (31.25–500 µg/mL). Thereafter, the spent culture medium was aspirated and replaced with 25 µL of the incubation medium (DMEM that had been diluted with 8 mM glucose, 0.1% Bovine serum albumin (BSA), and Phosphate-Buffered Saline (PBS) before it was incubated at 37°C for 3 h. Thereafter, 10 µL of the incubation medium was removed from all the wells and transferred into a new 96 well plate. The concentration of glucose in the medium was determined using the glucose assay kit (Sigma GAGO20, Johannesburg, South Africa) according to the manufacturer's instructions. The absorbance was then measured at 540 nm. The glucose used by the cells was calculated by subtracting the cell-containing wells from the cell-free wells (Odeyemi et al., 2019).

Statistical Analysis

All the experiments were executed in triplicate and repeated thrice, and deviations were calculated as the standard deviation of the mean.

Results

Cytotoxicity Assay

The cytotoxicity of both individual plant extracts and polyherbal formulations was evaluated using the MTT assay, and the IC_{50} values (the concentration of extract required to inhibit 50% of cell viability) were calculated (Table 6.1).

Among the individual plant species, the cytotoxicity results varied significantly. *Bauhinia variegata* exhibited the most pronounced cytotoxicity, with an IC_{50} value of 21.506 mg/mL, indicating that this extract had the strongest inhibitory effect on cell viability. On the other hand, *Cajanus cajan* demonstrated the least cytotoxicity, with an IC_{50} of 139.98 mg/mL, implying that this plant extract was the safest in terms of its effect on VERO cells. The IC_{50} values for the other three plants were as follows: *Catharanthus roseus* (54.735 mg/mL), *Euphorbia hirta* (71.293 mg/mL), and *Abroma augustum* (115.79 mg/mL). These results suggest that while some plant extracts, such as *Bauhinia variegata* and *Catharanthus roseus*, exhibit significant cytotoxic effects, others like *Cajanus cajan* and *Abroma augustum* show much lower toxicity, making them potential candidates for safer therapeutic applications.

Table 6.1: Cytotoxic Activity (IC_{50}) and Cell Viability of Selected Plant Species and Polyherbal formulations at 500 µg/mL concentration

S.No	Sample species	% Cell viability as compared with control (at 500 µg of sample conc.)	Cytotoxic activity (IC_{50}) (mg/mL)
1	*Abroma augustum* (L.) L.f.	26.67	115.79
2	*Bauhinia variegata* L.	40.00	21.506
3	*Cajanus cajan* (L.) Millsp.	47.84	139.98
4	*Catharanthus roseus* (L.) G. Don	29.22	54.735
5	*Euphorbia hirta* L.	39.41	71.293
6	**AB** (*Abroma augustum* + *Bauhinia variegata)*	51.18	46.828
7	**BC** (*Bauhinia variegata* + *Cajanus cajan)*	34.51	44.978
8	**CC** (*Cajanus cajan* + *Catharanthus roseus)*	32.94	47.054
9	**EA** (*Euphorbia hirta* + *Abroma augustum)*	34.90	34.803
10	**EC** (*Euphorbia hirta* + *Cajanus cajan)*	37.45	52.96
11	**A** (combination of all five plant species)	47.45	54.423

The cytotoxicity results for the polyherbal formulations revealed different trends compared to the individual extracts. The combination **EA** (*Euphorbia hirta* + *Abroma augustum*) displayed the lowest IC_{50} value among the formulations, with 34.803 mg/mL, indicating a higher cytotoxic effect than either plant extract alone. This suggests that the combination of these two plants may have synergistic effects, enhancing their cytotoxicity. In contrast, the **AB** formulation (*Abroma augustum* + *Bauhinia variegata*) exhibited an IC_{50} of 46.828 mg/mL, and the combination of all five plants (**A**) showed a moderate cytotoxicity with an IC_{50} of 54.423 mg/mL.

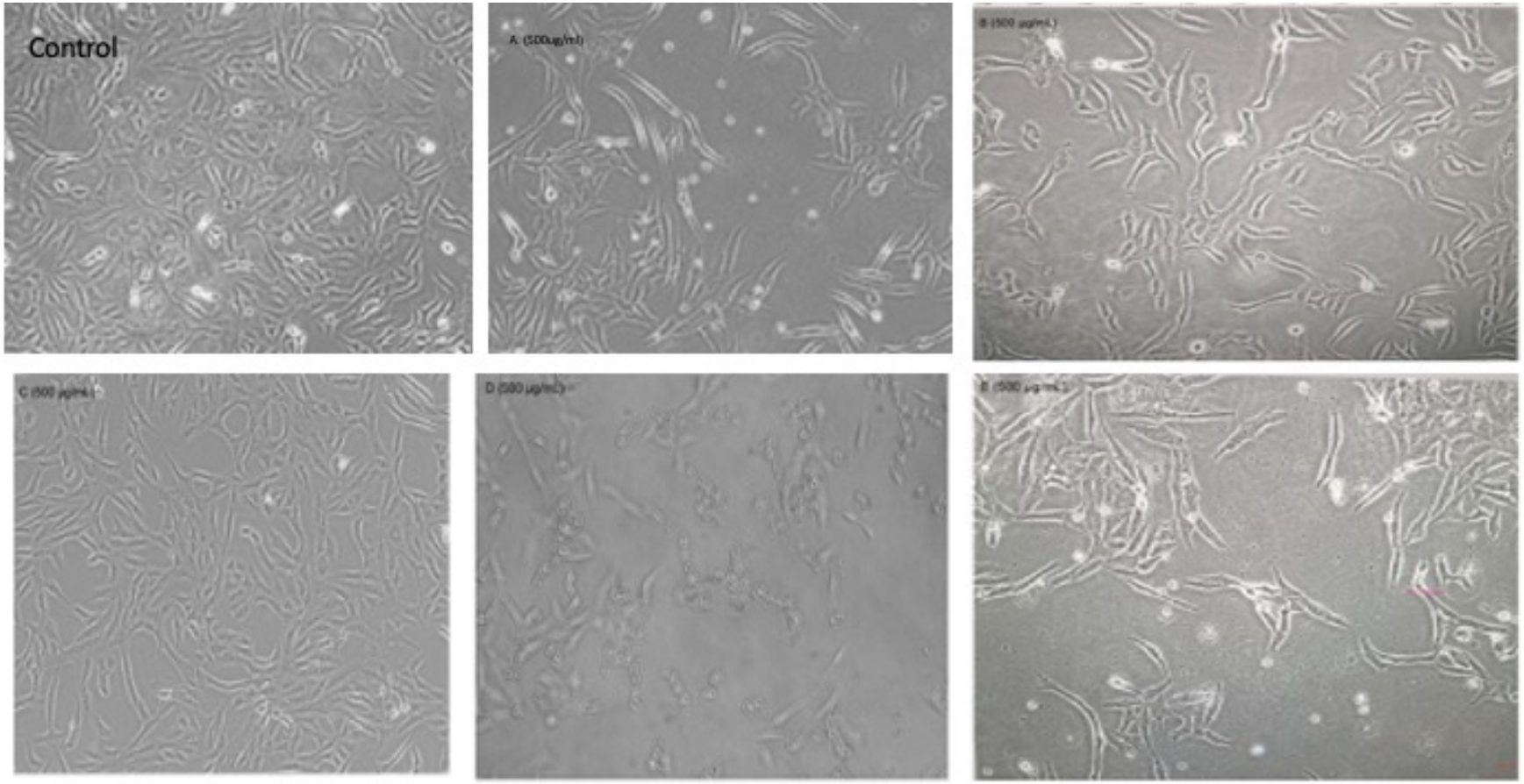


Figure 6.1: Morphological analysis of VERO cells treated with 500µg/ml sample. the *Abroma augustum* (A), *Bauhinia variegata* (B), *Cajanus cajan* (C), *Catharanthus roseus* (D) and *Euphorbia hirta* (E) extract.

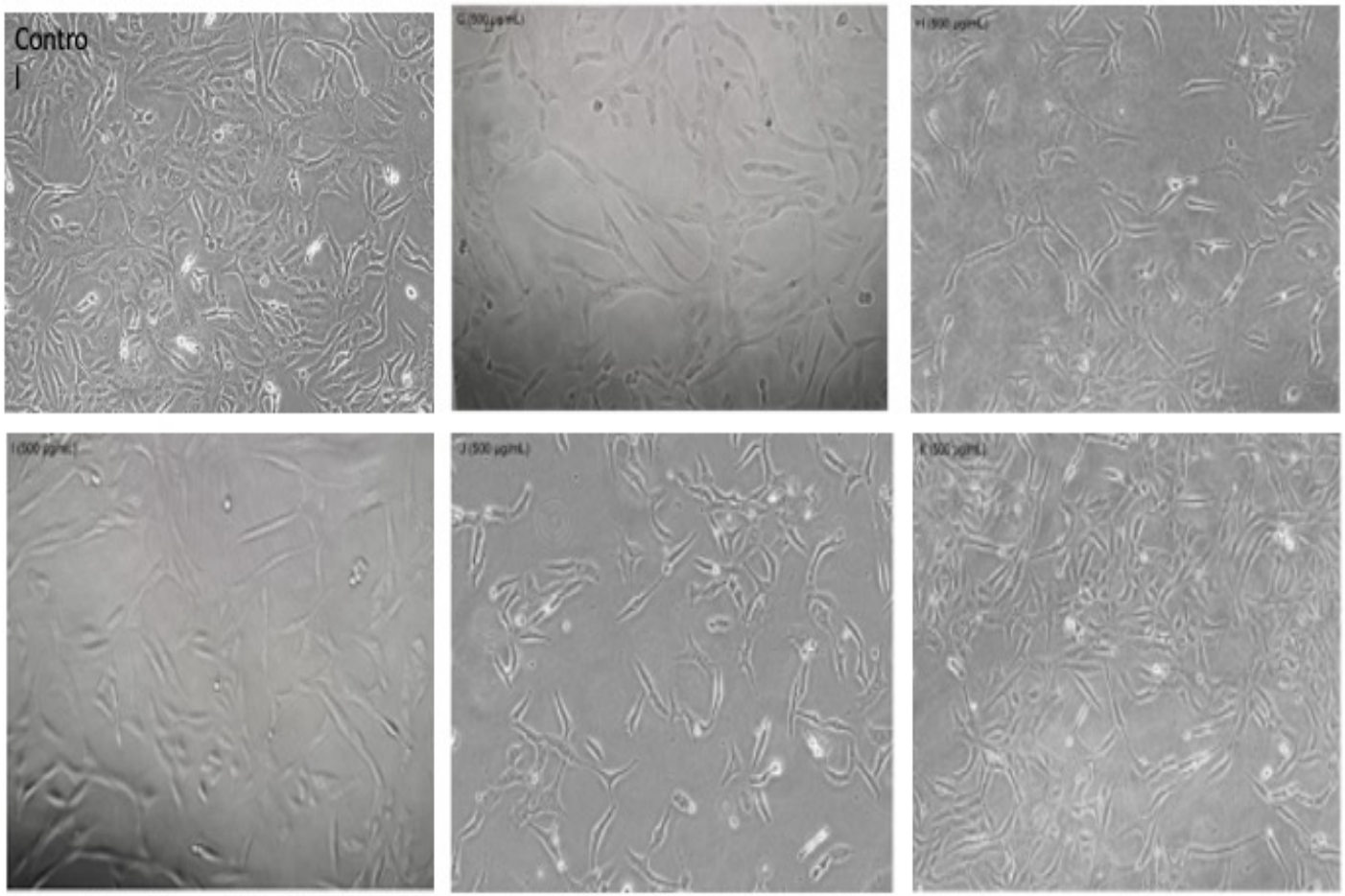
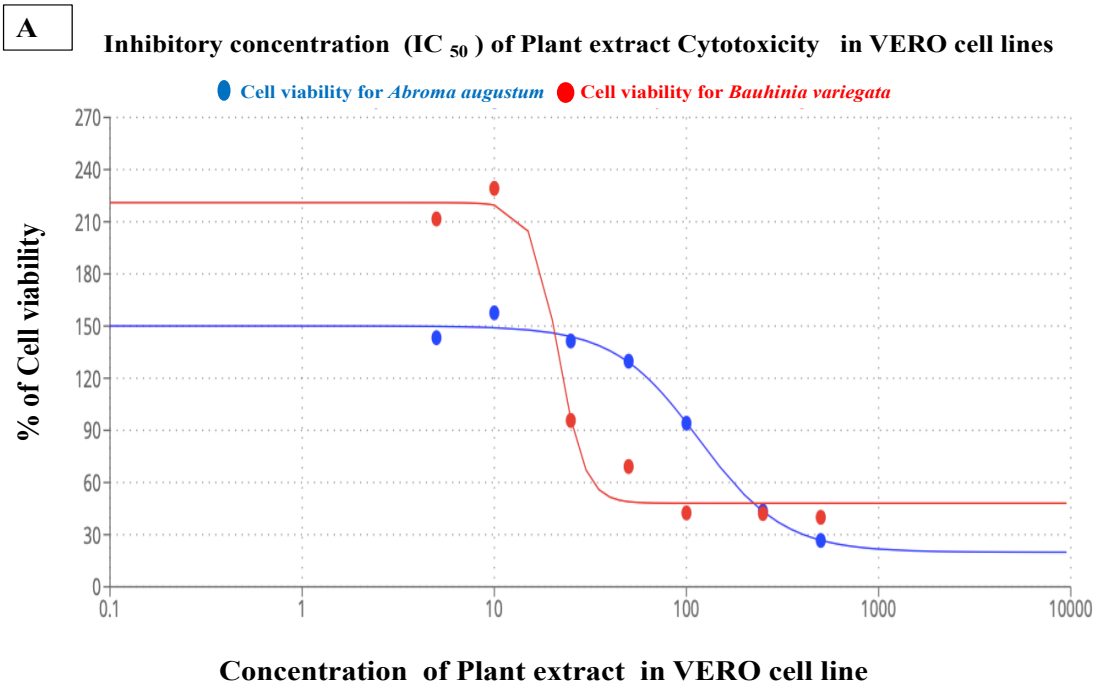
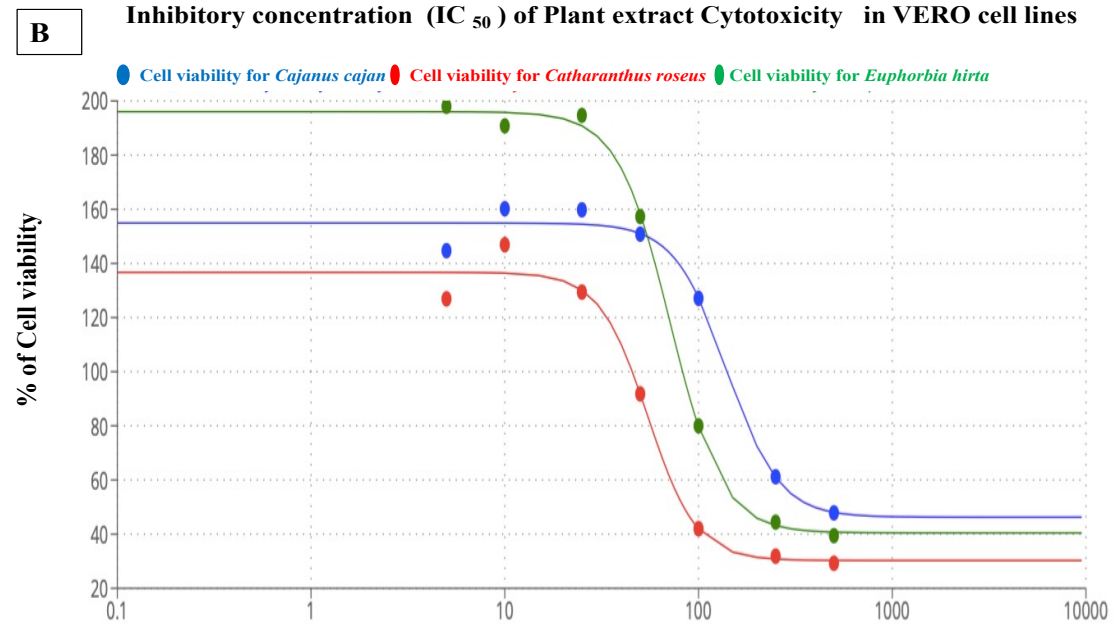


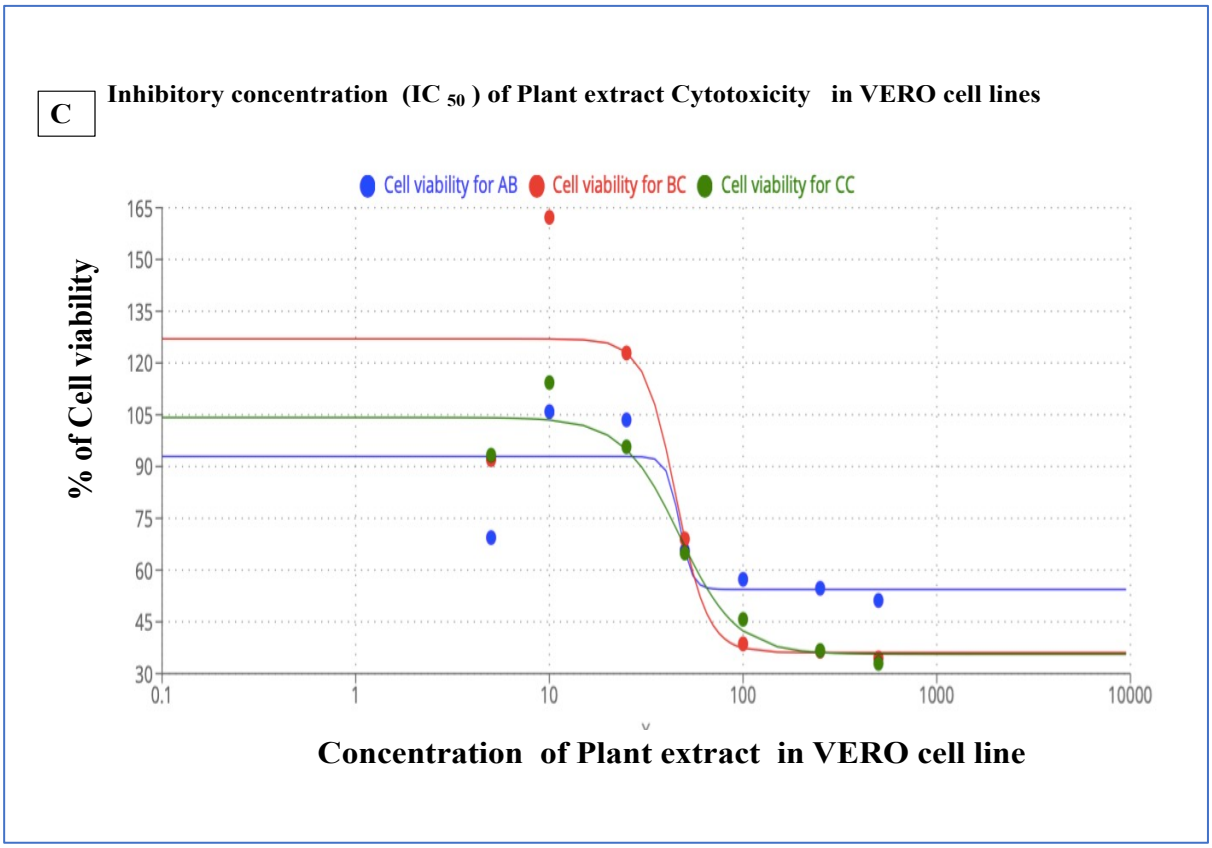
Figure 6.2: Morphological analysis of VERO cells treated with the *Bauhinia variegata+Cajanus cajan* (G), *Cajanus cajan+Catharanthus roseus* (H), *Euphorbia hirta+Abroma augustum* (I), *Euphorbia hirta+ Cajanus cajan* (J) and All the five sample (E) extract.

A


Inhibitory concentration (IC 50) of Plant extract Cytotoxicity in VERO cell lines
Cell viability for Abroma augustum
Cell viability for Bauhinia variegata
% of Cell viability
270
240
210
180
150
120
90
60
30
0
0.1
1
10
100
1000
10000
Concentration of Plant extract in VERO cell line

B


Inhibitory concentration (IC 50) of Plant extract Cytotoxicity in VERO cell lines
Cell viability for Cajanus cajan
Cell viability for Catharanthus roseus
Cell viability for Euphorbia hirta
% of Cell viability
200
180
160
140
120
100
80
60
40
20
0.1
1
10
100
1000
10000
Concentration of Plant extract in VERO cell line



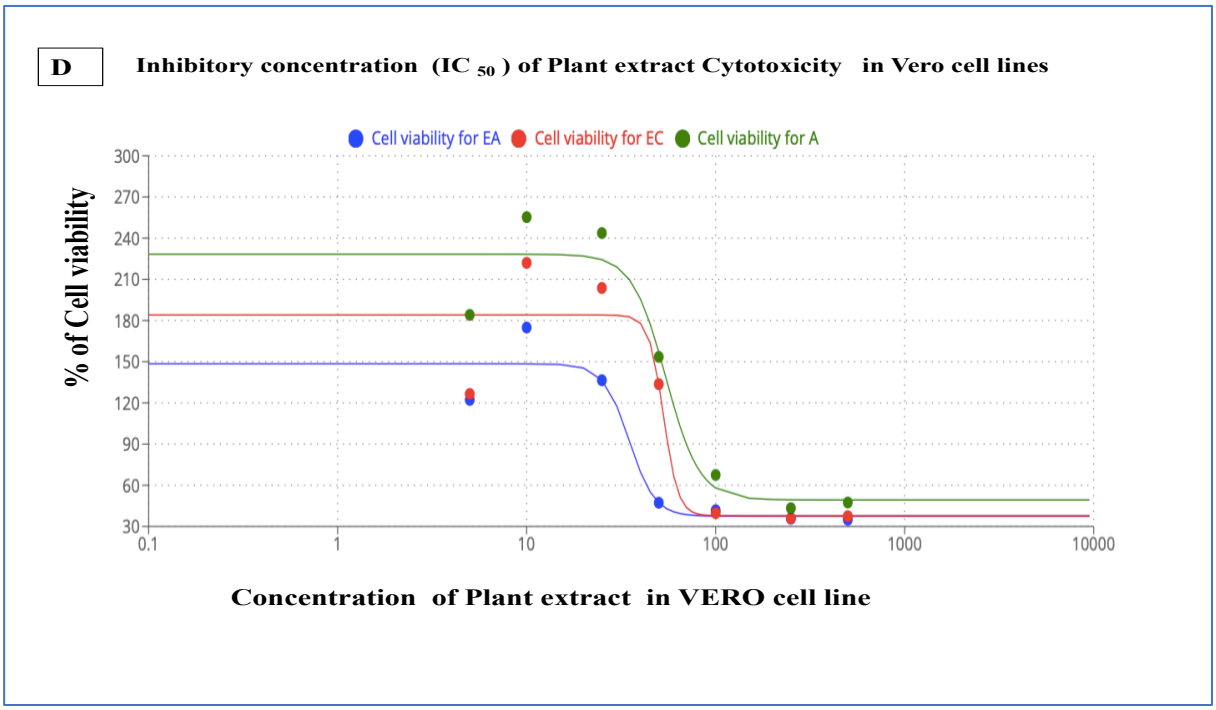


Figure 6.3: Inhibitory concentration IC_{50} of **A)** *Abroma augustum* and *Bauhinia variegata,* **B)** *Cajanus cajan, Catharanthus roseus* and *Euphorbia hirta,* **C)** Formulation **AB**, **BC**, **CC** and **D)** Formulation **EA, EC, A.**

The **A** formulation, which combined all five species, exhibited moderate cytotoxicity with an IC_{50} of 54.423 mg/mL. This value is higher than the individual cytotoxicity of *Catharanthus roseus* but lower than *Abroma augustum* and *Bauhinia variegata*, suggesting a balanced cytotoxic effect when all plants are used together. The result for formulation **A** indicates that the combination of all five plants may provide a more moderate cytotoxic profile, potentially reducing the risk of toxicity while maintaining efficacy across a broad range of plant constituents. Interestingly, the **AB** combination had higher cell viability (51.18%) compared to the individual extracts of *Abroma augustum* (26.67%) and *Bauhinia variegata* (40%), suggesting that combining these two plants may mitigate their individual cytotoxic effects. The combination **BC** (*Bauhinia variegata* + *Cajanus cajan*) also exhibited moderate cytotoxicity with an IC_{50} of 44.978 mg/mL. The **A** combination of all five plants also exhibited relatively high cell viability (47.45%) at 500 µg/mL, similar to the **AB** formulation. These findings highlight the potential advantage of polyherbal formulations in reducing cytotoxicity and improving safety profiles, offering a more favorable balance between efficacy and toxicity.

The cytotoxicity results demonstrate that individual extracts like *Bauhinia variegata* and *Catharanthus roseus* possess strong cytotoxic effects, which may raise concerns regarding their safety at higher concentrations. On the other hand, *Cajanus cajan* stands out for its low cytotoxicity, making it a safer candidate for further development. The polyherbal formulations, particularly **AB** and **A**, provide promising therapeutic options, as they exhibit lower cytotoxicity and higher cell viability compared to the more toxic individual extracts. The **EA** formulation, however, presents a potential synergistic cytotoxic effect that warrants caution in its therapeutic application. These results suggest that polyherbal formulations can alter the cytotoxic effects of individual

plants, either enhancing or mitigating their toxicity, and highlight the importance of careful combination strategies when developing plant-based treatments for diabetes or other therapeutic applications.

The Effect of Plant Extracts on Glucose Uptake Using HepG2 Cells

Cell Viability

HepG2 cells are widely used for biochemical studies as a cell culture model of human hepatocytes. Thus, this cell line has been extensively used for glucose uptake assay in order to evaluate antidiabetic properties (Huang et al., 2015).

HepG2 cell viability was dose-dependent after the treatment with extracts for 24 h of incubation. As the concentration increased, the inhibition of HepG2 cell proliferation increased significantly, especially at 250 and 500 µg/mL. Moreover, at lower concentrations, cell viability is more than or equal to 100 %, which indicates that all extracts are not cytotoxic.

Glucose Uptake Assay

The present study attempts to investigate the mechanisms of anti-diabetic actions of different extracts in *in vitro* assays. Our observations of increased glucose uptake in HepG2 cells could be considered relevant since the extract did not exhibit any potential toxicity. Glucose uptake was significantly raised compared to the cell control without treatment. The positive control, Metformin, however, produced slightly better stimulation, up to 250.83 % glucose uptake, compared to the untreated control. As shown in Table 6.2, the combination of all five plants (sample A) exhibited the highest glucose uptake (217.19%), significantly higher than the cell control (100%) and even higher than Metformin at 50 µg/mL (198.75%).

This suggests that polyherbal formulations can exhibit synergistic potential, where the combined effects of multiple plants result in greater efficacy than individual extracts. The enhanced glucose uptake in these combinations indicates that the bioactive compounds from different plants interact, amplifying their hypoglycemic properties.

Table 6.2: Glucose concentration and percentage glucose uptake of plant extracts and polyherbal combinations at 500 µg compared to Cell Control and Metformin

S.no.	**Samples**	**Glucose concentration (µg/mL) (at 500 µg of Sample conc.)**	**% glucose uptake compared to cell control (at 500 µg of sample Conc.)**
1	*Abroma augustum* (L.) L.f.	107.865	139.065±0.0035
2	*Bauhinia variegata* L.	129.749	167.278±0.0247
3	*Cajanus cajan* (L.) Millsp.	114.599	147.746±0.0134
4	*Catharanthus roseus* (L.) G. Don	133.116	171.618±0.0289
5	*Euphorbia hirta* L.	112.074	144.490±0.0240
6	**AB** (*Abroma augustum* + *Bauhinia variegata)*	156.683	202.002±0.0261
7	**BC** (*Bauhinia variegata* + *Cajanus cajan)*	136.483	175.959±0.0049
8	**CC** (*Cajanus cajan* + *Catharanthus roseus)*	153.316	197.662±0.0247
9	**EA** (*Euphorbia hirta* + *Abroma augustum)*	64.939	83.723±0.0212
10	**EC (***Euphorbia hirta* + *Cajanus cajan)*	57.365	73.957±0.0106
11	**A**(combination of all five plant species)	168.466	217.194± 0.0021
12	**Metformin**	194.558 (at 100 µg Conc.**)**	250.833±0.0169 (at 100 µg Conc.**)**
13	**Cell control**	77.565	100.00

Among individual plant extracts, *Catharanthus roseus* (171.61%) and *Bauhinia variegata* (167.27%) demonstrated the highest glucose uptake compared to the cell control. These results show the potential of these species as hypoglycemic agents, likely

due to the presence of bioactive compounds that improve glucose absorption. In contrast, species like *Euphorbia hirta* (144.49%) and *Abroma augustum* (139.06%) showed relatively lower glucose uptake, indicating that while they possess some hypoglycemic properties, they may be more effective in combination with other plants.Polyherbal combinations such as **AB** (*Abroma augustum* + *Bauhinia variegata*) and **CC** (*Cajanus cajan* + *Catharanthus roseus*) demonstrated enhanced glucose uptake (202.00% and 197.66%, respectively), further highlighting the potential synergistic effects. However, combinations like **EA** (*Euphorbia hirta* + *Abroma augustum*) and **EC** (*Euphorbia hirta* + *Cajanus cajan*) exhibited significantly lower glucose uptake (83.72% and 73.95%, respectively), as seen in Figure 6.4, suggesting that not all formulations are equally effective. This points to the need for optimization of plant combinations to fully harness their hypoglycemic potential.

Further analysis, illustrated in Figure 6.4, shows the glucose uptake of these plant extracts and polyherbal combinations across a range of concentrations (500 µg, 250 µg, 125 µg, 62.5µg, and 31.25µg). The trend in Figure 6.4 reveals that while all combinations demonstrated a dose-dependent increase in glucose uptake, the most effective combinations consistently outperformed others at higher concentrations, solidifying the concentration-dependent effects of these formulations.

The comparison with Metformin, a standard hypoglycemic drug, provides a benchmark for assessing the efficacy of the plant extracts. While, Metformin at 100 µg/mL showed the highest glucose uptake (250.83%), the plant combinations still demonstrated significant activity, especially at higher concentrations. This suggests that these extracts could be used as complementary therapies alongside standard hypoglycemic drugs, potentially reducing drug doses and minimizing side effects. The study revealed that certain polyherbal formulations exhibit strong anti-hyperglycemic

activity, particularly the combination of all five plants and pairs like AB and CC. These findings highlight the synergistic potential of plant extracts in managing glucose levels and suggest further exploration of their bioactive compounds. However, further studies, including dose-dependent *in vitro* and *in vivo* analyses, are necessary to confirm their efficacy as hypoglycemic agents and optimize their use for diabetes management.

The results from the cytotoxicity study and glucose uptake assay provide critical insights into the therapeutic potential and safety profiles of the selected plant extracts. *Bauhinia variegata* exhibited the highest cytotoxicity (IC_{50} = 21.506mg/mL), indicating strong inhibitory effects on cell viability, yet also showed significant glucose uptake, suggesting effectiveness at the cost of safety. Conversely, *Cajanus cajan* emerged as a safer option with the lowest cytotoxicity (IC_{50} = 139.98 mg/mL) and moderate glucose uptake (47.84%), marking it as a promising candidate for diabetes management.

In polyherbal combinations, **EA** (*Euphorbia hirta* + *Abroma augustum*) displayed increased cytotoxicity (IC_{50} = 34.803mg/mL), highlighting a potential synergistic interaction that could enhance glucose uptake but may compromise safety. Meanwhile, combinations like **AB** (51.18% cell viability) and **A** (47.45% cell viability) exhibited higher cell viability than some individual extracts, indicating protective effects when used together. This suggests that certain formulations may mitigate toxicity while preserving or enhancing glucose uptake.

The relationship between cytotoxicity and glucose uptake emphasizes that species with effective glucose-lowering properties do not always correlate with lower toxicity. For instance, while *Catharanthus roseus* shows good glucose uptake, it also has moderate cytotoxicity, necessitating careful evaluation in therapeutic applications. Overall, while individual extracts like *Bauhinia variegata* may be effective antihyperglycemic agents, their higher cytotoxicity raises safety concerns. In contrast, *Cajanus cajan* presents a

favorable safety profile, and polyherbal formulations like **AB** and **A** may offer effective therapeutic options with lower toxicity, highlighting the importance of combination therapy in developing safer and more effective hypoglycemic agents and for the management of diabetes. Further studies on the active compounds and their mechanisms are essential for optimizing these plant extract's use in clinical applications.

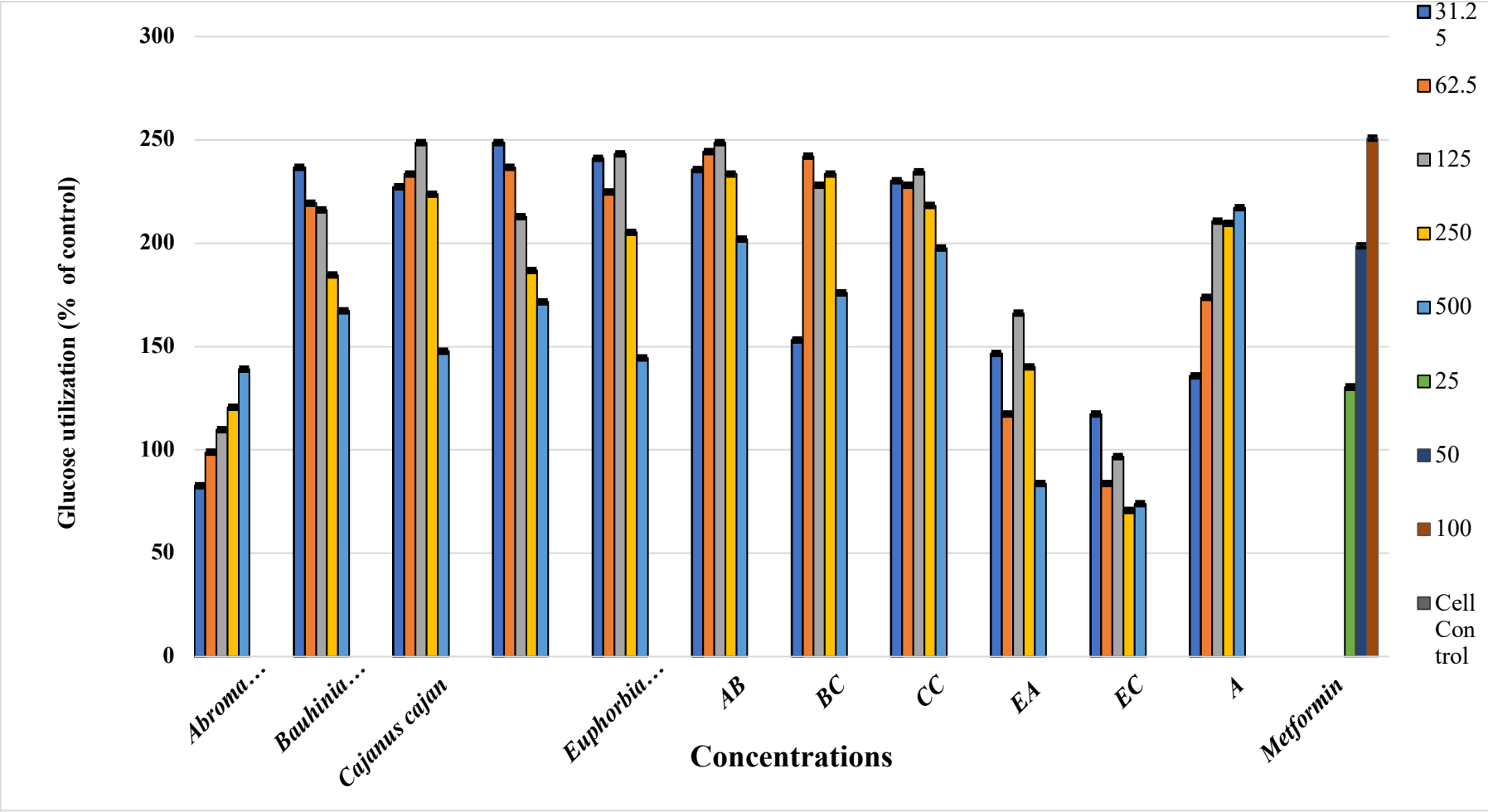


Figure 6.4: Percentage Glucose Uptake of Plant Extracts and Polyherbal Combinations at 500µg, 250, µg 125µg, 62.5µg and 31.25µg compared to Cell Control and Metformin.

Discussion

The current investigation offers significant insights into the cytotoxic properties and anti-hyperglycemic capabilities of selected medicinal flora and their polyherbal formulations. The results emphasize the critical necessity of assessing both the therapeutic efficacy and safety when evaluating plant extracts for medicinal use, particularly in the context of diabetes management.

In the present study, *Bauhinia variegata* demonstrated the highest level of cytotoxicity among the individual botanical species, presenting an IC_{50} value of 21.506 mg/mL, which indicates a considerable inhibitory effect on cellular viability. This observation is consistent with prior findings by Rajkapoor et al. (2003), which illustrated that *Bauhinia variegata* extract exhibited pronounced cytotoxicity against *Ehrlich ascites* carcinoma cells, with an IC_{50} of 625 mg/mL, thereby suggesting its potential utility as an anticancer therapeutic. Likewise, *Bauhinia variegata* was recorded to significantly impede cell viability across various neoplastic cell lines, as demonstrated by Arnould et al. (1990). These results illuminate the dualistic nature of *Bauhinia variegata*, unveiling its therapeutic promise while concurrently showing the imperative for meticulous safety assessments in non-cancerous applications due to its cytotoxic properties. Conversely, *Cajanus cajan* exhibited the lowest cytotoxic profile, with an IC_{50} of 139.98 mg/mL, signifying its potential as a safer alternative among the species evaluated. This minimal cytotoxicity positions *Cajanus cajan* as a promising candidate for combination therapies aimed at diabetes, where safety considerations are of utmost importance. Its safety and therapeutic promise have been substantiated by numerous investigations. For example, Oyewole et al. (2010) reported that methanolic extracts of *Cajanus cajan* leaves possess significant medicinal attributes, including antimicrobial properties, without demonstrating appreciable toxicity in animal models. Nonetheless, *Cajanus cajan* has shown

vulnerability to arsenic exposure, exhibiting a 50% reduction in growth at 0.93 mgL^{1}, thus accentuating the influence of environmental factors on its safety profile. These findings imply that *Cajanus cajan* merits further investigation to comprehensively elucidate its cytotoxicity and efficacy under varying conditions.

Our investigation revealed that *Abroma augustum* resulted in a cell viability of merely 26.67% when assessed at a concentration of 500 µg/mL, corresponding to an IC_{50} of 115.79 mg/mL. This marked reduction in cell viability signifies a robust inhibitory effect, which aligns with the findings of Sujaye et al. (2023), who documented an IC_{50} of 150 µg/mL for *Abroma augustum* extract in a brine shrimp lethality assay. In a similar vein, the crude extract of *Catharanthus roseus* demonstrated substantial cytotoxicity, with an IC_{50} value of 54.735 mg/mL in this study. This is consistent with earlier research conducted by Abd Wahab et al. (2020), which reported that *Catharanthus roseus* exhibited cytotoxic effects characterized by a IC_{50} of 0.5 mg/mL. Despite this, lower concentrations of *Catharanthus roseus* have been shown to possess non-cytotoxic characteristics, indicating that dosage is a critical determinant of its safety and therapeutic applicability. Investigations by Pham et al. (2018) revealed that the n-butanol fraction of *Catharanthus roseus* exhibited strong cytotoxic activity against malignant cells, with IC_{50} values ranging from 5.2-21.0 µg/mL. Furthermore, Kumar et al. (2022) indicated that extracts and compounds derived from *Catharanthus roseus* can be deemed safe within specific dosage parameters, beyond which cytotoxicity may escalate. These findings reinforce the necessity for further research to ascertain safe dosage thresholds for *Catharanthus roseus*, particularly in non-cancerous scenarios.

The polyherbal formulations exhibited a noteworthy interplay between cytotoxicity and glucose absorption. The combination **EA** (*Euphorbia hirta* + *Abroma augustum*) manifested the lowest IC_{50} value (34.803 mg/mL), signifying a synergistic augmentation

of cytotoxic effects. In their 2021 study, Mohan et al. pointed out that polyherbal formulations possess the ability to enhance therapeutic and toxicological outcomes because of the interplay between bioactive elements from diverse plant sources. The heightened cytotoxicity observed in the **EA** combination indicates that while the therapeutic efficacy of polyherbal formulations may be amplified, the concomitant risks necessitate meticulous evaluation to avoid adverse outcomes.

Conversely, combinations such as **AB** (*Abroma augustum* + *Bauhinia variegata*) and **A** (a composite of all five species) exhibited greater cell viability (51.18% and 47.45%, respectively) in comparison to individual plant extracts, implying that certain polyherbal combinations may mitigate cytotoxicity. This protective phenomenon may stem from the equilibrium between cytotoxic agents and those that confer a protective function, as substantiated in other polyherbal investigations.

In glucose uptake assays, the polyherbal combination **A** demonstrated the highest glucose absorption (217.19%), exceeding that of Metformin (198.75%) at a concentration of 50 µg/mL. This notable enhancement in glucose uptake can be ascribed to the synergistic interplay of bioactive compounds present within the combination. Sabu and Kuttan (2002) observed that polyherbal mixtures frequently exhibit superior hypoglycemic effects relative to individual extracts, and similar observations were made by Salehi et al. (2019), who identified improved glucose metabolism upon the integration of multiple antidiabetic botanical species. Among the individual extracts, *Catharanthus roseus* and *Bauhinia variegata* demonstrated substantial glucose uptake (171.61% and 167.27%, respectively), thereby affirming their viability as antihyperglycemic agents. Kumar et al. (2022) attributed the glucose-lowering properties of *Catharanthus roseus* to compounds such as vindoline and vinblastine, which enhance both insulin secretion and sensitivity. Similarly, *Bauhinia variegata* has exhibited considerable

glucose-lowering activity, likely attributable to its capacity to inhibit enzymes responsible for carbohydrate digestion, as articulated by Rajkapoor et al. (2003).

The combinations of **AB** (202.00%) and **CC** (*Cajanus cajan* + *Catharanthus roseus*) (197.66%) further corroborate the hypothesis that the amalgamation of plant extracts can augment glucose uptake through synergistic interactions. *Cajanus cajan*, as elucidated by Oyewole et al. (2010), possesses a commendable safety profile and demonstrates substantial medicinal properties, thereby rendering it an optimal candidate for inclusion in polyherbal formulations. The heightened glucose uptake observed within the **CC** combination insinuates that the bioactive constituents present in *Cajanus cajan* may interact with those in *Catharanthus roseus* to elicit a more potent therapeutic effect than that afforded by either plant in isolation. In contrast, combinations such as **EA** (83.72%) and **EC** (*Euphorbia hirta* + *Cajanus cajan*) (73.95%) exhibited diminished glucose uptake, thereby emphasizing that not all polyherbal formulations yield equivalent efficacy. Investigations conducted by Vaou et al. (2021) have indicated that certain plant combinations may manifest antagonistic effects, wherein one extract mitigates the therapeutic efficacy of another. This signifies the imperative for meticulous optimization of plant combinations to realize the intended therapeutic objectives.

The relationship between cytotoxicity and glucose uptake provides additional insights into the therapeutic potential of the selected plant extracts and their combinations. Some formulations with lower cytotoxicity, such as *Cajanus cajan* and **AB**, demonstrated considerable glucose uptake, suggesting that they may activate glucose transport mechanisms without compromising cell viability. This could be attributed to specific phytochemicals within these plants, such as flavonoids and terpenoids, which are known to enhance glucose uptake by upregulating glucose transport proteins like GLUT4 or through insulin-sensitizing effects (Sayem et al., 2018). Conversely, some extracts with

higher cytotoxicity, such as *Bauhinia variegata* and *Catharanthus roseus*, also exhibited substantial glucose uptake. This suggests that their cytotoxic compounds, which may include alkaloids and phenolics, could influence glucose metabolism independently of their effects on cell viability (Shehadeh et al., 2021). Such compounds may have dual roles, simultaneously affecting glucose transport and cellular health. This dual activity is typical of certain phytochemicals, which can be cytotoxic at high doses while retaining therapeutic benefits at lower concentrations. For instance, compounds that affect cell cycle progression might reduce cell viability but can also enhance glucose uptake by acting on metabolic pathways (Kumar et al., 2022). Interestingly, the **EA** combination (*Euphorbia hirta* + *Abroma augustum*) showed high cytotoxicity and only moderate glucose uptake. This suggests that while the combination enhances cytotoxicity, possibly through synergistic interactions, it may lack specific compounds that robustly enhance glucose uptake. This highlights the importance of not relying solely on cytotoxicity profiles to assess a formulation's antidiabetic potential. Ideally, a balanced combination of compounds that optimizes glucose uptake with minimal cytotoxicity is most desirable for developing safe and effective antidiabetic treatments (Etsassala et al., 2020). The **A** combination displayed moderate cytotoxicity and the highest glucose uptake, suggesting that the complex interplay of bioactive compounds may contribute to a balanced therapeutic profile. The moderate cytotoxicity in this formulation could result from protective phytochemicals that counteract the effects of more toxic compounds, allowing enhanced glucose uptake without substantially compromising cell viability (Etsassala et al., 2020). This points to the potential of polyherbal formulations in providing effective glucose uptake enhancement with minimized safety concerns.

Overall, these findings emphasize that the relationship between cytotoxicity and glucose uptake is not strictly linear. Formulations with moderate cytotoxicity, such

as **AB** and **A**, exhibit promising glucose uptake potential while maintaining acceptable safety profiles, suggesting that they could be viable candidates for further development as antidiabetic agents. Further research should investigate the bioactive compounds responsible for these effects, aiming to optimize formulations that enhance glucose uptake while minimizing cytotoxicity.The results of this study highlight the therapeutic potential of selected medicinal plants and their polyherbal combinations for diabetes management. The findings suggest that some polyherbal formulations can offer enhanced efficacy with reduced cytotoxicity, showing the importance of synergistic interactions in plant-based therapies.

Conclusions

This study provides compelling evidence for the cytotoxicity and antihyperglycemic potential of selected medicinal plants and their polyherbal formulations, signifying the importance of both efficacy and safety in the evaluation of plant extracts for therapeutic applications, particularly in diabetes management. The findings illuminate the complexities associated with the use of individual plant extracts and their combinations, offering insights that could contribute significantly to the development of effective and safer management for diabetes.The cytotoxicity assays revealed notable differences among the individual plant extracts. *Cajanus cajan* demonstrated the least cytotoxicity at an IC_{50} of 139.98 mg/mL, making it a safer candidate for diabetes combination therapies due to its favorable safety profile.The cytotoxicity assessment of polyherbal combinations revealed varying effects. The **EA** combination (*Euphorbia hirta* + *Abroma augustum*) displayed increased cytotoxicity, suggesting possible synergistic interactions that may enhance therapeutic efficacy but also carry risks. In contrast, combinations like **AB** (*Abroma augustum* + *Bauhinia variegata*) and **A** (all five species) showed improved cell viability, indicating a protective

synergy that may mitigate toxicity while enhancing therapeutic benefits. This shows the need for careful evaluation when formulating polyherbal preparations.

The glucose uptake assays demonstrated that the combination of all five plants (combination **A**) resulted in the highest glucose uptake at 217.19%, outperforming Metformin, indicating strong synergistic effects. Individual extracts like *Catharanthus roseus* and *Bauhinia variegata* also showed significant glucose-lowering potential, while *Euphorbia hirta* and *Abroma augustum* were less effective on their own. The relationship between cytotoxicity and glucose uptake was complex, as *Catharanthus roseus*, although effective in reducing glucose levels, also exhibited moderate cytotoxicity. This emphasizes the need for careful examination of safe dosage thresholds for plants with known toxic effects. Overall, the findings highlight the importance of optimizing polyherbal formulations to maximize therapeutic efficacy while minimizing toxicity, as some combinations may not yield equally effective results.

In conclusion, this study highlights the therapeutic potential of selected medicinal plants and their polyherbal combinations in diabetes management, revealing that certain formulations can offer enhanced efficacy while mitigating toxicity. Future research should focus on isolating the active compounds from these plants, elucidating their mechanisms of action, and conducting *in vivo* studies to confirm safety and effectiveness. This exploration of the synergistic effects of plant extracts may lead to the development of novel, safer, and more effective hypoglycemic agents, paving the way for improved therapeutic strategies in managing diabetes. The findings of this study advocate for continued exploration into the therapeutic applications of plant extracts, emphasizing the need for a balanced approach that prioritizes both efficacy and safety in the formulation of antidiabetic therapies.

Chapter – 7

Summary and Conclusions

Diabetes mellitus (DM) is a persistent metabolic disorder with widespread implications for global health. Recent estimates indicate that India harbours one of the world's largest diabetic populations, primarily due to genetic predisposition and lifestyle factors. Despite the availability of pharmaceutical drugs, many individuals seek alternative treatments due to the side effects, costs, and varying accessibility of conventional medicine. Ethnomedicinal plants have therefore gained renewed interest as they offer natural, culturally integrated, and potentially less adverse options for managing diabetes. This study focused on identifying, characterizing, and scientifically validating the anti-diabetic potential of fifteen ethnomedicinal plants used by the indigenous tribes of Nagaland, a biodiversity-rich region in Northeast India. Traditionally, these plants have been used to treat various ailments, including diabetes, based on knowledge passed down through generations. Through rigorous exploration of molecular characterization, phytochemical profiling, enzyme inhibition assays, and cytotoxicity testing, this study aimed to build a scientific foundation for these traditional practices, thus paving the way for new, plant-based therapeutic options for diabetes.

The objectives of this research was structured to systematically explore and validate the anti-diabetic potential of ethnomedicinal plants used by the indigenous tribes of

Nagaland. The study set out several specific objectives to systematically investigate and validate the anti-diabetic potential of these ethnomedicinal plants. The first objective focused on the collection and documentation of these plants, with particular attention to species that have been traditionally used for diabetes management by local communities. This involved gathering ethnomedicinal plants from various regions of Nagaland to ensure a comprehensive representation of plants known for their therapeutic applications. Following collection, molecular authentication was employed, using DNA barcoding techniques to confirm plant identities and enhance the study's reliability and reproducibility. By establishing accurate genetic profiles, this step was crucial in eliminating misidentification, which could compromise the subsequent findings. Once identification was confirmed, the study proceeded with phytochemical profiling to analyze the plant's chemical constituents. This step aimed to identify and quantify specific compounds associated with anti-diabetic properties, such as flavonoids, alkaloids, tannins, and terpenoids. Subsequently, an enzyme inhibition analysis was conducted to evaluate the inhibitory effects of the plant extracts on α-amylase and α-glucosidase - key enzymes involved in carbohydrate metabolism. Inhibiting these enzymes can significantly impact glucose absorption and, therefore, plays an essential role in diabetes management. Finally, cytotoxicity and cell line studies were undertaken to assess the safety and efficacy of the extracts at the cellular level. These studies involved testing the extracts on relevant cell lines to examine their effects on cell viability and glucose uptake, thereby providing a well-rounded understanding of the therapeutic potential and safety of the plants under investigation.

To fulfil the objective of identifying and documenting ethnomedicinal plants used for diabetes management, fifteen plants were collected from different districts across Nagaland, each selected based on their documented traditional use by local tribes. The

chosen plants included *Abroma augustum, Bauhinia variegata, Cajanus cajan, Catharanthus roseus, Senna alata, Clerodendrum colebrookianum, Euphorbia hirta, Gynura crepidioides, Kalanchoe pinnata, Mucuna pruriens, Paederia foetida, Passiflora edulis, Perilla frutescens, Solanum nigrum*, and *Solanum trilobatum*. Each plant underwent detailed morpho-taxonomic identification, which involved examining features such as leaf shape, flower structure, stem formation, and overall growth habit. This process was fundamental to ensuring the authenticity of the selected species, which was essential for the validity of the subsequent phytochemical, enzyme inhibition, and cytotoxicity analyses. In addition to morpho-taxonomic methods, expert consultations and botanical references, such as the "Checklist of Flora of Nagaland" were utilized to confirm the identities of the plants. This step also provided the opportunity to document each plant's traditional uses, local names, and preferred preparation methods, which helped ground the scientific investigation in the cultural context of the local communities. By combining traditional knowledge with scientific methods, this collection and identification phase established a solid foundation for the study, supporting the relevance and authenticity of the plants chosen for their anti-diabetic potential.

A primary objective of this study was to accurately confirm the identity of the selected ethnomedicinal plants through molecular characterization, using DNA barcoding techniques. Accurate plant identification is crucial in ethnobotanical research, as it ensures that specific plant species are correctly classified, which is essential for reproducibility, reliability, and the safety of future therapeutic applications. This step also served to scientifically validate the traditional knowledge associated with these plants, establishing a firm foundation for subsequent analyses. To achieve this, the study employed three well-recognized DNA barcoding markers i.e. ITS, *rbc*L, and *mat*K each

of which plays a unique role in plant identification and phylogenetic analysis, collectively providing a robust framework for taxonomic authentication.

The molecular characterization phase was designed to fulfil several key objectives, including the authentication of plant species, the creation of a genetic record, and the exploration of genetic relatedness among the selected species. By confirming the identity of each plant, the study ensured that traditional ethnomedicinal knowledge was applied to the correct botanical specimens, thereby minimizing the risk of misidentification, which could otherwise lead to inaccurate results or potentially harmful applications. Additionally, by sequencing the DNA of each species, the study contributed valuable genetic data to the global botanical knowledge base, particularly for the relatively under-documented Nagaland region. This supports conservation efforts, as having a genetic record is critical for preserving biodiversity. Furthermore, the study explored genetic similarities and differences among the selected plants to understand their phylogenetic relationships. This exploration provides insights into shared or unique therapeutic properties and evolutionary adaptations that might influence the production of bioactive compounds, which are key to their anti-hyperglycemic effects.

The DNA barcoding process successfully authenticated all 15 ethnomedicinal plants collected from Nagaland, thus validating their traditional uses and reinforcing the relevance of indigenous knowledge. Molecular characterization revealed several significant findings. For example, plants such as *Catharanthus roseus* were confirmed to align with traditional medicinal uses, with genetic data reinforcing their role in local healthcare practices. The study also shows enhanced phylogenetic understanding among species, confirming, for instance, that *Solanum nigrum* and *Solanum trilobatum* are distinct yet closely related species within the Solanaceae family, helping to explain biochemical similarities observed in phytochemical and enzyme inhibition analyses. This

genetic relatedness often correlates with similar bioactive compounds shared across family lines. Additionally, the *ITS* region, known for its higher variability, provided insights into intraspecific variability. For instance, genetic differences noted within *Kalanchoe pinnata* suggest that plants from different regions may exhibit varied therapeutic efficacies, likely due to adaptive responses to local environmental conditions. Another key outcome of this molecular characterization was its contribution to genetic databases; by sequencing and submitting barcodes, the study added to the body of knowledge for plants from Nagaland, supporting future conservation and pharmacognosy research.

These findings were instrumental in ensuring that the bioactive compounds measured in the phytochemical analysis, and the observed effects in enzyme inhibition studies, were accurately attributed to the correct species. The molecular data not only authenticated the plants but also established a genetic record that aligns with the study's overarching goal of validating traditional knowledge through scientific means. This alignment corroborates the ethnomedicinal applications of each species, adding further weight to the study's findings and supporting the ongoing conservation of Nagaland's rich plant biodiversity. Furthermore, the genetic information provides a foundation for future research, allowing for comparisons of ethnomedicinal plants across regions to uncover additional therapeutic uses or genetic adaptations. It also supports more targeted pharmacological investigations into specific genes linked to bioactive compound production, facilitating the development of new therapeutic agents. The molecular characterization achieved in this study effectively bridges traditional knowledge with modern scientific validation. By confirming the identity of these plants, the study signifies the credibility of the phytochemical, enzyme inhibition, and cytotoxicity analyses, thereby strengthening the overall reliability and applicability of the research

outcomes. This integration of molecular techniques not only supports the current study but also opens new avenues for future ethnobotanical and pharmacognosy research in Nagaland and beyond.

Moving forward, the phytochemical evaluation of the fifteen ethnomedicinal plants selected from Nagaland is a critical aspect of this research, supporting the objective of scientifically validating the anti-diabetic/anti-hyperglycemic potential of plants traditionally used by the indigenous tribes. The phytochemical evaluation was conducted to identify key bioactive compounds contributing to the anti-diabetic properties of these plants. Secondary metabolites, which plants produce, are known for their therapeutic properties and play a crucial role in medicinal efficacy. In particular, flavonoids, alkaloids, tannins, and triterpenoids have demonstrated not only anti-hyperglycemic potential but also substantial antioxidant capabilities. This study aimed to quantify these bioactive compounds to explore their contributions to managing diabetes and its associated complications through various mechanisms, including oxidative stress reduction and enzyme inhibition.

Flavonoids were identified as a major component, especially in plants like *Bauhinia variegata, Cajanus cajan*, and *Euphorbia hirta*. Flavonoid content varied significantly across different extraction solvents, with methanol generally yielding higher concentrations than water. For instance, *Bauhinia variegata* had the highest flavonoid concentration, recorded at 266.87 mgQE/g in methanol extracts and 167.20 mgQE/g in ethanol extracts. Flavonoids contribute to anti-diabetic effects by enhancing insulin sensitivity, improving glucose metabolism, and scavenging free radicals. The antioxidant activity was strongly linked to flavonoid content, particularly for *Bauhinia variegata*, which exhibited an IC_{50} value of 109.02 mg/mL in the DPPH assay for its ethanol extract, indicating potent radical scavenging ability. This powerful antioxidant activity supports

the traditional use of *Bauhinia variegata* for managing diabetes by alleviating oxidative stress, which is a key factor in diabetes-related complications. Alkaloids were also present in high concentrations in *Mucuna pruriens, Kalanchoe pinnata*, and *Euphorbia hirta*. Alkaloid content was highest in ethanol extracts, with *Euphorbia hirta* showing the highest concentration at 43.58 mgAE/g in ethanol extracts, followed by *Bauhinia variegata* at 41.29 mgAE/g. Alkaloids are known to influence glucose metabolism by modulating AMPK (AMP-activated protein kinase) pathways, which enhances glucose uptake and supports glycemic control. The antioxidant profile of these plants also showed a correlation with alkaloid content; for instance, *Mucuna pruriens* demonstrated an IC_{50} value of 582.59 mg/mL in the DPPH assay for its ethanol extract, indicating a moderate level of radical scavenging activity. The presence of alkaloids supports the anti-diabetic properties of these plants by promoting glucose regulation and reducing oxidative stress. Tannins were abundant in plants like *Bauhinia variegata, Euphorbia hirta*, and *Clerodendrum colebrookianum*, with significant variations depending on the extraction solvent. For example, *Bauhinia variegata* showed a tannin content of 163.29 mgTAE/g in ethanol extracts, which was the highest among the studied plants. Tannins are known for their ability to inhibit carbohydrate-digesting enzymes, particularly α-amylase and α-glucosidase, thus reducing postprandial glucose levels. Additionally, tannins contribute to antioxidant activity, with *Bauhinia variegata* showing strong ABTS radical scavenging with IC_{50} values as low as 2.19 mg/mL in ethanol extracts. The enzyme inhibition and antioxidant properties of tannins reinforce their role in traditional diabetes treatments by helping to manage blood glucose spikes and protect pancreatic cells from oxidative damage. Triterpenoids were found notably in *Solanum nigrum* and *Bauhinia variegata*, with the highest triterpenoid content in ethanol extracts of *Solanum nigrum* at 227.41 mgUAE/g. Triterpenoids offer both insulin-sensitizing and anti-inflammatory effects,

which are beneficial for diabetic patients. The strong antioxidant activity of *Bauhinia variegata*, observed in the FRAP assay (66.78 mM Fe^{2+}/g) and 73.62 mM Fe^{2+}/g for *Cajanus cajan*, is consistent with the protective role of triterpenoids in reducing oxidative stress. By stabilizing blood sugar levels and protecting β-cells, triterpenoids contribute to the anti-diabetic efficacy of these plants.The antioxidant data collected using DPPH, ABTS, and FRAP assays highlighted that ethanol extracts generally yielded the highest antioxidant activity, with *Bauhinia variegata, Euphorbia hirta*, and *Cajanus cajan* standing out. For example, *Euphorbia hirta* had a FRAP value of 75.21 mM Fe^{2+}/g in ethanol, indicating a robust reducing power. This demonstrates the importance of solvent choice in optimizing antioxidant extraction and suggests that ethanol is particularly effective for isolating antioxidant compounds from these plants. The antioxidant capacity supports the hypothesis that these plants can combat oxidative stress, thus addressing a major complication in diabetes management.

The phytochemical study findings substantiate the traditional uses of these plants for diabetes management. The significant amounts of phenol, flavonoids, alkaloids, tannins, and triterpenoids indicate that these plants exert their anti-diabetic effects through a multi-targeted approach, including enzyme inhibition, antioxidant defense, and glucose regulation. The high antioxidant potential, particularly in flavonoid- and tannin-rich plants, shows their capacity to reduce oxidative stress and inflammation, both of which are linked to diabetes complications. These findings not only validate traditional knowledge but also establish a scientific foundation for using these plants as complementary therapies in diabetes management. By connecting traditional practices with scientific evidence, this research contributes valuable insights into the therapeutic potential of indigenous medicinal plants from Nagaland in modern healthcare.

The enzyme inhibition studies also provided valuable insights into the anti-diabetic potential of the selected ethnomedicinal plants from Nagaland, focusing on their effects on two key enzymes involved in carbohydrate metabolism: α-amylase and α-glucosidase. These enzymes play critical roles in the digestion of carbohydrates, breaking down complex polysaccharides into glucose, which is then absorbed into the bloodstream. Inhibiting these enzymes can effectively delay carbohydrate breakdown and absorption, thus reducing postprandial glucose levels which is a major therapeutic target in hyperglycemic/diabetes management.

The ethanol and methanol extracts of plants such as *Bauhinia variegata, Passiflora edulis*, and *Gynura crepidioides* demonstrated significant α-amylase and α-glucosidase inhibition, with IC_{50} values for α-amylase inhibition ranging from 286.41 µg/mL in *Bauhinia variegata* to 499.88 µg/mL in *Solanum nigrum*. Similarly, for α-glucosidase inhibition, IC_{50} values ranged from 332.72 µg/mL in *Bauhinia variegata* to 529.31 µg/mL in *Solanum nigrum*, highlighting the varying degrees of efficacy among different plant extracts. Notably, *Catharanthus roseus* exhibited an IC_{50} of 381.13 µg/mL for α-glucosidase inhibition, supporting its traditional use in diabetes management by indigenous communities. The high inhibitory activity observed in *Passiflora edulis* extracts, with up to 75.24% inhibition of α-glucosidase in ethanol extracts at 500 µg/mL, aligns well with the plant's ethnomedicinal application, indicating its potential in managing postprandial hyperglycemia. Mode of inhibition analysis via Lineweaver-Burk plots revealed that certain plants, such as *Bauhinia variegata* and *Cajanus cajan* exhibited competitive inhibition of both enzymes, indicating that their active compounds may bind to the enzyme active site, blocking substrate access and thus reducing enzyme activity. Other plants, such as *Euphorbia hirta* and *Clerodendrum colebrookianum*, showed non-competitive inhibition, where the extract binds to a different site on the

enzyme, leading to structural changes that reduce enzyme function. This diversity in inhibition modes emphasizes the potential for these plants to target different aspects of glucose metabolism, providing a multi-faceted approach to blood sugar management. For instance, *Kalanchoe pinnata* demonstrated competitive inhibition with an IC_{50} value of 359.45 μg/mL against α-amylase and 412.53 μg/mL against α-glucosidase, which points to its potential to interfere with glucose release from complex carbohydrates.

The findings in this study reinforce the traditional applications of these plants for diabetes management and highlight the role of bioactive compounds, such as flavonoids, alkaloids, and tannins, in enzyme inhibition. *Bauhinia variegata*, which exhibited one of the lowest IC_{50} values for both enzymes, was also one of the most effective plants in reducing glucose levels *in vitro*, with its methanol extract achieving 88.65% α-amylase inhibition at 500 μg/mL. This significant inhibitory effect suggests that *Bauhinia variegata* can delay carbohydrate digestion and reduce postprandial glucose absorption, aligning with its traditional use in diabetes care. Moreover, *Gynura crepidioides*, with an IC_{50} of 328.67 μg/mL for α-glucosidase inhibition, further supports the therapeutic relevance of indigenous knowledge in selecting plants with substantial anti-diabetic potential.The implications of these findings for diabetes management are considerable. By slowing down carbohydrate digestion and absorption, these plant extracts offer a natural means of controlling postprandial glucose levels, which is crucial for individuals managing diabetes. The variety in modes of enzyme inhibition indicates that these plants may act through multiple mechanisms, potentially increasing their effectiveness and providing a holistic approach to managing blood sugar levels. Additionally, the data suggest that ethanol extracts are particularly effective, likely due to better solubility and extraction of active compounds in ethanol. This information is vital for optimizing extraction methods in future pharmacological applications. Thus, the enzyme inhibition

studies validate the anti-diabetic potential of these ethnomedicinal plants from Nagaland. By targeting α-amylase and α-glucosidase through competitive and non-competitive inhibition, these plants demonstrate substantial promise in diabetes management, bridging traditional medicinal practices with modern therapeutic research.

After a thorough study of phytochemical content and enzyme activities of these fifteen plants, the cell line and cytotoxicity study focuses on five medicinal plants—*Abroma augustum*, *Bauhinia variegata*, *Cajanus cajan*, *Catharanthus roseus*, and *Euphorbia hirta* are selected for their ethnobotanical significance, phytochemical richness, and strong enzyme inhibition properties against α-amylase and α-glucosidase, both of which are key in carbohydrate metabolism. Additionally, polyherbal formulations were investigated to explore potential synergistic effects that could enhance therapeutic efficacy while minimizing toxicity. The study aims to comprehensively assess the anti-diabetic potential of these plants by evaluating their cytotoxicity in human cell lines and their glucose uptake capabilities in HepG2 cells.

The cytotoxicity of individual plant extracts and polyherbal formulations was evaluated using the MTT assay, with IC_{50} values indicating the concentration at which cell viability is reduced by 50%. Among the individual extracts, *Bauhinia variegata* exhibited the highest cytotoxicity (IC_{50}=21.506 mg/mL); while, *Cajanus cajan* showed the lowest cytotoxicity (IC_{50}=139.98 mg/mL), suggesting it has a relatively safer profile. The polyherbal formulation **EA** (*Euphorbia hirta* + *Abroma augustum*) displayed increased cytotoxicity, potentially due to synergistic effects, while the **AB** combination (*Abroma augustum* + *Bauhinia variegata*) and the combination of all five plants (**A**) showed moderate cytotoxicity and higher cell viability than some individual extracts.In glucose uptake studies using HepG2 cells, the polyherbal formulation **A**, which includes all five plant extracts, demonstrated the highest glucose uptake (217.19%), surpassing

that of individual extracts and nearly matching the control drug Metformin (250.83%). Among individual extracts, *Catharanthus roseus* (171.61%) and *Bauhinia variegata* (167.27%) showed the greatest glucose uptake enhancement. Certain polyherbal combinations, such as **AB** and **CC** (*Cajanus cajan* + *Catharanthus roseus*), also displayed strong glucose uptake, suggesting potential synergistic hypoglycemic effects. However, combinations like **EA** and **EC** (*Euphorbia hirta* + *Cajanus cajan*) had significantly lower glucose uptake, expressing the variability in efficacy among different combinations.

Overall, these results highlight the potential of polyherbal formulations to enhance glucose uptake while reducing cytotoxicity compared to individual extracts. The data suggests that while certain plants, like *Bauhinia variegata*, exhibit strong antihyperglycemic effects, their higher cytotoxicity necessitates caution. In contrast, safer options, such as *Cajanus cajan* and selected polyherbal combinations like **AB**, may provide balanced hypoglycemic effects with lower toxicity, emphasizing the promise of combination therapies in the development of effective, safer plant-based treatments for diabetes.

Collectively, these findings show the therapeutic potential of the selected ethnomedicinal plants in managing diabetes. The integration of traditional knowledge with modern scientific investigation has resulted in a comprehensive understanding of how these plants can function as effective natural remedies. The mechanisms followed in this study, including antioxidant activity, enzyme inhibition, and enhancement of glucose uptake, contribute to a multifaceted approach to diabetes management, addressing the condition from various angles. The implications of this research extend beyond the validation of traditional practices; they highlight the importance of conserving and utilizing these plant species within modern healthcare frameworks. As diabetes rates continue to rise globally, the need for safe, effective, and accessible treatments becomes

increasingly urgent. The ethnomedicinal plants identified in this study offer a promising avenue for developing complementary therapies that align with local practices and knowledge systems.

Future Prospects

This investigation provides a foundational framework for ongoing research into ethnomedicinal flora exhibiting anti-diabetic properties, stressing the necessity for subsequent endeavours aimed at the isolation and characterization of the specific bioactive compounds implicated in the noted therapeutic efficacy. The execution of *in vivo* investigations will be vital to ascertain the effectiveness and safety of these botanical extracts within living organisms, thereby connecting laboratory outcomes with practical therapeutic applications. Furthermore, the examination of polyherbal formulations potentially in conjunction with conventional diabetes treatments could yield improved therapeutic results and a more extensive array of treatment alternatives. The conservation of these plant species is vital for ensuring their availability for future generations, as they constitute an invaluable resource for sustainable healthcare and encapsulate a wealth of indigenous knowledge. In the context of escalating diabetes prevalence, these plants provide accessible, cost-effective, and efficacious treatment modalities that resonate with local traditions while fostering environmental sustainability. By integrating traditional knowledge with empirical research, this study not only supports healthcare innovation but also highlights the crucial significance of biodiversity preservation. Through this integrative approach, the anti-diabetic flora of Nagaland presents a promising pathway for sustainable, culturally relevant, and environmentally conscientious healthcare solutions.

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Annexure - I

CTAB DNA Extraction Protocol (Doyle and Doyle, 1987; Kamba and Deb, 2018)

Chemical reagents:

CTAB reagent	Isoamyle alcohol
β-mercaptoethanol	Isopropanol
NaCl	Ethanol
EDTA.Na_2	RNase
Tris-HCl	TE Buffer
Agarose	Ethidium bromide
TAE buffer	TBE buffer
Chloroform	Tris-Phenol

DNA extraction procedure:

1. Grind 500 mg of plant fresh leaf tissue into a fine paste using 10 ml of CTAB buffer.
2. Transfer the CTAB/plant mixture to acentrifuge tube, add 0.2% β-mercaptoethanol (v/v), and incubate the mixture in a water bath at 60°C for 1 hour, occasionally inverting the tube.
3. After incubation, centrifuge the mixture at 10,000 rpm for 10 minutes at room temperature (27°C).
4. Add an equal volume of chloroform: isoamyle (24:1) to the tube, mix by inverting for 5 minutes, and centrifuge again at 10,000 rpm for 10 minutes.

5. Carefully transfer the aqueous phase to a cleancentrifuge tube and repeat step 4 until the solution becomes clear.
6. The upper aqueous layer contains DNA. Add an equal volume of chilled isopropanol to precipitate the DNA, mix by inversion for 5 minutes, and incubate at -60°C for 10-30 minutes (depending on the precipitation process).
7. Centrifuge the mixture at 10,000 rpm for 15 minutes, and discard the supernatant.
8. Wash the DNA pellet with 70% chilled ethanol by inverting the tube, then centrifuge at 10,000 rpm for 5 minutes.
9. Allow the pellet to dry at room temperature, ensuring that the ethanol residue is removed but not over-drying, as this may make the pellet difficult to re-suspend.
10. Re-suspend the DNA pellet in 200-300 µl of TE buffer.
11. If needed, treat the sample with RNase A (10 mg/ml) by adding 3-5 µl and incubating at 37°C for 45 minutes to remove RNA.
12. Assess the DNA concentration, quality, and yield by running aliquots on a 0.8% TAE agarose gel stained with ethidium bromide, then visualize the bands using a gel documentation system.
13. Store the DNA samples at -20°C for future use.

Annexure – II

Internal Accession Nos., Genbank Accession No., and Sequences of the Gene

GenBank Accession Number	Organism Name & Accession No	Nucleotide Sequence
OQ913536	***Abroma augustum*** isolate **NU-BOT-TIS-AAI** 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	GTCTTTGAACGCAAGTTGCGCCCCAAGCCATTAGGCCGAGGGCACGTCTGCCTGG GTGTCACGCATCGTTGCCCCCCTCAACACTCGTGAGGGGCGGACAATGGCCTCCC GTGCGCTTCCCGCTCGCGGCTGGCCCAAATGCCAGGTCCCGGCGACCGGAGTGCC GCGACGATCGGTGGTAAGCATACCTATTCCCGCGTCGTGCGTGCTAGTCGTTCGT TCGGACCCATGCTCTAGACCCTGATGCGTCGCTTGTTCGATGCTCGCATCGCGAC CCCAGGTCAGGCGGGATCACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAA AAGAAACTTACCAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAAGAGCCCAA A (**384 bp**)
OQ916148	***Abroma augustum*** isolate **NU-BOT-TIS-AA** ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast.	AGAGTATAAATTGACTTATTATACTCCTGAATATGAAGTCAAAGATACTGATATC TTGGCAGCCTTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTGAGGAAGCAGGGG CCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACCGTGTGGACCGACGG GCTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTGTT GGAGAAGAAAATCAATATATATGTTATGTAGCTTACCCCTTAGACCTTTTTGAAG AAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAA GCCCTGCGCGCTCTACGTCTAGAGGATCTGCGAATCCCTCCTGCTTATATTAAAA CTTTCCAAGGCCCGCCTCATGGCATCCAGGTTGAAAGAGATAAATTGAACAAGTA TGGTCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAG AACTACGGTAGAGCAGTTTATGAATGTCTACGTGGTGGACTTGATTTTACCAAAG ATGATGAGAATGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATT TTGTGCCGAAGCTAT (**621 bp**)
OQ916152	***Abroma augustum*** isolate **NU-BOT-TIS-AM** maturase K (matK) gene, partial cds; chloroplast.	TTTCTTCTTCTTTGCATTTTTTACGGTTTTCTCTCTATGAGTATTGTAATTTGAAGA GTTTTTTTACTCCAAAGAAATCAATTTCGATTTTTAATCCAAGATTATTCTTGTTCT TATATAATTCTCATGTATGTGAATACGAATCCATTTTCCTTTTTCTCCGTAACCAA

		TCTTCTCATTTACGATCAACATCTTCTGGGGTCTTTCTTGAACGAATTTTTTTCTAT GGAAAAATAGAGCATCTTGTAGAAGTCTTTTCGAATGATTTGCAGAGCAACCT CTGGTTGTTCAAAGATCCTATCATACATTTTATTAGATATCAAGGAAAAGCAATT ATAGCTTCAAAAGATACGTCTCTTCTGATGAATAGGTGGAAATATTACTTTGTCG ATTTATGGCAATATTATTTTTACGTGTGGTCTCAATCAGGAAGAGTCCGTATAAA TCAATTATCTAAATATTCTCTCGACTTTCTGGGCTATCTTTCAAATGTGCGATTAA ATTCTTCGGTGGTACGGAGTCAAATGCTAGAGAATTCATTTATAATAGATAATGC TATGAAGAAGCTGGATACAAGAATTCCAATTATTTCTCTGATTGGATCATTGTCT AAAGCGAAATTTTGTAACACATTGGGGCATCCCATTAGTAAGCCGACGTGGGCC GATTCCTCAGATTCTGATATTATTGAACGATTTGTGCGTATATGCAGAAATCTTTC TCATTATCACAGTGGATCT (**740 bp**)
OP946324	***Bauhinia variegata*** isolate **NU-BOT-TIS-CRD-ITS-2-BV**internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence.	TTCACGGATGCTTGGCGTTGAAGATTCAAGAACTATCATTCCTCAAGATAACATA AAAGGGATCATTGTCGAAACCTCAACCAAAACCACCCGCGAACTTGTATAAATA CCCACTGGGGGAGGCGGAGGGTGTCCCCACCCCGAGCCTCCCCTGCGCCCGGGC GGGGGCGCGTCGGGTGACCCCCGGTGCGTGCTCGTTCGGGCAAACTAACAAAAC CACGGCGCCAGACGCGTCAAGGAATCAAAACATAGAGGCACGCCCTCGTCGGCC CGGGAACGGTGATCGTGCGGGGTGCGTCGCCGATATTTTTACACAAAACGACTCT CGGCAACGGATATCTCGGCTTGCTCTATAAAA (**358 bp**)
OQ916147	***Bauhinia variegata*** isolate **NU-BOT-TIS-BV**ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	GCAAGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTC CTGACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCA ACCTGGAGTTCCTCCTGAAGAAGCAGGTGCCGCGGTAGCTGCTGAATCTTCTACT GGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAG GACGATGCTACCACATCGAGCCCGTTGCTGGAGAAGAAAATCAATATATTGCCTA TGTAGCTTATCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTT CCATTGTGGGTAATGTATTTGGATTCAAAGCCCTGCGCGCTCTACGTCTGGAGGA TTTGCGAATCCCTGTTTCTTATATTAAAACTTTCCAAGGTCCGCCTCACGGCATCC AAGTTGAGAGGGATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTAT TAAACCTAAATTGGGGTTATCCGCTAAGAATTACGGTAGAAAA (**540 bp**)
OQ916151	***Bauhinia variegata*** isolate **NU-BOT-TIS-BVM**maturase K (matK) gene, partial cds; chloroplast	ATTTAAATTATGTGTCAGATGTACAAATACCCTACCCTATCCATTTGGAAATTCTG CTTCAAACCCTTCGCTATTGGTTGAAAGATGTTTCGTCTCTTCATTTATTAAGGAT CTTTCTTCACCAATATTGTAATTGGAATAGTCTTATTACTCTAAGAAAATCGATTT CTACTTTTTCCAAAAGTAATCCAAGATTATTCTTGTTCCTATATAATTTGCATGTC TTCGAATATGAATCCATCTTCCTTTTTCTCCGTAACAAATCTTCTCATTTACGATT AACATCCTTTGGAGTCCTTTTTGAGCGAATATATTTCTATGGAAAAATAGAACAT

		CTTGTAGAAGTCTTTGCTAAAGATTTTCTGTCGACCTTATGGTTATTTAAGGATCC TTTCATTCATTATGTTAGATATCAAGGAAAATCGATTCTGGCTTCAAAGAATACG CCTCTTTTGATGAATAAATGGAAATACTATCTTATCAATTTATGGCAATGTCATTT TTATGTTTGGTCTCAACCAGGAAGAATCCATATAAACCAATTATCTGAGCATTCA TTCTACTTTTTGTTTTTGGGCTATTTTTCAAGTGTGCGACTAAATCCTTCAGTGGTA CGGAGTCGAATGCTGGAAAATTCATTTATAATAGAAAATGTTAGGAAAAAGCTT AATACAATAGTTCCAATTATTCCTCTAATGAAATTATTGGCTAAAGCGAAATTTT GTAACGTATTAGGCCATCCTATTAGTAAGCCGGTCTGGGCCGATTCATCTGATTTT GATATTATTGACCGATTTGTGCGGATATGCAGAAATTTTTATCATTATAAA (**830 bp)**
OQ915149	***Cajanus cajan*** isolate **NU-BOT-TIS-CCI** small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.	TGTTTGGTAAAAAAGTCGTAACAAGGTTTCTGTAGGTGAACCTGCGGAAGGATCA TTGTCGATACCTGCGAAGCAGAACGACCCGCGAACACGTTTACAAACTCTCGGCT GTGTGTACGGGCCCTTCGGGACCCTTAAACTGGCCTCGTGTCGGGGGCCTCGTCG GCCGCCGACTCAATAAACAAACCCCGGCGCGGTCTGTGCCAAGGAAATAACAAA GAAAGTAGCTTTGCCCCCGCGGTACCAGAAATGGTGTCAGCCTCGGGGTGCGTCG CTCGTTCGATATAATATGTCACAACGACTCTCGGCAACGGAGATCTAGGCTCTCG CATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATAGCAGAATCCCG TGAACCATCGAGTCTTTGAACGCAAGATGCGCCCGAAGCCGTCAGGCGGAGGGC ACGTCTGCCTGGGCGTCACGCACCGTCGCCCCCCCCGCGCAACCCGCTGTGCGGG TTCGTCACCGGGGGCGGACAATGGCCTCCCGTGCGCCCGGCGCGCGGTTGGCCCA AAATTGAGTCCTCGGCTTCGTTCGCCGTGACGATCGGTGGTTGTCGAGTTATCGG CGCCCCGTCGCGCGCGAGACCGCAGCAGGCGGACTCATCTCTCGACCCCAAATG GCAGCGACGACGGATTCTATCCGCCGACGCGCCATCGACGCGACCCCAGTCAGC GGACAGCCCAGATGGGGAAA (**730 bp**)
OQ913364	***Cajanus cajan NU*-BOT-TIS-CRD-5** ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds; chloroplast	GTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACGCCTCAGT ATCAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGG AGTTCCGCCTGAAGAAGCAGGTGCCGCGGTAGCTGCAGAATCTTCTACTGGTACA TGGACAACTGTGTGGACTGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGAT GCTACCACATCGAACCTGTTGCTGGAGAAGAAAATCAATTTATTGCTTATGTAGC TTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTG TCGGTAATGTATTTGGGTTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTGCG AATCCCTATTTCTTATGTTAAAACTTTCCAAGGTCCGCCTCATGGTATCCAAGTTG AGAGAGATAAATTGAACAAGTATGGCCGTCCCCTATTAGGATGTACTATTAAACC TAAATTGGGGTTATCTGCTAAGAATTACGGTAGAGCTGTTTATGAATGTCTTCGC

		GGGGGACTTGATTTTACTAAAGATGATGAAAATGTGAATTCCCAACCATTTATGC GTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCGCTTTTTAAAGCACAGGTTGA AACTGGTGAAATCAAAGGGCATTACTTGAATGCAACTGCAGGTACATGCGAAGA GATGATAAAAAGAGCTGTATTTGCCAGAGAATTAGGCGTTCCTATCATAATG CATGATTATTTAACAGGGGGATTCACTGCAAATACTAGCTTGGCTCATTATTGCC GAGATAATGGTCTACTTCTTCATATACATCGTGCAATGCATGCAGTTATAGACAG ACAAAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGTTACGTTTGTCT GGTGGAGATCATGTTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGGAAAGA GAAATCACTTTAGGTTTTGTTGATTTACTACGTGATGATTTTATTGAAAAAGATCG AAGTCGCGGTATTTATTTCACTCAGGATTGGGTTTCTCTACCAGGTGTTCTGCCTG TTGCTTCTGGAGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGATCTTTGGA GATGATTCTGTACTCCAATTTGGCGGAGGAACTTTAGGACATCCTTGGGGAAATG CACCCGGTGCTGTAGCTAATCGAGTAGCTCTTGAAGCATGTGTACAAG CTCGAAATGAAGGACGTGATCTTGCTCGTGAAGGTAATGAAATTATCCGTGAGGC TAGCAAATGGAGTCCTGAATTAGCTGCTGCTTGTGAAGTATGGAAGGCGATCAA ATTTGAATTCGAAGCAGTAGATACAATTTAG (**1398 bp**)
OP966870	***Cajanus cajan*** maturase K gene, partial cds; mitochondrial	CATCTGGAAATCTTGGTTCAAATCCTTCGATATTGGATAAAAGATGTCTCTTTCTT TCATTTATTAAGGTTGTTTTTTTATTACTATTGTAATTGGAATAGTCTTTTTACTCC AAAAAAATGGATTTCTACTTTTTTTTCAAAAAGTAATCCAAGATTTTTCTTGTTCT TATATAATTTATACGTCCGGGAATCAGAATCTATCTTTCTTTTTTTACGTAACAAA TCCTCTCAGTTACGATTAAAATATTTTCACGTTTTTTTTGAGCGAATTTTGTTCT ATGAAAAAATAGAATATCTTGTAGAAGTATTTACTACGGATTTTTCATATACCTT ATCATTCTTCAAGGATCCTTTCATCCATTATGTTAGATATCAAGGAAAATCCATTC TGGTTTCAAAGAATACTCCTCTTTTGATAAATAAATGGAAATACTATTTTATCTAT TTATGGCAATGTCATTTTGATATTTGGTCTCAATCAGTAACGATTCATATAAACCA ATTATCCCAGCATTCATTTCACTTTTTGGGCTATTTTTTAAGTATTCGGCTAAATA TTTCAGTGGTACGAAGTCAAATGTTGCAAAATTCATTTCTAATAAAAATTGTTAT AAAAAAGCTTGATACAATAGTTCCAATTTTTCCTCTAATTAGATCATTGGCAAAA GCAAAATTTTGTAATCTATTGGGTCATCCCATTAGTAAGTCGGTTTGGGCCAATTT ATCTTATTTTGATATTATTGACCGATTTTTGCGTATATGTAGAAATTTTTCTCATTA TTACAATGGATCTGCAAAAAAAAAGAGTTTGTATCAAATAAAATATATACTTCGG CTTTCTTGTATAAAAACTTTGGCTCGTAAACACAAAAGTACT (**879 bp**)
OQ915155	***Catharanthus roseus*** isolate **NU-BOT-TIS-CRI** internal transcribed spacer 1,	AAGGATCATTGTCGAATCCTGTAAAACAAACCGGCAAACTTGTTTTTAACTCGGG CCTCGAGCAAGGGGTCTCTAGGGACTACCTGCTCGTTGCCCCCTGGGCCTGCCGA

	partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	GTGCCCTTGGGCAACCGGTCGTGCCTAACAACAAACCCGGGCGCGGAAAGCGCC AAGGACTACTCAAGTGGGATTGCCTTCCCTAGGTCGGCCCGTTCCCGGTGCTGTC CTTGGGAGCTAAGGCACCTTTGTAAACCAAAACGACTCTCGGCAACGGAT ATCTAGGCTCTGGCATCGATGAAGAACGTAGCAAACTGCGATACTTGGTGTGAAT TGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCTGAAGCCATT AGGCCGAGGGCACGTCTGCCTGGGCGTCAAGCATCCCGTCGCCCTCCCCTCGCCC GTCCATCTGTGGATGACTCGGCGCTTGAGGAAGGACGTAGATTGGCCTCCCGTGC ATTACTCGCGGTTGGCCTAAATCTTGGTCCCTTGCTGCGGACGTCACGACAAGTG GTGGTTGAAATCCTCAACTCGAATGCGAGTCGTGACGAGAACCGCGGTCTAGGT GTCCGAACGACCCCTGGTGCTAGCCCTTCCCCCTGTA (**636 bp**)
OQ913366	***Catharanthus roseus NU-BOT-TIS-CRD-TI*** ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds; chloroplast	AAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCT GAATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAAC CTGGAGTTCCACCCGAAGAAGCAGGGGCTGCGGTAGCTGCTGAATCTTCTACTGG TACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCG TTACAAAGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGAAGATCAATA TATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACA TGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGT CTGGAAGATTTGCGAATCCCTACGGCTTATATTAAAACCTTCCAAGGCCCGCCTC ATGGGATCCAGGTTGAGAGAGATAAATTGAACAAATATGGTCGTCCCCTGTTGG GATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAAAACTACGGTAGGGCATG TTATGAATGTCTTCGTGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAAC TCCCAACCGTTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAATTTA TAAAGCACAGGCTGAAACCGGTGAAATCAAAGG (**684 bp**)
OP966869	***Catharanthus roseus*** maturase K gene, partial cds; chloroplast	TTGAATTTGAATTTTGTGTTAGATATACTAATACCCCACCCCGTTCATCTGGAAAT CTTGGTTCAAACCCTTCGCTATTGGGTAAAAGATGCCCCTTCTTTGCACTTATTAC GATTCTTTCTCCGCGAGTATTGGAATTGGAATAATCTTATTGCTACAAAGAACCT CAGTTTTGATTTTTTAATAAAAAGAAATCAAAGATTCTTCTTCTTCTTATATAATT TTTATGTATGTGAATACGAATCCATTTTCGTCTTTCTCTATAACCAATCTTCTCATT TACGATCAACATCTTTTGGGGTCCTTCTTGAACGAATCCATTTCTATGGAAAAAT AGAACGTCTTGTCGAAGTATTTGCTAAGGATTTTCTGGCCAACTTAGGCTTGTTCA AAGATCCTTTCATGCATTATGTTAGGTATCAAGGAAAATCCATTTTGGTTTCAAA AGGGCCGTCTCTTTGGATAAATAAATGGAAATCTTACCTTGTCAACTTTTGGCAA TGTTATTTTGACCTGTGGTTTCACTCGGAAAGGGTCTATATAAAACAATTGTCCA ATCATTCTCTTGACTTTATGGGTTATCTTGTAAATGTGCGACTAAACCCTTCAATG

		GTACGGGGTAAAATGCTAGAAAATGCATTTCTAATCAATAATGCTATTAAGCAAT TCGATACCCTTGTTCCAATTCTTCCTCTGATTAGATCATTGGCTAAAGCGAAATTT TGTAACCTATTAGGACATCCTATTAGTAAGCTGGTTCGGACTGATTTATCAGATTC TGATATTCTGGACAGATTTGGGCGGATATGCAGAAACCTTTCTCATTATCATAGT GGATCTTCCAAAAAAAAGAGTTTG (**858 bp**)
OQ915142	***Senna alata*** *isolate* **NU-BOT-TIS-SAI** internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	TCGTTCGGGGCCAGCAGCGAAAATGATGTCTAGAATGACTCTCGGCAACGGATA TCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATT GCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTA GGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCAAAACCCTGTCGT CCCTCCGGTCAATCGGAGGCGGCGAGGTGCTTGGGCGGAAGCTGGC CTCCCGTGAGCATTGCCTTGCGGATGGCCGAAATTAGAGCCTGTGAGGGGCAATC GCCACGTTCCACGGTGGTTGAGCAGACGCCTCGAGGCCGACCGTGCGCGAGTTGT CCCCACGACAAGGCTGCGAGACCCTTGCAAA (**406 bp**)
OQ913370	***Senna alata*** **NU-BOT-TIS-7** ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds; chloroplast	AAGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCT GACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAAC CTGGAGTTCCGCCTGAAGAAGCAGGTGCCGCGGTAGCTGCTGAATCTTCTACTGG TACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGA CGATGCTACCACATTGAGCCCGTTGCTGGAGAAGAAAATCA ATATATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTA ACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTA CGTCTGGAGGATTTGCGAATCCCTACTTCTTATATTAAAACTTTCCAAGGTCCGCC TCACGGCATCCAAGTTGAGAGAGATAAATTGAACAAGTACGGCCGTCCCCTATTG GGATGTACTATTAAACCTAAATTGGGGTTATCTGCTAAGAATTATGGTAGAGCAG TTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAAGATGATGAGA ATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTT (**626 bp**)
OQ913371	***Senna alata*** isolate **NU-BOT-TIS-CRD-SA** maturase K gene, partial cds; chloroplast	CCATCTGGAAATCTTGGTTCAAACCCTTCGATACTGGGTGAAAGATGCCTCTTCTT CTCATTTATTAAGGCTCTTTCTTTATGAGTATTTTAATTGGAATAGTCTTATTACTC CAAAAAAATGGATTTCTACTTTTTCAAAAAGGAATCCAAGATTATTCCTGCTCCT ATATAATTTTTATGTATGTGAATACGAATCTATCTTTCTTTTTCTCCGTAACAAAT CTTCTTATTTACGATTAACATCTTCTGGAGTCCTTTTTGAGCGAATCTATTTCTATG CAAAAATAGAACATTTTGTAGAAGTCTTTGATAAAGATTTTCCGTC CACCTTATGGTTCTTCAAGGACCCTTTCATTCATTATGTTAGATATCAAGGAAAAT CCATTTTGGCTTCAACGAATACGCCCTTTTTGATGAATAAATGGAAATACTATCTT ATCCGTTTATGGCAATGTCATTTTTATGTTTGGTCTCAACCAGAAAAGATCCATAT

		AAACCAATTATCTGAGCATTCATTTTACTTTTTGGGCTATTTTTCAAATGTGCGGC TAAATCCTTCAGTGGTACGGAGTCAAATGCTGGAAAATTCATTTCTAATTGAAAA TGTTATGAAAAGGCTTGATACAATAATTCCAATTATTCCA CTAATTAGATCATTGGCTAAAGCGAGATTTTGTAATGTATTAGGGCATCCCATTA GTAAGCCGGTCTGGGCCGATTCATCCGATTTGGATATTATTGACCGATTTTTGCG GAGATGCAGAAATCTTTCTCATTATTACAATGGATCCTCAACAAAAAAGAGTTTG TATCGAATAAAATATATACTTCGGCTTTCTTGTATTAAA (**850bp**)
OP001888	***Clerodendrum colebrookianum*** voucher **NU-BOT-LUM-ITS-CRD-1** internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	CTGAAACACACACCCGCGAACACGTGTTTACCAATCGGGGCTGCGGTCCCTTTCT CGCCGGCGTGCGCCCCCGCGTCGCCGGGCGGGCTAACAAAATCGGGCGCGAAAT GCCCCAAGGAATACATAAAAAAGCGTTCCCCTCCCCAGGGCCCGGGCGCGAAAA TCGGGGGGAAGGTTGGAATGCCGGTCTAATAAAAAAAGAACTCTCGGCAACGAA TATCTCGGCTCTCGCATCAATAAAAAACGTACCAAATGGCAATACTGGGGGTGA ATTGCAAAATCCCGGGAACCATCAATCCTTTAAACGCAGTTTGCCCCCAAA CCCTTTAGGCCAAGGGCACTTCGGCCGGGGGGTCACGCTTCGGGTCACCTCCCTC AACACACAAGGTTGTTGAGGAAGGTGAATATGGCCCTCCCGTGCTTCATTCCCCC CGGCGGGCCCAATTTCAATCCTTCGGCAACAAAGTTCCCGACAAGTGTTGTTTGA ATTATCACTTCGGTTCTTGTCTGGCCACAAAACTCCTTTCAATCGGAATTCATTAT AAACCCAAGGGCCCTGGCTGGCTTCGGGCCTCCAACGGCAACCCCGGGTCAGGC GGAATTACCCGCTAATTTTAACCTTATCATTAACCGAAGGAAGAAAAACTTACA (**651 bp**)
OP020130	***Clerodendrum colebrookianum*** isolate **NU-BOT-TIS-CRD** maturase K gene, partial cds; chloroplast	TGCTTCTTCTTTACATTTACTACGAGTCTTTCTCGATGAATATTGTAATTGGAATA GTCTTATTATTCCAACGAAAGCTGGCTCCTCTTTTTCAAAACGAAATCAAAGACT ATTCTTATTCTTATATAATTCTCATGTATGTGAATATGAATCCGTTTTCGTCTTTCT ACGTAACCAATCTTTTCATTTACGATCAACAGCTTTTGGAGTTCTTCTTGAACGAA TCTATTTCTATGTAAAAGTAGAACGTCTTGTGAACGTCTTTGGTAAGATTAACAA TTTTCGGGCGAACTCGTGGTTGGTCAAGGAACCTTTCATGCATTATATTAGGTATC AAAGAAGATCCATTCTGGCTTCAAAGGGAACATCTTTTTTCATGAAAAAATGGCA ATTTTATCTTGTCACTTTTTGGCAATGGCATTTTTCGCTGTGGTTTCATCCAAGAA GGATTTATATAAACCAATTATACAATTATTCCCTTGAATTTTTGGACTATCTTTCA AGCGTGCGAATGAACCCCTCCGTGGTACGGAGTCAAATTCTAGAAAATGCATTTC TAATCAATAATGCTATTAAGAAGTTTGATACCCTTATTCCAATTATTCCAATGATT GCGTCATTGGCTAAAGCGAAATTTTGTAACGTATTTGGGCATCCTGTTAGTAAGC CGATTTGGGCTGATTTATCCGATTCTAATATTATTGACCGATTTGGTCGTATATGC AGAAATCTTTCTCATTATCATAGCGGATCTTCCAAAAAAAAGAGTT

		TGTATCGAATAAAGTATATACT (**792 bp**)
OP035401	***Clerodendrum colebrookianum*** voucher **NU-BOT-TIS-CRD** chloroplast sequence	GGAAAGGCCTTTGATTACCTGTTTTCAGCCTGTGATTTATAAATTGCTTCGGCACA AAATAAGAAACGATCCCTCCAACGCATAAATGGCTGGGAGTTTACGTTCTCATCA TCTTTGGTAAAATCAAGTCCGCCACGAAGACATTCATAAACCGCTCTACCATAGT TTTTAGCGGATAACCCCAATTTCGGTTTAATAGTACATCCCAACAGAGGACGACC GTACTTGTTCAATTTATCTCTTTCAACTTGGATCCCATGAGGTGGGCCTTGGAAAG TTTTAATATAAGCAGTGGGGATTCGCAGATCTTCCAGACGTAGAGCACGTAGGGC TTTGAATCCAAATACATTTCCTACAATGGAAGTAAACATGTTAGTAACAGAACCT TCTTCAAAAAGATCTAAAGGATAAGCTACATAACAGATATATTGATCTTTTTCTC CAAGAACGGGCTCGATGTCGTAGCATCGACCTTTGTAACGATCAAGGC TGGTAAGGCCATCGGTCCACACAGTTGTCCATGTACCAGTAGAAGATTCGGCAGC TACCGCGGCCCCTGCTTCTTCAGGCGGAACTCCAGGTTGAGGAGTTACTCGGAAT GCTGCCAAGATATCAGTATCTTTGGTTTTGTATTCAGGAGTATAATAAGTCAATTT GTACTCTTTAACACCCGCTTTGAATCCAACACTTGCTTTAGTTT CTGTGTGGGGGACAAAAATAAAAA (**724 bp**)
OP002115	***Euphorbia hirta*** voucher **NU-BOT-LUM-TIS-CRD-2** small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	ATACCCCTTTCGGTAGGGGGACCTGCGGAAGGATCATTGTCGAAACCTGCCCAGC AGAACGACTTGTGAACGTGTTCATAAAACGAGGGGTCCGAAGCGGGTTTTACCA GCCTTGGCCCTTCACCAGGCCTTGATCGGGGTATCCGGGCGTGCAGGCTTGTCCT TCAATCCCGGACCGTCCCGGTCTTGGCTTGATAACAAACCCCCGGCGCGGAAAGC GCCAAGGAATTGCAAACAGAAAGATCGGACACTCTGACCGCCCCGG AAACGGTGTGCAACAGGAATGCTCCGTGCTTTTAAAATCTAAACGACTCTCGGCA ACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACTTGGT GTGAATTGCAGGATCCCGCGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAA GCCTTTTGGCCGAGGGCATGTCTGCCTGGGTGTCACTCAACCGTCGCCCCAACCT CCTCATTGCAGGAGGGGGGCGGAACATGGCCTCCCGT GTGCTCATCCCACGCGGTTGGCCCAAATGTCCGGTCCACGGCAACGACGCCACGG CAATCGGTGGTTGTATGGCCCTCGCTAAATGTCGTGAGTGATGCGGGTGCTGAGG GACTTAAAAGGCCCCGTAGCGTTTCTGATGAGGCGCTCGCAATGCGACCCCCAGG TCAGGCGGATCCCGGT (**703 bp**)
OQ913367	***Euphorbia hirta*** **NU-BOT-TIS-CRD-EU** ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds; chloroplast	TTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTG GCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGAGCT GCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACTGATGGGC TTACCAGTCTTGATCGTTATAAAGGACGATGCTACCACATC GAGCCCGTTGCTGGAGAAGAAAATCAGTATATTGCTTATGTAGCTTACCCCTTAG

		ACCTTTTTGAAGAAGGTTCTGTTACTAATATGTTTACCTCCATTGTGGGTAATGTA TTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTGGAGGATTTGCGAATCCCTCCTG CTTATACTAAAACTTTCCAAGGGCCACCTCATGGTATCCAAGTTGAAAGAGATAA ATTGAACAAGTATGGCCGCCCTCTATTGGGTTGTACTATTAAACCAAAATT GGGGCTATCCGCTAAGAATTACGGTAGAGCGGTTTATGAATGTCTTCGCGGGAT (**531 bp**)
OQ913374	***Euphorbia hirta*** isolate **NU-BOT-TIS-EH** maturase K gene, partial cds; chloroplast	GACCCTTCTTATTTGCATTTTTTACGACTCTTTCTTCATCAGTATTGGAGTTGGAA CAGTCTTATTATTCCACAGAAATCAATTTCTATTTTTCGAAAAAAAAATCCAAGA TTTTTCTTGTTCCTATATAATTTTCATATATATGAATATGAATCCATCTTCTTTTTT CTCCGTAATCAGTGCTTTCATTTACGATCAACATTTTCTCGC GTCTTTCTTGAACGAATTTTTTTCTATGGAAAAATAGAACATTTTGCAGAAGGTTT TGCTAATCATTTTCAGACCCTCCTATGGTTGGTCAAGGATCCTTTGATGCATTATG TTAGATATCAAGGAAAATCAATTCTGTCTTTAAAAGATAAGCCCTTTCTGATGAA AAAATGGAAATATTACCTTGTCAATTTATGTCAATGTCATTTT TATGTGTGGTTTCAACCAGAAAAGATCTATATCAATTCATTATCCAAAAATTTTCT CTCGTTTTTGGGCTATCTTTCAAGTGTACAAAGCAATTCTTTGGTAGTACGGAGTC AAATGCTAGAAAATTCATATCTAATAGATAAAGATAATACTATGAAGAAACTCG ATACAATAGTTCCAATTCTTCCTTTAATTAGATTATTGGCAAAAACGAAATTTTGT AACGCAGTAGGACATCCTATTAGTAAAGCGATCCGGACTCATTCATCCGATTCTG ATATTATCGACCAATTTGTCCGTATATGCAGAAATTTTTCTCATTATTATAGT (**750 bp**)
OQ915163	***Gynura crepidioides*** isolate **NU-BOT-TIS-GCI** internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	TCGAAGCCTGCGTAGCACAACGACCCGTGAACATGTAACAACAACYGGGTGTCC GTTGTATCGAGCCCTTGCTTGGTTCTTTGGATGCCATGTTGATGTGTGTCTTTGGT ACTCCTCTTGGGTTCCCTTAGGCGTCACATTGACACAAGAACAAACCCCCGGCAC GGCATGTGCCAAGGAAAAATTAACGTAAAAAGGGCTGGTATCGTGCGTCATCGT TYGTGATGATTGCATGGAATCTTGCTTCTTTATAATCATAAACGACTCTCGGCAA CGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTG GTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCG AAGCCTTTTGGTTGAGGGCACGTCTGCCTGGGCGTCACATATCGCGTCGCCTCCT ATCATACCTCTTGACGGGGATGCTTGGATGGRGGCGGAGATTGGTCTCCCGTCCT TAAGGTGCGGTTGGCCTAAACAGGAGTCCTCTTCGAAGGATGCACGATTAGTGGT GGTTGAAATGACCCTCTTATCGAGTCGTGTGTTCCAAGGAGTAGGGAAGATCCCT TYGATGACCCTAACGTGTCGTCTCATGACGATCCTT CGACTACTAAA (**648 bp**)

OQ913368	***Gynura crepidioides* NU-BOT-TIS-2** ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds; chloroplast	GCATTTTTTATGTCCCCCCCAACAGAAACTAGAGCGAGTGTTGGATTCAAAGCTG GTGTTAAAGATTATAAATTGACTTATTATACTCCTGAGTATGAAACCAAGGATAC TGATATCTTGGCAGCATTTCGAGTAACTCCTCAACCAGGAGTTCCGCCTGAAGAA GCAGGGGCCGCAGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGA CCGATGGACTTACGAGCCTTGATCGTTACAAAGGCCGATGCTATGGAATCGAGCC TGTTCCTGGAGAAGAAAATCAATTTATTGCTTATGTAGCTTACC CATTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGT AATGTATTTGGGTTCAAAGCCCTGCGTGCTCTACGTCTGGAAGATTTGCGAATCC CTACTGCGTATGTTAAAACTTTCCAAGGTCCGCCTCACGGTATCCAAGTTGAGAG AGATAAATTGAACAAGTATGGTCGTCCTCTATTGGGATGTACTATTAAACCTAAA TTGGGTCTATCCGCTAAAAACTACGGTAGAGCTGTTTATGAGTGTCTTCGTGGTG GCCTTGATTTTACTAAAGATGATGAAAACGTAAACTCCCAACCATTTATGCGTTG GAGAGACCGTTTCTTATTTTGTGCCGAAGCTATTTATAAATCACAAGCTGAAACA GGTGAAATCAAAGGGCATTACTA (**728 bp**)
OQ658381	***Gynura crepidioides*** maturase K gene, partial cds; chloroplast	TTCTGTATATACGCCCAAAGTGCTCAATAATATCAGAATCTGAGAAATCGGCCCA AATCGCCTTACCAATAGGACGCCCCACTGCGTTACAAAATTTAGATTTAGCCAAT GATCCAATCATTTCTAGCATTTGACTGCGTACCATTGAAGGCTTTAGCCGCACAC TTGAACGATAACCCAGAAAGTCAAGGGAATGATTGGATAATTGGGTTATATAAA TCCTTTCTGGTTGAGACCACAGGTAAAAATGGGATTTCCAGAAATTGACAAAGTA ATATTTCCATTTATTCATCAAAAGAAACGTCCCTTTTGAAGCAATAATTGATTTTC CTTGATACCTAACACAATGCATGAAAGGATCTTTGAACAACCATAAATTCGCTTG AAAAGTCCTGGCAAAGACTTCTGCAAGATGCTCCATTTTTACATAGAAATATATT CGTTCAATAAGGGCTACAGAAGATGTTGATCGTAAGTGAGAAGATTGGTTACGG AGAAATAGGAAGCTAGATTCATATTCACATACATGAGAAGTATATAGGAAGCAG AATAGTCTTTTATTTATTTTTGAAAAAGAAGAACTGGCTTTCCTTGAATTTGAAGT AATAAGACTATCCCAATTATGACACTCATGGAAAAAGAATCTTAATAAATGCAA AGAGGAAGCATCTTTTATCCAATAGCGAAGAGCCTG (**694 bp**)
OP010046	***Kalanchoe pinnata*** voucher **NU-BOT-LUM- TIS- CRD-6** internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence	GACCGCGGACAGTTGTTAACCGGTGAAGCGTCGGGATCCCTCGGGCTCCCATCGC CGACCCGCTCGGCCGGCGCGGAGTGCCGCACCCCGCGCCCGCCGAATCTAACGA ACCCGGAGCGGACAGCGCCAAGGATTAAGAAAGGAAGAGTTTGTCCCCCGCGCC CGTCCGCGGGCGTCGGAGGGGAGAGGGTCTGTTCAGAAAACAGTAACGACTCTC GGCAACGGATATCTCGGCTCTCGCCTCGATGA (**249 bp**)
OQ913365	***Kalanchoe pinnata* NU-BOT-TIS** ribulose 1,5-bisphosphate	AGTATGAAACCAAGGATACTGATATCTTAGCAGCATTCCGAGTAACTCCTCAACC TGGAGTTCCACCTGAGGAAGCAGGGGCTGCGGTAGCTGCTGAATCTTCTACTGGT

	carboxylase/oxygenase large subunit gene, partial cds; chloroplast	ACATGGACAACTGTGTGGACTGATGGACTTACCAGCCTTGATCGTTACAAAGGAC GATGCTACCACATCGAGCCCGTTGCTGGAGAAGAAAATCAATATATTGCTTATGT AGCTTATCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCA TTGTGGGTAATGTCTTTGGGTTCAAAGCTCTGCGTGCTCTACGTCTGGAGGATCTG CGAATTCCTGTTGCTTATGTTAAAACTTTCCAAGGACCACCTCATGGTATTCAAGT TGAAAGAGATAAATTGAACAAGTATGGGCGTCCTCTATTGGGATGTACTATTAAA CCGAAATTGGGATTATCTGCTAAGAACTATGGTAGAGCAGTTTATGA ATGTCTACG (**499 bp**)
OQ913373	***Kalanchoe pinnata*** isolate **NU-BOT-TIS-KP** maturase K gene, partial cds; chloroplast	GTATGATAATTGTAATAGCCTTATTACACTAAATAAATCCATTTCTTTTTTTTTTTT TAAAAGGAATCCAAAATGTTTCTTGTTCCTCTATAATTATCATGTATGTGAATCCG AATCCATCCTCGGTTTTATTTGTAACCAATCTTTTCATTTACGATCAACATCTTTTG GAGTTTTTTTTGAGCGAATCCATTTTTATGGAAAAATCAAAAAACTTCTTGTAGA AGTCTTTTCTATTCAAACCAAGCTAGGGTTGTTCAAGGATCCTTTCATCCATTATG TTAGGTATCAAGGAAAAGCCTTTTTGGGCTCAAAAGGAACGCCTTTGCT GATGAGTAAATGGAAATATTACCTTATCAATTTATGGCAATGTCATTTTTACGTGT GGGTTCAACCGGGAAGCGTTCATATAAACCAGTTATCCAAACATTCTTTAGACTT TATGGGTTATTTGTCGAATCCGGGACTAACTCTTTCAGTTGTACGGAGTCAACTAT TAGACAATTCATTTATAATTAATAATGGTATTAAGCAGTTATCTATTATAGTTCCC ATTTTTTCTTTTATTGCATCATTGGCTAAAGCAAAATTTTGTAATGTATTTGGGCA TCCCATTAGTAAACCCGCCTGGACTGATTCAGCTGATTCGGATATTATCGACCGA TTTGTGTGGCTATGCCGAAATCTTTCTCATTAT (**697 bp)**
OP954650	***Mucuna pruriens*** voucher **NU-BOT-TIS-CRD-ITS-1-MP** small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence	TGAACGGGTTCGCCGGTCGAACTTGTTGTGAGGCAACGATAACAAGGTTTCCGTA GGTGAACCTGCGGAAGGATCATTGTCGTTGCCTCACAACAAGTTCGACCGGCGA ACCCGTTCATCACAGGACAAGTTCGATCGGGGCTGGCTCGGGGGAGCTGTTCTCG AACACCGACCCCGTCCTCCCCGACCCGAGCTGGCGAGAGGCGGTCGCCCCGCGC ACCTCCTCTCGCCAAAACACAAACCCCGGCGCTTCGTGCGCCAAGGAACTCGA AACTGTTAAGTTGCAATGGTTCGCGGGCCCGGAGACGGCGACCCCGCGGACCT TGCCACGACACACAACATACAAAATGACTCTCGGCAACGGATATCTCGGCTCTT GCATCGAT (**386 bp**)
OQ916146	***Mucuna pruriens*** isolate **NU-BOT-TIS-MPR** ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	GTTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTT GGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTGAAGAAGCAGGTGC CGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTATGGACCGATGGG CTTACCAGTCTTGATCGTTACAAAGGACGATGCTACCACATCGAACCTGTTGCTG GAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGA

		AGGTTCTGTTACTAACATGTTTACTTCCATTGTCGGTAATGTATTTGGGTTCAAGG CACTGCGCGCTCTACGTCTGGAGGATTTACGAATCCCTACTTCTTATATTAAAACT TTCCAAGGTCCGCCTCAGGGCATCCAAGTTGAGAGAGATAAATTGAACAAGTAT GG (**442 bp**)
OQ916150	***Mucuna pruriens*** isolate **NU-BOT-TIS-MPM** maturase K (matK) gene, partial cds; chloroplast	GTAATCCAAGATTATTCTTGTTCCTATATAATTTATATGTATGGGAATACGAATCT ATCTTTCTTTTTCTTCGTAACAAATCCTCTCAGTTACGATTCAAATATTTTCGCGTT TTTTTTGAACGAATTTTTTTCTATGAAAAAATAGAACATCTTGTAGAAATATTTGT TAAGGATTTTTCGTATACCTTATCATTCTTCAAGGATCCCTTCATCCATTATGTTA GATATCGAGGAAAATCGATTCTGGTTTCAAAGAATACGCCTCTTTTGATAAATAA ATGGAAATACTATTTTATCTATATATGGCAATGTCATTTTAATATT TGGTCTCAACCAGGAACGATTGATATAAACCAATTATCTCAGCATTCATTTCGCC TTTTGGGTTATTTTTTAAGTATTCGGCTAAATCTTTCAGTGGTACGAAGTAAAATG TTGCAAAATTCATTTCTAATTCAAATTGTTATGAAAAAGCTTGATACAATAGTTCC AAAAAA (**499 bp**)
OQ915232	***Paederia foetida*** isolate **NU-BOT-TIS-PFI** small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	GTTTCCGTAGGTGACCTGCGGAAGGATCATTGTCGTATCCTGCGAACCACCGCGA ACGCGTTAACCAAACCGTCGGGGCGCCCTCGGGCGTCCCGGCCCCAAACGAACT CTCCGGCGCGAGAAGCGCCAAGGACCACTTGAAACGGACCGCCCGCCCCTCCCG CGGATTCCGCGGGGGGAGCGACGGCGTCTGAGTTGTAACAAAAACGACTCTCGG CAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTT GGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCC GAAGCCATTAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCTCGTCGCCACC CCCTCCCTCACCCCTCGCGGGACAAGGAAGGGGGCGGCGGAGGATGGCCTCCCG TGCCACTCGGCGCGGCCGGCCCAAACGAGAGTCCCCGGCGCAGGACGCCGCGAC GATGTGGTGGTTGAACTCCTCAGCACGATCGACGTCGCGGCCCGGCCCGGCGGA ACGCCGAGACCCCGAGGCCTCCCCTCCCGGAAG GGAGGAAGGCCCCTCGAACGCGACCCCAGTAAA (**609 bp**)
OQ913369	***Paederia foetida*** **NU-BOT-TIS-3** ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds; chloroplast	TCACCACAAACAGAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAG TACAAATTAACTTATTATACTCCTGAATACGAAACCAAAGACACTGATATCTTAG CAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCACCGGAAGAAGCAGGGGCCG CGGTAGCTGCCGAGTCTTCTACTGGTACATGGACAACTGTATGGACGGATGGACT TACCAGTCTTGATCGTTACAAAGGGCGATGCTACCATATTGAGCCAGTTCCTGGA GAAGAAGATCAATTTATTGCTTATGTAGCTTACCCATTAGACCTTTTTGAAGAAG GTTCTGTTACTAACATGTTTACTTCCATCGTAGGTAATGTATTTGGGTTCAAAGCC CTGCGCGCCCTACGTCTGGAAGATTTGCGAATTCCCATTGCTTATGTTAAA

		ACCTTCCAAGGACCGCCTCATGGCATTCAGGTCGAGAGAGATAAATTGAACAAG TATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAAATTAGGTTTATCTGCTAA AAACTACGGTAGAGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACCAAA GATGATGAAAACGTGAACTCCCAACCATTTATGCGTTGGAGAGATCGTTTCTTAT TTTGTGCCGAAGCACTTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGC ATTTTT (**715 bp**)
OQ913372	***Paederia foetida*** isolate **NU-BOT-TIS-4** maturase K gene, partial cds; chloroplast	ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAGCCAGTCTTTC TATATCCACTTATCTTTCAGGAGTATATTTACGGACTTGCTCATGATTATAGTTTA AACCGATCTCGTTTGTTGGAAAATCCAGGTTATGATAATAAATACAGTTTCCTAC TTGTGAAACGTTTAATTACTCGAATGTATCGACAAAATCATTTT ATTATTTTTGCTAATGATTCTAATAAAAATCTATTTTTTGGTCGCAACAAGAATTT CTACCCTCAAACGATATCAGAAGGATTTGCATTTATTGTGGAAATTCCATTTGAT ATACGATTAATATCTTCTCAAGAAGGGAAAAGAATATTCAAATCTCACAATTTAC GATCAATTCATTCCCTATTTCCTTTCTTAGAGAACCATTTTTTC CATTTTAATTCTGTATTAGATATATTAATACCGCGGGCGGTTCATCTGGAAATTAT GGTTCAAACCCTTCGTTATTGGGTAAAAGATGCCTCCGCCTTGCATTTATTACGAT TATTTTTCCACGAGTATTGGAGTTGGAATACTCTTAGTGTTACAAAGAAACTCCA TTTTGATTTTTCACCAAAAAGAAATCAAAGATTTTTTTTCTTATTATATAATTCTC ATGCATATGAATACGAATCCATTTTGGACTTTCTGCGTAACGAATCTTCTCATTTG CGATCAACATCTTTTGTATTTTTTCTTGAACGCCTCTCTTTTTATGGAAAAAAAGA ACGTCTTGTAGAAGTCGTTGCTAAGGATTTTGGGGTGAGTCTATGGCTGTTCATA GACCCTTTCGTGCATTATGTGAGATATCAAGGAAAATCCATTTTGGTTTCAAAGG GTACACCTCTTTTGATGAATAAATGGAAATCTTATCTTGTCAATTTTTGGCAATAT CACTTTGATCTGTGGTTTCACTCGGGAAGGGTTTGTATAAATAAATTTCCCAACC ATTCACTTTACTTTATGGGTTATCTTTCAAGTGTGCGACTAAATCCGGTAATGGTA CGGGGTCAAATGCTAGAAAATGCATTTCTAATCCATAATGCTATTAAGAAATTGG ATACACTTGTTCCAATTATTCCTCTTATTCGATCGTTGTCTAAATCTAAATTTTGTA ACCCATTAGGACATCCCATTAGTAAGGCGGTTTGGACTGATTTATC AGATTCGGATATGATTGACCGATTTGGGTATATATGCAGAAACCTTTCTCATTATT ATAGTGGGTCTTC (**1259 bp**)
OQ915147	***Passiflora edulis*** isolate **NU-BOT-TIS-PEI** internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal	GGGCGGCCCGTCGCGTGCCGGAAACGGATCTCTCGCGGGCGGCCCTTTCTCCTTC GGAAACAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAA CGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTC TTTGAACGCAAGTTGCGCCCGAAGCCTTCGGGCCAAGGGCACGTCTGCCTGGGTG

	transcribed spacer 2, partial sequence	TCATGCATCGTCGCCCCCCCATCCTTCCGTCTCCCCCGAGGGGAAAGGGGGTACG GGGCGGGCGGAGATTGGTCTCCCGTGCGCTCCCGCTCGCGGTTGGCCGAAATACG AGTTGTTGGCGGCCGAGAGCGCCACGGCAAGCGGTGGTTGTCAAAGACCTTCGG AGATTGCCGCCGGCGAGGCTGTCACGGGGGGG (**415 bp**)
OQ916149	***Passiflora edulis*** isolate **NU-BOT-TIS-PER** ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	ATGTCACCACAAACAGAGACTAAAGCAAGTGTTGGATTCAAGGCTGGTGTTAAA GATTATAAATTGACTTATTATACTCCTGAATATAACCCCAAAGATACTGATATCTT GGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCCGCTGAGGAAGCGGGAGC TGCAGTAGCTGCTGAATCTTCTACTGGTACATGGACAACCGTGTGGACCGATGGG CTTACCAGTCTTGATCGTTATAAAGGACGATGCTACGATATTGAACCCGTTGCTG GAGAAGAAAGTCAATTTATTGCTTATGTAGCGTACCCCTTAGACCTTTTTGAAGA AGGTTCCGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG CCCTACGCGCTTTACGTCTGGAAGATTTGCGAATCCCCACTGCTTATACTAAAAC GTTTCAGGGCCCACCTCATGGCATCCAAGTTGAGAGAGATAAGTTGAACAAGTAT GGTCGCCCTCTATTGGGTTGTACTATCAAACCTAAATTGGGGTTATCCGCTAAGA ATTACGGTAGAGCTGTTTATGAGTGTCTCCGCGGTGGACTTGATTTTACAAA (**602 bp**)
OQ921094	***Passiflora edulis*** isolate **NU-BOT-TIS-PEM** maturase K (matK) gene, partial cds; chloroplast	CATTTATTACGACTCTTTCTTCACGAGTATGGGAATTGGGACCCGTTTATTATTTC AAAGAAATTGAGTTCGATTTTTACGAAAAGTAATCCAAGATTTTTCTTATTCCTAT ATAACTCTTATATATATGAATACGAATCCGTCTTATTTTTTCTTCGTAAGCAATCC TCTCATTTACGATCAACATTTTATCGGGTCTTTCTTGAGCGAATATTTTTCTATGG AAAAATAGAATATTTTGCGGAAGTCATTGCTAATGATTTTGGGGCCACCCTATCC TTGTTCAAGGATTTTTTCACACATTATATTAGATATCAAGGCAAATCCATTCTGGC TTCAAAGAATCCCCCTCTTCTGATGAAAAAATGGAAATATTATCTTGTCATTTTCT GTCAATGTCATTTTGATGTGTGGTTTCCACCGGAAAAGATCCATATAAACTCATT ATCCAAACATTCTTTTAGCTTTTTGAGCTATCTTTCCAGTGTACGACTAA ATCTTTCAGTAGTACGGAGTCAAATGCTAGAAATTTCATTTCTAATAGATAATGC TATGAATAACCTAGATACAGTAGTTCCAATTATTCCTTTAATTGGATCTTTGGCAA AAATGAAATTTTGTAATGCCGCGGGACATCCTATTAGTAAACCGATCCGGACTGA TTCATCGGATTCTGATATTATTCATCGA TTTGTACGAATATGCAGAAATCTTTCTCA (**719 bp**)
OQ915234	***Perilla frutescens*** isolate **NU-BOT-TIS-PFFI** small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal	CTTATCATTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCG GAAGGATCATTGTCGAAACCTGCAAAGCAGACCGCGAACACGTGTTTAACATCA TCGGACACGGCGTGGGGGAGACTCCCGTCGTGCACCGCTCCCGCCGGAGTGCGC CCTCGGGCGTCGCACCGTGCGGGCTAACGAACCCCGGCGCGGCAAGCGCCAAGG

	RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	AAAACAAAATTTAGCGCCCGCCTTCCGCATCCCGTTCGCGGGGTGTGCGGGGGG AATGGACGTCTATCGAATGTCATAACGACTCTCGGCAACGGATATCTCGGCTCTC GCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCC GTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGG CACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCTCCCCGCGCTGAGCGCTC GTGAGGGGGGCGGATATTGGCCCCCCGTGCGCCCTGGCGTGCGGTCGGCCCAAA TGCGATCCCTCGACGACTCGTGTCGCGACTAGTGGTGGTTGAATAGCTCAATCTC GTGTCTTGTCGTGCTACCGCGTCGTCCGAATGGGAATCGAACAACGACCCAACGG TGTTCGTGCGTTACCGCACCGCACCTTCGACCGCGACCCCAGGTCAG GCGGGATTACCCGCTGAGTTTAAGCATATCACTTAAA (**737 bp**)
OQ921088	***Perilla frutescens*** isolate **NU-BOT-TIS-PFR** ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	TTGACTTATTATACTCCTGAATACGAAACCAAAGATACTGATATCTTGGCAGCAT TTCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGTAG CTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAG CCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTATTGGAGAAAAA GATCAATATATCTGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGT TACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGCCCTACGTG CTCTACGTCTGGAAGATCTGCGAATTCCTACTGCTTATATTAAAACTTTCCAAGGC CCGCCTCATGGGATCCAAGTTGAGAGAGATAAATTAAACAAGTACGGTCGTCCTC TGCTGGGATGTACTATTAAACCTAAATTGGGGTTATCT GCTAAAAACTATGGTAGAGCGGTTTATGAATGTCTTC (**517 bp**)
OQ921093	***Perilla frutescens*** isolate **NU-BOT-TIS-PFM** maturase K (matK) gene, partial cds; chloroplast	GTTTCTTCTTTGCATTTATTACGAGTCTTTCTCAATGAATATTGTAATTGGAATAG TCTTATTACTCCAAAGAAAGTAAGCTCCTCTTTGTCAAAAAGAAATCAAAGACTC TTTTTTTTCTTATATAATTCTTACGTATGTGAATACGAATCTGTTTTCGTCTTTCTA CGTAACCAATCTTTTCATTTACGATCAACATCTTCTGGAGTTCTTCTTGAACGAAT CTATTTTTATATAAAAATAAAAACAGAACGTCTTGTGAACGTCTTTGTTTTTGTTA AGGATTTGCGGGCGAACCTAGGGTTGCTCGAGGAACCCTGTATGCATTATATTAG GTATCAAAGAAAATCCATTCTGGCTTCAAAAAGGACATCTCTTTTCATGAATAAA TGGAAATTTTACCTTGTCACTTTTTGGCAATGGCATTTTTCGGTGTGGTTTCATCC AAGAAGGATTTGGATAAATCAATTTTCCAAGCATTCCCTTGAAATTTTGGGCTAT CTTTCAAACGTGCAAACGAACCCTTCCGTCGTGGTACGGAGTCAAATTCTAGAAA ATTCATTTCTAATCAATAATACTATTAAGAAGCTCGATACCCTTGTTCCAATTATT CCTCTGATTGCGAAATTGGCTAAAGCGAAATTTTGTAATGTATTGGGGCATCCTA TTAGTAAGCCGGTTCGGGCTGATTTATCAGATTCTAATATTATTGACCGATTTGGG CATATATGCAGAAATCTTTCTCATTATTATAGCGGATCTTCCAAAAA

		AAAGAGTTTGTATCGAATAAATTATAT (**797 bp**)
OQ915353	***Solanum nigrum*** isolate **NU-BOT-TIS-SNI** small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	TGGATAGTAAAAAAGCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCA TTGTCGAAACCTGCAAAGCAGAACGACCCGCGAACACGTTCAAACACCGGGGG AGCAGCGCGGCGCGGGTGCTTCGGCGTCCCTCCGCGCGCGTTCCCCCTCGTCCCC GGCTCGTTCCGGGCGACTAACGAACCCCGGCGCGAAAAGCGCCAAGGAATACTT AAACTGAGAGCCCTCCCCTCGCGCCCCGTCCGCGGAGTGTGCGGGGGGATGCGC GCTTCTTTTGAAACCAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG ATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAAC CATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACGTCT GCCTGGGCGTCACGCATCGCGTCGCCCCCCGAACGCCGCAAGGCGTCGTGGGGC GGATACTGGCCTCCCGTGCGCCTCGAGCTCGTGGCTGGCCTAAATGCGAGTCCA CGTCGACGGACGTCGCGGCAAGTGGTGGTTGAAACTCAACTCTCTTTGTGTCGCG GCTACAGCCCGTCGCGCGTCCGGACTCCAGACCCTCTAAGCGCTTAGGCGCTCCG ACCGCGACCCCAGGTCAGGCGGATTCTAAGTTACGCTTTTTTACTATC CAAAACTT (**708 bp**)
OQ921089	***Solanum nigrum*** isolate **NU-BOT-TIS-SNR** ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	AAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCT GAGTACCAAACCAAGGATACTGATATATTGGCAGCATTCCGAGTAACTCCTCAAC CTGGAGTTCCACCTGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGG TACATGGACAACTGTATGGACCGATGGACTTACCAGTCTTGATCGTTACAAAGGG CGATGCTACCGCATCGAGCGTGTTGTTGGAGAAAAAGATCAATATATTGCTTATG TAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCCGTTACCAATATGTTTACTTCC ATTGTAGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTGGAAGATC TGCGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGTCCGCCT CATGGGATCCAAGTTGAAAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGG GATGTACTATTAAACCTAAATTGGGGTTATCTGCTAAAAACTATGGTAGAGCTGT TTATGAATGTCTT (**553 bp**)
OQ921092	***Solanum nigrum*** isolate **NU-BOT-TIS-SNM** maturase K (matK) gene, partial cds; chloroplast	AACATTCCTTTTTTAGAGGACAATTTTTCACATCTAAATTATGTATTAGATATACT AATACCCTACCCCGTTCATCTGGAAATCTTGGTTCAAACTCTTCGCTATTGGGTAA AAGATGCCTCTTCTTTACATTTATTACGATTCTTTCTCCACGAATATTGTAGTCTT ATTACTTCAAAGAAGCCCGGTTACTCCTTTTCAAAAAAAACT CAAAGATTCTTCTTCTTCTTATATAATTCTTATGTATATGAATGCGAATCCACTTT CGTCTTTCTACGGAACCAATCTTCTCATTTACGATCAACATCTTTTGGAGCCCTTC TTGAACGAATATATTTCTATGGAAAAATAGAACGTCTTGTAGAAGTCTTTGCTAA GCATTTTCAGGTTACCCTCTGGTTATTCAAGGACCCTTTCATCCATTATGTTAGGT

		ATGAAGGAAAATCAATTCTGGCTTCAAAAGGGACGTTTCTTTTGATGAATAAATG GAAATTTTACCTTGTCAATTTTTGGCAATGTCATTTTTCTATGTACTTTCACACAG GAAGGATCCATATAAACCAATTATCCAACCATTCCCGTGACTTTATGGGCTATCT TTCAAGTGTGCGACTAAATCATTCAATGGTACGTAGTCAAATGTTAGAAAATTCA TTTCTAATCAATAATCCAATTAAGAAGTTCGATACCCTTGTTCCAATTATTCCTTT GATTGGATCATTAGCTAAAGCACACTTTTGTACCGTATTAGGGCATCCCATTAGT AAACCGGTTTGGTCCGATTTATCAGATTCTGATATTATTGACCGATTTGGGCGTAT ATGCAGAAATCTTTTTCATTATTATAGCGGATCTTGCAAAAAAAAGACTTTATAT CGAATAAAGTAT (**888 bp**)
OQ915375	***Solanum trilobatum*** isolate **NU-BOT-TIS-STI** internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	TCGAAACCTGCACAGCAGAACGACCCGCGAACACGTTCAAACACCGGGGGAG CCGCGCGGCGCGGGGCGCTCCGGCGCCGCCCCGCGCGTCTCCCCCTCGCCCCCT CCTCGGGGGGCCAAACGAACCCCGGCGCGAAAAGCGCCAAGGAATACTCAAAC GAGAGCCCTCCGCCCGTGCCCCGTCCGCGGGGCGTGCGGGCGGATGCGTGCTT CTTTCGAAACCAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGA AGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATC GAGTCTTTGAACGCAAGTTGCGCCCGAAGCCGTCAGGCCGAGGGCACGTCTGCCT GGGCGTCACGCATCGCGTCGCCCCCCCGCACGCCGCTCGGCGTCGCGGGGGCGGA TACTGGCCTCCCGTGCGCCTCGCGCCCGCGGCCGGCCTAAATGCGAGTCCACGTC GACGGACGTCGCGGCAAGTGGTGGTTGTAACTCAACTCTCTTGGTGCCGCGGCCA CAGCCCGTCGCGCGTGCGTGCTCCACGACCCTGCCGGCGCCAGCGCGCTCCGACC CCC (**598 bp**)
OQ921090	***Solanum trilobatum*** isolate **NU-BOT-TIS-STR** ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplas*t*	AAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATA CTCCTGAGTACCAAACCAAGGATACTGATATATTGGCAGCATTCCGAGTAACTCC TCAACCTGGAGTTCCACCTGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCT ACTGGTACATGGACAACTGTATGGACCGATGGACTTACCAGTCTTGATCGTTACA AAGGGCGATGCTACCGCATCGAGCGTGTTGTTGGAGAAAAAGATCAATATATTG CTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCCGTTACCAATATGTTT ACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTGG AAGATCTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGTCCGCCTCATGG GATCCAAGTTGAAAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGT ACTATTAAACCTAAATTGGGGTTATCCGCTAAAAACTACGGTAGAGCTGTTTATG AATGTCTTCGCGGTGGACTTGATTTTACCAAAGATGATGAGAACGTGAACTCACA ACCATTTATGCGTTGGAGAGATCGTTTCTGCTTTTGTGCCG AAGCCCTTTTTAAAGCACAGGCTGAAACAGGTGAAATCAAAGGACATTACTTGA

		ATGCTA (**706 bp**)
OQ921091	***Solanum trilobatum*** isolate **NU-BOT-TIS-STM** maturase K (matK) gene, partial cds; chloroplast	GAATATTGTAATTCGAATAGTCTTATTACTTCAAAGAAGCCCGGTTACTCTTTTTC AAAAAAAAATCCAAAATTCTTCTTCTTCTTATATAATTCTTATGTATATGAATGCG AATCTACTTTCGTCTTTCTACGGAAACAATCTTTTCATTTACGATCAACATCTTTT GGAGCCCTTCTTGAACGAATATATTTCTATGGAAAAATAGAACGTCTTGTAGAAG TCTTTGCTAAGGATTTTCAGGTTACCCTATGGTTATTCAAGGATCCTTTGATGCAT TATGTTAGGTATGAAGGAAAATCAATTCTGGCTTCAAAAGGGACGTTTCTTTTGA TGAATAAATGGAAATTTTACCTTGTCAATTTTTGGCAATGTCATTTTTCTATGTAC TTTCACACAGGAAGGATCCATATAAACCAATTATCCAACCATTCCCGTGACTTTA TGGGCTATCTTTCAAGTGTGCGACTAAATCATTCAATGGTACGTAGTCAAATGTT CGAAAATTCATTTCTAATCAATAATCCAATTAAGAAATTC GATACCCTTGTTCCAATTATTCGTTTGATTGGATCATTAGCTAAAGCACACTTTTG TACCGTATTAGGGCATCCCATTAGTAAACCGGTTTGGTCCGATTTATCAGATTCTG ATATTATTGACCGATTTGGGCGTATATGCAGAAATCTTTTTCATTATTATAGCGGA TCTTCCAAAAAAAAGACTTTATATCGAATAAAGTATATACTTCG (**752 bp**)

List of Publications

1. Deb, C.R. and Sharma, T.I. 2021. Ethnomedicinal plants with anti-diabetic properties used by tribes of Nagaland, India: A review. Journal of Pharmacognosy and Phytochemistry. 10(6), 216-219.
2. Deb, C.R., Sharma, T.I. and Jamir, N. 2023. Ethno-medicinal anti-diabetics plants of Northeast India: A review. Journal of Pharmacognosy and Phytochemistry. 12(3), 86-110. https://doi.org/10.22271/phyto.2023.v12.i3b.14668.
3. Sharma, T.I., and Deb, C.R. 2024. Molecular characterization of some potential ethnomedicinal plants used for treatment of diabetes in Nagaland, India. South African Journal of Botany. 172, 140-150. https://doi.org/10.1016/j.sajb.2024.07.024.
4. Sharma, T.I., and Deb, C.R. Exploring the antidiabetic potential phytochemicals and antioxidant activities of some ethnomedicinal plants used for management of diabetes in Nagaland. Phytomed Plus. (Under Revision).
5. Sharma, T.I., and Deb, C.R. *In vitro* evaluation of α- amylase and α- glucosidase inhibitory assays of ten ethnomedicinal plants: insights into their hypoglycemic properties. Phytomed Plus. (Under Revision).
6. Sharma, T.I., Giri, B., Mootapally, C., Nathani, N., Desai, R., Mahajan, M., Deb, C.R. Comprehensive *in vitro* assessment of *Abroma augustum* and *Bauhinia variegata* extracts: enzyme inhibition, cytotoxicity, and glucose uptake studies. Phytomedicine. (Under Communication).
7. Sharma, T.I., Giri, B., Mootapally, C., Nathani, N., Desai, R., Mahajan, M., Deb, C.R., Exploring α-amylase and α-glucosidase inhibition, cytotoxicity, and glucose uptake in extracts of three ethnomedicinal plants of Nagaland, India: An *in vitro* assessment Journal of Ethnopharmacology. (Under Communication).

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Ethnomedicinal plants with anti-diabetic property used by tribes of Nagaland, India: A review

Chitta Ranjan Deb and T Ibeeka Sharma

Abstract
Nagaland is a state of North Eastern region of India and a part of Indo-Myanmar biodiversity hotspot. The state is very rich in flora and fauna due to favorable agro-climatic condition. Tribes of Nagaland have been using various plants to treat different kind of diseases including diabetes. Since herbal drugs have lesser or no side effect, plant based herbal drugs are very popular in the state. This communication intended to review of the past researches in the state on use of different plant and plant parts for treating/controlling diabetes. Systematic review of literatures reveals that till date a total of 47 plants belonging to 29 families are used as anti-diabetic by different tribes in the state. Though, these plants/parts are being used for anti-diabetic ethnomedicinal purpose, there is no or very limited information available on characterization of anti-diabetic potential active molecules/compounds from these plants, thus warrants isolation and identification of anti-diabetic compounds from these plants.

Keywords: Ethnomedicinal plants, tribes, diabetes, Nagaland

1. Introduction
Diabetes is a chronic, complex metabolic disorder with numerous acute and chronic consequences [1]. The body cells cannot metabolize carbohydrate properly and there is abnormal increase in blood sugar level resulting from insulin deficiency. The pathogenic process of development of diabetes includes autoimmune destruction of beta cells of pancreas and abnormalities in metabolism [2]. Failure of organ systems such as heart, blood vessels, eyes, kidney and nerves are the long-term effect of diabetes which can ultimately leads to death [3]. Sign and symptoms of diabetes include weight loss, polyuria, hypertension, blurred vision, polydipsia, polyphagia, tachycardia [4]. Here are two type of diabetes: Type 1 is absolute deficiency of insulin. Here, Islets of Langerhans in pancreas that produce β cell are destructed autoimmune. Type 2 is relative deficiency resulting from impaired insulin secretion and resistance its action [5]. By 2020, 34.2 million have been diagnosed with Diabetes [6]. The severe complicacy as a result of diabetes includes cardiovascular diseases, retinopathy, nephropathy, peripheral vascular disease, neuropathy, stroke etc. Out of 14166 patient, a total of 356 death occurred every year [7]. According Centers for Disease Control and Prevention, the cause for Type 1 diabetes is unclear but a having them in family history is a risk factor and there are no preventive measures for Type 1 diabetes. For type 2 diabetes, risk factors include overweight, older than 45 year, physically inactive for less than 3times a week or have a history of diabetes in the family. Type 2 diabetes can be prevented with losing weight, eating healthier and getting regular physical exercise. By the year 2000, India has the highest record in diabetes with 31.7 million diabetes patients [8].
The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. Type 1 is usually treated with exogenous insulin and Type 2 with oral hypoglycaemic agents [9]. Plants based diet are rich in fiber, antioxidants and they reduce insulin resistance, inhibit glucose absorption, enhance glucose uptake and also promote weight loss. Limiting the intake of red meat and increasing plant in diet reduce the risk of cardiovascular disease leading from diabetes and since they are low in saturated fat, accumulation of toxic fat in hepatic and muscle cells that cause impairment in insulin signaling is also reduced [10]. Herbal drugs have lesser or no side effects and are less expensive as compared to synthetic drugs. Herbal medicinal plants with antidiabetic properties can induce release of insulin in Islets of Langerhans in pancreas and also act as insulin sensitizer [11]. Therefore, identification and isolation of anti- hyperglycemic compounds from the plants has become more and more important these days. The ethno-botanical information reports about 800 plants that may possess anti-diabetic potential [12]. Several anti-diabetic plants has been confirm for its hypoglycemic effect and mechanism of hypoglycemic effect are being studied [13]. Glycosides, alkaloids, terpenoids, flavonoid, carotenoids etc. are frequently implicated to

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Ethno-medicinal anti-diabetics plants of Northeast India: A review

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Abstract
With its large population, lifestyle and socio-economic transition due to urbanization, India is becoming the epicentre for diabetes. Diabetes is one of the leading reasons for world economic loss. Abnormal production of lipids and proteins and oxidative stress in the body are the primary etiology of diabetes. With the high cost of treatments and relatively large side effects, many patients are suffering. Utilizing and properly addressing herbal medicinal plants have thus become the need of the hour. Plants contain various compounds which are effective in increasing the performance of insulin secretion as well as reducing glucose levels in the human body. People of the northeast have been depending on herbal treatment for various diseases since time immemorial. Documenting and studying of ethnic expertise of medicinal plants of northeast India will be a new dimension in managing diabetes. This review aims to compile all published works from North East India on using various ethnomedicinal plants as anti-diabetes.

Keywords: Anti-diabetic plants, diabetes, ethnomedicinal plants, tribes, Nagaland, North East India

1. Introduction
Diabetes is a disorder with various metabolic impairments known to humankind for ages. However, still, it is one of the most challenging medical conditions which need to be adequately addressed medically for better treatment of patients with diabetes. Around 62 million people have been affected by diabetes in India only [1]. As per WHO 2014 report, about 422 million people globally are affected by diabetes [2]. Diabetes is described as the state of disequilibrium of glucose in body metabolism due to defects in insulin secretion or glucose metabolism.

1.1 Types of Diabetes
Insulin-dependent diabetes mellitus, also known as Type 1, is characterized by the absolute deficiency of insulin [3]. The blood has an unusually high sugar level due to the loss of pancreatic β-cell. The T-cell of the body destructs the β-cell, leading to the loss of insulin. Islets targeting autoantibodies, 65kDa glutamic acid decarboxylase, zinc transporter 8, insulinoma associated protein 2 are biomarkers associated with Type1diabetes, and it is known to be found in the body system even before the body shows symptoms like polyuria and thirst [4]. Other than genetic roles in the etiology of Type 1 diabetes, environmental factors also contribute to its development. Exposure to a group of viruses called Enterovirus induces the destruction of β-cell, and early exposure to dietary cow's milk protein contributes to the development of Type 1 diabetes [5]. It is more common in children than in adults. The patient needs lifelong insulin administration [6]. There is no cure for Type1 diabetes, but targeting T-cells, inducing β-cell tolerance, and β-cell replacement are some strategies for controlling and preventing Type1 diabetes [7].
While noninsulin diabetes mellitus, also known as Type 2, is characterisedbyimpaired insulin secretionorresistance to insulin or both [8]. Environmental factors such as lack of physical exercise, irregular diet, obesity, and genetic factors are the leading cause of Type 2 diabetes [9]. The pancreas's β-cell is impaired with insulin production. The patient is first affected by prediabetes characterized by impaired fasting glucose levels, glucose tolerance, or increased haemoglobin HbA1c levels [8]. Type 2 diabetes increases the chance of microvascular complications like neuropathy and retinopathy and macrovascular complications such as heart attack, blindness, amputations, renal failure etc. It can develop other deadly conditions, chronic liver disease, cancer, accelerated arthritis etc [10]. Lifestyle interventions to decrease weight, increasing physical activities, proper diet care, and a definitive study of molecular etiology are practical tools in fighting Type2 diabetes [11].

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Molecular characterization of some potential ethnomedicinal plants used for treatment of diabetes in Nagaland, India

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ABSTRACT

The use of medicinal plants for managing diabetes and related conditions has been an integral part of traditional healthcare systems throughout the world including Nagaland, India. Authentic identification of these medicinal plants is crucial for their safe and effective use. A study was aimed for authentic identification of some anti-diabetic potential ethnomedicinal plants from Nagaland, India using DNA sequence based molecular characterization by amplifying and sequencing of ITS, *rbcL* and *matK* barcode regions. DNA barcode loci from the nuclear and chloroplast genomes from 15 different ethnomedicinal anti-diabetic plants of Nagaland, India, were studied for molecular characterisation. Based on the ITS, rbcL and *matK* region sequences, three main discrimination methods, i.e., BLAST, Phylogenetic tree and genetic distance, were adopted to identify the species. Utilising DNA markers for identification is both highly advantageous and efficient due to its reliability and rapidity. Successful amplification across species and high blast hits (100 % identity) highlighted their potential for molecular characterisation. Phylogenetic trees constructed using various models showed distinct clades and subclades, with *matK* revealing hypervariability and *rbcL* demonstrating consistent matches. Genetic distances revealed varying levels of similarity, with certain species establishing close relationships based on pairwise comparisons. GC% variations were detected, potentially indicating nucleotide composition differences and functional constraints among species genomes. The findings of this study highlight that the ITS, rbcL, and *matK* regions serve as effective markers for verifying the authenticity of anti-diabetic medicinal plants indigenous to Nagaland, India.

1. Introduction

Diabetes Mellitus is a pervasive ailment stemming from aberrations in carbohydrate, protein, and lipid metabolism, coupled with insulin secretion anomalies or physiological malfunctions (Kavishankar et al., 2011; Lovic et al., 2020). India is the second largest diabetes affected population next to China and ~77 million people in India are living with diabetes (Kangra and Singh, 2023) and this number is anticipated to surge over 134 million by the year 2045 (Kansra and Oberoi, 2023). It manifests in three distinct forms. Type 1 diabetes involves an autoimmune response, wherein the body's immune system targets and dismantles insulin-producing cells, necessitating insulin injections or an insulin pump for glycemic management. Typically, type 1 diabetes emerges in early life, constituting 5–10 % of diabetes cases. Type 2 diabetes is most common and accounts for 90–95 % of cases, and it is due to insufficient insulin production or cellular insensitivity (insulin resistance) (Yedjou et al., 2023). Lifestyle variables, encompassing obesity, sedentary habits, and genetics, frequently underlie type 2 diabetes (Yun and Ko, 2021). Lifestyle alterations, oral medications, or insulin injections can mitigate it. While gestational diabetes is a transient affliction occurring during pregnancy due to insufficient insulin production, gestational diabetes often subsides postpartum, albeit women affected remain predisposed to type 2 diabetes later in life.

Diabetes can result in severe complications like heart disease, stroke, kidney and nerve damage, and eye and foot problems, necessitating urgent and effective treatments due to its widespread occurrence and diverse causes (Kooti et al., 2016). Available therapies include insulin, pharmacotherapy, and dietary management, utilising glucose-lowering drugs that act through distinct mechanisms. However, these drugs may yield side effects such as gastrointestinal disturbances, weight fluctuations, and liver concerns.

Abbreviations: ITS, internal transcribed spacer; rbcL, ribulose-bisphosphate carboxylase/oxygenase large subunit; matK, maturase K; DNA, deoxyribonucleic acid; BLAST, basic local alignment search tool; GC, guanine-cytosine; mtDNA, mitochondrial deoxyribonucleic acid; COI, cytochrome C oxidase; rpoC1, ribonucleic acid polymerase C1; CTAB, cetyl trimethyl ammonium bromide; PCR, polymerase chain reaction; dNTPs, deoxynucleotide triphosphates; MUSCLE, multiple sequence alignment; MEGA, molecular evolutionary genetics analysis; NCBI, national center for biotechnology information; ML, maximum likelihood

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List of Seminar, Conferences Attended and Presented Papers

1. Workshop on 'Research Ethics, Paper Writing & IPR' Organized & Sponsored by UGC-SAP (DRS-III), Department of Botany & Department of Biotechnology, Govt. of India sponsored Advance Level Institutional Biotech Hub, Nagaland University, Lumami. November 14-15, 2019.
2. Hands on Training on "Techniques in Applied Biology" [Under DST Sponsered 'Synergistic Training Program Utilizing The Scientific and Technological Infrastructure' (STUTI)] April 21-27,2022. Organized by Advance Level Institutional Biotech Hub Department of Botany, Nagaland University, Lumami-798627, Nagaland.
3. International Conference on "Bioresource and Bioeconomy" (ICBB-2022). Organized by Department of Botany, Nagaland University, Lumami, in collaboration with Nagaland Forest Management Project, Department of Environment, Forest and Climate Change, Govt. of Nagaland, India, from September 19-21,2022.
4. Oral presentation on the paper entitled "Analysis of certain nutraceutical phytochemical compounds and antioxidant activities of selected anti-diabetic potential plants of Nagaland, India" in the International Conference on "Bioresource and Bioeconomy" (ICBB-2022). Organized by the Department of Botany, Nagaland University, Lumami, in collaboration with Nagaland Forest Management Project, Department of Environment, Forest and Climate Change, Govt. of Nagaland, India, from September 19-21, 2022.
5. International Conference on "Metallomics, Biodiversity and Human Health: Recent Advances and Synthesis (ICMBHH, 2023). Organized by Department of Life Sciences

(Zoology), School of Life Sciences, Manipur University, Canchipur-795003, India, from March 3-4, 2023.

6. Oral presentation on the paper entitled “Internal Transcribed Spacer (ITS) Based Molecular Characterization and Analysis of Certain Phytochemicals of Some Antidiabetic Plants of Nagaland, India’. In the international conference on Metallomics, Biodiversity and Human Health: Recent Advances and Synthesis, ICMBHH, 2023. Organized by the Department of Life Sciences (Zoology), School of Life Sciences, Manipur University, Canchipur-795003, India, from March 3-4, 2023.
7. Science Lecture Workshop on “Traditional Knowledge, Medicinal Plants and Biodiversity Conservation” organized by the Department of Forestry, Nagaland University, Lumami, Nagaland-798627, India, sponsored by INSA- Indian National Science Academy, New Delhi; IASc- Indian Academy of Sciences, Bengaluru; NASI-The National Academy of Sciences, Prayagraj on March 13-14, 2024.
8. International Conference on “Science, Technology and Medicine” (ACSTM-2024). Organized by the Asian Council of Science Editors held at Khalidia Palace Hotel, Deira Dubai, UAE from 17-18th August 2024.
9. Oral presentation on the paper entitled “Molecular Insights and Therapeutic Potential: Exploring Some Anti-diabetic Ethnomedicinal Plants of Nagaland, India”. The 5th Asian Conference on Science, Technology and Medicine hosted from 17-18th August 2024. Khalidia Palace Hotel, Deira Dubai, UAE.

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