ROLE OF *PAEDERIA FOETIDA* TWIGS EXTRACT ON HEART AND BIOCHEMICAL PROFILE IN STREPTOZOTOCIN-INDUCED TYPE-2 DIABETIC AND HIGH-FAT DIET RATS

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ROLE OF *PAEDERIA FOETIDA* TWIGS EXTRACT ON HEART AND BIOCHEMICAL PROFILE IN STREPTOZOTOCIN-INDUCED TYPE-2 DIABETIC AND HIGH-FAT DIET RATS

By

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LIST OF ABBREVIATIONS

Advanced glycation end products	AGEs
AMP-activated protein kinase	AMPK
Analysis of variance	ANOVA
Animal Research and Service Centre	ARASC
Antibody	Ab
Apoptotic protease-activating factor	APAF
Avidin-Horseradish Peroxidase	HRP
Balanites aegyptiaca	BA
B-cell lymphoma 2	Bcl-2
B-cell lymphoma-extra large	Bcl-xL
BCL-2 associated agonist of cell death	BAD
BCL-2 Interacting Killer	BIK
Bcl-2-associated X protein	BAX
Benzyladenine	BAP
Beta cell apoptosis	β-cell apoptosis
Beta cells	β-cell
BH3 Interacting Domain Death Agonis	BID
Blood glucose	BG
Body mass index	BMI
Body surface area	BSA
Butanol fraction of a methanol extract	BMEL
Cadherin 1	CDH1

Calcium	Ca^{2+}
Carbon tetrachloride	CCI4
Cardiovascular	CV
Cardiovascular disease	CVD
Caspase-3-activated DNase	CAD
Catalase	CAT
c-Jun N-terminal kinase	JNK
Complete Fruend’s Adjuvant	CFA
C-reactive protein	CRP
Death-inducing signaling complex	DISC
Deoxyribonucleic acid	DNA
Diabetes Mellitus	DM
Diabetic cardiomyopathy	DCM
Dipeptidyl peptidase- 4	DPP-4
Diphenyl-2-picryl-hydrazyl	DPPH
Disability-adjusted life-years	DALYs
End stage renal disease	ESRD
Endoplasmic reticulum	ER
Enzyme-linked immunoassay	ELISA
Ethylbenzothiazoline-6-sulfonic acid	EBTS
Extracellular matrix	ECM
Fasting blood glucose	FBG
Fasting plasma glucose	FPG
Glucagon-like peptide-1	GLP-1

Glucose transporter -2	GLUT- 2
Glutamic-oxaloacetic transaminase	GOT
Glutathione peroxidase	GPx
Glycemic Variability	GV
Guanosine 5'-diphosphate	GPD
Guanosine triphosphate	GTP
Heart failure	HF
Heat shock protein 90	Hsp90
Hematoxylin and eosin	H&E
Hemoglobin A1c	HbA1c
Hepatitis B virus	HBV
Hepatitis C virus	HCV
High-density lipoprotein	HDL
High-fat diet	HFD
Hydrogen peroxide	H_2O_2
Hypochlorous acid	HOCI
IkappaB kinase-beta	IKKbeta
Immunohistochemistry	IHC
Immunoreactive score	IRS
Indole butyric acid	IBA
Institute of biodiversity	IBS
Insulin receptor substrate 1	IRS-1
Interferon gamma	IFN-g
Interleukin-1-beta	IL-1b

Interleukin-2	IL-2
Interleukin-6	IL-6
Interleukin-8	IL-8
Intracellular adhesion molecule 1	ICAM-1
kilocalories	kcal
Laryngopharyngeal	LP
Left ventricle	LV
Left ventricular ejection fraction	LVEF
Left ventricular failure	LVF
Low-density lipoprotein	LDL
Magnesium^{+2}	Mg^{+2}
Malondialdehyde	MDA
Masson’s trichrome	MT
Milliliters	mL
Monocyte chemoattractant protein	MCP
Murashige and Skoog	MS
Nicotinamide adenine dinucleotide phosphate	NADPH
Nitric oxide	NO
N-methyl-N-nitrosourea	MNU
Non-communicable Diseases	NCD
Non-insulin dependent diabetes mellitus	NIDDM
Normal control	NC
Normal pellet diet	NPD
Nuclear factor kappa-B	NF-κB

One-way analysis of variance	ANOVA
Optical density	OD
p38 mitogen-activated protein kinases	p38-MAPK
Paederia foetida	PF
Paederia foetida extract	PFE
Phosphate-buffered saline	PBS
Piper sarmentosum	PS
Plasminogen activator inhibitor -1	PAI-1
Potassium	K^+
Potassium chloride	KCl
Potassium hydrogen phosphate	KH_2PO_4
Probability value	P value
Protein carbonyl	PCO
Protein diaphanous homolog 1	DIAPH1
Protein kinase B	Akt substrate
Protein kinase C	PKC
Purging index	PI
Quantitative nuclear magnetic resonance	qNMR
Reactive nitrogen species	RNS
Reactive oxygen species	ROS
Receptor for advanced glycation end products	RAGE
Respiratory therapist	RT
Simulated patient	SP
Single nucleotide polymorphisms	SNPs

Sodium chloride	NaCl
Sodium phosphate	Na_2PO_4
Sodium, potassium adenosine triphosphatase	Na^+K^+-ATPase
Standard deviation	SD
Statistical Package of Social Science	SPSS
Streptozotocin	STZ
Stress-activated protein kinase	SAPK
Superoxide Dismutase 1	SOD1
Superoxide radical	O^{-2}
Thiobarbituric acid reactive substances	TBARS
Toll-like receptor	TLR
Total cholesterol	TC
Tracheobronchial	TB
Transforming growth factor beta	TGF-β
Transverse Myelitis	TM
Triglycerides	TG
Tumor mutational burden	TMB
Tumour necrosis factor-alpha	TNF-α
Type 1 Diabetes Mellitus	T1DM
Type 2 Diabetes Mellitus	T2DM
Ultraviolet-visible	UV-vis
Universiti Sains Malaysia	USM
Vascular cell adhesion molecule-1	VCAM-1
Vascular endothelial growth factor	VEGF

von Willebrand factor	vWF

LIST OF APPENDICES

PERANAN EKSTRAK RANTING *PAEDERIA FOETIDA* TERHADAP JANTUNG DAN PROFIL BIOKIMIA TIKUS DIABETES JENIS 2 ARUHAN STREPTOZOTOSIN DAN DIET TINGGI LEMAK

ABSTRAK

Diabetes melitus (DM) merupakan penyakit tidak berjangkit yang sangat membimbangkan disebabkan impak sosio-ekonomi yang besar ke atas negara terutamanya Malaysia di mana prevalen mengatasi angka global. Diabetes Jenis 2 (T2DM) adalah sejenis penyakit metabolik kronik yang bercirikan hiperglisemia dan penghasilan tekanan oksidatif yang berlebihan dan menyebabkan komplikasi kardiovaskular. *Paederia foetida* (Rubiaceae) adalah tumbuhan yang boleh dimakan yang didapati di negara Asia, digunakan secara tradisional untuk merawat pelbagai penyakit, termasuk diabetes kerana ia mempunyai ciri anti-radang, antioksidan, dan antidiabetik. Walau bagaimanapun, peranan ranting *Paederia foetida* ke atas hiperglisemia terhadap sistem kardiovaskular dalam diabetes belum dilaporkan lagi. Kajian ini dijalankan untuk menentukan kesan antioksidan, anti-radang dan antiapoptosis ranting *Paederia foetida* dalam tikus *Sprague Dawley* T2DM yang diaruh diet tinggi lemak dan streptozotosin. Sejumlah 48 ekor tikus *Sprague Dawley* jantan telah menjalani penyesuaian selama seminggu. Daripada 48 ekor tikus tersebut, 8 ekor tikus ialah kawalan normal, manakala 40 ekor tikus diinduksi obesiti dengan aruhan diet tinggi lemak selama 4 minggu. Daripada 40 tikus obes, 32 darinya telah diinduksi menjadi tikus T2DM dengan menggunakan streptozotosin 40mg/kg dan 32 tikus diabetes tersebut dibahagikan lagi kepada diabetes kawalan, dan tiga kumpulan diabetes yang dirawat (300mg/kg metformin, 50mg/kg dan 100mg/kg *Paederia*

foetida). Rawatan diberikan secara gavaj oral kepada tikus selama 28 hari sejurus selepas induksi T2DM berjaya. Berat badan, glukosa darah semasa puasa, dan pengambilan makanan diambil setiap minggu. Selepas empat minggu, tikus akan dimatikan dengan 200 mg/kg sodium pentobarbitol. Sampel darah dikumpul melalui tusukan jantung dan serum dianalisis untuk profil lipid dan paras glukosa darah. Jantung dikeluarkan secara pembedahan, dan homogenat disediakan dalam larutan kalium klorida 10% yang disejukkan dengan ais. Tiga enzim antioksidan (Superoxide dismutase (SOD), Glutathione peroxidase (GPx) dan Catalase (CAT)) dan dua penanda stres oksidatif (Malondialdehid (MDA) & karbonil protein (PCO) diukur bersama dengan penanda proapoptotik (BAX dan caspase-8) dan antiapoptotik (Bcl-2). Pewarnaan *Haematoxylin & Eosin* dan *Trichome Masson* dilakukan untuk menggambarkan perubahan histomorfologi dalam tisu jantung tikus diabetes. Pemberian gavaj oral 50mg/kg ekstrak *Paederia foetida* sekali sehari pada tikus diabetes selama 28 hari menunjukkan dos ini sebagai yang paling berkesan dalam menurunkan glukosa darah (27.2%), yang setanding dengan 300mg/kg metformin (23.1%). Aktiviti antioksidan ekstrak tersebut adalah ditunjukkan oleh perencatan peroksidasi lipid dan peningkatan SOD, GPx dan CAT. Jantung tikus DM menunjukkan perubahan ciri-ciri kardiomiopati diabetik seperti hipertrofi kardiomiosit, fibrosis dan fibrosis perivaskular. Pengambilan *Paederia foetida* atau metformin membantu melindungi daripada perubahan histopatologi dan biokimia yang berkaitan dengan kardiomiopati diabetik. Secara ringkasnya, kajian ini mencadangkan bahawa *Paederia foetida* berjaya menghasilkan data positif tentang kardiomiopati diabetes pada tikus melalui sifat antihiperglisemik dan antioksidannya.

ROLE OF *PAEDERIA FOETIDA* TWIGS EXTRACT ON HEART AND BIOCHEMICAL PROFILE IN STREPTOZOTOCIN-INDUCED TYPE-2 DIABETIC AND HIGH-FAT DIET RATS

ABSTRACT

Diabetes mellitus (DM) is a concerning non-communicable disease worldwide that has great socio-economic impact especially in Malaysia where the prevalence beats global figure. Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterised by hyperglycaemia leading to overproduction of oxidative stress that causes multiple complications. Oxidative stress is associated with its pathogenesis and hyperglycemia may cause cardiovascular complications. *Paederia foetida* (Rubiaceae) is an edible plant found in Asian countries, traditionally used to treat various diseases, including diabetes as it has anti-inflammatory, antioxidant, and antidiabetic properties. However, the role of *Paederia foetida* twigs in diabetic cardiomyopathy has not been reported yet. This study was conducted to determine the antioxidant, anti-inflammatory, and antiapoptotic effects of *Paederia foetida* twigs in high-fat diet streptozotocin-induced type-2 diabetes mellitus in Sprague Dawley rats. Forty-eight (48) male Sprague Dawley rats were acclimatized for a week. Out of 48 rats, 8 rats were assigned to normal control, while 40 rats were induced with obesity using a high-fat diet for 4 weeks. Out of 40 obese rats, 32 were induced with Type 2 diabetes Mellitus (T2DM) rats using 40mg/kg streptozotocin (intraperitoneally). The thirty-two (32) diabetic rats were further sub-divided into 4 groups; diabetic control, and three groups treated with 300mg/kg metformin, 50mg/kg, and 100mg/kg *Paederia foetida* respectively. *Paederia foetida* and metformin were orally administered to the rats for 28 days. Body weight, fasting blood glucose, and food intake were taken every week. The rats were sacrificed after four weeks of treatment using 200 mg/kg of

sodium pentobarbital. The blood samples were collected by cardiac puncture and serum was prepared and analyzed for lipid profile and blood glucose levels. The heart was surgically removed, and the homogenate was prepared in an ice-chilled 10% potassium chloride solution. Three antioxidant enzymes (Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (CAT) and two oxidative stress markers (Malondialdehyde (MDA) & protein carbonyl (PCO) were measured along with proapoptotic (BAX and caspase-8) and antiapoptotic (Bcl-2) markers. Hematoxylin & Eosin and Masson's Trichrome staining were done to visualize the histomorphological changes in the heart tissues of diabetic rats. Daily single oral administration of 50mg/kg *Paederia foetida* twigs on diabetic rats for 28 days revealed this dosage as the most effective in lowering blood glucose (27.2%), which is comparable to 300mg/kg metformin (23.1%). The antioxidant activity of the extract is demonstrated by its ability to inhibit lipid peroxidation and enhance levels of SOD, GPx, and CAT. In diabetic rats, the heart exhibit typical signs of diabetic cardiomyopathy, including cardiomyocyte hypertrophy, fibrosis, and perivascular fibrosis. Supplementation of *Paederia foetida* or metformin helps to safeguard against the histopathological and biochemical alterations associated with diabetic cardiomyopathy. In a nutshell, this study suggests that *Paederia foetida* managed to produce positive data on diabetic cardiomyopathy in rats through its antihyperglycaemic and antioxidative properties.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Diabetes mellitus (DM) is a major global health concern and one of the most common chronic illnesses worldwide (Hossain et al., 2024). It is a group of metabolic diseases characterized by persistent hyperglycemia (blood glucose level >11.1mmol/L), which is caused by abnormalities in insulin secretion, insulin action, or both (Feldman et al., 2023). There are three main types of diabetes: (i) Type 1 diabetes mellitus (T1DM), (ii) Type 2 diabetes mellitus (T2DM), and (iii) Gestational diabetes mellitus. T1DM is an autoimmune condition that results in the destruction of pancreatic beta cells., (ii) T2DM, also known as non-insulin-dependent diabetes mellitus, is increasingly common because of worsening glucose control caused by malfunctioning pancreatic beta cells and insulin resistance, either individually or in combination. The third type is the DM in pregnancy, also called gestational DM, which resolves once the pregnancy ends (CPG - Management of Type 2 Diabetes Mellitus (6th Edition)). Gestational DM can be due to formerly prevailing diabetes in the pregnant woman, rising insulin resistance or insistent hyperglycaemia during the pregnancy (Davies et al., 2022).

Globally, the non-communicable diseases (NCD) burden, related to infectious causes, resulted in a significant number of disability-adjusted life-years (DALYs) which was about 130 million, comprising 8·4% of all NCD DALYs (Al-Quwaidhi et al., 2014; Peykari et al., 2017). The 2019 National Health and Morbidity Survey by the Ministry of Health shows that the prevalence of diabetes among adults in Malaysia increased from 13.4% in 2015 to 18.3% in 2019, with a blood sugar level of 7.0

mmol/L or above. It anticipated that 3.9 million adults in Malaysia, 18 years of age and older, were diagnosed with diabetes, up from 3.5 million in 2015 (Ministry of Health Malaysia, 2020). It has been a major concern in Malaysia, resulting in substantial mortality and morbidity (CPG - Management of Type 2 Diabetes Mellitus (6th Edition)).

Complications associated with DM are quite prevalent and account for considerable morbidity and mortality (Roth et al., 2018). Complications can be classified as microvascular and macrovascular, with microvascular complications being more prevalent (Martín-Timón et al., 2014). Unlike conventional microvascular complications, atherosclerosis of large vessels can result in the development of diabetes (Harreiter & Roden, 2023). It demonstrates that rather than being an additional manifestation of diabetes, atherosclerosis and diabetes share genetic and environmental origins (Feijóo-Bandín et al., 2020; Morana et al., 2022). It indicates that they arise from a "common soil," meaning that the underlying mechanisms such as inflammation, metabolic dysregulation, and oxidative stress contribute to the development of all conditions (Bajaj, 2018; Buss et al., 2020). Cardiovascular disease (CVD) and T2DM are typically ranked among the top five most frequent and widespread chronic illnesses.

Even though diabetes is linked with an elevated likelihood of complications of cardiovascular, involving coronary artery disease, hypertension, along the advancement of heart failure (HF) (Schmidt, 2019), diabetes is not a cause of cardiovascular complications. The occurrence of a primary cardiac disease process known as "diabetic cardiomyopathy" is increasing (Malek et al., 2019). It causes ventricular dysfunction in diabetic people without coronary, valvular, or hypertension disease that is clinically significant. Diabetic cardiomyopathy (DCM) is defined as by

either systolic or diastolic left ventricular dysfunction in otherwise healthy diabetic patient (Dandamudi et al., 2014). According to Rubler et al., in 1972, it is believed that DCM is secondary to primary hyperglycemia based on post-mortem findings. The outcome includes many adverse effects such as decreased myocyte calcium handling, increased renin-angiotensin-aldosterone system activation, microangiopathy, and myocardial fibrosis (Fein & Sonnenblick, 1985). However, his perspective was contradicted by a few baffling factors surrounding the study topics. Mitral regurgitation, anaemia, and renal insufficiency are examples (Kannel et al., 1974; Sies et al., 2017). Even though several pathological mechanisms causing diabetic cardiomyopathy are being proposed (Miki et al., 2013) oxidative stress has been recognised as one of the primary causes of the disease (Sweeney et al., 2020). Hyperlipidemia, hyperglycemia, hypertension, and inflammation-induced oxidative stress are major risk factors for microvascular complications in the diabetic heart. These factors lead to abnormal gene expression, changes in signal transduction, and activation of pathways that result in programmed cell death in the heart muscle (Davies et al., 2022).

Oxidative stress is thought to be associated with both the development and consequences of diabetes (Opara, 2004). Diabetes is characterised by an increase in oxidative stress because persistently elevated blood sugar causes auto-oxidation of glucose and glycation of proteins (Butt, 2022). Oxidative damage caused by reactive oxygen or nitrogen species (ROS and/or RNS) produced by high blood sugar has a significant role in damaging organs in individuals with diabetes (Kanamori et al., 2015). Consequently, the connection between oxidative stress and DCM is a key topic of study at present (Bhatti et al., 2022). Oxidative stress is the primary contribution to DCM (Slatter et al., 2000). Therefore, antioxidant prevention and treatment are crucial

for preventing or delaying the onset of diabetic cardiovascular complications (Tee & Yap, 2017).

Malondialdehyde (MDA) is generated through lipid peroxidation of arachidonic, eicosapentaenoic, and docosahexaenoic acid in reaction to oxidants or oxidative stress (Go et al., 2021). Accumulation of MDA in tissue is associated with problems in diabetes mellitus. Classically, MDA reacts irreversibly and completely to protein and phospholipid, causing significant cardiovascular damage. AGEs can create cross-links between protein and lipid molecules. Antioxidant therapy can prevent the worsening effect (Sies, 2015).

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the primary antioxidant defense mechanisms found in all cells (Nikooyeh & Neyestani, 2016). They convert free radicals into stable and innocuous compounds (Negre-Salvayre et al., 2008). Superoxide ions and singlet oxygen radicals are transformed into hydroxyl radicals by SOD in cells and subsequently turned into water and oxygen by CAT and GPx. A reduction in the antioxidative defense system might lead to an increase in lipid peroxidation (Weseler & Bast, 2010). Ultimately, the disturbance of metabolic and antioxidative processes in diabetes mellitus leads to increased oxidative stress, which causes diabetic cardiomyopathy (Salvatore et al., 2021).

T2DM has been defined as a state of systemic inflammation (Khalili et al., 2021). Numerous cytokines and chemokines collaborate, making it difficult to evaluate the impact of mediators on the phenotypes observed in DCM (Belali et al., 2022). The primary inflammatory markers include tumor necrosis factor-alpha (TNF-α), interleukin (IL-6, IL-8, IL-1), and C-reactive protein (CRP) (Feijóo-Bandín et al., 2020). Certain mediators manifest in the subsequent secretion of other mediators;

therefore, it is unclear which of these cytokines directly cause detrimental myocardial alterations (Ru & Wang, 2020). However, it was previously shown that inflammation in diabetes mellitus triggers the activation of nuclear factor kappa B (NF-κB). Development of this transcription factor leads to myocardial and vascular injury mediated by cytokines. NF-κB is a common pro-inflammatory signalling pathway (Zhang et al., 2021).

Apoptosis has been the consequence of cardiomyocytes in response to diabetic hyperglycemia, inflammation, hyperlipidemia, and ER stress (Aravani et al., 2020). Apoptosis is also the fundamental instigating factor in promoting resident cardiomyocyte hypertrophy and fibroblast proliferation, both of which ultimately result in cardiac remodeling and dysfunction (Yang et al., 2022). T2DM is regulated by β-cell apoptosis. Apoptosis is a complex biological process marked by chromatin condensation, cell shrinkage, internucleosomal DNA fragmentation, and breakdown into membrane-enclosed vesicles known as apoptotic bodies (Ma et al., 2021). The pathophysiology of T2DM is significantly influenced by apoptosis. T2DM has a complex etiology involving obesity-related insulin resistance, impaired insulin secretion, and the reduction of β-cell mass caused by β-cell death (Akhtar et al., 2016). β-cell apoptosis in T2DM is regulated by numerous components of the caspase family cascade (Yuan et al., 2022). Recent studies have concentrated on examining the equilibrium between pro-apoptotic Bcl-2 proteins (Bad, Bid, Bik, and BAX) and anti-apoptotic Bcl family members (Bcl-2 and Bcl-xL) in hyperglycemia-induced β-cell death using isolated islets and insulinoma cell culture in vitro (Al-Damry et al., 2018). Apoptosis occurs when the amount of pro-apoptotic Bcl-2 at the mitochondrial membrane in the intrinsic route is higher than that of anti-apoptotic proteins (Xiong et al., 2018).

Caspases are proteases that specifically cleave aspartic acid residues and include cysteine. They are present as inactive precursors called zymogens in various cellular compartments such as the cytosol, endoplasmic reticulum, mitochondrial intermembrane space, and nuclear matrix (Othman et al., 2017). Apoptosis is triggered by the binding of cell surface receptors like Fas (CD 95) or tumor necrosis factor (TNF) receptors, known as "death receptors," and is a mechanism regulated by caspases (Williamson et al., 2010). When a ligand binds to the receptor, it triggers the formation of a group of proteins known as the death-inducing signaling complex (DISC), which then activates procaspase-8 (Jubaidi et al., 2021), an apical caspase. The subsequent cascades of events follow caspase-8's is the activation of caspase-3 (Belali et al., 2022; Liu et al., 2021).

Perivascular and interstitial fibrosis is a histological feature of diabetic cardiomyopathy (DCM), and the amount of fibrosis is associated with cardiac mass (Liu et al., 2021). In addition to a rise in collagen deposition, diabetes may also increase the cross-linking of collagen filaments, contributing to a decrease in ventricular compliance (Abdel-Hamid & Firgany, 2015). An investigation (Khan et al., 2005) indicated that glycation of collagen fibers is raised in the hearts of diabetic patients.

Treating diabetes mellitus involves precise management of blood sugar levels, educating patients on health, promoting physical activity, encouraging healthy lifestyle choices, and utilizing pharmaceutical medications. Ongoing research is being conducted in this field (Morana et al., 2022). There are different categories of oral diabetes medicines and injectable insulin. Insulin replaces the patient's naturally produced insulin and is crucial for those with T1DM (Ozturk et al., 2021). Exogenous insulin may be necessary for patients with T2DM to manage blood glucose levels

during periods of hyperglycemia. For example, individuals may experience complications and may require insulin in addition to oral drugs to achieve optimal glycemic control (Li et al., 2020). The prevalence of diabetes patients and associated comorbidities is increasing despite the use of medications (Hossain et al., 2024). Therefore, thorough research should be carried out to explore all possible origins, development processes, and treatments of DM problems, supported by scientific evidence.

Medicinal plants are important for developing new healing chemicals and are recognized for their biologically active properties, such as antioxidants, antihyperglycemic, and antihyperlipidemic agents (Kumar et al., 2014). In recent decades, plants have been utilized for treating diabetes and associated consequences (Ozturk et al., 2021). Several natural products include *Terminalia chebula, Allium cepa, Camellia sinensis, Artemisia herba-alba, Solanum americanum, Citrullus lanatus, Cocculus hirsute, Danae racemosa, Ficus glumosa,* and *Musa paradisiaca* serve as a valuable source of bioactive phytochemical substances. These phytochemical substances may decrease blood glucose levels and help reduce oxidative stress in a diabetic animal model (Greenwell & Rahman, 2015). In a separate investigation involving diabetic rats caused by streptozotocin-nicotinamide, the methanolic extract of Albizzia lebbeck bark notably reduced serum glucose, creatinine, urea, total cholesterol, triglycerides, low-density lipoprotein, and very low-density lipoprotein levels, while increasing high-density lipoprotein levels (Salehi et al., 2019).

Paederia foetida has a long history of being used in Chinese, Ayurvedic, and other traditional medicine systems for treating various conditions such as arthritis, inflammation, asthma, diarrhea, dysentery, piles, diabetes, and other health issues (Tan et al., 2019). The plant is utilised as a vegetable because of its abundant nutritional

properties. The pulverised twigs of *Paederia foetida* were extracted independently with chloroform, hexane, and methanol (Osman et al., 2009). The gas chromatography-mass spectrometry examination of twig extracts identified 12 bioactive chemicals, such as DL-alpha-tocopherol, 2-hexyl-1-decanol, n-hexadecanoic acid, and stigmastanol. Stigmasterol and n-hexadecanoic acid were identified as metabolites of *Paederia foetida* that may bind with α-glucosidase and α-amylase to form α-amylase-stigmasterol and α-glucosidase-n-hexadecanoic acid complexes, respectively. Metformin, or *Paederia foetida* supplementation, corrected the clinical signs and symptoms of T2DM, but *Paederia foetida* supplementation reduced biochemical alterations T2DM more effectively than metformin (Agbaire, 2011). However, additional intervention studies are needed to give sufficient proof based on science on the safety and effectiveness of this natural substance as well as its phytochemical makeup (Husna et al., 2023).

1.2 Justification of Study

T2DM comes with many comorbidities and the incidence is on the rise. Among the incidence of diabetic with co-morbidities, the CVD is the most common diabetic comorbidity and is the leading cause of mortality in individuals with T2DM, accounting for over two-thirds of deaths related to T2DM (Buddeke et al., 2019).

Anti-diabetic drugs come in various classes, including sulphonylureas, insulin analogues, biguanides, and α-glucosidase inhibitors, each with distinct mechanisms for controlling blood glucose levels. However, some of these medications associated with the use of conventional drugs include high-cost, severe hypoglycaemia, weight gain, and other side effects (Greenwell & Rahman, 2015).

Natural products, especially those derived from plants, include diverse chemical components that may provide a treatment option for treating T2DM through various methods (Ríos et al., 2015). Antioxidants such as polyphenols and flavonoids are abundant in natural products and can protect against cardiovascular diseases (Peng et al., 2015).

There is increase in the evidence supporting the role of natural products supplementation in the management of diabetes and its complications e.g., Stingless bee propolis, Chamomile, honey (Ozturk et al., 2021; Rocha et al., 2023; Srivastava et al., 2010; Ahmed et al., 2018). On the other hand, natural products generally have minimal adverse effects and can be more effective and affordable for treating diseases.

Our aim is to determine the cardioprotective effects of twig part of *Paederia foetida* on diabetic rats. *Paederia foetida* is an edible plant with in-vivo and in-vitro antioxidant and anti-diabetic properties. In Asian countries, *Paederia foetida* is used to treat numerous diseases, particularly diabetes. The twigs of *Paederia foetida* are extracted independently with chloroform, hexane, and methanol (Osman et al., 2009). Thus, this study provides new scientific evidence on cardioprotective activity of *Paederia foetida* twigs in diabetic heart is yet to be investigated.

1.3 Research Objectives:

1.3.1 General Objectives

This study investigated the effect of *Paederia foetida* twigs on cardiovascular parameters in high fat-diet and streptozotocin induced T2DM.

1.3.2 Specific Objectives

1. To determine the effects of *Paederia foetida* twigs on physical parameters (food intake, body weight) in HFD and STZ-induced T2DM rats.
2. To determine the effects of *Paederia foetida* twigs on biochemical profiles such as fasting blood sugar, blood lipid profiles including total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides (TG) in HFD and STZ-induced T2DM rats.
3. To determine the effect of *Paederia foetida* twigs on inflammatory markers which include interleukin-6 (IL- 6) and tumor necrosis factor-alpha (TNF-α) of heart tissues in HFD and STZ-induced T2DM rats.
4. To determine the effects of *Paederia foetida* twigs on oxidative stress markers [malondialdehyde (MDA and protein carbonyl (PCO)], and antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] in HFD and STZ-induced T2DM rats.
5. To determine the effects of *Paederia foetida* twigs on anti-apoptotic markers (Bcl-2) and proapoptotic markers (BAX, caspase-8) in heart tissues in HFD and STZ-induced T2DM rats.
6. To determine the effects of *Paederia foetida* twigs on histomorphological changes in heart tissues of HFD and STZ-induced T2DM rats.

1.4 Research Hypothesis:

1. *Paederia foetida* twigs supplementation improve physical parameters in diabetic-induced rats.
2. *Paederia foetida* twigs supplementation improve biochemical profiles in diabetic-induced rats.
3. *Paederia foetida* twigs supplementation decrease the levels of inflammatory markers in diabetic-induced rats.
4. *Paederia foetida* twigs supplementation decrease the oxidative stress in the heart of diabetic-induced rats.
5. *Paederia foetida* twigs supplementation decrease the apoptosis in the heart of diabetic-induced rats.
6. *Paederia foetida* twigs supplementation improve the histomorphological changes of cardiomyocytes in diabetic-induced rats.

1.5 Null hypothesis

1. There was no effect of *Paederia foetida* twigs supplementation on physical parameters in diabetic-induced rats.
2. There was no effect of *Paederia foetida* twigs supplementation on the biochemical profiles in diabetic-induced rats.
3. There was no effect of *Paederia foetida* twigs supplementation on the levels of inflammatory markers in diabetic-induced rats.
4. There was no effect of *Paederia foetida* twigs supplementation on the oxidative stress markers in the heart of diabetic-induced rats.

5. There was no effect of *Paederia foetida* twigs supplementation on the apoptotic markers in the heart of diabetic-induced rats.
6. There was no effect of *Paederia foetida* twigs supplementation on the histomorphology of heart tissue in streptozotocin-induced diabetic rat.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a chronic disease that happens when there are high levels of glucose in the blood due to pancreas is unable to produce insulin or sufficient insulin or the body is not able to use insulin effectively (Hossain et al., 2024; Saeedi et al., 2019). Diabetes is also a series of metabolic disorders of protein, carbohydrate and fats (Markiewicz et al., 2017). Deficiency of insulin affects the metabolism of carbohydrates, protein, and fat, and causes disturbance in water and electrolyte homeostasis (Reddy et al., 2018). These can lead to high blood glucose levels, or hyperglycemia, which is the characteristic of diabetes. Diabetes mellitus causes various long-term damage to the body organs, development of life-threatening complications such as cardiovascular disease, neuropathy, nephropathy and retinopathy (Saeedi et al., 2019) . However, diabetic complications can be prevented if the appropriate management of diabetes is attained. The most common forms of diabetes mellitus are Type 1 and Type 2 Diabetes mellitus (You and Henneberg, 2016).

2.1.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is an autoimmune disease in which the body immune system attacks the insulin-producing β-cells in the islets of the pancreatic gland (CPG - Management of Type 2 Diabetes Mellitus (6th Edition)). So, the pancreas produces low insulin, β-cell becomes starved of energy and produces extra amount of glucose in the blood. This was then followed by life-threatening circumstances of hypoglycemia and hyperglycemia. Due to hypoglycemia, the cells did not get enough glucose, and patients suffer because of confusion, loss of consciousness, collapse, and

death. Lack of insulin and hyperglycemia could contribute to ketoacidosis, an accumulation of ketones in the blood that causes the body to use fat instead of glucose for energy. The causes of this destructive mechanism are not well known, although a sequence of genetic vulnerability and environmental factors have been implicated (You and Henneberg, 2016). Although T1DM can be developed at any age, but the children and adolescents are the common group with T1DM. The insulin injections are needed every day to keep the blood glucose level in the normal range. People with T1DM can live a healthier life and delay or eliminate the complications associated with diabetes with daily insulin injections and regular blood glucose monitoring. People with T1DM have symptoms of frequent urination, abnormal thirst, fatigue, continual hunger, a sudden loss of weight, and vision blurred (Fatima et al., 2016).

2.1.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is the most common type of diabetes, accounting for 90% of all cases of diabetes. T2DM results from the body's ineffective use of insulin (CPG - Management of Type 2 Diabetes Mellitus (6th Edition)). An increase in insulin production initially will lead to a decrease in blood glucose levels. However, a state of relative insufficient production may grow over time. Obesity and overweight are significant factors in the development of insulin resistance and glucose tolerance impairment (Feldman et al., 2023). These contributors occur when there are hormone concentrations imbalances, increased cytokine concentration, cytokine signaling suppressors, presence of other inflammatory signals, and possibly retinol-binding protein. People with T2DM have the symptoms of drowsiness, increased thirst, frequent urination, slow-healing wounds, persistent infections, and numbness in hands and feet (Alam, 2021). However, the beginning of T2DM is typically slow and its

normal appearance without the acute metabolic disruption seen in T1DM indicates that it is difficult to determine the exact time of onset (Harreiter and Roden, 2023). As many as one-third to one-half of the population with T2DM may not know that they have diabetes since they may persist for many years without symptoms. When unrecognized for a longer time, the chronic hyperglycemia complications can be developed. In some people with T2DM, it was first diagnosed when they have the complications due to hyperglycemia such as foot ulcers, vision changes, and renal failure (Cloete et al., 2022). The roots of T2DM were not well known, but there was a clear correlation with obesity, age, ethnicity, and family background. The obesity, unhealthy diet, lack of physical activity, and smoking are several major modifiable risk factors (Punthakee et al., 2018).

2.2 Natural products as a food source: Ecology, propagation, and plantation of *Paederia foetida*

Natural products are valuable sources of nutritious foods that are beneficial for maintaining good health and preventing disease (Upadhyaya, 2013). Approximately 10% of the globe's higher plants are considered weeds (Odhav et al., 2007). Even though they can be raised at comparatively reduced management costs, they have been underutilised due to a lack of knowledge regarding their nutritional value (Han et al., 2019).

Natural products are chemical substances derived from plants, animals, marine organisms, and microorganisms (Smetanska, 2008). The natural products' plant metabolites are composed of primary and secondary metabolites (Bennett et al., 2018). Due to energy metabolism, primary metabolites including carbohydrates, amino acids, ethanol, along with lactic acid are produced during the growth process (Brahimaj et

al., 2017). Secondary metabolites are organic compounds produced by an organism that are not essential for its basic functions such as development, growth, or reproduction (Ahn et al., 2019). There are five main classes of secondary metabolites, including phenolics, alkaloids, saponins, terpenes, as well as lipids (Chanda et al., 2013).

Diabetes treatment with medicinal plants has been documented since antiquity (Chanda et al., 2013). A natural product known as *Paederia foetida*, also known locally as "Pokok Sekentut", is a semi-woody climber belonging to the Rubiaceae family that grows in India, Malaysia, China, Japan, the Philippines, as well as other Asian countries. The plant's foliage and branches can treat DM (Hassan et al., 2013).

Paederia foetida is one of the prevalent and less recognisable wild green vegetables of the globe (Elsayed et al., 2023). *Paederia foetida* is commonly found growing in disturbed areas within deep forests, woodlands, forest edges, secondary perennial to deciduous forests, and primary forest clearings. The plant also grows well in mountainous vegetation above 3000 meters, on steep wooded slopes, or on gravel or rocky seashores (Thirupathi et al., 2013).

Paederia foetida is primarily propagated via seeds. Despite being a pest, *Paederia foetida* germinates slowly (Fahim et al., 2019). At times, branches develop adventitious roots when they touch the earth, enabling them to be propagated (PROSEA: Plant Resources of South-East Asia | NHBS Academic & Professional Books, 2023). Micropropagation was successful, and after 7 days of cultivation, multiple shoots were obtained from shoot tips (1–2 cm long) of field-grown *Paederia foetida* plants in Murashige and Skoog (MS) media supplemented with 1 mg/l benzyladenine (BAP). Root induction was observed within 12 days of growth in MS

media supplemented with 0.25 mg of BAP and 0.5 mg of indole butyric acid (IBA). Around 70% of these seedlings were successfully transferred to soil (Chu et al., 2017).

2.3 Biology of *Paederia foetida*

Paederia foetida, a member of the Rubiaceae family, is a consumable plant found in various Asian nations, such as Malaysia. The plant exhibits rapid growth and demonstrates a great degree of adaptability to different light, soil, and salt conditions (Pemberton et al., 2005). The plant can thrive and grow beyond the frost line, but some leaves may turn reddish-yellow or drop after a freeze. Occurs most frequently in West Central (Chanda et al., 2013), is a major and prevalent weed in Hawaii and Brazil and is a significant weed in New Guinea. The leaves emit a distinctive odour of carbon bisulfide when pulverised, hence the name. The Latin word foetida means "stinky" or "foul-smelling" (Patel, 2017). The climbing plant *Paederia foetida* is extensively distributed in China, India, Bangladesh, Japan, Malaysia, Cambodia, Myanmar, Nepal, Thailand, and Vietnam (Afroz et al., 2006). The plant can reach heights of 1500–1800 metres. Because of the existence of methyl mercaptan (Fedchenko & Reifenrath, 2014), it emits a characteristic musk odour. *Paederia foetida*'s common name differs from location to region. For instance, the English names are King's tonic or skunk vine (Koerdt et al., 2017), whereas the Chinese and Malaysian names are ji shi teng and akar sekentut (Tan et al., 2019), respectively. It develops naturally in Malaysia on branches over shrubs and trees. The plant prefers moist, bright sunshine environments and is tolerant of various soil types. The leaf can be eaten whether it is raw or cooked. Malays eat the leaves with 'nasi ulam' (rice mixed with various chopped herbs), whereas native Tripurans eat them with dried fish (Satyavati et al., 1969). Besides that, the plant can boost sex energy by increasing the quantity of semen and strength for

men and is good for women after childbirth as it can remove wind from the body (Upadhayaya, 2013). Tribal communities in some areas of India used the powder form for the treatment of weakness and rheumatoid joint pains (Chanda et al., 2013). Also administered to the abdomen raw or following being pummelling to treat bloating.

Other applications include enhancing appetite and protecting children recovering from illness from disease (enhances immunity) (Srianta et al., 2012). The plant has been used to treat several conditions such as dysentery, toothaches, lesions, enterosis, enteromagaly, rhinosis, rheumatism, edema, night blindness, gastritis, diarrhea, and ulcer. Moreover, it has been stated to be beneficial for women after childbirth (Sarma et al., 2023). *Paederia foetida* has anti-inflammatory, antinociceptive (Morshed et al., 2012), antidiarrheal (Afroz et al., 2006), antioxidant (Khamphaya et al., 2022), antihepatotoxic, antidiabetic (Husna et al., 2023; Kim et al., 2020), antitussive (Husna et al., 2023), and gastroprotective properties (Chanda et al., 2015), according to bioassays. Several types of research done on phytochemical investigations of leaves; however, there are few findings on twigs and their effect on diabetic cardiomyopathy (Pradhan et al., 2019). Previous studies reported *Paederia foetida* twigs chloroform extract has good antioxidant and antidiabetic activity in silico and in vitro (Tan et al., 2019). *Paederia foetida* twigs exhibited strong 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and β-carotene bleaching effects. *Paederia foetida* twigs also showed good inhibition against α-amylase and α-glucosidase enzymes (Tan et al., 2020). A high content of scopoletin, coumarin, isolated from *Paederia foetida* twigs, showed high antioxidant and antidiabetic activities. Gas Chromatography-Mass Spectrometry revealed several antidiabetic metabolites of *Paederia foetida* twigs including tocopherol, fatty acid, steroids, ketone, amino acid, and glycerides (Tan et al., 2019).

In addition, *Paederia foetida* twigs also contains alkaloids, tannins, saponin, terpenoids, flavonoids, and phenol that show antioxidant properties based on DPPH and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays (Dutta et al., 2023). The evaluation of *Paederia foetida* acute toxicity confirmed that no mortality or sign of toxicity was seen in the animals up to a dose of 2000mg/kg (Das et al., 2013).

Paederia foetida has medicinal value in India. It is typically found in the Himalayas, and has been reported to treat gout, vesicle calculi, diarrhoea, dysentery, piles, liver inflammation, and emetic (Srianta et al., 2012). Native leafy vegetable *Paederia foetida* is ingested traditionally in Indonesia, according to PROSEA and Thumbuhan Berguna, Indonesia (Afroz et al., 2006). In Ayurvedic medicine, the decoction of the entire plant is commonly used to cure a variety of diseases. According to Ayurveda, it has antispasmodic, antiarthritic, diaphoretic, expectorant, and stomachic properties. In addition, it is utilized for asthma, gastrointestinal complaints, diarrhoea, diabetes, and seminal insufficiency. Additionally, the extract is used for neuralgia along with in the making of ayuvedic medication. It is also utilised for gout, vesical calculi, diarrhea, dysentery, ulcers, liver inflammation, and emetic (Kumar et al., 2014). In Bangladesh, it has been used as a remedy for diarrhea and dysentery (Wong and Tan, 1994), and to inhibit intestinal movement. Phytochemicals such as iridoid glycosides, paederolone, paederone, and paederenine have been found in this plant (Hou et al., 2021).

Many steroids and terpenoids have been found in the volatile compounds of the stems, leaves, and flowers of *Paederia foetida* (Dubey et al., 2017). According to reports, *Paederia foetida* is a potential source of fibre, sodium, calcium, potassium, iron, and vitamin C. The green vegetable is a potential source of antioxidants (Afroz

et al., 2006). The main chemical ingredients found in this plant are iridoid glycosides, sitosterol, stigmasterol, alkaloid compounds, protein, carbohydrates, amino acids, and volatile oil (Meite et al., 2009).

Paederia foetida is a small genus consisting of thirty unique species found in Asia (16 species), Africa and Madagascar (12 species, with 11 exclusives to Madagascar), and America (Al-Quwaidhi et al., 2014). *Paederia foetida* comprises 3 subgenera, with all species of the subgenus *Paederia foetida* and most species of the subgenus Alatopaederia located in South-East Asia. Five species in Africa and Madagascar belongs to the subgenus Lecontea, and six species in Madagascar belong to the subgenus Alatopoederia. Alatopaederia is the sole species found in the United States, with one native to Mexico and the other to South America (Marles and Farnsworth, 1995).

The scientific name *Paederia foetida* was originally applied to two distinct species, that are remarkably comparable vegetatively as well as in flower but notably distinct in fruit. *Paederia foetida* has round fruits and is widely disseminated in Southeast Asia. On the other hand, *Paederia foetida* cruddasiana Prain has flattened oval fruits that are specifically dispersed through the air and is in northern India, Bhutan, Bangladesh, Nepal, southwestern China, and Thailand (Pedersen et al., 1999).

2.4 Bioactivity and chemical composition of *Paederia foetida*

Paederia foetida possesses pharmacological potential consisting of antioxidant, antimicrobial, anticancer, and anti-inflammatory properties. The main chemical components of this plant are sitosterol, iridoid glycosides, stigmasterol, alkaloids, protein, carbs, amino acids, and volatile oil (Thirupathi et al., 2013). This fragrant climbing plant has edible leaves that can be consumed fresh or steamed

(Panmei et al., 2019). In Bangladesh, it is also used to treat diarrhea and dysentery and to suppress intestinal motility (Mollick et al., 2013).

Numerous studies have revealed that the volatile oils of *Paederia foetida*'s stems, leaves, and flowers contain numerous steroids and terpenoids in addition to 77 constituents. Its leaves are abundant in vitamin C and carotene (Kumar et al., 2011).

2.4.1 Antidiabetic properties of *Paederia foetida*

The anti-diabetic properties of *Paederia foetida* were confirmed by in-vitro testing of plant extracts (Tan et al., 2023). The identification of the phytochemicals responsible for the anti-diabetic properties of the plant carried out using assay-guided isolation and metabolomics techniques. Finally, standardisation of the plant extract done by using quantitative nuclear magnetic resonance (qNMR) and Ultraviolet-visible (UV-vis) spectrophotometer. Verification of the plant's anti-diabetic properties employing an animal model (Radenković et al., 2016).

Diabetes induced by alloxan has been characterised as a valuable experimental model for studying the physiological effects of hypoglycemic agents (Singh et al., 2024). On an alloxan-induced diabetic mouse model, *Paederia foetida* had a dose-dependent glucose-lowering effect, where *Paederia foetida* extract (PFE) 300mg/kg and PFE-500mg/kg demonstrated a highly significant antidiabetic effect. The level of activity was equivalent to that of metformin HCl at 150 mg/kg body weight (Ahmed, 2014). The fasting blood glucose (FBG) level did not significantly increase in the normal control group that received only citrate buffer solution, highlighting the influence of *Paederia foetida* extract (Ferderbar et al., 2007). This antidiabetic effect may be attributable to the occurrence of both glycosides and steroids in the leaf extracts, as these secondary metabolites have been shown to possess antidiabetic properties. In DM, oxidative stress is implicated in the production of free radicals. As

secondary metabolites, flavonoids and tannins exhibit significant antioxidant properties that allow them to deactivate free radicals via various mechanisms, thus decreasing the diabetic condition caused by alloxan injection (Ojha et al., 2018).

Due to STZ-induced cell dysfunctionalities, STZ-induced diabetic rats had elevated levels of blood glucose and reduced insulin levels (Davies et al., 2022). The study results demonstrate that the methanolic leaf extract of *Paederia foetida* lowered blood sugar levels in normal, glucose-induced, and STZ-induced diabetic rats (Kumar et al., 2014). In normoglycemic and STZ-induced diabetic rats, *Paederia foetida* leaf extract exhibited dose-dependent hypoglycemic activity, with a protracted hypoglycemic effect at higher doses (Graham et al., 2011). Methanolic leaf extract of *Paederia foetida* (MEPF) treatment alleviates oxidative stress and decreases renal inflammation through the inhibition of NF-kB in diabetic kidney in the early stage of diabetic nephropathy (Borgohain et al., 2017). Like the synthetic drug glibenclamide, the *Paederia foetida* dose demonstrated the greatest results. The likely mechanism of action of *Paederia foetida* leaf extract may involve stimulating the release of insulin and regeneration of pancreatic β-cells or increasing the cellularity of islet tissue and regeneration of β-cell granules (Wu et al., 2017). Based on the results, it is possible to hypothesise that *Paederia foetida* reduced blood glucose levels and increased pancreatic insulin levels. Histopathology examinations of the pancreas indicated that the pancreas is protected from the harmful effects of microbial streptozotocin. The leaf extract exhibited the same mode of action as glibenclamide, i.e., insulin secretion stimulation (Graham et al., 2011; Kumar et al., 2015).

2.4.2 Anti-inflammatory property of *Paederia foetida*

The study discovered that *Paederia foetida*, a plant native to India, possessed significant anti-inflammatory properties (Khushbu et al., 2010). Therefore, several

investigations were carried out to evaluate the anti-inflammatory characteristics of *Paederia foetida*. In rat cotton pellet granuloma models, the n-butanol fraction of the methanolic extract of *Paederia foetida* leaves exhibited significant anti-inflammatory activity (De et al., 1994). In addition, regular use of the extract did not affect the liver weight of rodents, while it caused an increase in spleen weight. It suggested that the extract may have an impact on the body's immune system (Thirupathi et al., 2013).

Similarly, to rheumatoid arthritis, "Amavata" in Ayurveda had a tremendous response to *Paederia foetida*, according to a 2009 study (Fitra et al., 2009). In India, routinely *Paederia foetida* is utilized to treat joint disorders. The leaf preparation is administered topically to rheumatic body parts until they are completely cured. The leaves are additionally prepared with rice, which has been utilised to treat rheumatism and gout, whereas the extract of the plant is employed to treat joint pain when taken orally for 8 to 10 days (Das et al., 2013) .

2.4.3 Antioxidant property of *Paederia foetida*

β-carotene bleaching and the 2, 2'-azinobis (3-ethylbenzothiazole-6-sulfonic acid) (ABTS) radical cation assay (Thirupathi et al., 2013) were utilised to determine the antioxidant activity of *Paederia foetida*. Like DL-alpha-tocopherol, the antioxidant properties in newly harvested *Paederia foetida* (78.1%) had been the highest. In addition, fresh *Paederia foetida* had 78% activity as an antioxidant, whereas desiccated samples only had 65%, which was verified and supported by the increased concentration of phenolic compounds in the new samples (Negre-Salvadare et al., 2008). Along with, Srianta et al. (2012) demonstrated that *Paederia foetida* has a high antioxidant activity in the methanolic extract of its leaves, along with an increased capacity for 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging and phenolic content (Woo et al., 2021). Upadhyaya (2013) found that the ethanolic extract of

Paederia foetida leaves possesses antioxidant and antimicrobial properties. Antioxidant activity was evaluated using four tests: total phenolic content, total flavonoid content, 2,2-azino-bis (3-ethylbenthiazole-6-sulphonic acid (ABTS) free radicals, and DPPH (Ghosh et al., 2021). An ethanolic extract of *Paederia foetida* leaves showed anti-inflammatory and antioxidant effects in albino rats with acetic acid-induced colitis (Ferdous et al.,2019). The extract of methanol of *Paederia foetida* leaves has a high antioxidant capacity.

In silico and in-vitro, the chloroform extract of *Paederia foetida* twigs has strong antioxidant and anti-diabetic properties. Strong 2,2-diphenyl-1-picrylhrazyl (DPPH) free radical scavenging and β-carotene bleaching effects were observed in *Paederia foetida* branches (Chanda et al., 2014). Moreover, *Paederia foetida* branches inhibited α-amalyase and α-glucosidase enzymes effectively. In addition, the presence of alkaloids, tannins, saponin, terpenoid, flavonoids, and phenol in *Paederia foetida* was reported to exhibit antioxidant properties based on DPPH and 2,2-azino-bis (3-ethylbenzothiazole-6-sulphonic acid) (ABTS) radical scavenging effect (Mollick et al., 2013).

The leaf extract exhibited substantial antihyperglycemic, antihyperlipidemic, as well as antioxidant properties in streptozotocin-induced diabetic Swiss albino rats, according to Kumar et al (2014). Additionally, it was stated that the methanolic extract of leaves possessed significant antioxidant activity. Moreover, Sadino (2018) demonstrated that the leaf extract possessed less cytotoxic, effective anti-diabetic, and intermediate thrombolytic effects.

2.4.4 Other medicinal uses of *Paederia foetida*

The phytochemical participants, antioxidant and antimicrobial activity, and nutrient content of *Paederia foetida* leaves from three Assam districts were analysed