






Diabetes and *Vernonia amygdalina* Delile (Asteraceae)

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Abstract: *Vernonia amygdalina*, is a perennial tropical shrub from Asteraceae with a height ranging from 1 to 10 m tall. In Tropical African Countries, *V. amygdalina*, known as bitter leaf because of the bitter taste of the leaf, is propagated for consumption as a vegetable due to its medicinal properties.

In this paper, the use of the plant *V. amygdalina* for the treatment of diabetes mellitus is reviewed by searching scientific databases such as Frontiers, HealthSTAR, MDPI, MEDLINE, Pubmed, Taylor and Francis, Science Direct, Scopus, Springer, Wiley, American Diabetes Association from 2000 to January 2021. Herbal medicine is a form of healthcare that has been used in diabetes treatment. One such herbal plant is *V. amygdalina*, a multipurpose plant with many uses, health benefits, and bioactivities. *V. amygdalina* is identified as the most medically beneficial plant in the genus *Vernonia*. *V. amygdalina* possesses several activities, including the anti-diabetic effect. This review discusses classifications and treatments (conventional and herbal) of diabetes mellitus, the phytochemical profile of *V. amygdalina* and its uses in the treatment of diabetes mellitus, and their mechanisms of action of the plant. The published literature used for the present work supports anti-diabetic properties of *V. amygdalina*.

Keywords: Diabetes mellitus; medicinal plants; *Vernonia amygdalina*.

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1. Introduction

Diabetes mellitus is a metabolic disorder where the blood glucose level becomes elevated if the pancreas is unable to produce enough insulin or its activity of promoting glucose uptake is resisted by the cells. Many synthetic drugs are available for the management of diabetes, but these therapies seem to be insufficient to prevent diabetic complications in type-2 diabetes, with a likelihood of developing cardiovascular events. Because of these limitations, there is a continuous need to develop novel health promotion strategies and therapeutic modalities. Despite the large armamentarium presently available, the progressive deterioration of diabetes control is such that treatment is still insufficient. The majority of type-2 diabetes patients eventually require insulin therapy to achieve targeted glycemic levels, and an estimated 75% dying diabetes-related complications from cardiovascular disease. Insufficiency of current therapies for the treatment of diabetes, combined with side effects of conventional medical treatment and an inability of the economy to absorb the cost of pharmaceuticals, have created a growing public interest in dietary supplements and botanicals [1]. Thus, diabetes patients rely on alternative medicine.

Traditional medicine has been used for diabetes management in many countries. Compared to prescription drugs, the herbs required are readily available, cost-effective, and have very low side effects. The plants present their anti-diabetic activity by either inhibiting enzymes involved in glucose generation or promoting insulin secretion. Secondary metabolites such as glycosides, alkaloids, terpenoids, and flavonoids present in plants contribute to the anti-diabetic activity. Over the years, the use of traditional herbal medicine for the treatment and prevention of various diseases, including diabetes, has become popular. The techniques of herbal medicine for use requires preparation of decoctions, syrups, extracts, or tincture using plant roots, leaves, barks or flowers [2].

For the management of diabetes mellitus, more than 800 medicinal plants have been studied and are being used for treatment globally. This is due to their effectiveness, low side effects compared to conventional pharmacotherapy, and relatively low costs [3]. *Vernonia amygdalina* (VA) is one such herb that is known for its anti-diabetic qualities. This review will stimulate further research on *V. amygdalina* and diabetes treatments.

2. Materials and Methods

The data for the current article were obtained by searching scientific databases such as Frontiers, HealthSTAR, MDPI, MEDLINE, Pubmed, Taylor and Francis, Science Direct, Scopus, Springer, Wiley, American Diabetes Association from 2000 to January 2021 using the following keywords: Diabetes, monotherapy, and combination therapy for diabetes, *Vernonia* species, *Vernonia amygdalina*, phytochemicals of *V. amygdalina*, the anti-diabetic effect of *V. amygdalina*. A total of 150 published articles were studied, and 85 articles were used for this review. About 6% of the published articles used for the present study have digital object identifiers (DOI).

3. Results and Discussion

3.1. Diabetes.

Diabetes mellitus is listed among the top 10 causes of death in adults, and it is estimated to cause millions of deaths globally every year. According to the International Diabetes Federation (IDF), there were estimated record of 425 million people who had diabetes (Type 1 diabetes, T1D and Type 2 Diabetes, T2D combined) in 2017, and in the same year, the global health expenditure on diabetes was estimated to be USD 727 billion [4].

Diabetes mellitus is a metabolic disorder associated with high blood sugar levels, where one's body cannot effectively control glucose metabolism. A major source of energy for the cell is glucose. For the glucose to enter the cells, it requires insulin produced from the endocrine pancreas. The blood glucose level becomes elevated if the pancreas is unable to produce sufficient insulin, or its activity of promoting glucose uptake is resisted by the cells. Hyperglycemia, increased β adrenergic stimulation, and intestinal hormone secretin affects insulin release [1, 5]. Changes in the activities of defining proteins or enzymes of glucose metabolism or transport in target tissues – liver, muscle, and adipose lead to alterations in the metabolism of the energy molecules. The complications of diabetes are not only limited to chronic metabolic disorders, including alterations in the metabolism of major energy molecules such as carbohydrates, fats, and proteins. Important checkpoints are constituted by the key proteins and/or enzymes in endogenous and exogenous glucose homeostasis. Thus, their mechanism can be exploited in the study of conventional and potential anti-diabetic drugs [6].

In its advanced stages, diabetes mellitus affects other metabolic pathways of lipids leading to hypercholesterolemia and hyperlipidemia, which are risk factors in atherosclerosis. Diabetes affects the other vital organs in the body, such as the eyes, liver, and kidneys and the degree of organ involvement depends on the duration and severity of the disease. This leads to retinopathy with a potential vision loss, increased gluconeogenesis, ketogenesis, diabetic ketoacidosis, non-ketotic syndrome, polyuria, and nephropathy leading to renal failure [5, 7]. Many secondary complications associated with diabetes, such as impaired wound healing, inflammation, foot ulcer, nerve disorders, and sexual depression, are caused by an increase in the concentration of advanced glycation end products (AGEs). Patients with diabetes have a higher chance of getting atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are commonly found in people with diabetes [5, 8, 9].

3.1.1. Classification of diabetes.

Diabetes can be classified into two main types; Type-1 Diabetes (T1D) and Type-2 Diabetes (T2D). T1D requires one to be injected with insulin due to the body's failure to produce insulin. This can be caused due to an autoimmune response by the body where the immune system mistakenly attacks and kills the pancreatic beta cells. Serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers can be used to identify the possibility that individuals are at increased risk of developing this type of diabetes. T1D accounts for about 5 – 10% of those with diabetes [5,8]. While the other ~90-95% of the diabetic patients are diagnosed with Type-2 Diabetes, also referred to as non-insulin-dependent diabetes or adult-onset diabetes. T2D is a condition where the availability of insulin in the body leads to a state of fasting hyperglycemia due to the cells becoming insensitive/resistant to the action of insulin. The deficient or diminished effectiveness of endogenously synthesized insulin causes the increase of glucose concentration in the blood and urine. For a long period, pathologic and functional changes in various target tissues can occur with a slight degree of hyperglycemia; however, diabetes won't be detected without clinical symptoms. This is because the hyperglycemia gradually over the years, and it is often not severe in the earlier stages for the patient to notice any symptoms. Many of the patients diagnosed with type-2 diabetes are at risk of developing macrovascular and microvascular complications. T2D patients do not require insulin treatment to survive, and there are many different causes for this type of diabetes. The specific etiologies are unknown; however, autoimmune destruction of pancreatic β - cells does not occur. Many patients with this type of diabetes are obese, and some degree of insulin resistance is caused by obesity. Even if the patients are not obese by the traditional weight criteria, they may have a high body fat percentage, mainly in the abdominal region [5, 8, 10].

As per the Expert Committee on Diagnosis and Classification of Diabetes Mellitus, individuals whose glucose levels are higher than those considered normal but do not meet the criteria for diabetes are defined as having impaired fasting glucose or IFG. A patient is to be considered as IFG if the fasting plasma glucose (FPG) level ranges from 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L), while those with 140 mg/dL (7.75 mmol/L) to 250 mg/L (11.1 mmol/L) in a 2 – hour postprandial plasma glucose (PPG) test are said to have impaired glucose tolerance or IGT. An individual is considered to have prediabetes, associated with an increased risk of developing T2D in the future if they are IFG and/or IGT. Both IFG and IGT should be considered as risk factors for future development of diabetes ad well as

cardiovascular diseases. A fasting plasma glucose reading above 126 mg/dL (7.0 mmol/L) suggests diabetes mellitus [1, 8].

3.1.2. Treatments of diabetes.

To date, there is no drug that can cure diabetes completely [9]. Therefore, T1D patients are required to take insulin for life to control blood glucose levels. Ingestion of high-calorie foods, family history of the disease, obesity, race, genetic disorders, smoking, inactivity, viral infections, and drug or chemicals are all associated with Type-2 Diabetes and can be managed with pharmacological and/or non-pharmacological means. The pharmacological means are drugs/ oral hypoglycemic, while non-pharmacological means are diet and exercise [3, 5].

Many individuals diagnosed with T2D can achieve their target blood sugar levels with interventions in their lifestyle alone; however, some may require medication to manage their glucose levels. These medical treatments can manage the blood glucose levels by either activating chemicals that enhance insulin secretion or suppressing hepatic glucose output. The decision on the medication depends on many factors, including the individual's blood sugar level and any other health problems they may have. There is also the possibility of combining drugs from different classes to help control glucose levels. Specific organs or metabolic pathways are targeted by most of the conventional and herbal treatments designed for diabetes. Table 1 illustrates the organs targeted by both conventional and herbal drugs in general for diabetes management in patients [5, 11, 12].

3.1.3. Pharmacotherapy.

All anti-diabetic drugs, except for insulin, are considered pharmacological agents that have the ability to treat hyperglycemia in type 2 diabetes mellitus. Pharmacological treatment with drugs is administered when lifestyle modifications such as weight loss, dietary modification, and exercise do not reduce blood glucose levels [13]. The therapeutic treatment begins with monotherapy and progresses to dual therapy or multi-agent therapy followed by insulin administration in combination with other anti-diabetic drugs. Due to the increase in demand for anti-diabetic drugs, there are regularly new drugs in the market [14]. The orally diagnosed anti-diabetic medications consist of many classes of drugs, including biguanides, sulfonylureas, meglitinide, thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter (SGLT2) inhibitors, and α -glucosidase inhibitors.

Combination therapy with two oral reagents or insulin is considered when the hemoglobin A1C level rises to 7.5% while on medication or if the initial HbA1C is $\geq 9\%$. These medicines are applicable to be used by all patients; however, before usage, some factors should be considered, such as the drug's ability to reduce blood sugar levels, its effect on body weight, any side effects, its reaction to other drugs, and the price [15, 16].

Table 1. Therapeutic targets for the management of diabetes mellitus [5].

Therapeutic Target	Functions
Adipose Tissue	<ul style="list-style-type: none">• Adipocyte function• Adipocyte differentiation• Glucose uptake• Oxidative Stress
Intestine	<ul style="list-style-type: none">• Inhibition of starch digestion• α - amylase inhibitors• β - glucosidase inhibitors• DPP-IV inhibitors

Therapeutic Target	Functions
Liver	<ul style="list-style-type: none">• Hepatic glucose metabolism• Glucose utilization• Gluconeogenesis• Lipotoxicity
Muscle	<ul style="list-style-type: none">• Myocyte function• Glucose uptake• Lipotoxicity• Oxidative stress
Pancreas	<ul style="list-style-type: none">• β - cell function• Insulin secretion• β - cell proliferation• Oxidative stress

The most commonly used drug in diabetic treatments is metformin. It has a beneficial effect on glucose metabolism and also aids in weight loss or weight stabilization. Based on numerous studies, it was found that metformin reduced mortality and the risk of complications. For a certain patient, if metformin is not tolerated and does not control the blood glucose levels as expected, they should be administered another class of anti-diabetic medicine [13]. Even though the safety of anti-diabetic drugs is assured, they have multiple modes of action and have unknown effects throughout the body [14]. Many anti-diabetic drugs are not recommended, or patients with moderate or severe renal failure or any other significant comorbidities should be instructed to use them with caution. During pregnancy or breastfeeding, the use of oral anti-diabetic drugs is prohibited [13].

The classes of anti-diabetic medications (oral) that are currently in use were analyzed with the help of literature (Table 2). Biguanide and its derivatives were discovered in the middle ages and were introduced in the 1920s [14, 16]. This was by discovering *Galega officinalis*, a herb that has blood glucose reducing capabilities and was found to contain guanidine, galegine, and biguanide [16]. Until the 1950s, these agents were forgotten, which was when biguanides were re-investigated for diabetes treatment. Three biguanides were reported for anti-diabetic action by the late 1950s: phenformin, buformin, and metformin. In many countries, both phenformin and buformin were discontinued due to a high incidence of lactic acidosis. Thus, the only biguanide currently on the market is metformin, the go-to medicine for diabetes patients [14]. Metformin reduces blood glucose levels by activating the adenosine monophosphate-activated protein kinase in the liver. The complex effect on the mitochondrial enzymes causes hepatic glucose uptake and inhibits glucose gluconeogenesis [16]. It is known to slow down the progress of type-2 diabetes in patients. It is also known to have only mild side effects such as the risk of hypoglycemia, reduced vitamin B12 absorption, gastrointestinal complaints (e.g., diarrhea, abdominal cramps), and low chances of weight gain [13, 16].

Sulfonylureas became the first pharmacological option in treating non-insulin-dependent diabetes by 1955. The first generation of sulfonylureas was: tolbutamide, chlorpropamide, acetohexamide, and tolazamide which were replaced by the second generation. The newer agents include glimepiride, gliclazide, glipizide, and glibenclamide (glyburide) [14]. Sulfonylureas control blood glucose levels by stimulating insulin production in the pancreas and improving the efficacy of insulin in the body. Usually, sulfonylureas are not recommended for overweight or obese patients due to weight gain [17]. Other side effects include the risk of hypoglycemia, hematological changes such as agranulocytosis, hemolysis [13]. Meglitinides are another class of anti-diabetic drugs. They act similar to sulfonylureas by increasing the insulin secretion from the pancreatic β – cells. However, unlike sulfonylureas, they are associated with a low risk of developing hypoglycemia [13]. Until present, there have

been three meglitinides used in clinical practice: nateglinide, repaglinide, and mitiglinide [14]. The side effects are also similar to sulfonylureas, such as weight gain, risk of hypoglycemia, and liver failure [13].

Fixed-dose combination therapy (FDC) is common in anti-diabetic management since monotherapy does not provide an acceptable permanent benefit or glycaemic control in many patients. Moreover, concurrent use of several anti-diabetic medications results in poor treatment adherence. FDC products for diabetic management improve patient compliance, reduce the frequency of drug administration, reduce risk of events and provide the synergistic effect as compared to administration of two drugs independently [18]. For most patients with type 2 diabetes, the most prevalent multiple drug therapy is a combination of metformin and sulfonylurea, followed by thiazolidinediones or α -glucosidase inhibitors [19, 20]. Sulfonylurea acts upon the insulin released from the beta cells of the pancreas, and metformin acts by improving the insulin sensitivity of muscle and liver, whereas pioglitazone improves adipose tissue insulin sensitivity. Voglibose is an α -glucosidase inhibitor that acts by reducing the postprandial blood glucose by regulating glucose absorption. For FDC, currently, in the market, there are an array of commercial pharmaceutical tablets available. These combined metformin and sulfonylurea medications are available under several brands (Table 3) [18].

FDC of glibenclamide and metformin improved HBA1c levels in diabetic patients who swapped from monotherapy to dual anti-diabetic therapy [9].

Table 2. Overview of orally administered anti-diabetic drugs.

Class	Selected drugs (Brand name)	Mechanism of action	Reference
Biguanide	Metformin (Glucophage)	Decreases blood glucose levels by decreasing hepatic glucose production (gluconeogenesis), and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization.	[13, 16]
Sulfonylureas	First-generation: Chlorpropamide Tolazamide Tolbutamide	Increase insulin secretion from the liver through the stimulation of pancreatic beta cells.	[21]
	Second-generation: Glyburide (Diabeta, Micronase) Glimepiride (Amaryl) Glipizide Gliclazide		
Meglitinides	Nateglinide (Starlix) Repaglinide (Prandin)	Increase insulin secretion by regulating ATP-sensitive potassium channels in pancreatic beta cells.	[22]
Gliptins or Dipeptidyl peptidase 4 (DPP-IV) inhibitor	Alogliptin Anagliptin Linagliptin Saxagliptin Sitagliptin Teneligliptin Vildagliptin	Prevent degradation of GLIP – 1. Increase insulin secretion, with an increase in blood sugar. Suppress glucagon production in response to a carbohydrate-containing meal. Decrease the risk of hyperglycemia and weight gain.	[13, 14, 16]
Sodium-glucose cotransporter (SGLT-2) inhibitor (canagliflozin, dapagliflozin, empagliflozin)	Canagliflozin, Dapagliflozin (Daploz, daplozmet) Empagliflozin (Jardiance) Ertugliflozin.	By inhibiting SGLT-2 in the kidney, glucosuria is increased Block glucose reabsorption by the kidneys resulting in increased urinary excretion of glucose.	[13, 16]
Thiazolidinediones	Pioglitazone (Actos) Rosiglitazone (Avandia)	Improve pancreatic β -cell function and insulin sensitivity. Increase glucose uptake by skeletal muscle	[13, 16]
Alpha-glucosidase inhibitors	Acarbose Miglitol (Glyset) Voglibose (Starvog)	Inhibition of carbohydrate absorption in the gastrointestinal tract (small intestine)	[21]

Table 3. Fixed-dose commercial pharmaceutical tablets of metformin [18].

Brand Name	Drug combination (composition of Tablet)
Glucovance	Glibenclamide/ Metformin, 2.5mg/500 mg

Brand Name	Drug combination (composition of Tablet)
Metaglip	Glipizide/ Metformin
Diapred-m2	Glimipiride/ Metformin
Jardiance Duo	Empagliflozin/ Metformin, 12.5 mg/500 mg
Trajenta Duo	Linagliptin/Metformin, 2.5 mg/500 mg
Istamet	Sitagliptin/metfomin
Pioglu	Pioglitazone/metformin
Volix	Voglibose/metformin

3.1.4. Herbal treatment.

In recent years, the use of herbal products for preventive and therapeutic purposes has been increasing among the population. This is mostly due to the belief that ‘natural’ implies safety. Many patients with diabetes use natural herbs alone or alongside the prescribed drugs in disease management. This is noted mainly in severe cases of type-2 diabetes patients. This might be the difficulty in attaining adequate glycemic control despite the pharmacotherapy present because of the decline in β – cell function. Leading to long treatment plans, making patients seek out alternative forms of treatment [23]. Since the use of herbal medicine in diabetes treatment is becoming widespread, a study was conducted by Ezuruike *et al.*, 2016, where the potential herb-drug interaction among Nigerian diabetic patients was assessed. This literature analysis was done based on 112 patients with type-2 diabetes, and fifty percent of the participant's used herbs alongside their prescription drugs. A large number (60%) of the patients did not know the type of herbs they were using, whereas the most prominently used plant was VA followed by *Ocimum gratissimum L.* and *Musa paradisiaca*. It was concluded due to the lack of clinical predictors that the influence for herb use was due to cultural factors [23].

Improvement of glucose and lipids by using cinnamon in people with type-2 diabetes was determined by Khan *et al.*, 2003. In the study, 60 people with type-2 diabetes were randomly given cinnamon or placebo, daily for 40-days, followed by a 20-day washout period. It was found in the results that cinnamon reduced serum glucose, triglyceride, LDL, and total cholesterol in patients with type-2 diabetes [24]. In 2016, a study was conducted by Attanayake *et al.*, using *Coccinia grandis* (Linn.) Voigt (Cucurbitaceae) leaf extract. It investigated the antihyperlipidemic, antioxidative effect of leaf extract in streptozotocin induced diabetic rats and standardized the leaf extracts by a standard analytical method. There was significant improvement ($P < 0.05$) in total cholesterol, triglycerides, and antioxidation potential. It was proven that *C. grandis* leaf extract possesses the ability to manage diabetic complications [25].

A study was conducted using different doses of cyclohexane and ethanol extracts of walnut leaf on diabetic male rats. The increased activity of aldose reductase (AR) was targeted in order to study the prevention of diabetes complications. The AR activity in the lens and testis of diabetic rats was analyzed in this study. Compared to the control group, the AR activity in both the extracts reduced significantly ($P < 0.05$). Thus, it was concluded by the study that walnut leaf extract can reduce the activity of AR. However, further studies are required to establish its effect on other species or humans [26]. A systematic review of the published literature on the efficacy and safety of 36 herbal therapies (single or combination) and 9 dietary supplements for glycaemic control in 4,565 patients with diabetes or impaired glucose tolerance was reported by Yeh *et al.*, 2003. It was concluded in the study that there was insufficient evidence on the herbs and supplements to draw a definite conclusion. However, they were found to be generally safe [27]. A review of medicinal potentials of plants of the

genus *Vernonia* based on results from a combination of in vitro and in vivo efficacy and toxicity studies reported showed that *Vernonia amygdalina* is the most promising species for development into a nutraceutical against diabetes [28].

3.2. *Vernonia amygdalina*.

The *Vernonia* genus has about one thousand species, and members are widely used as food and medicine. VA is scientifically classified into the kingdom Plantae that is an angiosperm of the order Asterales. VA belongs to the Asteraceae family and genus *Vernonia*. The *Asteraceae* family consists of herbs, shrubs, or, less commonly, trees. They are considered the largest family of flowering plants with approximately 1620 genera and about 23,600 species [29]. *Vernonia* species can easily adapt to their habitat according to different environments. In forests, they can be found next to water sources but also in forest margins, woodlands, grasslands up to 2800 m in altitude, and a mean annual rainfall of 750-2000 mm [30]. *Vernonia* is the largest genus with approximately 1000 species of shrubs, out of which VA is the most prominent [29, 31].

VA is usually cultivated by stem planting, and it doesn't produce seeds [31]. VA is a perennial soft wooded shrub with a height of about 2 to 10 m with stem diameter up to 40 cm. The shrub's bark is densely pubescent at the early stage; when the plant gets matured, the bark turns from grey to brown. The leaves are petiolate in shape arranged alternatively to each other [30, 31]. Flowers of VA are regular and bisexual, and then they develop into fruits. The fruits are shaped into 10-ribbed achene lengths up to 1.5 – 3.5 mm [30]. The most frequently used member of the *Vernonia* genus is *Vernonia amygdalina* (VA). VA is commonly known as a bitter leaf due to the bitter taste of the leaves. The shrub is commonly found in tropical Africa, where it is domesticated. It can also be found in some regions of Asia, including Malaysia. VA is consumed as a green leafy vegetable throughout West and Central Africa as a source of due for its nutritional and medicinal properties [28].

3.2.1. Pharmacological effects/ethnopharmacological activity.

Extracts of VA have been used traditionally as a tonic and in treating sexually transmitted diseases, feverish conditions, cough, constipation, and hypertension. Traditionally, in different parts of Africa, VA is used as a remedy for many medical purposes. A decoction of its roots and leaves is used in ethnomedicine to treat fevers, hiccups, kidney problems, and stomach discomforts, among others [29]. In Nigeria, a tonic is made from the plant for medicinal purposes. The bitter taste is removed by boiling the leaves and then using them as a soup condiment.

Similarly, a popular Nigerian dish known as 'Onugbo' is made [30]. It is also used in the treatment of diabetes, diarrhea, dysentery hepatitis, and cough. In addition, the leaves are used against nematodes in humans [29].

The pharmacological properties of VA have been studied to validate its therapeutic effects VA is identified as the most medically beneficial plant in the genus *Vernonia*. VA possesses several activities, including anti-diabetic, anti-plasmodial, cathartic, antioxidant, antimicrobial, hypolipidemic [2, 28, 29, 30, 32].

The antioxidant property of crude VA extracts was studied for years. Aqueous extracts of VA leaf showed a decrease in serum malondialdehyde levels in streptozotocin-induced diabetic rats that underwent oxidative stress [32]. In research for evaluation of phytochemicals,

proximate and antioxidant composition in conjunction with its effect *in vivo* on diabetes and obesity biomarkers, antioxidant and hematological profiles. This study carried out by Imaga and Bamigbetan [33] increases the level of the antioxidants, glutathione (GSH), superoxide dismutase (SOD), catalase, and malondialdehyde (MDA) of the test rats as compared to control, which is shown in the *in-vivo* antioxidant. The injection of VL into the rat's system leads to improvement in the functionality of the antioxidant system, possibly due to the effect of the phytochemical antioxidants in the extract.

The ethanol extract of VL can suppress parasitemia very easily during the early stages of injection. Sesquiterpene lactones are responsible for anti-malarial activity. In the same study by Audu *et al.*, the anticancer properties of VA leaves were studied. The exposure of BT-549 to three different VA volumes (10, 100, and 1000 µg/mL) led to cell growth by approximately 14%, 22%, and 50% in order [35]. A study was done to assess the anticancer effects of VA leaves fractions on 4T1 breast cancer cells. The Ethylacetate fraction (EAF) of VA leaves contains many bioactivity compounds capable of performing anticancer activity in the 4T1 cancer cells [34].

The lipid-lowering effects of ethanolic leaf extract of VA on high cholesterol diet rats were conducted by Adaramoye *et al.* The values for total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, lipid peroxidation (LPO), phospholipid, and glutathione (GSH) were all measured for the plasma and liver of the rats. There was a significant ($P < 0.05$) decrease in the cholesterol levels indicating the new potential of VA for hyperlipidemia treatment [36]. The effect of aqueous VA leaves on hypoglycemic and hypolipidemic were analyzed using alloxan-induced diabetic rats. In the duration of two weeks, rats were treated with different concentrations of VA extract. In the end, there was a significant ($P < 0.05$) decrease in the fasting glucose level respective of the alloxan-induced diabetic levels [2].

3.2.2. Toxicology.

There have been studies conducted on mice for the toxicology of VA. These studies showed no clinical signs of toxicity or toxicological effects in the treated animal groups. The only signs are of decrease in red blood cell count and an increase in serum bilirubin as per dosage [31]. Lower doses of ethanolic leaf extract, around 100mg/kg per oral, show no effects in male albino rats' testis. However, higher dosages (300-600 mg/kg) per oral shows toxicity [29]. In the paper by Adefisayo *et al.*, oral toxicity was done regarding the effect of methanolic VL extract on aspirin-induced gastric ulcers in Wistar rats. In the study, it was found that methanolic extract up to 3400 mg/kg didn't cause any toxicity. Thus, it can be said that VA causes no clinical signs of toxicity or toxicological effects when given in amounts [37]. VA has been reported to be safe and considered as a lead herbal drug candidate with good efficacy, fewer side effects, and reduced toxicity for clinical trials [38].

3.2.3. Anti-diabetic properties/ the use of VA in the management of diabetes.

Vernonia amygdalina (VA) is cultivated in tropical African and Southeast Asia countries, and the leaves are consumed as a vegetable in the traditional management of diabetes. Diabetes patients use pulverized fresh leaves that are soaked in water and are taken orally. In addition, the leaf extract of VA has been explored as an alternative therapeutic and preventive option in managing diabetes in many studies. Numerous research studies

demonstrated that VA leaves can be developed as an effective and safe drug for diabetes management due to their anti-diabetic properties [39]. Studies to investigate the hypoglycemic properties of *Vernonia amygdalina* Del. (VA) and its possible mechanisms of action in streptozotocin-induced diabetic rat models have been conducted.

Ong *et al.* conducted a dose-response study to determine the optimum dose for the hypoglycemic effect of VA in STZ-induced diabetic rats. The optimum dose (400 mg/kg) was used throughout the 28-day chronic study. The ethanolic extract of VA showed a significant improvement in glucose tolerance of the STZ-induced diabetic rats [40]. A similar study of anti-diabetic properties of different extracts of VA showed that the chloroform extract has the highest blood and serum glucose-lowering effects compared to methanol and petroleum ether extracts [41]. The effect of methanol crude VA extract on streptozotocin treated rats of type-2 diabetes was carried out by Okoduwa *et al.* to validate the anti-diabetic effects of fractions of methanolic leaf extracts of VA. The methanol crude extract was fractionated with *n*-hexane, chloroform, ethyl-acetate, *n*-butanol, and water. After a 28-day post oral treatment of the diabetic rats with the fractions, the results showed a significant decrease in the fasting blood glucose levels ($P < 0.05$). The diabetic rats treated with chloroform fraction showed the maximum glucose tolerance with a fasting blood glucose reduction of 66.7% [39]. The effect of VA (methanolic extract) capsules with a combination of anti-diabetic drugs glibenclamide or metformin was studied in male streptozotocin-induced diabetic rats has been reported [3]. The aqueous leaf extract of VA is also reported to exhibit a reduction in blood glucose in fasted normal and alloxanized rabbits. The effect was comparable with those of tolbutamide and chlorpropamide, which are standard drugs used in the management of diabetes, and it also improves the repair of the pancreas [42].

Studies to investigate the anti-diabetic mechanism of action of VA have been reported. Atangwho *et al.* studied the anti-diabetic mechanism of VA leaves and its impact on the transcription of key enzymes involved in cellular modulation of glucose in streptozotocin-induced diabetic rats for a period of 14 days. After an administration period of 200 mg/kg, 400 mg/kg, and metformin, the results showed a significant decrease in diabetic levels. However, it was suggested by the study that there was little or no effect on glycolysis. Instead, the VA achieves its anti-diabetic effect by simultaneously suppressing gluconeogenesis and potentiating glucose oxidation through the pentose phosphate pathway [6]. Another study on the RNA content of the pancreas of the diabetic animal model to elucidate the possible mechanism of action of VA was conducted by Asanga *et al.* The results showed the aqueous extract caused an increase in the cell mass of the pancreas, enhanced proliferation of the β -cells of islets of Langerhans of the pancreas as evidenced in the RNA content of the post-mitochondrial supernatant and whole homogenate obtained by the fractionation of the pancreas [43].

The anti-diabetic effect of the combination of metformin (50 mg/kg) and aqueous extracts of VA leaf (100 mg/kg) indicated that the combination of the aqueous leaf extract and metformin was efficacious, additive, and safe for the management of diabetes mellitus [44]. The blood sugar lowering effect of VA leaf has been confirmed by several published studies. Table 4 summarizes the literature reports of the anti-diabetic effect of VA. Each of the published articles mentioned in this review highlights the benefits of the leaf of VA in managing diabetes and other blood sugar-related conditions.

The proximate components present include proteins, ash, carbohydrates, minerals, and crude fiber among others. Bioactive compositions include Stigmastane-type saponins,

flavonoids, sesquiterpene lactones, and others such as terpenes, coumarins, phenolic acids, etc. Many studies have been conducted for the identification of bioactive compounds in VA by isolating and characterizing them. The phytochemical studies show many phytochemicals such as saponins, flavonoids, alkaloids, tannins, and several types of sesquiterpene lactones [30, 31, 45–47]. Some of the isolated bioactive compounds from VA leaves that are reported in the literature are shown in Table 5.

The bioactive compounds composition for VA leaves was found to be flavonoids (0.85%), tannins (0.37%), saponins (2.2%), polyphenols (0.35%), alkaloids (2.13%), and HCN (12.25%) in a study by Nwaoguikpe *et al.*, where the effect of bitter leaf extract on blood glucose levels of diabetic rats was studied [48]. Similar amounts of bioactive were found by Atangwho *et al.* when a comparative study on the composition of leaves from anti-diabetic plants; *Azadirachta indica*, *Vernonia amygdalina*, and *Gongronema latifolium* was done. VA leaves had the highest content of flavonoids, saponins, and polyphenols among the three plants used [49]. The content of bioactive components in VA according to Ezekiel *et al.*, was tannin (3.34 mg/100g), phenol (1.78 mg/100g), phytate (19.69 mg/200g), Oxalate (3.78 mg/100g), Saponin (3.76 mg/100g), Alkaloids (3.59 mg/100g) and flavonoids (4.09 mg/100g) [50]. The presence of oxalates, phytates, and tannins was also found in reports by Kadiri and Olawoye, Zakaria *et al.*, and Adefisayo *et al.* [37, 46, 47]. The bitterness present in the VA leaves is due to the presence of the A – series saponins, alkaloids, tannins, and glycosides [20, 45]. Sesquiterpene lactones are another bioactive compound found abundantly in VA leaves (Table 5). Primary screening of ethanolic extract of old and young VL leaves showed the presence of alkaloids, saponins, tannins, glycosides, and terpenoids. However, flavonoids were absent in the young leaf extract and only present in the old leaves [10].

Table 4. Studies on anti-diabetic effects of VA in alloxan or streptozotocin-induced Type II diabetic rat model.

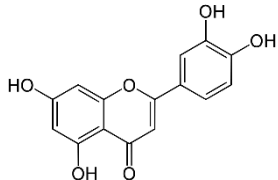
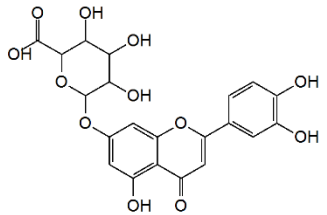
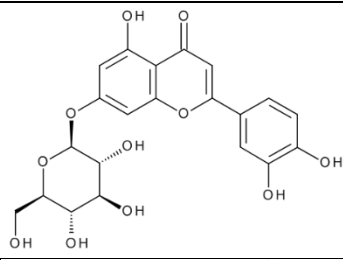
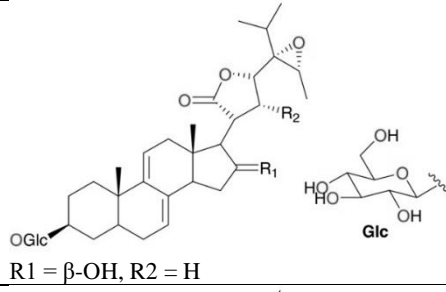
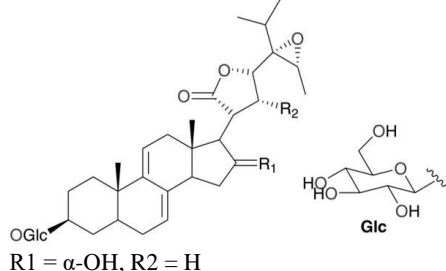
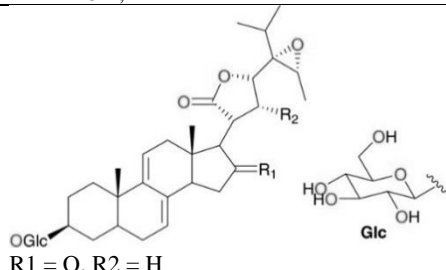
S. No	Extract	Outcomes ^a	References
1.	Aqueous	Hypoglycemic effect	[1]
2.	Aqueous	Hypoglycemic and hypolipidemic effect	[32]
3.	Aqueous	Hypoglycemic and hypolipidemic effect	[51]
4.	Aqueous	Anti-diabetic effect Increase in the cell mass of the pancreas. Enhance proliferation of the β -cells of islets of Langerhans of the pancreas.	[43]
5.	Aqueous	Hypoglycemic effect	[52]
6.	Aqueous	Hypoglycemic and hypolipidemic effect	[53]
7.	Chloroform	Anti-diabetic effect Suppression of gluconeogenesis and potentiation of glucose oxidation via PPP pathway in the liver	[6]
8.	Ethanol	Hypoglycemic and hypolipidemic effect	[54]
9.	Ethanol	Hypoglycemic and hypolipidemic effect	[10]
10.	Ethanol	Hypoglycemic and hypolipidemic effect The protective effect over pancreatic β -cells Increase in insulin level. Stimulation of skeletal muscle's glucose uptake Restoration in skeletal muscle glycogenesis	[40]
11.	Ethanol	Hypoglycemic effect Reduction in serum urea, chloride, and sodium concentrations Elevation of serum potassium and creatinine concentrations	[55]
12.	Ethanol	Hypoglycemic effect	[56]
13.	80% methanol	Hypoglycemic effect	[39]
14.	80% methanol	Hypoglycemic effect	[3]
15.	70% methanol	Hypoglycemic and hypolipidemic effect	[57]
16.	Methanol	Hypoglycemic effect	[58]
17.	Methanol	Hypoglycemic and hypolipidemic effect	[59]

S. No	Extract	Outcomes ^a	References
18.	Methanol	Hypoglycemic effect and hypolipidemic effect	[60]
19.	Methanol	Hypoglycemic effect and hypolipidemic effect	[61]
20.	Methanol	Hypoglycemic	[62]

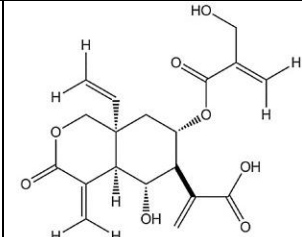
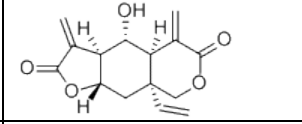
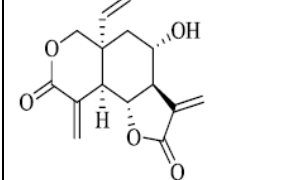
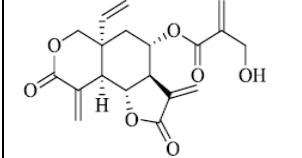
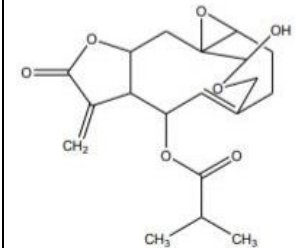
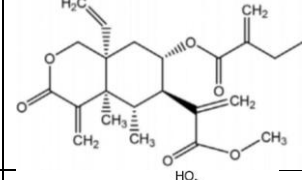
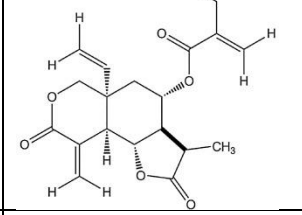
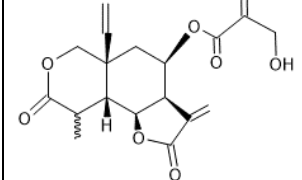
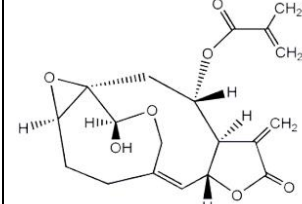
^aThe results of diabetic animals treated with the extracts (test group) compared to the values of their normal control groups.

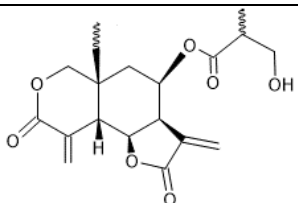
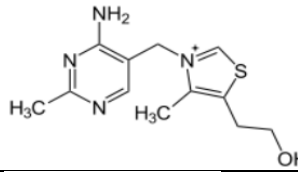
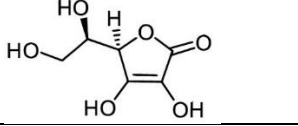
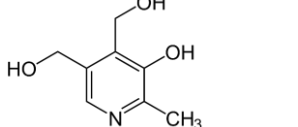
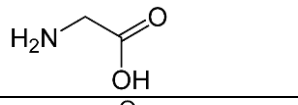
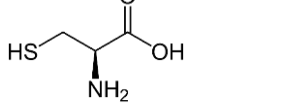
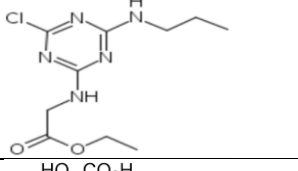
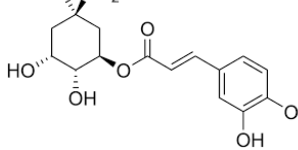
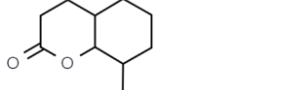
3.2.4. Bioactive constituents.

Table 5. Major bioactive compounds isolated compounds from *Vernonia amygdalina* leaf.

S. No	Isolated compound	Chemical structure	Reference
1.	Luteolin		[28, 45]
2.	Luteolin 7-O-β-glucuronide		[28, 45]
3.	Luteolin 7-O-β-glucoside		[28, 45]
4.	Vernonioside A1	 R1 = β-OH, R2 = H	[45, 63]
5.	Vernonioside A2	 R1 = α-OH, R2 = H	[45, 63]
6.	Vernonioside A3	 R1 = O, R2 = H	[45, 63]

S. No	Isolated compound	Chemical structure	Reference
7.	Vernonioside A4		[45, 63]
8.	Vernonioside B1		[45, 64]
9.	Vernonioside B2		[28]
10.	Vernonioside D		[65]
11.	Vernocuminoside G		[65]
12.	Vernolide		[45, 64]
13.	Vernodalol		[45, 64]

S. No	Isolated compound	Chemical structure	Reference
14.	Vernodalinol		[65]
15.	Vernomenin		[31]
16.	Vernolepin		[45, 64]
17.	Vernodalin		[45, 64]
18.	Vernomygdin		[45, 66]
19.	Epivernodalol		[65]
20.	11,13-dihydrovernodalin		[65]
21.	4,15-dihydrovernodalin		[31]
22.	Hydroxyvernolide		[31]

S. No	Isolated compound	Chemical structure	Reference
23.	1,2,2',3'-tetrahydrovernodalin		[31]
24.	Thiamin		[31]
25.	Ascorbic acid		[31]
26.	Pyridoxine		[31]
27.	Glycine		[31]
28.	Cysteine		[31]
29.	Casein hydrolysate		[31]
30.	Chlorogenic acid		[67]
31.	8-methyloctahydrocoumarin		[68]

Flavonoids are another group of bioactive compounds found in VA leaves. They play an important role in the taste, color, protection of plant vitamins and enzymes and stop fat oxidation. Various flavonoids isolated from VA leaves using organic solvents are most commonly three flavones: luteolin, luteolin 7-O- β -glucuroniside and luteolin 7-O- β -glucoside [31,45]. From the identified flavones exhibited the strongest oxidant activities comparable to synthetic butylated hydroxytoluene (BHT). The most abundant compound in VA leaves was luteolin 7-O- β -glucuroniside, and along with luteolin, 7-O- β -glucoside both possess antioxidant activity (lower compared to luteolin). According to Yeap *et al.*, the most effective method for luteolin extraction was found to be using ethanol [20]. Luteolin has been scientifically established as an antioxidant, anti-inflammatory, anti-estrogenic, anti-tumorigenic, anti-mutagenic, anti-apoptotic, anti-allergic, and anti-diabetic [69].

The stigmastane-type saponins vernoniosides A1, A2, A3, and B1 were first isolated from VA leaves in 1992 [70]. Later in 1993, three stigmastane-type steroid glucosides, vernonioside A4, B2, and B3 were isolated from VA [71]. It was observed by Ohgashi *et al.*

that these saponins had weak antischistosomal, plasmodial, and leishmanicidal activities. However, vernonioside B1 displayed antischistosomal activity [72]. Other stigmastane-type saponins isolated from VA include vernoniosides C, D, and E [73]. Several steroidal saponins such as vernoniamyoside A–D, and vernoamyoside D were found in VA leaves. They demonstrate anti-malaria and antitumor activity, especially anti-breast cancer activity. However, further research on the topics is required [63, 73, 74]. Saponins isolated from VA were also observed to possess antioxidant and hypolipidemic effects [31, 41]. The saponin's content reduces blood glucose levels by reducing oxidative stress and has an anti-diabetic activity to stimulate insulin secretion and action and pancreatic beta-cell regeneration [75].

Terpenoids are another important phytochemical that is responsible for the interaction of plants with their environment. Terpenoids have been shown to have a wide range of biological activities such as antibiotic, cytotoxic, anti-malarial, antifeedant, insecticidal, molluscicidal, and herbicidal properties. Subdivisions of terpenoids include monoterpenes, sesquiterpenes, diterpenes, triterpenes, and carotenoids. In essential oils obtained from the Asteraceae family, including the *Vernonia* genus, the main constituents are monoterpenes and sesquiterpenes [28]. The pharmacological properties of terpenoids isolated from VA include anticancer, anti-inflammatory, hepatoprotective, antioxidant, antibacterial, antileukemia, analgesic, and anti-nociceptive activities [20, 31, 76]. Some triterpenes isolated from VA are thiamine, ascorbic acid, pyridoxine, glycine, cysteine, casein hydrolysate, eucalyptol, beta piene, myrtenal, and alpha-muurolol (Table 5).

Sesquiterpene lactones are a compound among terpenoids that was found in VA. Some known sesquiterpene lactones include vernolide, vernodalol, vernolepin, vernodalin, vernomygdin, hydroxyvernolide, vernodalinol, vernomenin, and vernolic [29, 45]. They possess pharmacological properties such as antibacterial, anesthetic, antifungal, anti-inflammatory, and antimicrobial [31]. These sesquiterpene lactones are also reported to inhibit breast cancer cell growth and possess antitumoral properties.

In 1993, a study was conducted by Jisaka *et al.*, where the antitumoral and antibacterial activities of 10 sesquiterpene lactones were conducted. These studies were carried out *in vitro* antitumoral activity against mouse leukemia cells, P-388 and L-1210, and the antibacterial activity against Gram-positive bacteria, *Bacillus subtilis*, and *Micrococcus lutea*. From the study, it was reported that vernodalin and vernolide exhibit potent antitumor activity while the activity of hydroxyvernolide and vernodalol was weak [77]. In another study vernolide and vernodalol were tested by agar dilution method against 10 bacteria strains (gram-positive and negative) and 5 fungi species. It was found that the two compounds exhibited significant bactericidal activity against 5 Gram-positive bacteria while lacking efficacy against the Gram-negative strains. In the antifungal test, vernolides exhibited high activity (LC₅₀ values of 0.2, 0.3, and 0.4 mg/ml against *Penicillium notatum*, *Aspergillus flavus*, *Aspergillus niger*, and *Mucor hiemalis*, respectively). However, vernodalol showed moderate inhibitions against *Aspergillus flavus*, *Penicillium notatum*, and *Aspergillus niger* with LC₅₀ values of 0.3, 0.4, and 0.5 mg/ml, respectively. Both compounds were ineffective against *Fusarium oxysporum* [78]. Vernolepin also exhibits antiplatelet activity against ADP, arachidonic acid, and collagen-induced platelet aggregation in rabbits [20].

3.2.5. Nutritional value.

As a food, vegetable, and culinary herb *Vernonia* species are grown in many parts of the world. The nutritional content VA has been researched by many researchers. Based on the <https://biointerfaceresearch.com/>

past literature, the presence of protein, carbohydrate, moisture, ash, fiber, and fat in VA is established [46]. A comparative study of chemical compositions of leaves of *Azadirachta indica*, *Vernonia amygdalina*, and *Gongronema latifolium* was carried out in 2009. This study done by Atangwho *et al.* [49] compared the proximate compositions of the three diabetic plants: crude fiber, ash, fat, and protein. The composition for VA was crude protein (23.3%), crude fiber (16.05%), fat (3.53%), and ash (10.01%). When compared with the other two plants, VA had the lowest ash and fat content.

The analyzed crude fiber content by Kadiri and Olawoye is 8.78% which is the normal amount found in vegetables found in Nigeria [46]. The ash content is 4.28%, which along with the crude fiber, is lesser than the values reported by Asaolu *et al.* [19]. According to Nwaoguikpe *et al.*, the composition of VA revealed the presence of protein (62.2%), crude carbohydrate (22%), ash (9.95%), crude fiber (16%), and crude fat (3.45%). The values obtained by Asaolu *et al.* for crude protein (50.64%) and ash content (9.56%) are similar [19, 48]. The nutritional composition of VA, varies from study to study due to different geographical locations, genetic, environmental, harvest conditions, and ecology of the plant used [31]. The ash in the leaves confirms the presence of minerals. Analyses of literature data show that the major minerals present in VA are Potassium (973), Sodium (819), Manganese (710), Calcium (166), and Magnesium (88), while copper, phosphorus, and iron are least detected (Table 6). It should be noted that the old and young age of leaves does have a significant difference in nutrient content [20]. The presence of mineral contents is beneficial in the treatment of diabetes.

Calcium and potassium are beneficial for human health since these two elements play essential roles in many physiological activities, such as the regulation of enzyme activity and blood pressure regulation. Calcium stimulates enzymes in the digestive process and coordinates the functions of all other minerals in the body. Potassium maintains the water-acid balance and in the transmission of nerve impulses to muscle [79]. In controlling the glucose level in the body, potassium and calcium are important as they maintain normal glucose tolerance levels [46].

Magnesium is an important co-factor that is actively involved in metabolic reactions, including carbohydrate metabolism and glycaemic control. Literature report of cross-sectional and longitudinal research indicates that reducing dietary magnesium intake is a risk factor for the incidence of impaired glucose regulation and type 2 diabetes [80]. The benefits and safety of oral magnesium supplementation on glycaemic control in patients with Type 2 diabetes are yet to be ascertained. However, researchers have found that oral magnesium supplementation for 1- 4 months can effectively reduce plasma fasting glucose levels and raise HDL cholesterol in patients with Type 2 diabetes [81].

Manganese is an essential element that plays an important role in various physiological functions. It is important for regulating blood sugar by the metabolism of glucose and insulin secretion [82]. Manganese deficiency is reported to be associated with impaired glucose tolerance and increased risk of metabolic syndrome through impaired glucose and lipid metabolism [83]. Gong *et al.* examined the association between manganese intake and the risk of type 2 diabetes in postmenopausal women. Their research founding showed that dietary manganese was associated with a lower risk of type 2 diabetes among postmenopausal women. Therefore, consuming food groups rich in manganese could potentially target intervention against type 2 diabetes risk in postmenopausal women [84].

The presence of these essential minerals in the leaves of VA highlights the plant's usefulness for managing diabetics.

Table 6. Mineral composition of VA (mg/100g dry weight).

Mineral	Reference			
	[19]	[50]	[46]	[31]
Calcium (mg/100g)	71.50	166.372	145	67.39
Sodium (mg/100g)	52.76	819.469	-	8.48
Iron (mg/100g)	16.43	6.637	5.0	14.20
Zinc (mg/100g)	18.15	3.423	85.0	8.05
Potassium (mg/100g)	73.25	973.451	-	60.90
Phosphorus (mg/100g)	12.52	-	6.7	60.90
Magnesium (mg/100g)	61.08	19.604	-	88.10
Manganese (mg/100g)	3.16	0.401	710.0	5.56
Copper (mg/100g)	1.06	2.521	-	6.01

4. Conclusions

In conclusion, diabetes mellitus is a global phenomenon that is spreading around the world rapidly. There are many conventional drugs for diabetes maintenance in the market. However, most of them have some serious side effects or are not suitable for long usage periods. Herbal medicine is a form of healthcare that has been known to humankind for centuries, and in many countries, it is used in diabetes treatment. One such herbal plant is *Vernonia amygdalina*, a multipurpose plant with many uses, health benefits, and bioactivities. The full potential of this plant for diabetes drug development is still not fully exploited. Thus, this literature review will stimulate further scientific research, possibly discovering novel or lead pharmaceutical agents.

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Conflicts of Interest

The authors declare no conflict of interest, financial or otherwise.

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