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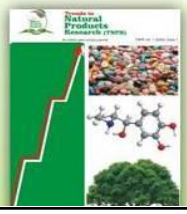

Anti-Diabetic Activity of Ethanol Stem Bark Extract of *Dacryodes Klaineana* (Pierre) H. J. Lam (Burseraceae) On Alloxan Induced Diabetic Rats.

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Abstract

There is paucity of scientific knowledge concerning the general pharmacological activity of *Dacryodes klaineana*. Conventional anti-diabetic drugs are associated with adverse drug reactions and several side effects. The aim of this study was to screen the phytochemical composition and the anti-diabetic properties of ethanol extract of the stem bark of *Dacryodes klaineana* in alloxan-induced diabetic rats. The powdered stem bark was macerated in ethanol for 72hrs and two third of the total weight of the extract obtained was fractionated with n-hexane, ethyl acetate and n-butanol using column chromatography. The toxicity study, phytochemical screening and analytical evaluation of the powdered sample were done using standard methods**.** The albino Wistar rats were randomly divided into groups of four rats each and diabetes was induced in the rats intraperitoneally using 120 mg/kg of alloxan monohydrate. Groups of diabetic rats were treated with oral doses of 200 and 400 mg/kg of the ethanol crude extract (ECE), 200 mg/kg of each fraction and 5 mg/kg of glibenclamide (standard) for 14 days. Blood glucose levels of the rats were measured every 3 days for 14 days. The macroscopic evaluation revealed a reddish-brown stem bark with a characteristic smell when powdered, a rough texture when touched and is brittle and quill shaped when dried. The analytical evaluation of the powdered sample produced total ash value of (6.50%), acid insoluble ash (0.67%), water soluble ash (5.00%), and moisture content (7.83%), ethanol extractive value (4.00%), n-hexane extractive value (0.50%) and ethyl acetate extractive (1.00%). Significant reductions ($p<0.05$) in blood sugar level by the crude extract and fractions as well as the standard drug groups were evident. The result of the phytochemical analysis revealed the presence of glycosides, tannins, flavonoids, proteins, saponins, reducing sugars. carbohydrates, steroids and terpenoids in the crude extract. The ethanol extract of *Dacryodes klaineana* stem bark has potential anti-diabetic properties that could be explored in the development of new anti-diabetic agent.

Keywords
Dacryodes klaineana, glibenclamide, alloxan,hyperglycemia, diabetes mellitus

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Introduction

Throughout the ages people have turned to herbal medicine for healing. All cultures have folk medicine traditions that include the use of plants and plant products. In Nigeria, herb sellers and farmers are known to treat human diseases such as diabetes and malaria with herbs and other traditional practices before the advent of orthodox medicine. They were able to distinguish between useful herbs with beneficial effects and those that were inactive or toxic (Kunle *et al.*, 2012).

Diabetes mellitus describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long term damage, dysfunction, and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death (WHO, 1999). Diabetes mellitus has been identified as the most common endocrine disorder that currently affects 200 million people of the world's population (Wais *et al*., 2012). It is projected to rise to over 366 million in the year 2030 (Wild *et al.*, 2004). In 2019, according to International Diabetes Federation (IDF) report, 463 million adults (20-79 yrs) were living with diabetes and by 2045 this will rise to 700 million. 79% of adults with diabetes were living in low and middle-income countries and 1 in 5 people who are above 65 years have diabetes (IDF, 2019). The number of individuals with diabetes is rising rapidly throughout the world and it is predicted that about 366 million people are likely to be diabetic by the year 2030 (Oyedemi*,* 2011).

The Burseraceae are a moderate-sized family of 17-19 genera and about 540 species of flowering plants. The actual numbers differ according to the time and period in which a given source is written describing this family. The Burseraceae are also known as the torchwood family (Dimmitt, 2016). The frankincense and myrrh family, or simply the incense tree family, includes both trees and shrubs, and is native to tropical regions of Africa, Asia, Australia, and the Americas. *Dacryodes klaineana* is an evergreen perennial tree within the Burseraceae family. It is locally called monkey plum and African cherry fruit because of its edible pulp. It was reported that *D. klaineana* leaves are used to treat tachycardia and cough (Brink, 2008). The ground leaf is used as enema applied against painful menstruation. The fruit is eaten raw or cooked and it has a high demand in the Eastern part of Nigeria. In many populations, conventional anti-diabetic drugs are often unaffordable and/or inaccessible.

Consequently, these problems and the adverse effects associated with the use of the existing conventional anti-diabetic drugs, necessitated the search for new drugs as an alternative to combat diabetes. The aim of this study was to screen the phytochemical composition and the anti-diabetic activity of ethanol extract of the stem bark of *Dacryodes klaineana* and its fractions using *in-vivo* techniques.

Materials and Methods

Plant Collection

Dacryodes klaineana stem bark was collected from Nsukka Local Government Area of Enugu State, Nigeria in November 2020. It was authenticated by Mr. Alfred Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD). A voucher specimen with number ESUT/COG/202011 was deposited at the herbarium of the Department of Pharmacognosy, Enugu State University of Science and Technology, Agbani., Enugu, Nigeria.

Animals

Wistar albino rats (79-123 g), 6 to 12 weeks of age were used. The mice were bred at the Animal House of the Department of Pharmacology, Enugu State University of Science and Technology, Enugu State under standard conditions. They were housed in aluminium cages in soft wood shavings as beddings in a room, at room temperature, 24.5-26 humidity, with free access to drinking water and rat chow (Grand Cereals Ltd, Enugu Nigeria). The experimental protocols were in accordance with an established Ethical guideline of the Enugu State University of Science and Technology (approved ref. no.: ESUT/AEC/0102/ AP117).

Plant Preparation and extraction

The stem bark was washed with water to remove adhering dirt and air-dried for 14 days under room temperature (25±0.5º C) and then pulverized using a milling machine to coarse uniform. The powdered crude sample was then evaluated for ash value and extractive value using standard methods described by Obi *et al.* (2021). The powdered stem bark (800 g) was macerated in 2000 ml of ethanol for 72 hours. The extract was first filtered using a muslin cloth and then further filtered using Whatman No. 1 filter papers. The filtrate was concentrated using a rotary evaporator, at 40º C and further heated in a water bath at 40º C for 12 hours. The percentage yield of

the extract was then determined. The dried ethanol extract was fractionated with n-hexane, ethyl acetate and n-butanol using column chromatography. The fractions were concentrated with rotary evaporator set at 40º C and 120 rpm and the resultant extracts were further dried at 40º C in the oven. The dried extract/fractions in airtight containers were stored in a refrigerator.

Phytochemical Analysis

Phytochemical tests were carried out on the ethanol stem bark extract using standard methods (Harbourne, 1973; Evans, 2009).

Acute toxicity test

The acute toxicity test of the extract was estimated in mice using the method of Lorke (1983).

Induction of diabetes

The albino rats were fasted overnight (12-14hrs) and their weights recorded. Their fasting blood glucose levels were recorded using Accu-check glucometer (Roche Diagnostic Corporation, Mannheim, Germany). Diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan monohydrate solution (Sigma-Aldrich, USA), (120 mg/kg) and 10% glucose solution, was kept in their cages for the next 24 hrs to prevent hypoglycaemia. Food was allowed 30 minutes after administration of alloxan. After 48 hrs of alloxan injection, blood glucose level of each animal was determined by taking blood from the tail vein and animals with a fasting blood glucose level above 200 mg/dl were used for the study (Jamal *et al*., 1997; Sabu and Subburaju, 2002).

Experimental design

Sub-acute antidiabetic study

Eight (8) groups of diabetic rats (group B to H) and one group of non-diabetic rats (group A) (n=5) were

selected randomly. Group B (negative control) received no treatment, while group C received glibenclamide (5 mg/kg), group C and D received 200 and 400 mg/kg of the crude extract respectively. Group E received 200 mg/kg of n-hexane fraction, group F received 200 mg/kg of ethyl acetate fraction and group H received 200 mg/kg of n-butanol fraction. The treatments were administered orally, daily for 14 days and their blood glucose level was measured every 3 days of treatment for 14 days.

Statistical Analysis

Data obtained from the study were expressed as the mean values ± SEM (n=5). Differences among means of control and tested groups were determined using one-way analysis of variance (ANOVA) using IBM SPSS software followed by Turkey comparison test for significance. A probability level of less than 5% ($p < 0.05$) was considered significant.

Results

Macroscopic evaluation of the stem bark of D.

Klaineana

The macroscopic evaluation revealed a reddish-brown stem bark with a characteristic smell when powdered. The stems bark showed a brittle and quill shape when dried. These parameters can be used in qualitative evaluation of the plant during collections to avoid adulteration.

Analytical evaluation of the powdered stem bark

The results of physicochemical evaluations of *D. klaineana* stem bark (Table 1) relatively high moisture content, ash values and extractive values.

Table 1. Physicochemical characteristics of the powdered stem bark sample

S/N	Parameter	Value (% w/w)
1.	Moisture content	7.83±0.17
	Ash values	
2.	Total ash	6.50± 0.00
3.	Water soluble ash	5.00 ± 0.00
4.	Acid insoluble ash	0.67 ± 0.17
	Extractive values	
5.	N-hexane soluble extractive value	0.50±0.17
6.	Ethyl acetate soluble extractive value	1.00±0.02
7.	Ethanol soluble extractive value	4.00±0.60

Phytochemical Analysis

The preliminary phytochemical screening showed that the crude extract contained glycosides, flavonoids, saponins, tannins, proteins, steroids, terpenoids and alkaloid. Glycosides, alkaloids, steroids and terpenoids were detected in n-hexane fraction. Glycosides, flavonoids, tannin, carbohydrate and steroids in ethyl acetate while glycosides, flavonoids, saponins, tannins and steroids were present in the n-butanol fraction (Table 2).

Table 2. Phytochemical constituents of the ethanol extract and fractions

S/n	Constituents	Ethanol Extract	N-Hexane	Ethyl Acetate	N-Butanol
1.	Glycosides	+	+	+	+
2	Flavonoids	+	-	+	-
3	Alkaloids	+	+	+	-
4	Saponins	+	-	-	+
5	Tannins	+	-	+	+
6	Steroids	+	+	-	-
7	Terpenoids	+	+	-	-

Key: + = Present - = negative

Acute toxicity test

Oral administration of the extract up to 5000 mg/kg produced no significant changes in behavior of the mice. No death occurred in any of the group 24 hrs after treatment suggesting an LD_{50} of above 5000 mg/kg.

Effects of the extract and fractions on diabetic rats

There was a significant reduction ($p<0.05$) in FBG by all doses of the crude extract and fractions within 14 days of administration (Figure 1). The n-hexane fraction (200 mg/kg) exhibited the fastest onset of significant anti-diabetic activity than the other treatments when compared with the negative control. On the 6^{th} to 14^{th} day, all the treatment groups exhibited significant reduction ($p<0.05$) in blood glucose except the 200 mg/kg of the crude extract that produced significant reduction only at the 9^{th} day (Figure 2). The n-hexane fraction evoked the highest reduction in blood glucose (Figure 3).

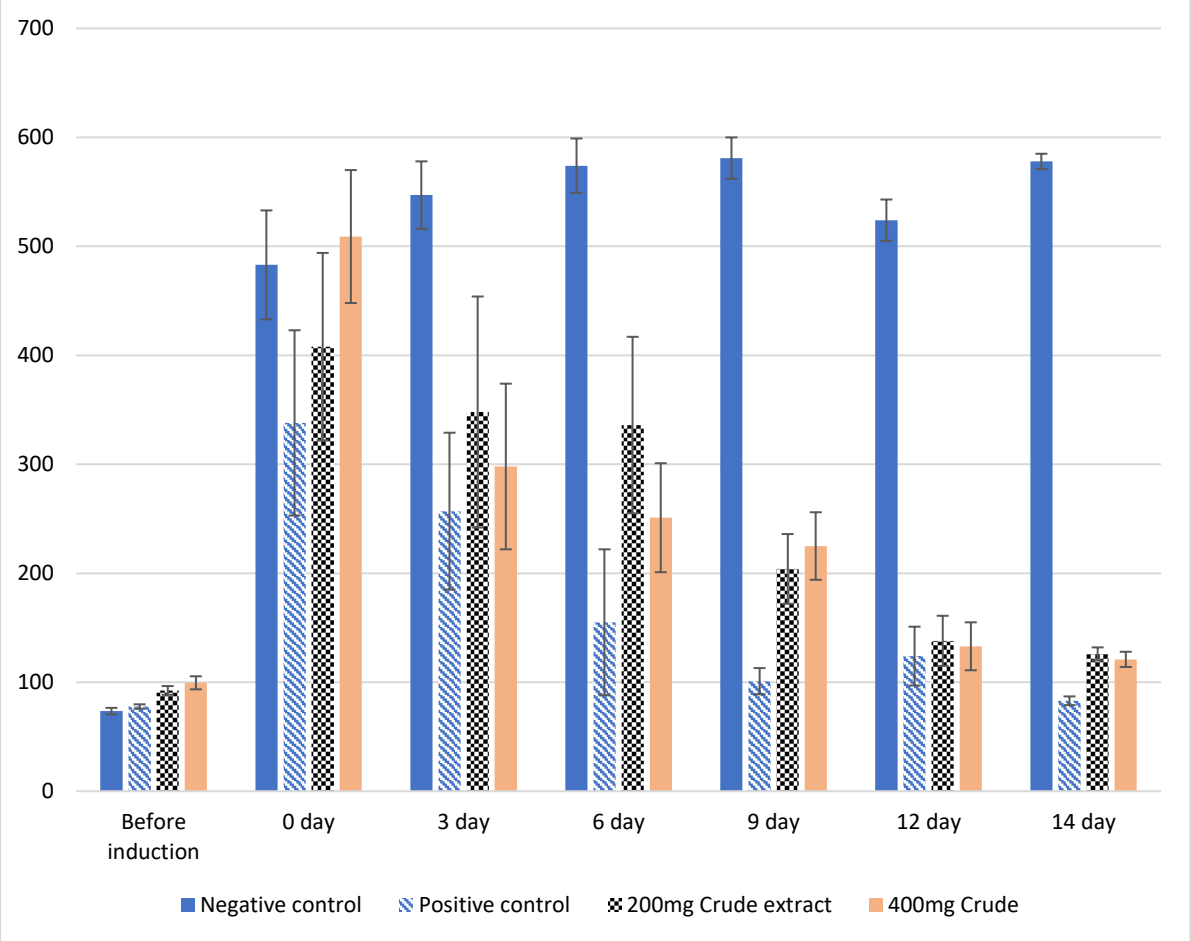

Figure 1. Effect of the crude extract on diabetic rats

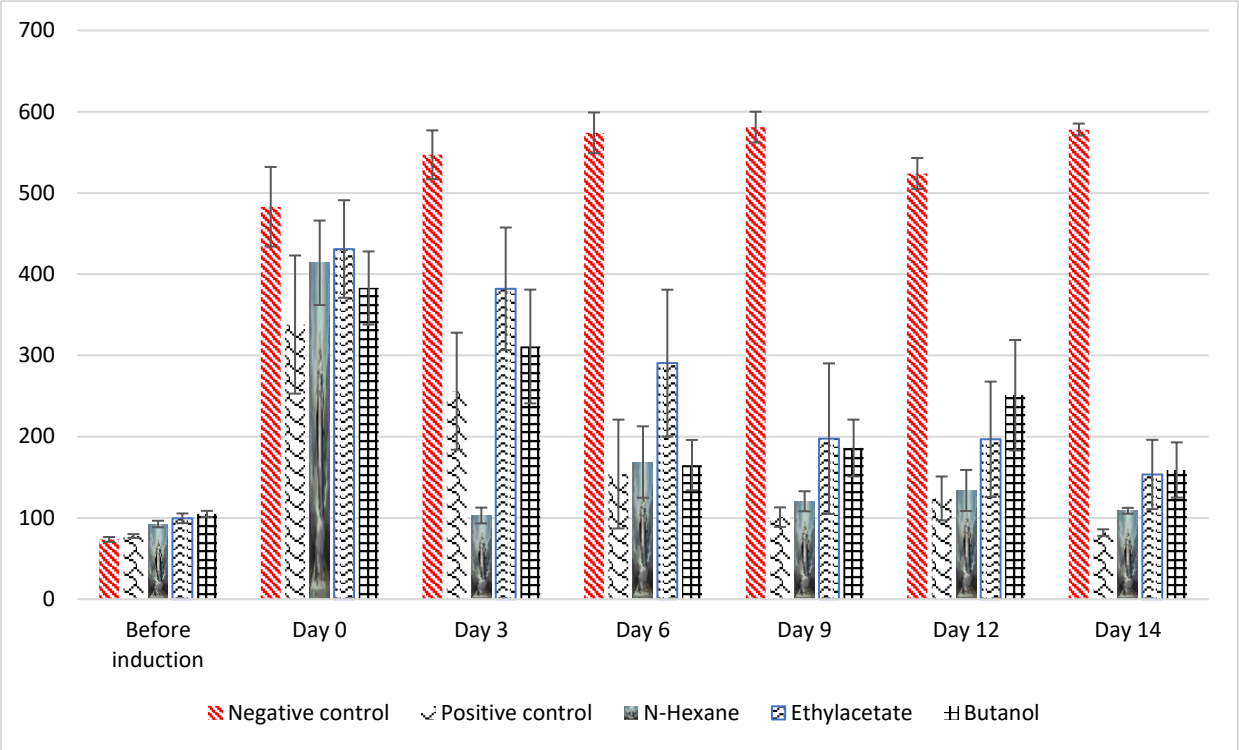

Figure 2. Effects of the fractions on diabetic rats

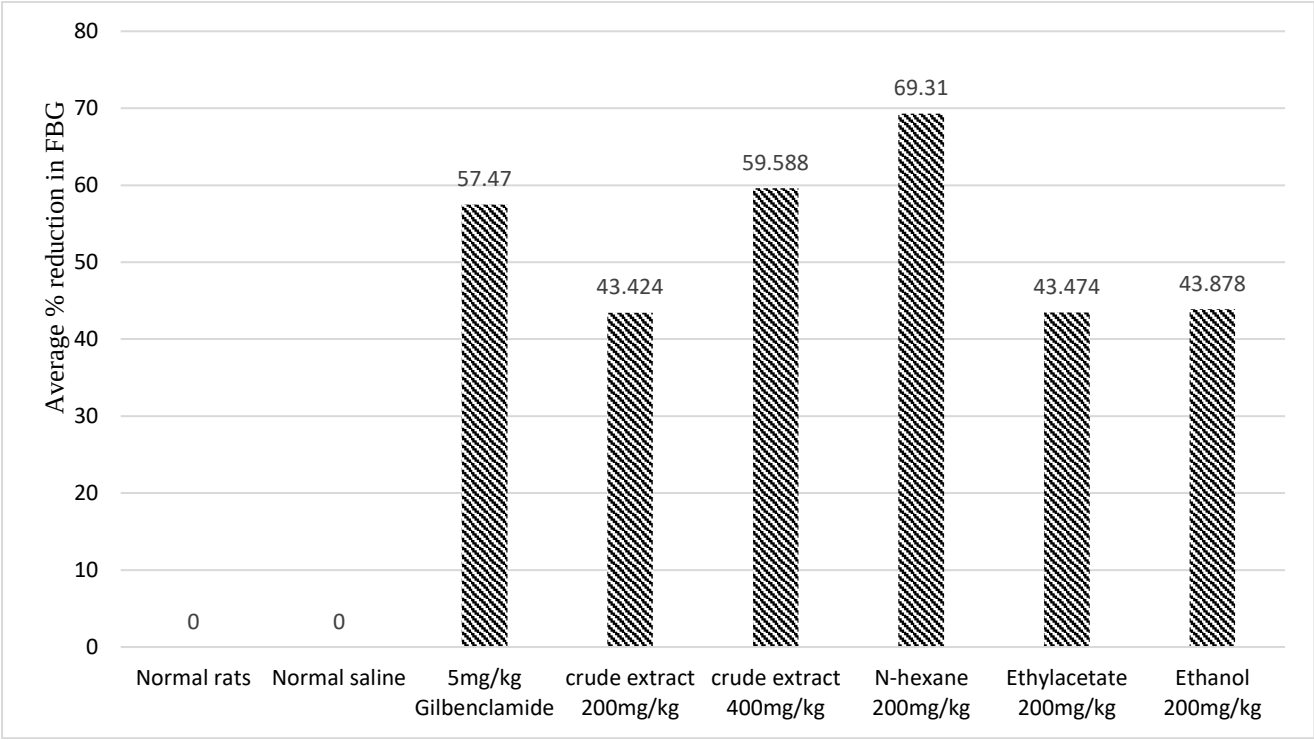

Figure 3. Percentage blood glucose reduction by the extract and fractions.

Discussion

The macroscopic evaluation parameters can be used in qualitative evaluation of the plant during collections to avoid adulteration. The extractive value of the stem bark sample showed that water gave the highest extractive value followed by ethanol.

The powdered sample had low moisture content (7.83%) and therefore will have high stability on storage, and it will not be prone to some storage factors due to high water activities. High water activities in pharmaceutical products encourage microbial infestation that may eventually result to microbial degradation. There were insignificant amount of extraneous earth matters like siliceous earth matters, sand and water-soluble salts in the powder stem bark based on the result obtained from the total ash, acid insoluble and water-soluble ash. These parameters can help to identify any adulteration in the product.

The phytochemical screening showed that the stem was rich in saponins, tannins, proteins, carbohydrates, reducing sugars, steroids, flavonoids, terpenoids and alkaloids. These compounds are known to show medicinal activity as well as exhibit physiological activities (Sofowara, 1993). Thus, the anti-diabetic effect of the extract could be due to the presence of these phytoconstituents which could act synergistically or independently to enhance the activity of glycolytic enzymes. Flavonoids such as chrysin and isoquercitrin have been shown to be involved in insulin secretion (Roman-Ramos *et al.,* 1995). Other mechanisms of anti-diabetic activity of flavonoids include to target glycogen synthesis, glycolysis, and gluconeogenesis (Jung *et al.*, 2004), restoration of pancreatic cell and insulin secretions (Waltner-Law *et al.*, 2002), enhancing lipid and glucose metabolism by activation and increasing of peroxisome proliferator activated receptors (PPARs) and decrease insulin resistance (Howes *et al.*, 2003). Saponins are bioactive compounds present naturally in many parts and known to possess potent antihyperglycemic activity. Saponins (such as Stigmasterol, quercitol and gymneic acid) and glycosides are effectively involved in regeneration of pancreatic β cells, insulin secretion and free radical scavenging (Ma *et al.,* 2008). Glycosides are also involved in glucose transport and carbohydrate metabolism. Certain carbohydrates also have considerable hypoglycemic effect. They have been shown to be involved directly and indirectly in insulin secretion, carbohydrate digestion and absorption (Kar *et al.,* 2003). According to Wang *et al.* (2010) some alkaloids are known hypoglycemic agents. For instance, berberine, a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids has been used successfully in experimental models of diabetes mellitus and in clinical studies (Ikuta and Itokawa, 1988).

The acute toxicity study showed that the plant extracts had no observable adverse effect at the doses tested, which implies that the crude extract is not lethal up to 5000 mg/kg in mice. According to Khan and Shechter (1991), a 25 % reduction in blood glucose levels is considered a significant anti-hyperglycaemic effect. The plant extract significantly reduced the blood glucose level in alloxan induced treated mice in a 14-day observation, in a dose dependent manner, which implies that the plant extract reduces glucose level more effectively.

The n-hexane fraction gave the highest anti diabetic activity which may be due to the secondary metabolites such as terpenoids and alkaloids that were mostly found in n-hexane fraction. Terpenoids have been implicated for most of the reported biological activities in previous studies on the *Dacryodes* genus (Okolo *et al.,* 2016). It is an established fact that antioxidant compounds bear metabolic relationship with diabetes mellitus due to their ability to reduce oxidative stress associated with diabetes. Terpenes are large class of plant's secondary metabolites with large percentage of their members having reported antioxidant activity (Okolo *et al.,* 2016). Alkaloids have shown no less efficacy in lowering blood glucose level as they have been shown to activate free radical scavenging enzymes (Kar *et al.*, 2003; Van *et al.*, 2008), regeneration of pancreatic beta cells maintain proper amount of insulin by increasing expression of insulin gene, increasing secretion of insulin, and inhibiting their degradation (Sudhanshu *et al.*, 2018).

Though the exact mechanism of action of the extract has not been exhaustively investigated, the extract possibly acted by potentiating the insulin effect either by increase in pancreatic secretion of insulin from beta cells of pancreatic islets of Langerhans, regeneration of pancreatic cells and by increase in peripheral glucose uptake. They could be acting similar to glibenclamide which secretes insulin from beta cells in type II.

Conclusion

In conclusion, the ethanol stem bark extract of *Dacryodes klaineana* has an anti-diabetic activity as revealed by reductions in fasting blood glucose. Hence, this study supports the use of the plant in the treatment of diabetes.

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