Chemical Constituents and Effect of Ethanol Leaf Extract of *Hibiscus articulatus* Hochst. ex A. Rich. (Malvaceae) on Glucose- and Streptozotocin-Induced Hyperglycaemia using Wistar Rats

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Abstract

Hibiscus articulatus is an herbaceous plant that is consumed over decades as diet, in management stomach pain and diabetes in north central Nigeria. The aim of the study is to determine the chemical constituents and evaluate the effect of ethanol leaf extract of *Hibiscus articulatus* (ELEHA) on glucose- and streptozotocin-induced hyperglycaemia using Wistar rats. Preliminary phytochemical screening, proximate and gas chromatography-mass spectrophotometric (GC-MS) analyses was conducted on ELEHA. Acute toxicity, blood glucose levels of ELEHA in Wistar rats was evaluated using; glycaemic index (GI) test, oral glucose tolerance test (OGTT) and streptozotocin-induced hyperglycaemia test. Secondary metabolites; alkaloids, tannins, saponins, flavonoids, triterpenes and primary metabolites; carbohydrates, proteins and fats, were present in ELEHA. Ascorbic acid, linoleic acid, coumaran, phytol hexadecanoic acid are some chemical constituents present in ELEHA. The oral median lethal dose was estimated to be > 5000 mg/kg body weight in rats. The glycaemic index and glycaemic load of ELEHA were calculated to be 41.60 % and 17.83 respectively. There was significant ($p < 0.05$) decrease in the blood glucose level (250 and 500 mg/kg of ELEHA administered) in OGTT and streptozotocin-induced hyperglycaemia test compared with diabetic group. Superoxide dismutase, catalase, malondialdehyde and glutathione reductase were evaluated however, only superoxide dismutase levels (250 and 500 mg/kg) were significantly ($p < 0.05$) increased. Moreso, the level of low density lipoprotein (125 mg/kg) and HOMA IR values (125 and 250 mg/kg) were significantly ($p < 0.05$) decreased when compared with diabetic group. In summary, the ethanol leaf extract of *Hibiscus articulatus* possesses important phytochemicals with anti-hyperglycaemic activity on Wistar rats.

Key Words: *Hibiscus articulatus,* Acute toxicity, Anti-hyperglycaemia, Glycaemic index, Chemical constituents

Introduction

Hibiscus articulatus (ex A. Rich) is a greenish leafy herb with fibrous stem 60 cm - 2 m high, that grows in grassy and tropical forests (Burkill, 1997; Kunatsa *et al.,* 2020; Hyde *et al.,* 2022). *Hibiscus articulatus* grows in Nigeria in the following states; Adamawa, Kogi, Kwara, Ondo and Ekiti [Herbarium Section Obafemi Awolowo University (O.A. U.), 2017; Herbarium Section, Ahmadu Bello University (A. B. U.,) 2017]. The local names of *Hibiscus articulatus* are; "ware"/ "selekiya" in Hausa/Fulfulde, "isapa eluju" in Yoruba and "akuku" in Ebira (Herbarium Section Obafemi Awolowo University (O.A.U.), 2017; Herbarium Section, Ahmadu Bello University (A.B.U.), 2017; Personal communication). The dried leaves are made as soup and used in the management of stomach aches and diabetes mellitus in North central Nigeria. (Burkill, 1997; Personal Communication; Kunatsa, *et al.,* 2020).

Diabetes mellitus is a complex, chronic metabolic disease, which constitutes a global burden that affects the public health as well as socio-economic development (Lin *et al.,* 2020; Galicia-Garcia *et al.,* 2020). Hyperglycaemia is a major symptom of diabetes mellitus and occurs primarily from insulin resistance and/or beta cells dysfunction, and it's responsible for the development, progression and complications of diabetes (Sornalakshmi *et al.,* 2016: Moon *et al.,* 2017; Wang *et al.,* 2018). Type 2 diabetes mellitus (T2DM) is the most common and accounts for 95 % of diabetes cases worldwide (WHO, 2022). Despite the advancements made in understanding the pathophysiology of diabetes mellitus and the various orthodox drugs used for its treatment, co-morbidity and mortality from the disease complications is still very high (Sadri *et al.,* 2017; Ekuro, *et al.,* 2019), with global prevalence estimated at 537 million and a projected increase in incidence to 643 million by 2030 (IDF, 2021). The high prevalence and complications of diabetes is partly due to unhealthy lifestyles (WHO, 2022), late diagnosis (Lin *et al.,* 2020), less attention and sub-optimal adherence to non-drug and drug therapy, particularly in type 2 diabetes management (Ogbera and Ekpebegh 2014; Davies *et al.,* 2018; Godman *et al.,* 2020). The standard management strategies encourage both non-pharmacological and pharmacological therapy, with major emphasis on non-drug (dietary, exercise and education) therapy, especially in T2DM (ADA, 2020; IDF, 2021). Although, there is no standard diet specified in diabetes management, the choice of diet is influenced by the disease knowledge and nutritional education, age, gender, educational qualification, socio-economic condition, financial status, religious and cultural beliefs of individuals (Colles *et al.,* 2013; Ogbera and Ekpebegh, 2014; Sami *et al.,* 2017; Tirfie *et al.,* 2020). Researches revealed that some plants have both nutritive and medicinal functions (Ojewumi and Kadiri, 2013), they are natural, potent, less expensive, available and accessible, with less side effects (Tran *et al.,* 2020). Some of these plants and their parts had been evaluated and used as diets to treat diabetes (Paswan *et al.,* 2016; Sunmonu, and Lewu, 2019; ADA, 2020). However, no scientific documentation regarding the efficacy and safety of *Hibiscus articulatus*, hence the need to evaluate the chemical constituents and effect of ethanol leaf extract of *Hibiscus articulatus* on blood glucose level, so as to provide scientific evidence and guide to support its acclaimed ethno-medicinal uses.

Materials and Method

Drugs and Chemicals

Ammonium solution (BDH LTD Poole, England), bromine water (JDH, China), citrate buffer (Sigma-Aldrich Germany), conc. sulphuric acid (BDH LTD Poole, England), ferric chloride solution (JDH, China), distilled water, sodium hydroxide (BDH LTD Poole, England), n-hexane (JDH, China), boric acid (BDH LTD Poole, England), 40 % sodium hydroxide (BDH LTD Poole, England), ammonium sulphate (BDH LTD Poole, England), concentrated nitric acid (BDH LTD Poole, England), concentrated hydrochloric acid (BDH LTD Poole, England), Molisch reagent (BDH LTD Poole, England), glacia acetic acid (JDH, China), diethyl ether (BDH LTD Poole, England), D (+) glucose (Shanghai, China), ethanol (Guangdong Guanghua Sci-Tech Co. China), formalin (10 %), metformin (Hovid), normal saline (Dana Pharmaceuticals Ltd), streptozotocin (Sigma-Aldrich Germany), Rat Insulin ELISA Kit (Biotech Co. Ltd, Shanghai). Randox enzymatic kit.

Equipment and Apparatus

Weighing scale "g" (Golden-Mettler USA), Mettler electronic balance "mg" (AE240 Switzerland), glucometer (Accu-check Roche-Germany), GC-MS machine (Shimadzu QP-2010), , microplate reader (RT-2100C Rayto, India), heating incubator 37˚C (DHP-9035A model), atomic absorption spectrum (Varian AA240FS), water bath, micro-pipette 10-100µL (Dragon Laboratory), desiccator (Monax-Scotland), mortar and pestle, syringe and needle, thermometer, Soxhlet apparatus, pipette.

Plant Material

The fresh plant of *Hibiscus articulatus* was collected in August from Upake, Adavi Local government of Kogi State, Nigeria. It was identified and authenticated by Mallam Sanusi Namadi of the Department of Botany, Faculty of Life Science, Ahmadu Bello University, Zaria. The plant sample was compared with an existing library specimen and a voucher number (2267) was assigned to it.

Experimental Animal

Wistar rats (180-220 g) used for this study were obtained and kept in Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, A.B.U., Zaria. The guidelines for the care, handling and use of laboratory animals, as adopted and promulgated by A.B.U. Committee on Animal Use and Care (ABUCAUC) was studied for compliance and approval certificate (ABUCAUC/2018/073) was obtained from the Institution's Ethical Committee.

Preparation of Plant Material

The leaves of *Hibiscus articulatus* was separated from the stem, cleaned and air-dried under shade for fourteen days. The dried leaves were pulverized into coarse powder using mortar and pestle. Five hundred (500) g of the coarse powder was extracted using cold maceration with 1.5 liter of 70 % $^{v}/_{v}$ ethanol (in water) for 72 hours, with occasional stirring using a glass rod. The resultant mixture was then filtered using Whatman filter paper (No.1) and concentrated to dryness using evaporating dish over a water-bath, maintained at a temperature 30-40 ^{o}C until a constant weight of the extract was obtained. The extract was weighed, labelled as ethanol leaf extract of *Hibiscus articulatus* (ELEHA), kept in an airtight container and stored in a desiccator until required for further studies. The percentage yield of the extract was then calculated as shown in the formula below: Percentage yield= (weight of dried extract / weight of dried leaf used) × 100 %.

Preliminary Phytochemical Screening of Ethanol Leaf Extract of *Hibiscus articulatus*

Phytochemical screening tests for detecting presence of various secondary metabolites in the ELEHA was conducted using the standard procedures of Sofowora (1993) and Trease and Evans (2002).

Proximate Analysis of Ethanol Leaf Extract of *Hibiscus articulatus*

The qualitative and quantitative test for the primary metabolites presents in ELEHA was conducted according to the methods of A0AC, (1980) and Pearson (1976).

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of Ethanol Leaf Extract of *Hibiscus articulatus*

The GC-MS of ELEHA was conducted according to the method described by Okhale *et al.* (2018) using Shimadzu QP-2010 GC with Mass selective detector (MSD) [operated at electron energy = 70 eV, scan range = 45-700 amu, and scan rate = 3.99 scans/sec)] and Shimadzu GC-MS solution data system. One microliter (1 µL) of diluted ELEHA sample (500 µg/ml in ethanol w/v) was injected using auto-sampler and in the split mode with ratio of 20:80. Individual constituents were identified by comparing their mass spectra with known compounds present in the National Institute of Standards and Technology (NIST) Mass Spectra Library (NIST II). The percentage area of each components was reported as raw percentage based on the total ion current without standardization.

Acute Toxicity Study (Lorke method)

The oral median lethal doses (LD_{50}) of ELEHA was determined in rats using the method described by Lorke (1983). The Wistar rats was fasted overnight and the LD_{50} evaluation was carried out in two stages. In the first stage, nine Wistar rats were randomly divided into three groups of three mice each. Groups I, II and III were treated with the extract at doses of 10, 100 and 1000 mg/kg body weight orally respectively. In the second phase, three Wistar rats were placed in three different cages with one Wistar rat each. Groups I, II and III were administered the extract at doses of 1600, 2900 and 5000 mg/kg body weight respectively. In both phases, the Wistar rats were observed for 24 hours for signs of toxicity and mortality. The LD_{50} value was then calculated as the geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred).

Experimental Design

Glycaemic Index Test; consisted of 2 groups of 6 Wistar rats per group.
Group I (reference); received D (+) glucose 2g/kg body weight only,
Group II (test); received ELEHA 2g/kg body weight only

Oral Glucose Tolerance Test; consisted of 6 groups of 6 Wistar rats per group.
Group I [normal control (NC): non-diabetic]; received distilled water1 mL/kg only,
Group II [diabetic control (DC)]; received distilled water 1 mL/kg + D-glucose 2g/kg
Group III; received ELEHA 125 mg/kg + D-glucose 2g/kg
Group IV; received ELEHA 250 mg/kg + D-glucose 2g/kg
Group V; received ELEHA 500 mg/kg + D-glucose 2g/kg
Group VI; received metformin 250 mg/kg + D-glucose 2g/kg

Streptozotocin-induced Hyperglycaemia Test; consisted of 6 groups of 9 Wistar rats per group.
Group I [normal control (NC): non-diabetic]; received distilled water 1 mL/kg only,

Group II [diabetic control (DC)]; received streptozotocin 45mg/kg + distilled water 1 mL/kg
Group III; received streptozotocin 45mg/kg + ELEHA 125 mg/kg
Group IV; received streptozotocin 45mg/kg + ELEHA 250 mg/kg
Group V; received streptozotocin 45mg/kg + ELEHA 500 mg/kg
Group VI; received streptozotocin 45mg/kg + metformin 250 mg/kg

Experimental Procedure

Glycaemic Index Test in Wistar Rats

The experiment was conducted according to the method described by Wolever *et al.,* (1991), as modified by Ijarotimi *et al.*, (2015). Twelve (12) Wistar rats were divided into two (2) groups of six (6) Wistar rats per group. The basal blood glucose level (accu-chek glucometer) in mg/dL and body weight (weighing balance) in grams, of each rat was taken and recorded. Rats in group I received 2 g/kg each of glucose, while rats in group II received 2 g/kg each of ELEHA orally. Monitoring and recording of blood glucose level (mg/dL) was continued for each rat at 30, 60, 90 and 120 minutes after glucose and ELEHA administration. Blood glucose curves for each rat were constructed from blood glucose values of rats at time 0, 30, 60, 90- and 120-minutes intervals after consumption of the glucose (reference food) and ELEHA (test food). The Incremental Area Under Curve (IAUC) was calculated for each rat separately in the group that received 2 g/kg glucose, by using the trapezoidal rule, to reflect the total rise in blood glucose concentration after administration of glucose. Similarly, the IAUC for each rat that received 2 g/kg ELEHA (test) was calculated using the same method as shown below;

IAUC (using the formula for calculating area of a trapezium) = (a+b/2) × h, where a = length, b = breath and h = height. The glycemic index (GI) was then calculated by ratio of IAUC for ELEHA (test) to the IAUC for glucose (reference) multiply by 100 as shown below:

$$\text{GI} = \frac{Incremental\ area\ under\ the\ 2\ h\ blood\ glucose\ curve\ of\ 2\ g\ equiv.\ ELEHA}{Incremental\ area\ under\ the\ 2\ h\ blood\ glucose\ curve\ \ of\ 2\ g\ glucose} \times 100$$

The scale for measuring glycaemic index ranges from 0–100 [(glycaemic index ≤ 55 is low, 56–69 is considered medium and ≥ 70 is high)]. D (+) glucose has a glycaemic index of 100 and is used as standard for comparing sugar contents of diets (Eleazu, 2016; Campbell *et al.*, 2017).

Calculation of glycemic load (GL) for ELEHA was determined by the method of Salmeron *et al.*, (1997). Glycemic load was calculated by taking the percentage of carbohydrate content present in a typical 100 g ELEHA and multiplying it by its GI value as shown below:

$$\text{GL} = \frac{Net\ Carbohydrate\ (g) \times GI}{100}$$

The glycaemic load is classified on a scale as follows; < 10 = low-GL, 11-19 = medium-GL and > 20 = high-GL (Dona *et al.*, 2010: Oluwajuyitan and Ijarotimi, 2019). Note: Net Carbohydrate = Total carbohydrates in 100 g ELEHA = 42.85 % as presented in Table 2.

Oral Glucose Tolerance Test (OGTT) in Wistar Rats

The experiment was conducted according to the method described by Ernsberger and Koletsky (2012). Thirty-six (36) Wistar rats were grouped into 6 groups of 6 Wistar rats in each group. Rats were fasted overnight, the body weight (weighing balance) and basal fasting blood glucose (accu-chek glucometer) of each rat was taken and

recorded. Then extract and metformin were administered according to their body weights. Groups I and II Wistar rats were administered with distilled water 1 mL/kg, while Wistar rats in groups III, IV and V were administered each with ELEHA 125, 250 and 500 mg/kg respectively and Wistar rats in group VI received metformin 250 mg/kg body weight. Thirty (30) minutes after extract and metformin administration, glucose 2 g/kg was administered to each rat (groups II-VI) according to their body weight. Recording and monitoring of blood glucose levels was then continued at 30, 60, 90 and 120 minutes after glucose administration.

Streptozotocin-induced Hyperglycaemia Test in Wistar Rats

The experiment was conducted according to the method described by Siddiqqui *et al.,* 1987 (as modified by Radenkovic *et al.,* (2016). Streptozotocin was freshly prepared in 1ml of 100 mM iced cold citrate buffer (pH = 4.5) solution and injected to overnight fasted Wistar rat through the intraperitoneal route at a dose of 45mg/kg body weight. The negative control Wistar rats received 1 mL/kg body weight of distilled water. After seven days of streptozotocin administration, the blood glucose level was evaluated by tail tip cut with the aid of a scissors. A drop of blood was placed on glucose test strip attached to accu-chek glucometer. Rats with blood glucose of 250 mg/dL and above were considered hyperglycaemic and selected for the experiment. Fifty-four (54) rats were divided randomly into 6 groups of 9 Wistar rats per group. Groups I (normal control: non-diabetic) and group II (diabetic control) were administered with 1 mL/kg of distilled water, groups III, IV and V were administered with graded doses of the extract 125, 250 and 500 mg/kg body weight, while Group VI received metformin 250 mg/kg body weight. Blood glucose level in mg/dL was monitored and recorded on; day 0; at 0, 30, 60, 90 and 120 minutes and then continued on days; 7, 14 and 21. The animals were euthanized on 22nd day in diethyl ether chamber. Blood samples were then collected by cardiac puncture into plain bottles for biochemical analysis.

Biochemical assay of ethanol leaf extract of *Hibiscus articulatus* in streptozotocin-induced hyperglycaemia using Wistar rats

The blood samples in plain bottles were allowed to clot and then centrifuged at 3500 rpm for 10 minutes. The serum was separated and stored at -4 $^{\circ}$C until used. The serum was analyzed for lipid profile (low density lipoprotein, high density lipoprotein, total cholesterol, triglyceraldehyde) using Randox Manual Enzymatic Procedure. Also, superoxide dismutase (Misra and Fridovich, 1972), catalase (Pari and Latha, 2004), malondialdehyde (Ohkawa *et al.,* 1979), reduced glutathione (Ellman 1959), serum glucose (accu-chek glucometer) were evaluated, while insulin levels (Mathew *et al.,* 1985) was measured using kits obtained from Rat Insulin ELISA Kit by Biotech Co. Ltd, Shanghai. The homeostasis measurement assessment of insulin resistance (HOMA-IR) of ELEHA was then calculated as the product of the concentration of fasting serum glucose (mg/dL) and the concentration of fasting insulin obtained, divide by 405. HOMA-IR = [Fasting plasma glucose (mg/dl) × Fasting insulin (mU/L)] / 405 (Eissa *et al.,* 2015).

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS, version 20.0). Differences between means were analyzed using one-way analysis of variance (ANOVA) or repeated measure ANOVA followed by Bonferroni's post-hoc test where appropriate. Values of $p \leq 0.05$ were

considered statistically significant. Data were presented as Mean ± Standard Error of the Mean (S.E.M.) in charts, graphs and tables.

Results

Percentage Yield of the Extract

The extraction of 500 g of the dried leaf of *Hibiscus articulatus* yielded 47.52 g of extract. Hence, the percentage yield of ELEHA was calculated to be 9.50 % w/w.

Phytochemical Constituents Present in Ethanol Leaf Extract of *Hibiscus articulates*

The metabolites found in ELEHA are; alkaloids, cardiac glycosides, saponins, tannins, flavonoids, phenols, carbohydrates, triterpenes and steroids (Table 1)

Table 1: Phytochemical Constituents of Ethanol Leaf Extract of *Hibiscus articulatus*

Constituents	Tests	Inference
Alkaloids	Mayer	Present
Cardiac glycosides	Keller-Kiliani	Present
Carbohydrates	Molisch	Present
Flavonoids	Shinoda	Present
Phenols	Lead Acetate	Present
Saponins	Frothing	Present
Steroids	Salkowski	Present
Tannins	Ferric Chloride	Present
Terpenes	Liebermann Burchard	Present

Nutritional (proximate) Composition of Ethanol Leaf Extract of *Hibiscus articulatus*

Proximate analysis of ethanol leaf extract of *Hibiscus articulatus* showed the following primary plant nutrients and their percentage compositions; carbohydrate (42.85), protein (4.38), lipid (18.77), fiber (0.00), moisture (24.67) and ash (9.34) (Table 2)

Table 2: Proximate Composition of Ethanol Leaf Extract of *Hibiscus articulatus*

Constituents	Mean Percentage Composition (%)
Carbohydrate	42.85
Protein	04.38
Lipid	18.77
Fiber	00.00
Moisture	24.67
Ash	09.34
Total	100

Gas Chromatography-Mass Spectrophotometry (GC-MS) Analysis for Chemical Compounds Present in Ethanol Leaf Extract of *Hibiscus articulatus*

The ethanol leaf extract of *Hibiscus articulatus* on analysis using GC-MS machine revealed the following chemical constituents: Diethylnitrosamine, coumaran,

6-oxaheptanoic acid, dodecanoic acid, octadecatrienoic acid, 3-deoxy-d-mannoic lactone, stearic acid, ethyl alpha-D-glucopyranoside, beta-D-glucopyranoside, 4-O-beta,11-bromodecanoic acid, 2,4,6,8-tetramethyl-13-tetradeconoic acid, 3,4-dimethylcyclohexanol, palmitic acid, docosanoic acid, 1-(+)-ascorbic acid, linoleic acid, hexadecanoic acid, beta-monoglyceride, 2-amino-4-methy-oxazole, cyclopropyl-phytol, 1, 4-cyclohexanedimethanol (Table 3)

Table 3: Chemical Compounds Present in Ethanol Leaf Extract of *Hibiscus articulatus* on Analysis using Gas Chromatography-Mass Spectrophotometry Machine

Names of Compounds	Retention Time (minutes)	Retention Area (%)
Diethylnitrosamine	5.550	0.22
Coumaran	5.909	0.87
6-oxaheptanoic acid	6.214	0.96
Dodecanoic acid	7.177	0.48
Octadecatrienoic acid	9.720	11.57
3-deoxy-D-mannoic lactone	7.508	1.78
Stearic acid	9.785	2.86
Ethyl alpha-D-glucopyranoside	7.583	4.80
Beta-D-glucopyranose	7.633	10.51
4-O-beta,11-bromodecanoic acid	7.900	0.55
2,4,6,8-tetramethyl-13-tetradeconoic acid	8.136	0.20
3,4-dimethylcyclohexanol	8.252	0.31
Palmitic acid	12.509	0.67
Docosanoic acid	8.566	0.14
1- (+)-Ascorbic acid	8.732	10.18
Linoleic acid	9.667	9.05
Hexadecanoic acid	8.873	8.29
Beta-monoglyceride	12.903	0.46
2-amino-4-methy-oxazole	7.083	0.20
Cyclopropyl-phytol	9.543	6.35
1,4-cyclohexanedimethanol	11.488	0.35
Octadecatrienoate	9.879	11.57
1-hexyl-2-nitrocyclohexane	11.179	0.55
1,2-benzenedicarboxylic acid	12.903	0.46
2,2-bioxane	3.389	0.49
2,4-dihydroxy-2,5-dimethyl alanine	4.686	0.28
Ethylbenzene	6.337	0.41
Ethyl esther	9.972	1.69
1,2-benzenedicarboxylic acid	12.903	0.46
Cyclohexane	9.436	0.79

Median lethal dose of *Hibiscus articulatus*

The oral lethal median dose (LD_{50}) of ethanol extract of *Hibiscus articulatus* (ELEHA) was found to be greater than 5000 mg/kg.

Effect of Ethanol Leaf Extract of *Hibiscus articulatus* on Glycaemic Index and Glycaemic Load

The Incremental Area Under the Curve (IAUC) for 2g/kg glucose and 2g/kg ELEHA were calculated to be 7.83 and 3.27 respectively. Hence the glycaemic index of ELEHA was then calculated to be 41.60 %, while the glycaemic load of ELEHA was calculated to be 17.83.

Effect of Ethanol Leaf Extract of *Hibiscus articulatus* on Oral Glucose Tolerance Test (OGTT) in Wistar Rats

There was no statistically significant difference in mean blood glucose level at 30 minutes after extract administration, compared with diabetic control group. However, at 60, 90 and 120 minutes after extract and drug administration, there was statistically significant ($p < 0.05$) decrease in blood glucose levels for groups administered with ELEHA (250 and 500 mg/kg) and metformin 250 mg/kg when compared with diabetic control group (Figure 1).

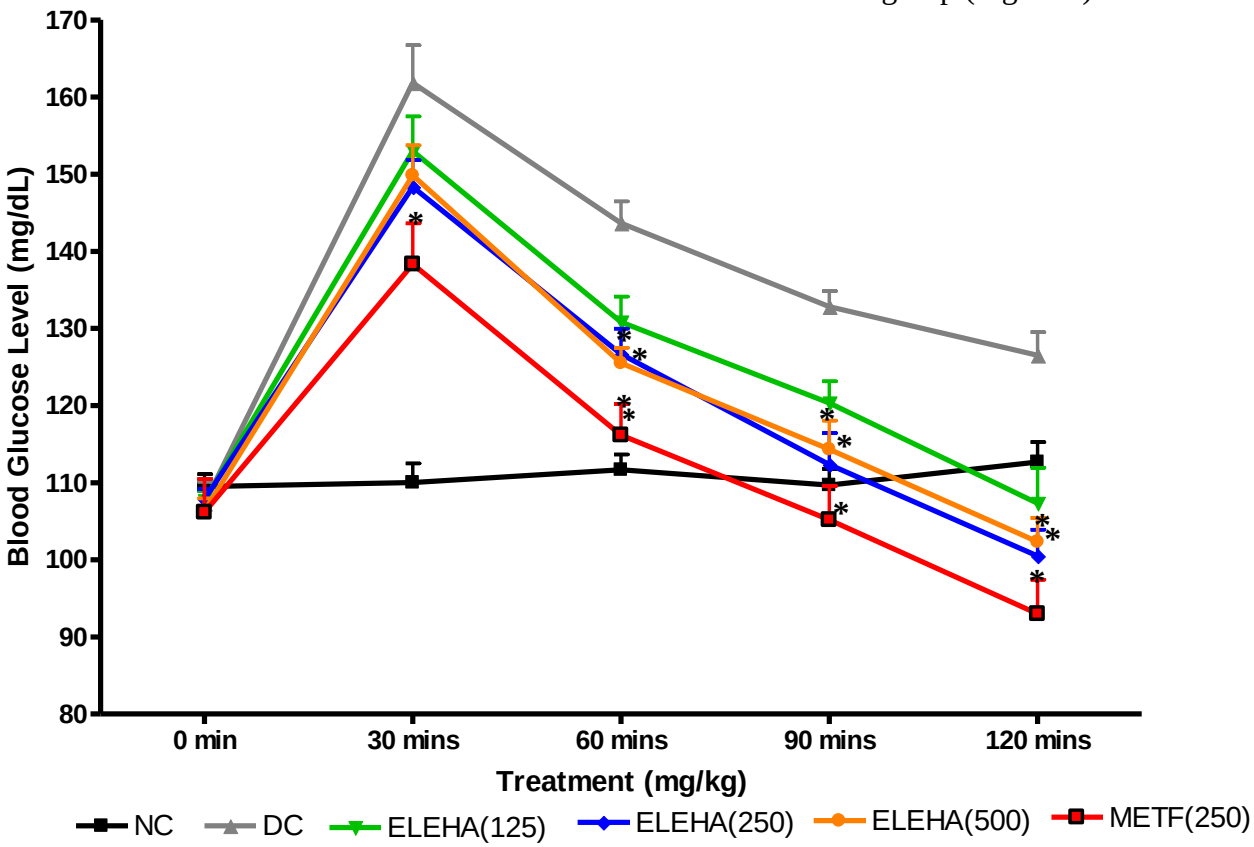

Figure 1: Effect of Ethanol Leaf Extract of *Hibiscus articulatus* on Fasting Blood Glucose Level using Oral Glucose Tolerance Test in Wistar Rats

Data were analyzed using repeated measure ANOVA with Bonferroni post-hoc test and presented as mean ± SEM. n = 6. Compared with diabetic control group: *= $p < 0.05$. NC = Normal Control, DC = Diabetic Control, ELEHA= ethanol leaf extract of *Hibiscus articulatus*, METF = metformin, mins = minutes

Effect of Acute Administration of Ethanol Leaf Extract of *Hibiscus articulatus* on Fasting Blood Glucose Levels using Streptozotocin-induced Hyperglycaemia in Wistar Rats

The ELEHA at 250 and 500 mg/kg, and metformin 250 mg/kg showed statistically significant ($p < 0.05$) decrease in blood glucose level at 90 ad 120 minutes after extract administration when compared with diabetic control group. Also, there was statistically significant decrease in blood glucose level for ELEHA 250, 500 mg/kg and metformin 250 mg/kg at times; 90 and 120 minutes when compared with 30 minutes after drug administration (Figure 2).

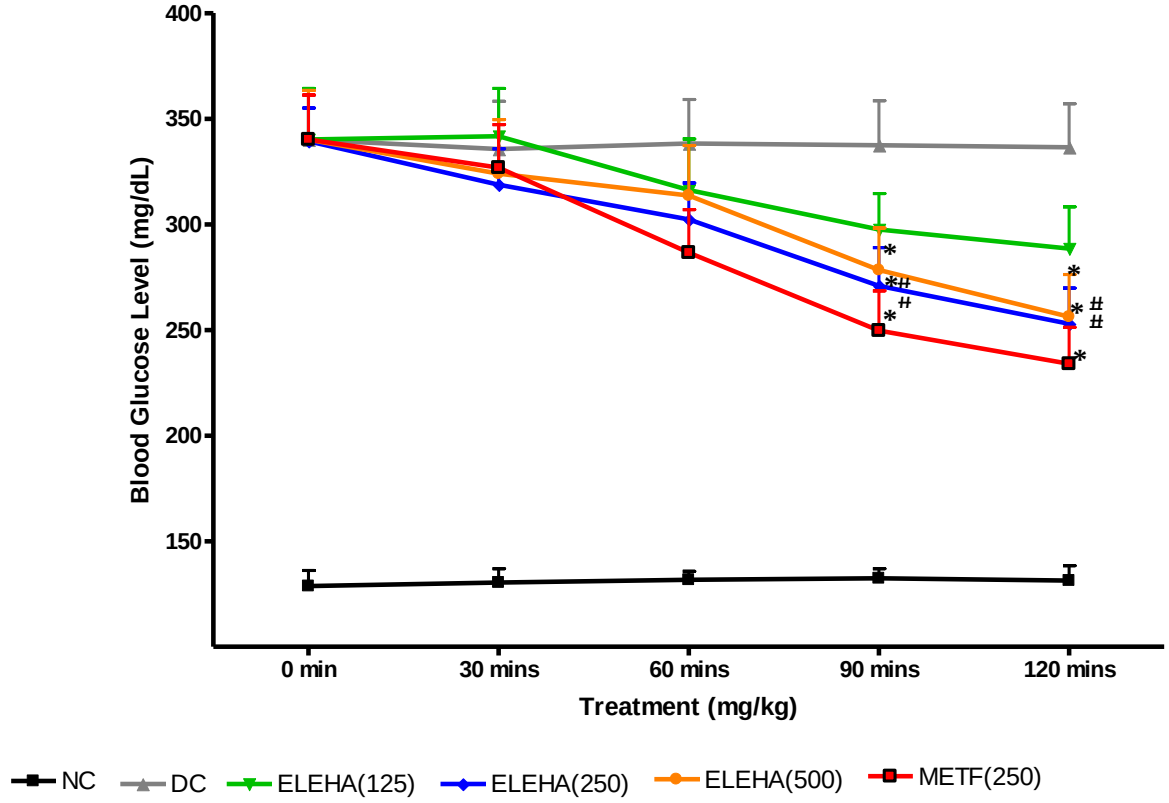

Figure 2: Effect of 2 hours (Acute) Administration of Ethanol Leaf Extract of *Hibiscus articulatus* on Fasting Blood Glucose Level in Streptozotocin-induced Hyperglycaemia using Wistar Rats

Data were analyzed using repeated measure ANOVA with Bonferroni post-hoc test and expressed as Mean ± SEM. n=9. Compared with DC group; *= $p < 0.05$. Compared with 30 min; # = $p < 0.05$. NC = Normal Control, DC = Diabetic Control, ELEHA = Ethanol leaf Extract of *Hibiscus articulatus*, METF = Metformin, mins = Minutes

Effect of Chronic Administration of Ethanol Leaf Extract of *Hibiscus articulatus* on Fasting Blood Glucose Level using Streptozotocin-induced Hyperglycaemia in Wistar Rats

The administration of ELEHA significantly decreased ($p < 0.05$) blood glucose level at doses of 250 mg/kg and 500 mg/kg for days 7, 14 and 21 after administration, when

compared with diabetic control group (Figure 3).

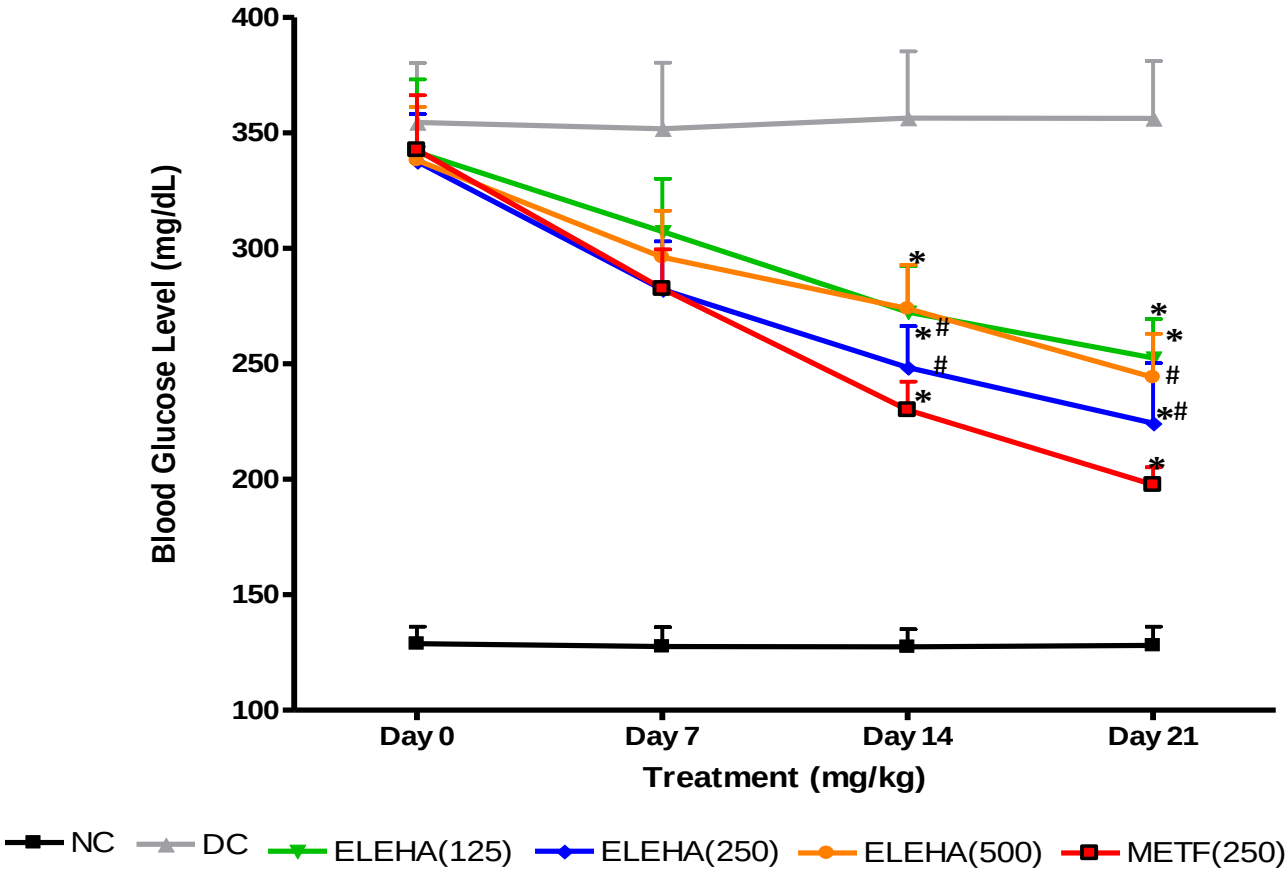

Figure 3: Effect of Chronic (21 days) Administration of Ethanol Leaf Extract of *Hibiscus articulatus* on Fasting Blood Glucose Level using Streptozotocin-induced Hyperglycaemia in Wistar Rats

Data were analyzed using repeated measure ANOVA with Bonferroni post-hoc test and expressed as Mean ± SEM. n=7-9. Compared with DC group; * = $p < 0.05$, Compared with Day 0; $^{\#}$ = $p < 0.05$. NC = Normal Control, DC = Diabetic Control, ELEHA = Ethanol Leaf Extract of *Hibiscus articulatus*. METF = Metformin.

Effect of Ethanol Leaf Extract of *Hibiscus articulatus* on Lipid Profile using Streptozotocin-induced Hyperglycemia in Wistar Rats

The analyses for lipid profile in streptozotocin induced hyperglycaemia showed no statistically significant difference in the parameters tested except for LDL where there was statistically significant ($p < 0.05$) decreased level at 125 mg/kg of ELEHA, when compared to the diabetic control group (Figure 4).

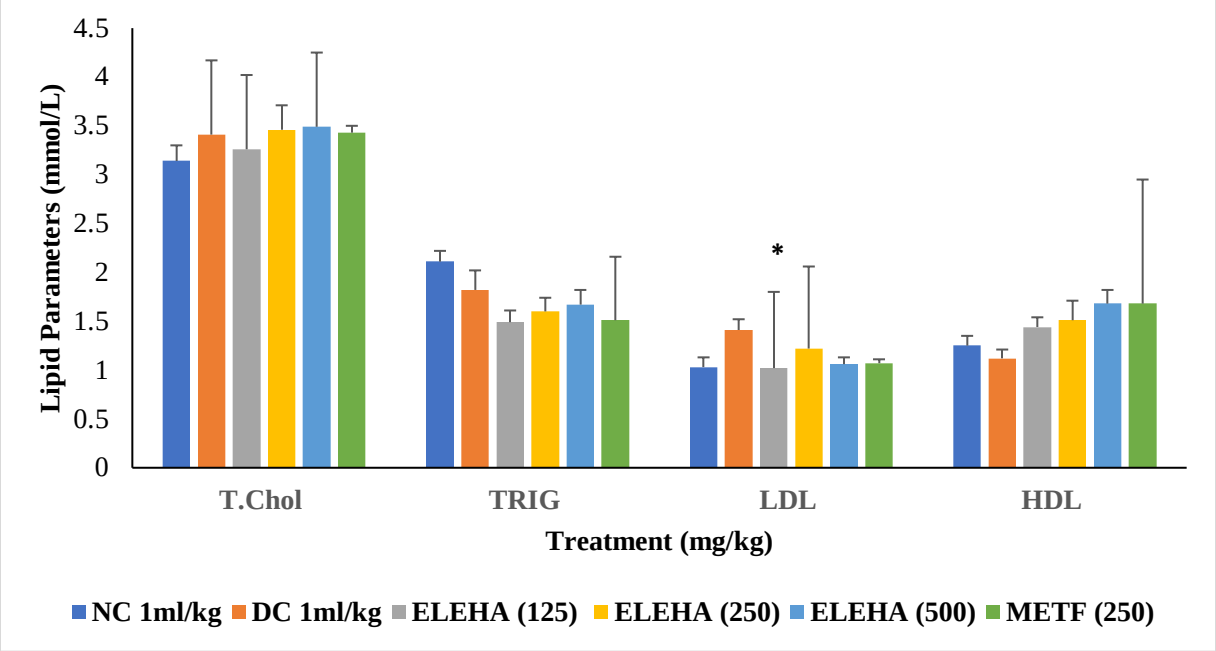

Figure 4: Effect of Ethanol Leaf Extract of *Hibiscus articulatus* on Lipid Profile using Streptozotocin-induced Hyperglycemia in Wistar Rats

Data were analyzed using one-way analysis of variance ANOVA followed by Bonferroni post-hoc test and expressed as Mean ± SEM. n = 5. Compared with diabetic control, $^{*}= p < 0.05$. NC = Normal Control, DC = diabetic control, ELEHA = ethanol leaf extract of *Hibiscus articulatus*, METF = Metformin, T.Chol = Total cholesterol, TRIG = Triglyceraldehyde, LDL = Low density lipoprotein, HDL = High density lipoprotein.

Effect of Extract on Antioxidant Profile using Streptozotocin-induced Hyperglycemia in Wistar Rats

The analyses for levels of catalase, malondialdehyde and reduced glutathione in streptozotocin-induced hyperglycaemia showed no statistically significant difference compared with the diabetic control group. However, level of superoxide dismutase (125 and 250 mg/kg of ELEHA) showed statistically significant ($p < 0.05$) increased mean when compared with the diabetic control group (Table 4).

Table 4: Effects of Ethanol Leaf Extract of *Hibiscus articulatus* on Antioxidant Parameters using Streptozotocin-induced Hyperglycemia in Wistar Rats

Treatment (mg/kg)	SOD (U/mL)	CAT (U/mL)	MDA (Um/L)	GSH (Umol/mL)
NC 1mL/kg	105.20±7.65	493.40±17.76	16.60±1.78	100.20±7.23
DC 1mL/kg	89.80±4.96	429.20±18.55	22.60±1.33	89.40±6.85
ELEHA (125)	115.20±4.25*	438.60±17.76	17.00±2.08	106.00±11.52
ELEHA (250)	116.00±5.86*	468.20±22.13	20.40±3.56	112.00±8.64
ELEHA (500)	111.60±5.86	433.40±19.44	19.00±3.08	112.00±9.09
METF (250)	104.00 ± 4.28	437.00 ± 22.27	16.20 ± 1.66	109.80 ± 8.04

Data were analyzed using one-way analysis of variance ANOVA with Bonferroni post-hoc test and expressed as Mean ± SEM. n = 5, compared with DC group; *= $p<0.05$. NC = Normal Control, DC = Diabetic Control, ELEHA = Ethanol Leaf Extract of *Hibiscus articulatus*, METF = Metformin, SOD = Superoxide Dismutase, CAT = Catalase, MDA = Malondialdehyde, GSH= Reduced Glutathione

Effect of Extract on HOMA-IR using Streptozotocin-induced Hyperglycemia in Wistar Rats

The administration of ELEHA and metformin showed statistically significant ($p < 0.01$) decrease in the mean level of HOMA-IR for all groups tested (except for ELEHA at 500 mg/kg) when compared with the diabetic control group (Table 5).

Table 5: Effect of Extract on HOMA-IR using Streptozotocin-induced Hyperglycemia

Treatment (mg/kg)	**Blood glucose level (mg/dL)**	**Insulin (mU/L)**	**HOMA-IR**
STD (Kit)	-	07.83±0.30	-
NC ml/kg	48.50±2.045	05.68±0.46	0.69±.08*
DC ml/kg	80.67±5.24	11.15±0.50	2.24±0.21
ELEHA (125)	55.67±2.36	08.31±0.22	1.14±0.05*
ELEHA (250)	51.17±2.69	09.26±0.28	1.17±0.08*
ELEHA (500)	62.50±3.37	09.99±0.30	1.54±0.09
METF (250)	52.83±3.64	09.01±0.52	1.18±0.11*

Data were analyzed using one-way analysis of variance ANOVA with Bonferroni post-hoc and expressed as Mean ± SEM. n = 5, *= $p < 0.05$. STD = Standard, NC = Normal Control, DC = Diabetic Control, ELEHA = Ethanol Leaf Extract of *Hibiscus articulatus*, METF = Metformin, HOMA-IR = Homeostatic Measurement Assessment of Insulin Resistance

Discussion

Ethanol was used as the extractive solvent based on its properties; preserves anti-oxidant components and possessed minimal toxic effect on plant tissues when used appropriately, as reported by Radzali *et al.,* (2020). Alkaloids, tannins, flavonoids, terpenes obtained from phytochemical screening of ELEHA had been reported to possess antioxidant activities, with proven relevance in the management of chronic diseases (Altemini *et al.,* 2017; Verma *et al.,* 2018; Vadivelan *et al*., 2019; Tonisi *et al*., 2020). Also, some of the chemical constituents like coumarans, ascorbic acid, hexadecanoic acid, cyclo-propyl phytol, linoleic acid and stearic acid are present in ELEHA and had been reported to possess antioxidant and anti-hyperglycaemia activities (Ighodaro and Akinloye 2018; Verma *et al.,* 2018).

Ascorbic acid is an important micronutrient obtained from diets and functions in various biological processes such as; antioxidant, wound and skin healing, immune booster, detoxification, co-factor, co-enzyme, synthesis of neurotransmitters, helps in body growth, development and maintenance of bone matrix (Santosh and David 2017; Praveen *et al.,* 2020). Its supplementation in diabetes was reported to decrease levels of fasting blood glucose, HbA1c, LDL and MDA (Santosh and David, 2017; Verma *et al.,* 2018; Wagh *et al.,* 2018). ELEHA possesses reasonable quantity of ascorbic acid and might be beneficial in the management of diabetes through scavenging of free radicals, decreased concentrations of fasting blood glucose and glycosylated hemoglobin, and thus preventing complications of T2DM. These conforms to the report of Praveen *et al.,* (2020).

Cyclo-propyl phytol is family of diterpene alcohol and are precursors for the synthesis of vitamin E and K. Phytols are reported to produce anti-oxidant activities by scavenging hydroxyl radical and nitric oxide via inhibition of peroxisome proliferator activated receptor (PPAR-ϒ) and retinol X receptor (RXR) (Verma *et al.,* 2018; Tonisi *et al.,* 2020). Cyclo-propyl phytol also possesses anti-cholesterol and anti-inflammatory activities making it relevant in the management of diabetes, obesity and cardiovascular diseases (Verma *et al.,* 2018). ELEHA possesses reasonable quantity of cyclo-propyl phytol and might be useful in the management of diabetes mellitus.

Hexadecanoic acid is family of hexanoic acid found in palmitic acid and are reported to regulate insulin sensitivity by phosphorylation of adenosine monophosphate (AMP)-activated protein kinase and activation of peroxisome proliferator activated receptor (PPAR-ϒ) (Verma *et al.,* 2018). Linoleic acid (a hexanoic acid) is the major component of polyunsaturated fatty acid (PUFA) and was reported to have anti-diabetic, anti-inflammatory and reduces gutamyl glutamate aminotransferase level in the liver, hence plays an important role in the prevention of T2DM and atherosclerosis (Pertiwi *et al.,* 2020). The hexadecenoic acid and linoleic acid present in ELEHA may offer advantage in the prevention and management of diabetes mellitus.

The oral LD_{50} of ELEHA was estimated to be greater than 5000 mg/kg as it did not cause any mortality or signs of toxicity on short term exposure (24 hours) in Wistar rats when tested. Hence, the ELEHA was found to be practically non-toxic on oral acute exposure in Wistar rats (Lork, 1983; Loomis and Hayes, 1996).

Glycaemic index is a measure of the response

(quality) obtained on the blood glucose level when carbohydrate containing diets are ingested, digested and absorbed into the blood stream (Jenkins *et al.,* 1981; Oputa and Chinenye, 2015), while the glycaemic load is the quality and quantity of carbohydrate present in the food that produced effect on glycaemia when consumed (Wolever *et al.,* 1994; Salmeron *et al.,* 1997; Eleazu, 2016). The glycaemic index of ELEHA was calculated to be of low value, which conformed to the report of Oputa and Chinenye (2015); Barkley *et al.,* (2021). Hence ELEHA may be considered as a suitable as a dietary component in the management and prevention of diabetes mellitus, as this conforms to the reports of Haque *et al.,* (2020) and Grant *et al.,* (2020), based on its low glycaemic index. Generally, low glycaemic index diets are usually recommended for diabetic patients as it had been reported to help control appetite, delay hunger and reduce post-prandial hyperglycaemia (Ijarotimi *et al.,* 2015; Campbell *et al.,* 2017; Barkley *et al.,* 2021).

The glycaemic load of ELEHA was calculated to be of medium value. The absence of fiber in ELEHA might be responsible for the observed increase in glycaemic load as this conforms to the report of Barkley *et al.,* (2021). Fiber containing diet have been reported to slow digestion and absorption of food due to slow intestinal transit time, leading to reduce spike on glycaemia and insulinaemia, reduced satiety and food intake, increases adipose tissue mobilization (lipolysis), inhibits lipogenesis (which favours oxidation of fats and hence decreases weight gain) and reduced cholesterol or blood glucose level (Pareira *et al.,* 2015; Haque *et al.,* 2020; Manullang *et al.,* 2020 and Barkley *et al.,* 2021).

Oral glucose tolerance test measures the ability of the body to utilize a form of sugar called glucose and it applied the principle of fasting plasma glucose in the diagnosis of T2DM (Ernsberger and Koletsky, 2012; Lages *et al.,* 2022). Results obtained from OGTT revealed that, the blood glucose level was highest at 30 minutes, post glucose administration and declined gradually up till 120 minutes of acute phase of the OGTT. The ability of the Wistar rats to metabolize the administered glucose, leading to decline in its concentration after 30 minutes of administration suggest the extract does not cause glucose tolerance nor affects the sensitivity of glucose to liver and other peripheral tissues (Ernsberger and Koletsky, 2012; Kuo *et al.,* 2021), hence this suggests that ELEHA may possess anti-hyperglycaemic activity.

Streptozotocin is a chemical used to induce necrosis on pancreatic β-cells in laboratory animals (Goud *et al.,* 2015; Dey *et al.,* 2022). The experimental administration of a single dose of 45 mg/kg streptozotocin produces hyperglycaemia and this was confirmed (using Accu-chek glucometer) on the 7 days after streptozotocin administration, as evident by hyperglycaemia, polydipsia, polyphagia, loss of body weight, which conformed to results obtained by Rajesh and Sreekala (2020). Hyperglycaemia involves decreased utilization of glucose by the liver and peripheral tissues and increased hepatic production of glucose (Jiang *et al.,* 2020). The ELEHA significantly decreased the blood glucose level (acute and chronic phases) in the groups treated, this conformed to the work done by Villas Boas *et al.,* (2020). The decreased blood glucose level observed may be due to ability of ELEHA to provide anti-oxidant effect, and thus causing the protection of beta cells, improve the sensitivity of tissues to insulin or decrease glucose absorption from the gastro intestinal tracts, as this conforms to the work of Sabu

and Kuttane (2002); Devi and Kumar (2017) and Koth El-Sayed *et al.,* (2020). Hence, ELEHA may possess anti-hyperglycaemic activity on Wistar rats.

Low density lipoprotein is reported among the lipids components whose aggregation and metabolism leads to blockage of capillary and tissues in the body, resulting in various cardiovascular (hypertension, heart attack) and metabolic (diabetes mellitus) disease (Sharma *et al.,* 2016; Galicia-Garcia *et al.,* 2020). The decreased levels of LDL in ELEHA treated groups might be beneficial in the management of diabetes mellitus associated with cardiovascular diseases. This conforms with the report of Athyros *et al.,* (2018) and Jomard and Osto, (2020), which stated that decreased LDL and triglyceraldehyde level accompanied with increased HDL will help reduce the incidence of insulin resistance (a hallmark in type 2 diabetes) due to diabetic dyslipidemia.

Oxidative stress occurs due to imbalance between the generation of free radicals and scavenging activities of endogenous antioxidant defense mechanism (Lushchak and Storey, 2021), and it's the major pathological condition involved in development of diabetes mellitus and its complications (Tabatabaei-Malazy *et al.,* 2017; Garcia-Sanchez *et al.,* 2020). Persistent hyperglycaemia was found to further induce oxidative stress by generating excess reactive oxygen species (Moraes *et al.,* 2015; Tabatabaei-Malazy *et al.,* 2017; Dey *et al.,* 2022) causing lipid peroxidation and oxidative cellular injuries, leading changes in cellular functions (Cruz *et al*., 2015) which further enhance the development of diabetic complications (Tabatabaei-Malazy *et al.,* 2017; Dey *et al.,* 2022). The harmful effects of free radicals can be modified by enzymatic or non-enzymatic anti-oxidant. The enzymes include; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), reduced glutathione (GSH), NOS, NOX, while the non-enzymatic anti-oxidants are; vitamins (A, C, E), minerals (copper, zinc, manganese, selenium), carotenoids, bioflavonoids, polyphenols and other molecules (folic acid, uric acid, vitamins B_1, B_2, B_6, B_{12}), albumin (Chikezie *et al.,* 2015; Tabatabaei-Malazy *et al.,* 2017).

The treatment with ELEHA produced statistically significantly increased level of SOD only. Although, the concentrations of catalase and reduced glutathione were raised, and malondialdehyde was decreased in ELEHA treated groups, but they are not statistically significant. Superoxide dismutase is the most important (Stephenie *et al.,* 2020; Garcia-Sanchez *et al.,* 2020) first line antioxidant (Ighodaro and Akinloye, 2018) defense against free radicals in cells. It produces its antioxidant effect by preventing the formation or suppressing the accumulation of free radicals (Ighodaro and Akinloye, 2018; Ogunmoyole *et al.,* 2022) via catalyzing the dismutation of superoxide anion into hydrogen peroxide and oxygen (Younus, 2018: Rajput *et al.,* 2021). Thus, ELEHA might be producing its anti-hyperglycaemia effect on Wistar rats via free radical mopping activities of SOD present in its constituents.

HOMA IR evaluation can be useful in understanding the pathogenesis, etiology, consequences as well as intervention appropriate for diabetes management (Singh and Saxena, 2010; Okita *et al.,* 2014). The value of HOMA IR calculated for ELEHA treated groups and metformin were found to be reduced compared with diabetic control. This result conformed with the work of Pitea *et al.,* (2009) which stated that subjects with HOMA IR of > 2 when calculated are

indicative of high risk of developing insulin resistance. The ELEHA treated groups showed a decreased value (< 2) of HOMA IR and thus might suggests that the anti-hyperglycemia effect observed may be due to increased sensitivity of insulin to peripheral tissue, leading to increased blood glucose uptake, inhibition of hepatic glucose production and lipolysis, enhance secretion of glycogen leading to decrease post-prandial hyperglycaemia as reported by Duan *et al.*, (2019).

Thus, ELEHA was found to possess polyphenolics, ascorbic acid, phytol, hexanoic acid, palmitic acid and linoleic acid, which may contribute to its anti-inflammatory, anti-cholesterol, antioxidant and thus might be responsible for the anti-hyperglycaemia activity observed in Wistar rats.

Conclusion

The ethanol leaf extract of *Hibiscus articulatus* possesses relevant chemicals with anti-hyperglycaemia activity in Wistar rats, and thus lends credence to its ethno-medicinal use in the management of diabetes mellitus.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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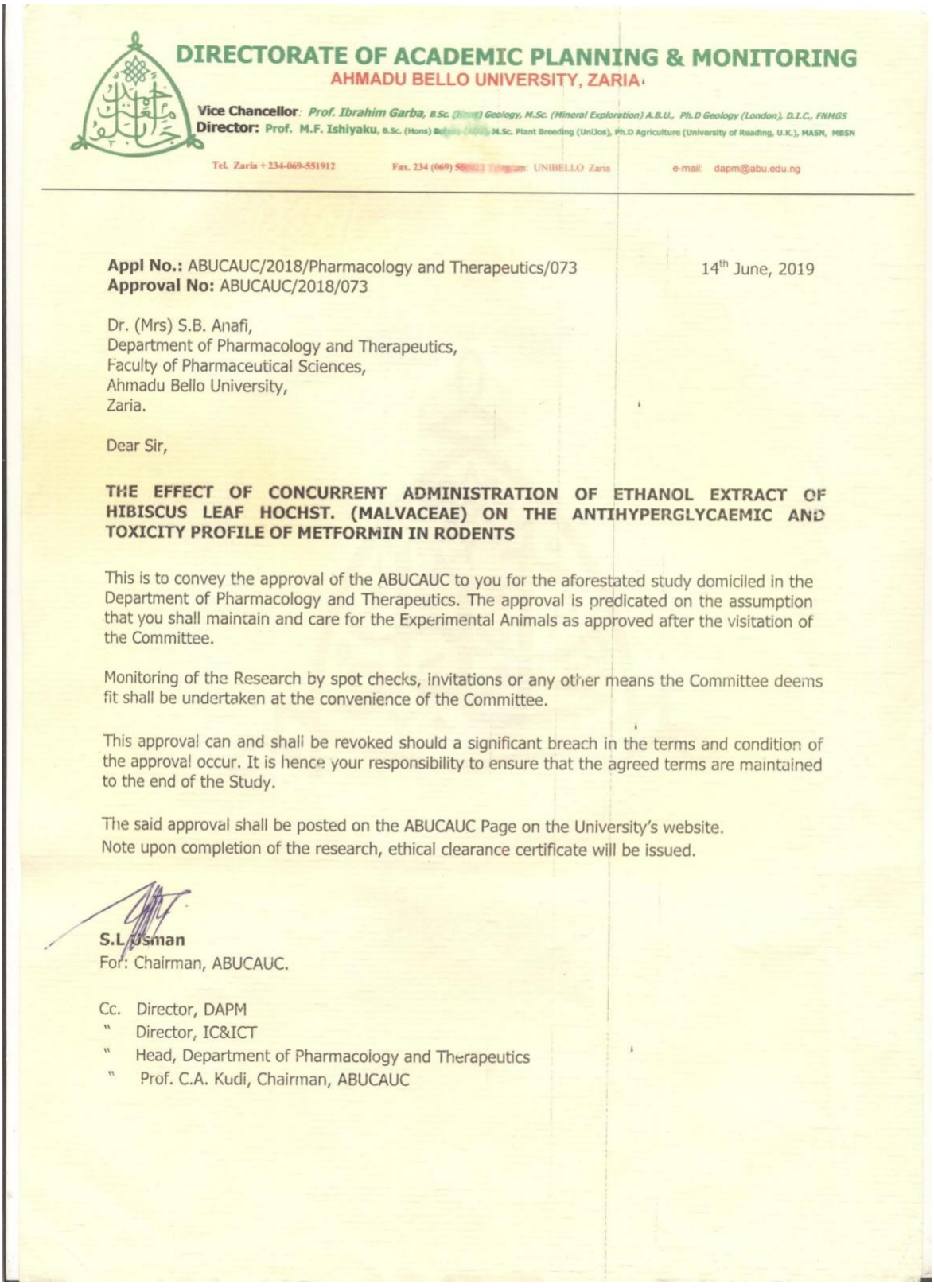

DIRECTORATE OF ACADEMIC PLANNING & MONITORING
AHMADU BELLO UNIVERSITY, ZARIA

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Appl No.: ABUCAUC/2018/Pharmacology and Therapeutics/073 14th June, 2019
Approval No: ABUCAUC/2018/073

Dr. (Mrs) S.B. Anafi,
Department of Pharmacology and Therapeutics,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria.

Dear Sir,

THE EFFECT OF CONCURRENT ADMINISTRATION OF ETHANOL EXTRACT OF HIBISCUS LEAF HOCHST. (MALVACEAE) ON THE ANTIHYPERGLYCAEMIC AND TOXICITY PROFILE OF METFORMIN IN RODENTS

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Pharmacology and Therapeutics. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

S.L. Usman
For: Chairman, ABUCAUC.

Cc. Director, DAPM
" Director, IC&ICT
" Head, Department of Pharmacology and Therapeutics
" Prof. C.A. Kudi, Chairman, ABUCAUC

DEPARTMENT OF MEDICINAL PLANT RESEARCH AND TRADITIONAL MEDICINE (MPR & TM)
National Institute For Pharmaceutical Research And Development (NIPRD)
Idu, Abuja

CERTIFICATE OF ANALYSIS

Client Name	Abbas Medinat Yakubu		
Client Address	Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaira, Kaduna State		
Study Title	Not applicable		
Sample Name(s)	*A. articulatus* and *E. tremula*		
Sample ID	01/03/15012019/410/0079A and 01/03/15012019/410/0079B		
Sample Description	***Physical appearance:*** Solid extracts ***Storage condition:*** Room Temperature		
Analysis requested	GC-MS analysis of the samples		
Sample receipt Date	15/01/2019	**Study Schedule**	***Start date:*** 6/2/2019 ***End date:*** *13/2/2019* ***Submission date:*** *13/2/2019*
Purpose of the study	Not applicable		

Methodology

The samples were analyzed by GC-MS using Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector [MSD, operated in the EI mode (electron energy=70 eV), scan range of 45-700 amu, and scan rate of 3.99 scans/sec], and Shimadzu GCMS solution data system. The Gas chromatography column was Optima-5 ms fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 mL/min. The program used for Gas chromatography oven temperature was 40- 240°C at a rate of 40°C/min, then held at 240°C for 2 min, followed by 240-280°C at a rate of 5°C/min, then again held at 280°C for 2 min. The injection port temperature was 250°C while detector temperature was 280°C. 1.0 µL of diluted sample (500µg/ml in ethanol, w/v) was injected using autosampler and in the split mode with ratio of 20:80. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11). The percentages of each component are reported as raw percentages based on the total ion current without standardization.

Result(s)

A. articulatus and *E. tremula* both have 35 peaks each. Find attached the chromatogram and compound table for both samples.

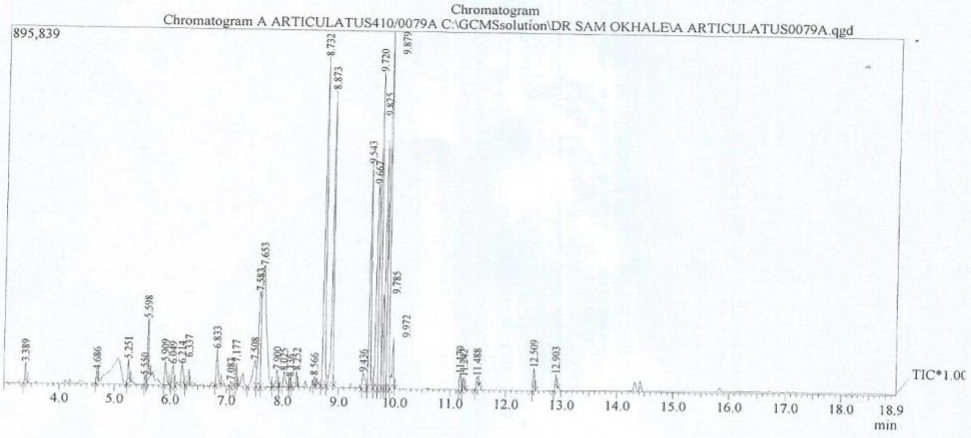
Comment/ Conclusion

No comment			
References Okhale SE, ugbabe GE, Oladosu PO,Ibrahim JA, Egharevba HO, Kunle OF, Elisha EP, Chibuike AJ, Ettah UO (2018). Chemical constituents and antimicrobial activity of the leaf essential oil of Ixora coccinea L (Rubaiceae) collected from north central Nigeria. International journal of bioassays 7(5); 5630-5637.			
HOD	Dr. Jemilat A. Loraliim	**Sign & Date**	18/02/19

NATIONAL INSTITUTE FOR PHARMACEUTICAL RESEARCH AND DEVELOPMENT
(NIPRD) IDU IDUSTRIAL AREA
ABUJA

Chromatogram

Chromatogram A ARTICULATUS410/0079A C:\GCMSsolution\DR SAM OKHALE\A ARTICULATUS0079A.qgd



Method

[Comment]

Analytical Line 1

[GC-2010]

Column Oven Temp.	:40.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Pressure
Pressure	:100.2 kPa
Total Flow	:47.4 mL/min
Column Flow	:1.78 mL/min
Linear Velocity	:48.2 cm/sec
Purge Flow	:10.0 mL/min
Split Ratio	:20.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	40.0	2.00
40.00	240.0	0.00
5.00	280.0	5.00

< Ready Check Heat Unit >
Column Oven : Yes
SPL1 : Yes
MS : Yes
< Ready Check Detector(FTD) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >
SPL1 Carrier : Yes
SPL1 Purge : Yes
< Ready Check APC Flow >
< Ready Check Detector APC Flow >
External Wait :No
Equilibrium Time :3.0 min

[GC Program]

[GCMS-QP2010 Plus]

IonSourceTemp	:200.00 °C
Interface Temp.	:250.00 °C
Solvent Cut Time	:2.50 min
Detector Gain Mode	:Relative
Detector Gain	:0.00 kV

Threshold :2000

[MS Table]
--Group 1 - Event 1--
Start Time :3.00min
End Time :19.00min
ACQ Mode :Scan
Event Time :0.50sec
Scan Speed : 526
Start m/z :45.00
End m/z :300.00

Sample Inlet Unit :GC

[MS Program]
Use MS Program :OFF

Peak Report TIC

Peak#	R.Time	l.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.389	3.358	3.433	79005	0.49	52369	0.75	1.51		2,2'-Bioxirane, (R*,R*)-(+/-)-
2	4.686	4.667	4.717	45670	0.28	29779	0.42	1.53		2,4-Dihydroxy-2,5-dimethyl-3(2H
3	5.251	5.225	5.300	96184	0.59	59275	0.84	1.62		l-Alanine, N-cyclobutylcarbonyl-,
4	5.550	5.533	5.575	35296	0.22	20040	0.29	1.76		Diethylnitrosamine
5	5.598	5.575	5.650	257193	1.59	154490	2.20	1.66	V	3,5-Dihydroxy-6-methyl-2,3-dihy
6	5.909	5.883	5.983	139994	0.87	57393	0.82	2.44		Coumaran
7	6.049	6.008	6.092	134769	0.83	53496	0.76	2.52		1-(2-Methyl-1,3-oxathiolan-2-yl)e
8	6.214	6.092	6.267	154806	0.96	55121	0.79	2.81	V	6-Oxoheptanoic acid
9	6.337	6.267	6.358	66587	0.41	41803	0.60	1.59	V	1-(4-Hydroxy-2-methylphenyl)eth
10	6.833	6.800	6.908	182148	1.13	89451	1.27	2.04		Benzene, 1-(bromomethyl)-3-nitr
11	7.083	7.067	7.158	32262	0.20	14505	0.21	2.22		2-Amino-4-methyl-oxazole
12	7.177	7.158	7.208	76937	0.48	59787	0.85	1.29	V	Dodecanoic acid
13	7.508	7.375	7.533	288425	1.78	66321	0.94	4.35		3-Deoxy-d-mannoic lactone
14	7.583	7.533	7.608	776539	4.80	240415	3.42	3.23	V	Ethyl .alpha.-d-glucopyranoside
15	7.653	7.608	7.817	1699677	10.51	304267	4.33	5.59	V	beta.-D-Glucopyranose, 4-O-.beta
16	7.900	7.817	7.933	89063	0.55	41865	0.60	2.13	V	11-Bromoundecanoic acid
17	8.025	7.933	8.117	103697	0.64	38639	0.55	2.68	V	Methyl 2,4,6,8-tetramethyl-13-tet
18	8.136	8.117	8.158	32241	0.20	23563	0.34	1.37	V	13-Tetradecenoic acid, 2,4,6,8-tet
19	8.252	8.158	8.275	49676	0.31	39329	0.56	1.26	V	3,4-Dimethylcyclohexanol
20	8.566	8.550	8.592	22773	0.14	22239	0.32	1.02		Docosanoic acid, methyl ester
21	8.732	8.683	8.775	1646515	10.18	823303	11.73	2.00		l-(+)-Ascorbic acid 2,6-dihexadec
22	8.873	8.775	8.908	1340975	8.29	741509	10.56	1.81	V	Hexadecanoic acid, ethyl ester
23	9.436	9.408	9.508	127322	0.79	40384	0.58	3.15		Cyclohexane, cyclopropyl-
24	9.543	9.508	9.617	1026926	6.35	563558	8.03	1.82		Phytol
25	9.667	9.617	9.692	1463209	9.05	512508	7.30	2.85	V	Linoleic acid
26	9.720	9.692	9.758	1870269	11.57	792832	11.29	2.36	V	9,12,15-Octadecatrienoic acid, (Z
27	9.785	9.758	9.800	461830	2.86	237710	3.39	1.94	V	Stearic acid
28	9.825	9.800	9.850	1339866	8.29	625175	8.91	2.14	V	Linoleic acid ethyl ester
29	9.879	9.850	9.917	1870980	11.57	895839	12.76	2.09	V	Ethyl 9,12,15-octadecatrienoate
30	9.972	9.917	10.008	273460	1.69	128681	1.83	2.13	V	Stearic acid, ethyl ester
31	11.179	11.150	11.217	89721	0.55	39100	0.56	2.29		1-Hexyl-2-nitrocyclohexane
32	11.242	11.217	11.275	53370	0.33	31635	0.45	1.69	V	1,4-Cyclohexanedimethanol
33	11.488	11.450	11.517	55916	0.35	28116	0.40	1.99		1,4-Cyclohexanedimethanol
34	12.509	12.475	12.542	108246	0.67	57636	0.82	1.88		Palmitic acid .beta.-monoglyceride
35	12.903	12.867	12.933	75013	0.46	37798	0.54	1.98		1,2-Benzenedicarboxylic acid, dii

Library
<< Target >>
Line#:1 R.Time:8.133(Scan#:617) MassPeaks:9
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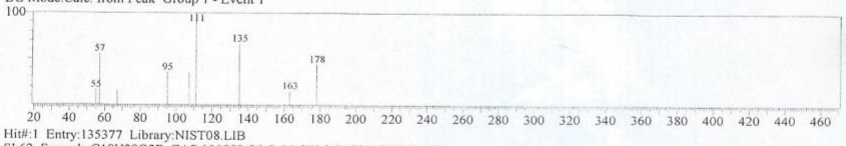
BG Mode:Calc. from Peak Group 1 - Event 1



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SI:62 Formula:C18H39O3P CAS:101883-26-5 MolWeight:334 RetIndex:0
CompName:Ethylphosphonic acid, di(2-ethylhexyl) ester $$ Bis(2-ethylhexyl) ethylphosphonate # $$