

Antidiabetic Activity of Bioactive-Rich Fractions of *Ficus exasperata vahl* Leaf in Fructose and Streptozotocin-induced Diabetic Rats

SOJI-OMONIWA, Omolola^{a,*} and OLOYEDE, Hussein Oyelola Bukoye^a

^a*Department of Biochemistry, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria.*

**Corresponding author Email: lolasojiomoniwa@gmail.com*

Abstract

The antidiabetic activity of *Ficus exasperata* Vahl leaf (FEL) has been previously reported but data on the metabolites responsible for this effect has not been established. The aim of this study was to conduct bioactivity-guided fractionation of FEL and identify the antidiabetic principles in fructose and streptozotocin-induced diabetic rats. Aqueous, butanol, ethyl acetate and n-hexane solvents extracts of FEL were used to treat diabetic rats in phase one of the study. The most potent crude solvent extract in phase 1 was then subjected to column chromatography in phase two which gave five different fractions which were further evaluated for efficacy in diabetic rats. In each phase, fasting blood glucose (FBG) and serum lipid profile concentrations were analyzed. Gas chromatography-Mass spectrometry (GC-MS) analysis of the most active fraction was subsequently carried out. FBG, serum cholesterol, triglycerides and low density lipoprotein concentrations of extracts and fractions-treated rats reduced significantly ($p < 0.05$) while high density lipoprotein concentration increased significantly ($p < 0.05$). GC-MS analysis showed that thymol, phytol acetate and eugenol were present predominantly in the butanol extract. In conclusion, the result revealed that butanol extracts of *Ficus exasperata* vahl leaf elicited antihyperglycaemic and antidyslipidaemic activities. The predominant constituents of butanol extract are known compounds with reported antioxidant activity, which might be one of the ways by which the plant extract elicits its antidiabetic effect.

Keywords: *Ficus exasperata*; chromatography; Diabetes mellitus; Streptozotocin

Introduction

The result of an ethnobotanical survey conducted in selected districts of Lagos State in Nigeria revealed that *Ficus exasperata* was one of the plants used traditionally for the management of diabetes mellitus (Gbolade, 2009). *Ficus exasperata vahl*

belongs to the family Moraceae, with 800 species occurring in the warmer part of the world (Odunbaku *et al.*, 2008). It is commonly known as sand paper tree. It is a deciduous tree of up to 20 m high, with smooth grey bark and very rough leaves, viscid non-milky sap and coppice shoots with lobed leaves. *F. exasperata vahl* is widespread in tropical Africa and the Arabian Gulf (Adewole *et al.*, 2011). The Yoruba-speaking people of western Nigeria often use decoctions and infusions of *F. exasperata* leaf traditionally for the management, control and/or treatment of an array of human diseases, including diabetes mellitus.

Diabetes mellitus is one of the leading cause of death worldwide (WHO, 2016). In 2014, 422 million people in the world were reported to have diabetes, with prevalence rate of 8.5% among the adult population (WHO, 2016). Also in the same report, the prevalence of diabetes mellitus was observed to have been steadily increasing for the past 3 decades and is growing most rapidly in both low and middle-income countries (WHO, 2016). Modern pharmaceutical industry relies mainly on the diversity of secondary metabolites in medicinal plants for the discovery of new compounds with novel biological properties. It was estimated that natural products and their derivatives and analogues represent over 50% of all drugs in clinical use (Gurib-Fakim, 2006; Muthia, 2008). The aim of this study was to conduct bioactivity-guided fractionation of *Ficus exasperata vahl* leaf and identify the antidiabetic principles in fructose and streptozotocin-induced diabetic rats.

Materials and methods

Collection and identification of *Ficus exasperata vahl* leaf

Fresh leaves of *Ficus exasperata* were collected in February, 2015, from Odo Eri in Kogi State, Nigeria. The specimen sample was deposited for identification and authentication at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria and was issued a voucher number UILH/001/883.

Animal procurement and housing

Wistar rats of *norvegicus* strain, weighing between 100 g to 150 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were housed in well ventilated cages and allowed to acclimatize to animal house conditions for 7 days. They were fed with normal rat pellet and tap water prior to the commencement of this experiment.

Ethical Clearance

Ethical clearance for this study was obtained from the University of Ilorin ethical committee with protocol identification code UERC/LSC 067 and UERC approval number UERC/ASN/2016/243 were obtained.

Preparation of aqueous, ethyl acetate and n-hexane extracts of *Ficus exasperata* vahl leaves

Two hundred grams (200 g) of pulverized *Ficus exasperata* leaves (FEL) were soaked separately in 1000 ml of distilled water, butanol, ethyl acetate and n-hexane for 48 hr. Each solution was filtered using muslin cloth and whatman (no. 1) filter paper afterwards. The resulting filtrate for the aqueous extract was concentrated on water bath to dryness at 40 °C, while the butanol, ethyl acetate and n-hexane were concentrated in a rotary evaporator at 40 °C and was further concentrated in a water bath to dryness at 40 °C. Twenty-five (25) mg/kg body weight of each of the prepared crude leaf extracts were reconstituted and administered orally to diabetic rats except the positive control group that received distilled water only for 13 days. At the end of the experiment, the most potent crude extract was butanol extract and it was further subjected to column chromatographic fractionation. The different fractions obtained from this phase were further administered to diabetic rats to determine the most potent fraction.

Column chromatographic fractionations of crude butanol extract (CBE)

Seven (7) g of CBE was mixed with 6 g of silica gel 60-120 mesh and air dried to constant weight. Eighty (80) grams of silica gel was then dissolved in 100 % dichloromethane to form a slurry. The slurry was carefully poured into the glass column and allowed to settle evenly in the column. The extract silica gel mixture was then poured gently into the packed column. Chromatographic elution was done by step gradient, starting with 100 % dichloromethane (DCM), followed by 70 % DCM : 30 % butanol (BUT) (v/v), 50 % DCM : 50 % butanol (BUT) (v/v), 30 % DCM : 70 % butanol (BUT) (v/v), and finally, 100 % Butanol (BUT) were obtained. The Eluates were concentrated to dryness on rotary evaporator at 37 °C, this was further dried on water bath at 40 °C. Five fractions (Fraction A, 100 % Dichloromethane (DCM); Fraction B, 70 % DCM: 30 % butanol (BUT); Fraction C, 50 % DCM: 50 % butanol (BUT); Fraction D, 30 % DCM: 70 % butanol (BUT) and Fraction E, 100 % Butanol. Each fraction was weighed and stored in the refrigerator at 4 °C for further use. All five fractions were tested for antidiabetic potency using fructose and streptozotocin induced diabetic rats. Biological screening was done by monitoring fasting blood glucose, cholesterol, triacylglycerol, low density lipoprotein, and high density lipoprotein concentrations.

Gas chromatography-mass spectrometry analysis (GC-MS) of crude butanol extract of *Ficus exasperata* leaves

The GC-MS analysis was carried out using a Hewlett packard gas chromatograph (Model 6890 series) equipped with a flame ionization detector (FID) and Hewlett Packard 7683 series injector, MS transfer line temperature of 250 °C. The GC was equipped with a fused silica capillary column- HP-5MS (30 x 0.25 mm) with film

thickness of 1.0 μm . The oven temperature was held at 50 °C for 5 mins holding time and raised from 50 to 250 °C at a rate of 2 °C /min. Helium gas (99.999 %) was used as a carrier gas at a constant flow rate of 22 cm/s. One (1.0) micron of extract (1 mg dissolved in 1 ml methanol), at a split ratio of 1:30 was injected. Qualitative data were obtained by electronic integration of FID area percent without the use of correction factor. MS analysis was carried out on Agilent Technology Network Mass Spectrometer (Model 5973 series) coupled to Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with NIST08 library software database. Mass spectra were taken at 70 eV/200 °C, scanning rate of 1 scan/s. Identification of compounds was conducted using the database of NIST08 Library. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database library.

Induction of diabetes mellitus

Experimental rats apart from those in the positive control group were administered with 10 % fructose solution *ad libitum* for 2 weeks, while those in the positive control group were given distilled water. At the end of the 2 weeks administration of fructose solution, animals were fasted overnight and each of the fructose-fed animals was injected with a freshly prepared 40 mg/kg b.wt. Streptozotocin (STZ), which was dissolved in 0.1 M citrate buffer, pH 4.5, intraperitoneally (Wilson and Islam, 2012). One week after administration of STZ, fasting blood glucose concentration of the animals were checked using AccuChek active glucometer and compatible strips by withdrawing blood from the caudal vein of the rats' tail. Rats showing glucose concentration above 200 mg/dl were considered diabetic.

Animal Groupings

Phase one

Forty-two (42) albino rats were randomly selected into 7 groups of 6 animals each as shown below:

- PC- Positive control i.e. non diabetic rats
- NC- Negative control i.e. diabetic untreated rats
- MT- Diabetic rats treated with 12.14 mg/kg body weight (b.wt.) of metformin
- AqET- Diabetic rats treated with 25 mg/kg b.wt. of aqueous extract of FEL
- BET- Diabetic rats treated with 25 mg/kg b.wt. of butanol extract of FEL
- HET- Diabetic rats treated with 25 mg/kg b.wt. of n-Hexane extract of FEL

EAT- Diabetic rats treated with 25 mg/kg b.wt. of ethyl acetate extract of FEL

Phase two

Forty-eight (48) albino rats were randomly selected into 8 groups of 6 animals each as shown below:

PC-	Positive control i.e. non diabetic rats
NC-	Negative control i.e. diabetic untreated rats
MT-	Diabetic rats treated with 12.14 mg/kg (b.wt.) of metformin
Fraction A- of CBE	Diabetic rats treated with 13.61 mg/kg b.wt. of 100 % DCM fraction
Fraction B-	Diabetic rats treated with 7.10 mg/kg b.wt. of 70 % DCM and 30 % BUT fraction of CBE
Fraction C-	Diabetic rats treated with 3.04 mg/kg b.wt. of 30 % DCM and 70 % BUT fraction of CBE
Fraction D- of CBE	Diabetic rats treated with 0.70 mg/kg b.wt. of 100 % BUT fraction
Fraction E-	Diabetic rats treated with 0.56 mg/kg b.wt. of 50 % DCM and 50 % BUT fraction of CBE

Biochemical determinations

Fasting blood glucose concentration determination

Fasting blood glucose (FBG) concentration of all experimental groups was determined using a glucose oxidase-based glucometer (AccuChek active, Roche Diagnostic). After induction of diabetes have been confirmed, FBG concentration was checked every 3 days throughout the experimental period.

Serum lipid profile determination

The method described by Roeschlan *et al.* (1974) was employed in the determination of cholesterol concentration. High density lipoprotein concentration was determined using the method described by Tietz (1994). Triglycerides concentration was determined using the method described by Tietz (1995). The method described by Friedewald *et al.* (1972) was employed for the determination of low density lipoprotein concentration.

Statistical analysis

Data were expressed as mean of 6 determinations $\pm$ SEM. The data were subjected to statistical analysis using the IBM[®] statistical package for social sciences (SPSS) software version 20. All significant differences were determined by one way analysis of variance (ANOVA). Post hoc multiple comparisons were done using Duncan's multiple range test. The level of significance was set at $p < 0.05$ (confidence level = 95 %).

Results

Fasting Blood Glucose Concentration in Diabetic Rats Treated with Different Crude Solvent Extracts

Table 1 presents the fasting blood glucose concentration in diabetic rats treated with different crude solvent extracts. The Fasting blood glucose concentration of the diabetic treated with the various solvent extracts reduced significantly ($p < 0.05$) compared to the negative control rats throughout the period of the experiment. However, the FBG concentration of *Ficus exasperata* butanol extract treated group showed that there was no significant difference ($p > 0.05$) by day 9 through day 13 when compared to the positive control rats.

Table 1: Fasting Blood Glucose Concentration (mg/dl) in Diabetic Rats Treated with Different Crude Solvent Extracts of *Ficus exasperata* Leaves

Group	Days of administration							
s	0	1	3	5	7	9	11	13
PC	63.50 $\pm$ 2.50 ^a	97.00 $\pm$ 7.90 ^a	89.00 $\pm$ 4.60 ^a	90.25 $\pm$ 8.0 ^a	76.50 $\pm$ 6.65 ^a	76.00 $\pm$ 4.93 ^a	77.50 $\pm$ 5.89 ^a	68.50 $\pm$ 2.32 ^a
NC	63.50 $\pm$ 3.57 ^a	351.00 $\pm$ 15.40 ^b	317.25 $\pm$ 11.21 ^b	373.25 $\pm$ 10.19 ^b	409.25 $\pm$ 14.88 ^b	464.25 $\pm$ 18.29 ^b	517.00 $\pm$ 19.12 ^b	536.00 $\pm$ 15.64 ^b
MT	60.25 $\pm$ 1.60 ^a	343.25 $\pm$ 11.12 ^b	138.00 $\pm$ 17.92 ^c	117.00 $\pm$ 8.69 ^c	103.75 $\pm$ 5.75 ^c	72.00 $\pm$ 2.71 ^a	70.25 $\pm$ 0.84 ^c	62.75 $\pm$ 2.19 ^c
AqET	65.00 $\pm$ 5.57 ^a	356.50 $\pm$ 17.40 ^b	138.00 $\pm$ 7.82 ^c	109.00 $\pm$ 2.79 ^c	94.50 $\pm$ 5.18 ^c	92.25 $\pm$ 4.03 ^c	90.5 $\pm$ 7.32 ^d	92.00 $\pm$ 2.12 ^d
BET	63.25 $\pm$ 3.09 ^a	351.00 $\pm$ 13.00 ^b	113.50 $\pm$ 12.00 ^d	102.50 $\pm$ 6.26 ^a	86.50 $\pm$ 1.03 ^d	74.00 $\pm$ 3.56 ^a	85.00 $\pm$ 2.89 ^a	69.00 $\pm$ 2.89 ^a
EAT	62.50 $\pm$ 0.29 ^a	364.25 $\pm$ 12.37 ^b	107.75 $\pm$ 1.49 ^d	88.00 $\pm$ 4.08 ^a	76.00 $\pm$ 2.44 ^a	67.00 $\pm$ 2.42 ^d	66.00 $\pm$ 0.40 ^a	55.75 $\pm$ 9.64 ^a
HET	63.00 $\pm$ 0.57 ^a	366.50 $\pm$ 3.50 ^b	169.25 $\pm$ 8.57 ^a	140.00 $\pm$ 7.07 ^d	120.00 $\pm$ 7.07 ^a	96.75 $\pm$ 4.61 ^c	97.75 $\pm$ 6.34 ^d	93.00 $\pm$ 2.89 ^d

Values are expressed as mean of 6 determinations $\pm$ S.E.M. and rows with different superscripts across a column are statistically different ($p < 0.05$)

Day 0 – FBG concentration before diabetes induction

Serum Lipid Profile Concentrations in Diabetic Rats Treated with Different Crude Solvent Extracts

Figure 1 shows the serum lipid profile in diabetic rats treated with different crude solvent extracts. There was significant reduction ($p < 0.05$) in the cholesterol, triglyceride and low density lipoprotein concentration of all the treated groups compared to the negative control group. High density lipoprotein of the various treatment group increased significantly ($p < 0.05$) compared to the negative control group.

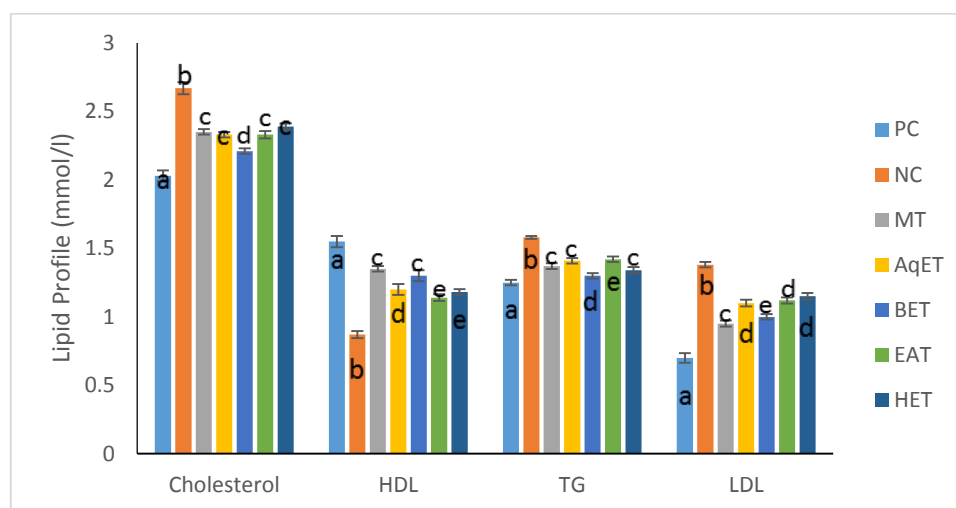


Figure 1: Serum Lipid Profile in Diabetic Rats Treated with Different Crude Solvent Extracts of *Ficus exasperata* Leaves

Values are expressed as mean of 6 determinations $\pm$ S.E.M. and bars with different superscripts are statistically different ($p < 0.05$)

Fasting Blood Glucose Concentration and Serum Lipid Profile in Diabetic Rats Treated with Fractions of Crude Butanol Extract of *Ficus exasperata* Leaves

Figure 2 shows the fasting blood glucose concentration in diabetic rats treated with different sub-fractions of butanol crude extract of *Ficus exasperata* leaves. The result shows that at the end of the experiment all the different fractions of the *Ficus exasperata* butanol leaves extract reduced the concentration of blood glucose. Figure 3 shows the comparative analysis of the results of crude butanol leaf extract and fractions of butanol crude extract upon fractionation. The FBG of crude butanol leaf extract performed better than the butanol fractions. Figure 4, shows the serum lipid profile in diabetic rats treated with different column chromatography fractions of crude butanol extract of *F. exasperata* leaves. Cholesterol and LDL concentrations of fractions treated groups were not significantly different ($p > 0.05$) compared to the negative control rats.

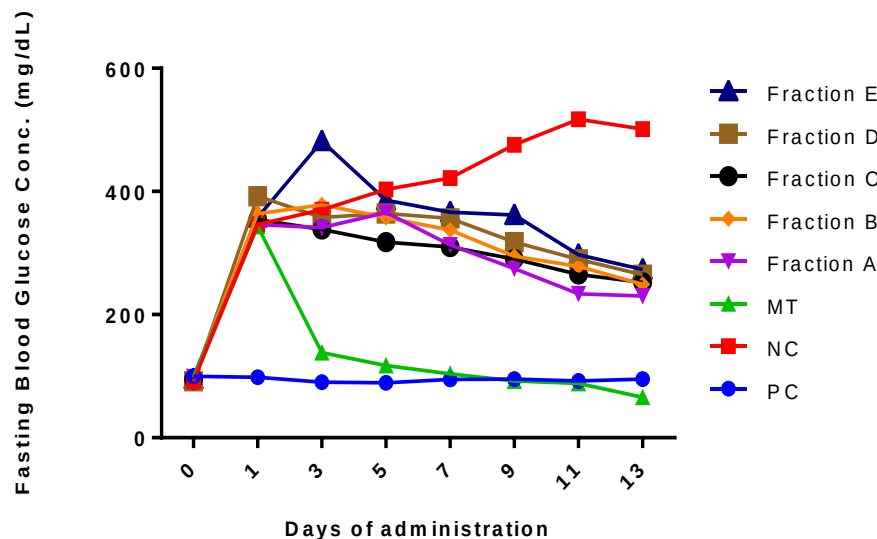


Figure 2: Fasting Blood Glucose Concentration in Diabetic Rats Treated with Fractions of Crude Butanol Extract of *Ficus exasperata* Leaves

Values are expressed as mean of 6 determinations $\pm$ S.E.M.

Day 0 – FBG concentration before induction of diabetes mellitus

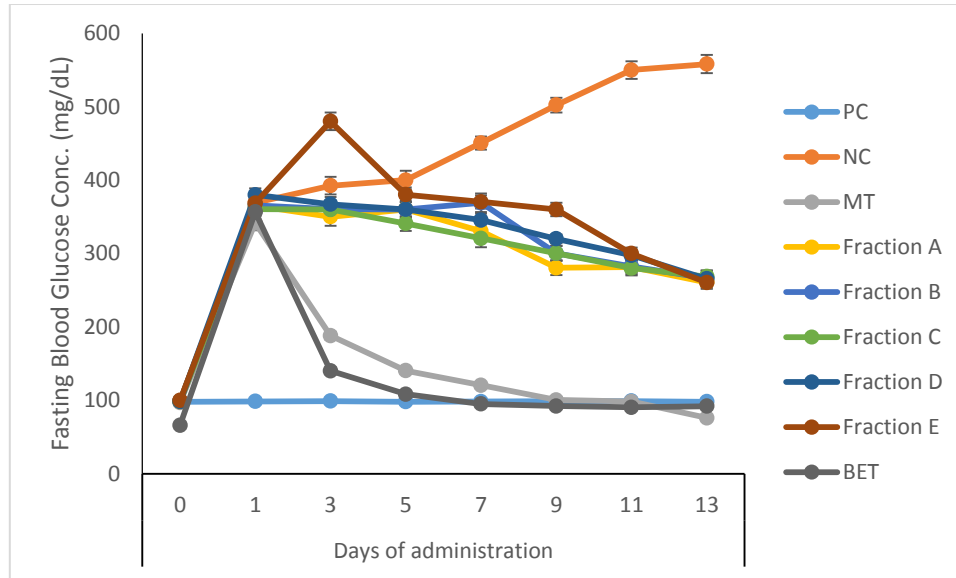


Figure 3: Butanol Extract Fractions and Crude Butanol Extracts of *Ficus exasperata* Leaves

Values are expressed as mean of 6 determinations $\pm$ S.E.M.

Day 0 – FBG concentration before induction of diabetes mellitus

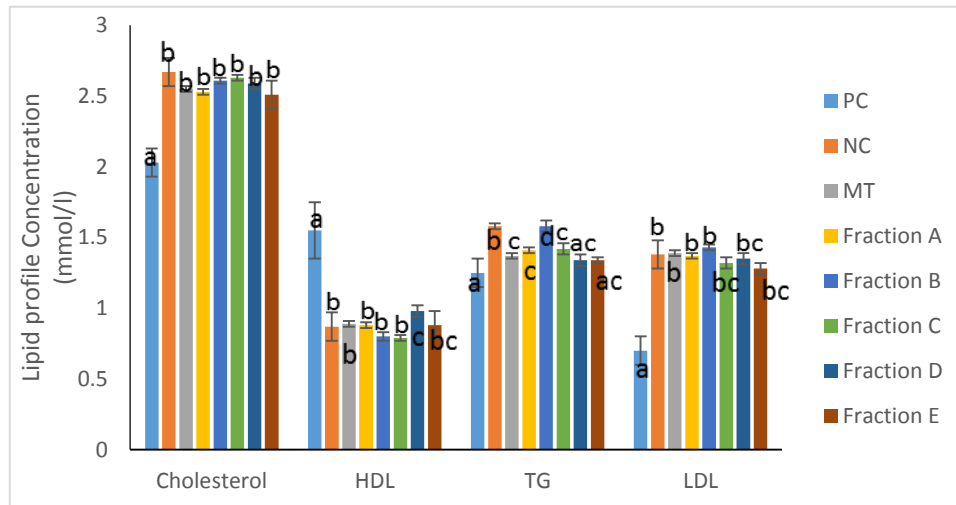


Figure 4: Serum Lipid Profile of Diabetic Rats Treated with Fractions of Butanol Crude Extract of *Ficus exasperata* Leaves

Values are expressed as mean of 6 determinations $\pm$ S.E.M. and bars with different superscripts are statistically different ($p < 0.05$)

Gas Chromatography-Mass Spectrometry Constituents of Butanol Crude Extract of *Ficus exasperata* Leaves

The constituents of butanol crude extract of *Ficus exasperata* leaf after GCMS analysis are presented in Table 2. The analysis revealed the presence of thymol, phytol acetate and eugenol predominantly.

Table 2: Gas Chromatography-Mass Spectrometry Constituents of Crude Butanol Extract of *Ficus exasperata* Leaf

S/n	RT	Area %	Constituents
1	51.639	27.42	Thymol
2	55.361	7.40	Eugenol
3	66.408	1.66	1-Methylpropyl propylphosphonofluoridate
4	68.778	3.38	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl
5	78.174	1.63	Benzene, 1,1'-(1,3-propanediyl)bis
6	83.885	6.79	Pentadecanal
7	87.044	5.92	2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, (R)
8	90.703	2.56	5-Hydroxy-3-methyl-1-indanone
9	92.367	26.91	Phytol, acetate
10	92.843	4.16	2-Hexadecene, 3,7,11,15-tetramethyl
11	94.031	5.36	9-Octadecyne
12	95.107	6.82	3,7,11,15-Tetramethyl-2-hexadecen-1-ol

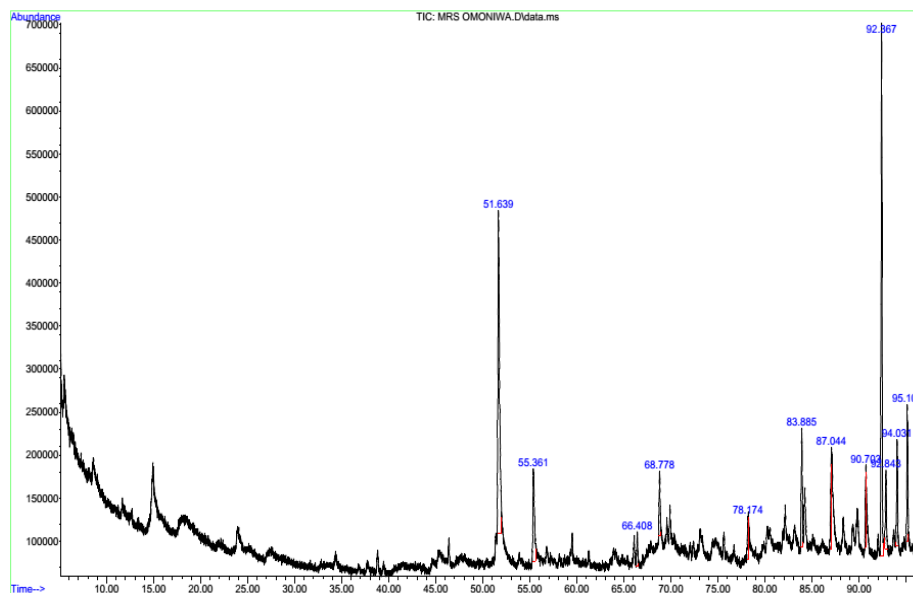


Figure 5: Gas Chromatography-Mass Spectrometry Chromatogram of Butanol Crude Extract of *Ficus exasperata* Leaves

Discussion

The significantly reduced ($p < 0.05$) fasting blood glucose concentration of diabetic rats treated with crude solvent extracts of *Ficus exasperata* leaves confirmed its antihyperglycemic activity. This result supports reports from previous studies and the folkloric claim that *Ficus exasperata* leaf extract could be used to treat diabetes mellitus (Ogunleye et al., 2003; Sonibare et al., 2006; Adewole et al., 2011; Yakubu et al., 2014). Diagnosis of type 2 diabetes mellitus is based most commonly on the presence of hyperglycaemia, that is, a fasting blood glucose concentration of equal to or greater than 126 mg/dl (Harvey and Ferrier, 2011). The existing orthodox drugs for type 2 diabetes mellitus and insulin, were developed to control homeostasis of blood sugar via different mechanisms, however, treatment has remained unsatisfactory due to undesirable side effects (Cubeddu et al., 2000).

The significant reduction ($p < 0.05$) in cholesterol, triglyceride, low density lipoprotein concentrations and significant increase ($p < 0.05$) in high density lipoprotein (HDL) concentration of all the treated groups irrespective of the solvent used for extraction compared to the negative control group confirmed the ability of the plant to manage dyslipidaemia in the treatment groups. Circulating free fatty acids derived from adipocytes are elevated in many insulin-resistant states and have been suggested to contribute to the insulin resistance of diabetes and obesity by inhibiting glucose uptake, glycogen synthesis and glucose oxidation, and by

increasing hepatic glucose output (Bergman and Ader, 2000). The ability of *F. exasperata* leaf to improve dyslipidaemia in the treatment groups in this study suggests that the plant would be beneficial in the management and prevention of cardiovascular diseases.

The significant decrease ($p < 0.05$) in the FBG and serum lipid profile concentrations elicited by the various fractions obtained from the crude butanol extract of *Ficus exasperata* leaves, suggests that the fractions also possesses antihyperglycaemic and dyslipidaemic activities. However, the reduction did not compare favourably with the standard drug (metformin) and the positive control group. Comparing the effect of the crude butanol extract of *Ficus exasperata* leaves on blood glucose concentration and lipid profile parameters of diabetic rats to that of the fractions showed that the crude extract had a better antidiabetic effect than the fractions. This suggests that the antidiabetic principles present in the crude extract might be acting in synergy which upon fractionation resulted in reduction of the antidiabetic activity observed. This observation is in agreement with previous reports which submitted that herbal medicines are by themselves multi-component mixtures and elicit effects through additive and/or synergistic mode of action (Kong *et al.*, 2009; Efferth and Koch, 2011; Gong *et al.*, 2012; Liu *et al.*, 2014; Wu *et al.*, 2014). The predominant constituents from GCMS analysis (eugenol, thymol and phytol acetate) are compounds with known antioxidant activities, which might be one of the ways by which the plant extract elicits its antidiabetic effect. Eugenol is a reported fat soluble antioxidant inhibiting the accumulation of fat peroxide products in red blood cells and maintaining the activities of the body's antioxidant enzymes at normal levels (Bendre *et al.*, 2016). Thymol is also a natural monoterpene phenol and studies have also confirmed the antioxidant properties of this compound (Undeger *et al.*, 2009; Zarrini *et al.*, 2010). Studies had indicated that oxidative stress, through the mechanism of superoxide production, is a common pathogenic factor leading to insulin resistance, β -cell dysfunction, impaired glucose tolerance and ultimately type 2 diabetes mellitus (Ceriello and Motz, 2004; Chandie *et al.*, 2006; El-Bahr, 2013).

Conclusion

Butanol leaf extract of *Ficus exasperata* vahl and its column chromatography fractions elicited antihyperglycaemic and antilipidaemic activities but upon fractionation, these activities were significantly reduced, suggesting that the antidiabetic constituents may be acting in synergy. The predominant constituents identified are compounds with known antioxidant properties which might be contributing to the antidiabetic effect elicited by the plant. Therefore, developing the crude plant into products aimed at managing type 2 diabetes mellitus is recommended.

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