WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 9, 56-72 Research Article ISSN 2278 – 4357

EFFECT OF FRACTIONS OF *BRIDELIA MICRANTHA* ON INSULIN RESISTANCE AND LIPID PROFILES IN HIGH SALT-INDUCED INSULIN RESISTANT RATS AND IDENTIFICATION OF PHYTOCONSTITUENTS BY GC-MS

Ajao Folasade O[1,2]* and Soladoye Ayodele O.[1]

[1]Department of Physiology, University of Ilorin, Ilorin, Nigeria.

[2]Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

Article Received on
27 June 2018,

Revised on 17 July 2018,
Accepted on 07 August 2018

DOI: 10.20959/wjpps20189-12235

***Corresponding Author**
Folasade O. Ajao
Department of Physiology, University of Ilorin, Ilorin, Nigeria.

ABSTRACT

This present study investigates the effect of fractions of *Bridelia micrantha* leaves on insulin resistance and lipid profiles in high salt-induced insulin resistant rats and its identification of phytoconstituents by Gas chromatography-mass spectroscopy (GC-MS). Crude methanol leaf extract of *Bridelia micrantha* was fractionated using silica gel column chromatography to yield 48 fractions. Pooling together of fractions with similar thin layer chromatographic (TLC) mobility profile afforded four major fractions (Fr A – Fr D). Bioactivity of the fractions obtained was evaluated in insulin resistant Wistar rats. Female Wistar rats were given (*p.o.*) normal diet (CON), high-salt diet (8%) daily (HS), Fr A-Fr D (HS rats treated with 10mg/kg bw of fractions A-D). Insulin resistance (IR) was estimated using the homeostatic model of assessment (HOMA). Results showed that high salt diet led to significant increases in insulin resistance, plasma insulin, total cholesterol (TC), triglyceride (TG), TC/HDL-cholesterol, and decreased glucose tolerance. Fraction B was the most effective in attenuating insulin resistance and dyslipidaemia. Fraction B of methanol extracts of *Bridelia micrantha* yielded 18 different phytochemicals confirmed by GC-MS analysis, some of which are known to have antidiabetic properties. In conclusion, the present study report the use of fractions of *Bridelia micrantha* leaves in the attenuating insulin resistance and dyslipidaemia in rats fed high salt diet and also identified the presence of compounds that could be responsible for these actions.

KEYWORDS: ***Bridelia micrantha,*** High salt, Insulin resistance, Dyslipidemia, GC-MS.

INTRODUCTION

Diabetes mellitus (DM) is a major health concern and the third greatest cause of death worldwide. According to the International Diabetes Federation (IDF), an estimated 415 million people had DM in 2015 which is predicted to increase to 642 million by 2040.[1] About 90% of diabetic patients have type 2 DM with insulin resistance playing a key role in the development of the disease.[2,3] DM and insulin resistance have been reported to be associated with increased serum triglycerides, decreased serum HDL and sometimes increased serum LDL.[4]

The present day diets termed Western diet are overloaded with salt and sugar.[5,6] Apart from the daily salt added to cooked food and natural sources of salt such as meat and plant matter, about 80% of daily salt intake comes from processed food alone.[7] Thus it can be inferred that frequent daily high salt intake occurs in individuals who are not often conscious of the amount of salt they consumed. The current estimate of salt consumption in human per day is about 8–12 g[8,9] which is much higher than the recommended daily intake of 1.5–2.0 g.[10] The contemporary dietary habit of processed food consumption has become a risk factor in the development of metabolic syndrome.[11,13] Indeed, diet high in sucrose and/or salt diet have been linked to increasing incidence of obesity, hypertension, stroke and type 2 diabetes.[8,14,15] Studies in both humans and animals have reported the link between high-salt diet intake and insulin resistance.[16,17] Glucose dysregulation and dyslipidaemia also play important roles in the pathogenesis of IR and CVD.[18]

Medicinal plants are commonly used in the management of diseases worldwide due to the high cost and adverse side effects of most pharmacological treatments.[19,20] Herbs peculiar to each sub-regions have been used to cure and control different diseases, Nigeria inclusive.[21] Until now, only a few of such medicinal plants have been scientifically validated.[22] In South Western Nigeria, a leaf decoction of *Bridelia micrantha* is used traditionally as part of recipe for the management of diabetes mellitus.[23] *Bridelia micrantha.* (Euphorbiaceae), commonly known in English as “coast gold leaf” or “ogaofia” (the boss of the bush) in Igbo or “igi-ira” in Yoruba, is a deciduous tree of about 20 meters tall with a dense rounded crown. The plant is indigenous to southern part of Nigeria. The leaf extract of the plant *Bridelia micrantha* has been reported to have several beneficial metabolic effects in animal models, including blood glucose lowering and antioxidant effects.[24] Bioassay guided fractionation is one the key

technique by which compounds with good biological activity has been isolated from medicinal plants.[25] This present study was carried out to separate *Bridelia micrantha* leaf extract into fractions, investigate the effect of fractions on insulin resistance and identify the bioactive compounds present in the plant extract.

MATERIALS AND METHODS

Plant material and extract preparation

Fresh leaves of *Bridelia micrantha* were collected from a farm settlement in Ogbomoso, Oyo State, Nigeria. The plant was identified, authenticated and registered with voucher specimen number (LHO 376) at the herbarium unit of the Biology department, Ladoke Akintola University of Technology, Ogbomoso. The *Bridelia micrantha* leaves were collected washed in tap water and air dried at room temperature. The dried leaves were ground into powder and the powdered sample was extracted using water and methanol as solvent.[26] The powder (800g) was macerated in 100% methanol at room temperature for 72 hrs. This was then filtered using a filter paper (Whatmann size no.1) and the filtrate was evaporated to dryness in water bath at 40^0c to a brown dried residue of 80.6g and kept in an air tight bottle until used.

Column Chromatography fractionation of Bridelia micrantha crude extract

Bridelia micrantha crude extract (20 g) was subjected to column chromatography to separate the extract into its component fractions using a column size of 3.5 x 50 cm. Silica gel (60 G) was used as the stationary phase while varying solvent (hexane, ethyl acetate and methanol) combinations of increasing polarity were used as mobile phase. The tap was then opened to allow the eluent to flow at a controlled rate of 40 drops per minute. Elution of the extract was done with solvent systems of gradual increasing polarity. The following ratios of solvent combinations were sequentially used in the elution process; Hexane: ethylacetate 100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100. The same ratios were used for Ethyl acetate: methanol combination. The eluted fractions were collected in aliquots of 20 ml in small sterile bottles. The eluent was recovered from fraction by rotary evaporation. Spectroscopic techniques (IR and UV) were used for preliminary characterization of active compounds.[27]

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) involves the use of silica gel sorbent spread on an inert sheet of glass as a stationary phase. The mobile phase was allowed to travel up the plate

carrying the sample that was iitially seen on the sorbent just above the solvent[27] (Kaur *et al*., 2014).

Animals and treatment

The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the University of Ilorin Ethical Review Committee (Reference no: UIL/UERC/12/68BK001). Every effort was made to minimize both the number of animals used and their suffering. Wistar rats weighing between 150-200g were randomly assigned into 6 groups (n=6/group). Rats were maintained under standard environmental conditions of temperature, relative humidity, and dark/light cycle. The control group (CON) received standard rodent diet, the high-salt diet group (HS) received 8% salt in diet only, HS group treated with 10 mg/kg bw fraction A of methanolic extract of *Bridelia micrantha* leaf (FrB), HS group treated 10 mg/kg bw fraction B of methanolic extract of *Bridelia micrantha* leaf (FrC), HS group treated with 10 mg/kg bw fraction C of methanolic extract of *Bridelia micrantha* leaf (FrD), HS group treated with 10 mg/kg bw fraction D of methanolic extract of *Bridelia micrantha* leaf (FrG). For the first three weeks, five groups (HS, FrA, FrB, FrC, and FrD) were fed with high-salt diet daily to induce IR. For the subsequent three weeks, FrA, FrB, FrC, and FrD groups received (*po*) fractions A, B, C, D of methanolic extracts of *Bridelia micrantha* leaf respectively. CON and HS groups continued with their previous diets.

Measurement of body weight, food, and water intake

Body weight of rats was measured weekly using a weighing balance. The body weight change was calculated as: (final body weight − initial body weight) in grams. Food and water intake was measured daily based on the weight and volume of leftover feed and water respectively.

Sample preparation

At the end of treatment period, the rats were anesthetized with sodium pentobarbital. Blood was collected by cardiac puncture into heparinized bottle and was centrifuged at 3000g for 5min. Plasma was stored frozen until needed for biochemical assay.

Oral glucose tolerance test (OGTT)

Glucose challenge test was performed 24 hrs before the end of the experiment. The rats had 12 hrs overnight fast. Glucose (2 g/kg *bw*) was given (*po*). Blood sample was obtained from

the tail before glucose load and then sequentially after 30, 60, 90 and 120 min. Blood glucose levels were determined with a glucometer (ACCU-CHEK Active- Roche Diagnostics, Germany). Glucose tolerance was expressed as a function of the area under the OGTT curve (AUC) as previously described.[28] Elevated 1-hr postload glucose level is also used as a reliable predictor of IR, pancreatic β-cell function, atherosclerotic CVD and renal dysfunction.[29,30]

Biochemical assays and IR

Plasma insulin was determined using ELISA kit from Ray Biotech, Inc. (Georgia, USA). Fasting plasma levels of total cholesterol (TC) and triglyceride (TG) were measured by standardized enzymatic colorimetric methods using assay kit obtained from Fortress Diagnostics Ltd. (Antrim, UK). High-density lipoprotein-cholesterol (HDL-C) was measured by enzymatic clearance assay (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) whereas low-density lipoprotein-cholesterol (LDL-C) was estimated using modified Friedewald's formula.[31] TC/HDL-C and TG/HDL-C ratios were estimated as marker of atherogenic lipid indices. IR was estimated using the homeostasis model assessment for IR (HOMA-IR). HOMA-IR is expressed as fasting glucose (mmol/l) * fasting insulin (μU/l)/22.5.

Gas chromatography-mass spectroscopic analysis of fraction C of methanolic extract of Bridelia micrantha leaf

GC-MS analysis of fraction C of *Bridelia micrantha* was performed using the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 μm film. The carrier gas used is Helium with at low of 1.0 ml/min. The injector was operated at 250°C and the oven temperature was programmed as follows: 60°C for 15 min, then gradually increased to 280°C at 3 min. The identification of components was based on Willey and NIST libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument and the results obtained have been tabulated.

Data analysis and statistics

All data were expressed as means ± SEM. Statistical group analysis was performed with SPSS statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the

significance of pair wise comparisons of mean values among the groups. Statistically significant differences were accepted at $p<0.05$.

RESULTS

Column Chromatographic Fractions of Bridelia micrantha Leaf Extract

Fractionation of *Bridelia micrantha* crude extract using column chromatography yielded 48 fractions. Pooling together of the fractions with similar TLC mobility profile; afforded four (4) major fractions.

Physiological parameters

In the high salt only treated rats, high-salt diet only caused a significant decrease in body weight (Table 1). Treatment with the fractions of extract for 3 weeks produced signs of recovery in body weight. High-salt diet only led to significant decrease in food intake (Table 1). The high-salt only fed rats consumed significantly more water but less food compared to the control (Table 1). Treatment with fractions of extract ameliorated these effects. The weights of the heart and pancreas were not affected in all experimental groups (Table 1).

Glucose regulation

Fasting glycemia was not affected in all experimental groups (Fig 1a). High-salt diet only led to significant elevated glycemia after ½-hour of glucose load. High salt diet only still caused elevated glycemia after 1-hour and 1½-hour of glucose load. However, treatment with FrA, FrB, FrC and FrD led to significant reduction in glycemia after 1-hour and 1½-hour of glucose load in the high-salt diet rats (Fig. 1b). Glucose tolerance was estimated by the area under the curve (AUC) of oral glucose tolerance test (OGTT). The values of AUC were significantly higher in high-salt diet only rats when compared to the control, whereas AUC was significantly lower in FrA, FrB and FrC treated rats when compared to high-salt diet only rats (Fig. 1c).

Insulin sensitivity

High-salt diet only led to significant increase in HOMA-IR, whereas treatment of the high-salt fed rats with FrB and FrC of methanolic extract of *Bridelia micrantha* leaf led to significant decreases in HOMA-IR (Fig. 2a). High-salt diet only resulted in significantly increased fasting plasma insulin level compared to control (Fig. 2b). After three weeks of treatment, only the high-salt fed rats that received 10 mg/kg bw of fraction B of methanolic

extract of *Bridelia micrantha* leaf had significantly reduced plasma insulin level compared with the high-salt diet only.

Circulating lipids and atherogenic dyslipidemia

Table 2 depicts the effect of fractions of methanolic extract of *Bridelia micrantha* leaf on TG, TC, HDL-C and LDL-C in IR rats. High-salt diet only led to significant increase in plasma levels of TC, TG and LDL-C in all experimental groups when compared with the control whereas plasma HDL-C level was significantly decreased in all experimental groups compared to control. After three weeks of treatment, only FrB treated group had significantly reduced plasma TC, TG and LDL-C levels and significantly increased plasma HDL-C level compared with the high-salt diet only. The significantly increased atherogenic indices in high-salt diet only rats were reduced significantly in FrB and FrC groups only. (Fig. 3a & b).

GC-MS studies

Out of 48 column fractions of methanolic extract, fraction B was chosen for GC-MS studies because it was the most effective in improving insulin sensitivity in our model of insulin resistance. In fraction B, 18 prominent compounds were identified by GC-MS studies (Table 3, Fig 4).

Figure Legend

Fig. 1: Effect of fractions of methanolic extracts of *Bridelia micrantha* leaf on oral glucose tolerance test (OGTT; a) and area under curve (AUC) of OGTT in salt induced IR rats (b). High salt diet only led to increase in 1hr postload glucose and AUC that was attenuated in FrA, FrB, FrC and FrD rats. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 6 rats per group (p*<0.05 *vs* CON; ***p*<0.01 *vs* CON; # *p*<0.01 *vs* HS; ##*p*<0.01 *vs* HS).**

Fig. 2: Effect of fractions of methanolic extracts of *Bridelia micrantha* leaf on IR marker. HOMA-IR. High salt diet only led to increase in HOMA-IR that was attenuated in FrB and FrC rats. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 6 rats per group (p*<0.05 *vs* CON; #*p*<0.05 *vs* HS).**

Fig. 3: Effect of fractions of methanolic extracts of BM on atherogenic indices (a & b). IR led to an increase in TC/HDL-C and TG/HDL-C ratios. Elevated TC/HDL-C and

TG/HDL-C ratios were attenuated on treatment with FrB and FrC. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 6 rats per group (p*<0.05 *vs* CON; #*p*<0.05 *vs* HS, ##*p*<0.01 *vs* HS).**

Fig. 4: Gas chromatography-mass spectroscopic analysis of fraction B of methanolic extract of *Bridelia micrantha* leaf.

Table 1: Effects of fractions of methanolic *Bridelia micrantha* leaf extracts on body weight change, food and water intake in high salt-induced IR.

PARAMETERS	CON	HS	Fr A	Fr B	Fr C	Fr D
Body weight change(g)	10.6±0.6	-1.2±0.3	1.0±0.3	6.3±0.9	2.2±0.3	4.3±0.8
Water intake (ml/kg)	118.4±2.4	150.9±3.0*	131.4±3.4	117.8±3.3$^{\#}$	125.1±4.5	123.0±8.3
Food intake (g/kg)	121.8±2.5	92.8±3.1*	102.5±4.8	118.6±6.6$^{\#}$	114.0±3.5	99.6±2.3

Data are expressed as mean ±SEM of 6 rats per group. Data were analyzed by one-way ANOVA followed by Bonferroni *posthoc* test (*p<0.05 *vs* CON; **p<0.01 *vs* CON; $^{\#}$p<0.05 *vs* HS, $^{\#\#}$p<0.01 *vs* HS).

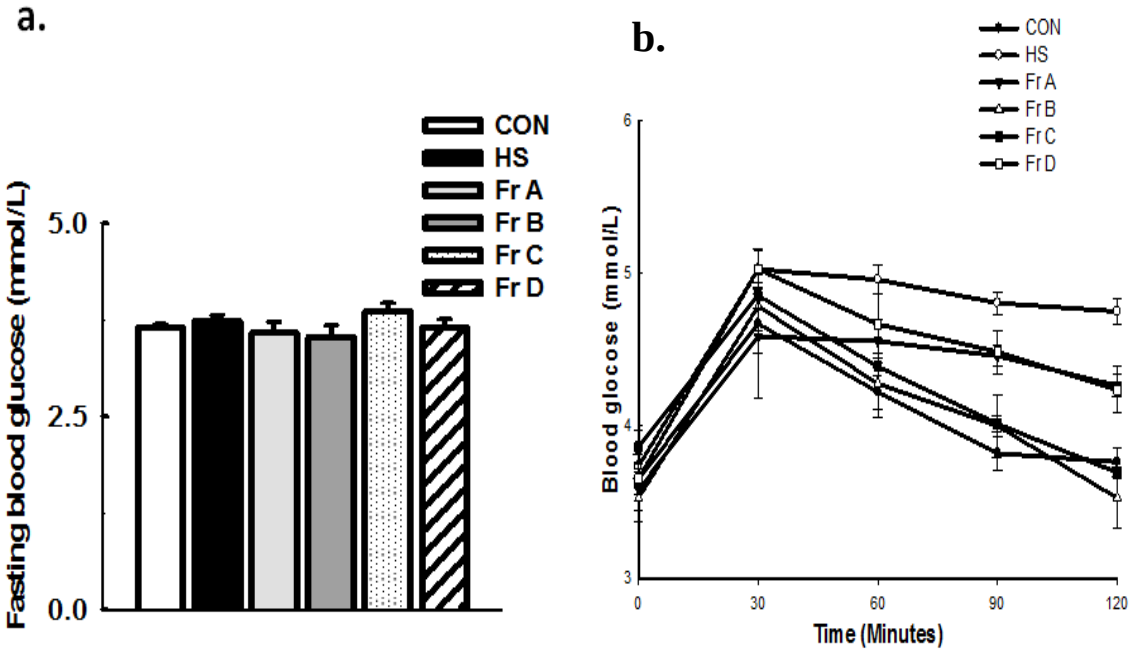
Table 2: Effects of fractions of methanolic extract of *Bridelia micrantha* leaf on lipid profile in high salt-induced IR.

PARAMETERS	CON	HS	Fr A	Fr B	Fr C	Fr D
Triglyceride (mg/dl)	68.4±4.4	180.9±6.0**	132.4±23.4*	61.8±7.3$^{\#\#}$	115.1±11.5*$^{\#\#}$	123.0±28.3
Total cholesterol (mg/dl)	82.8±7.1	111.8±5.5*	102.5±4.8	69.6±4.3$^{\#\#}$	74.0±2.5$^{\#\#}$	88.6±6.6$^{\#}$
HDL-cholesterol (mg/dl)	34.2±2.6	18.4±1.1**	20.1±4.5*	29.9±1.2$^{\#\#}$	22.2±1.8**	18.6±2.4**
LDL- cholesterol (mg/dl)	34.8±7.1	57.3±3.6*	55.9±5.7*	27.3±4.5$^{\#\#}$	28.9±0.7$^{\#\#}$	45.4±2.2$^{\#}$

Data are expressed as mean ±SEM of 6 rats per group. Data were analyzed by one-way ANOVA followed by Bonferroni *posthoc* test (*p<0.05 *vs* CON; **p<0.01 *vs* CON; $^{\#}$p<0.05 *vs* HS, $^{\#\#}$p<0.01 *vs* HS).

Table 3: GC-MS spectral analysis of fraction B of methanolic extract of *Bridelia micrantha* leaf.

No	Retention Time (min)	Name of compound	Mol. formular	Mol. weight	Peak area %
1	15.032	Ethanol 2-octadecyloxy-	$C_{20}H_{42}O_2$	314.6 g/mol	0.98
2	18.064	Cyclooctane, methyl	$C_{12}H_{22}$	168.3 g/mol	1.38
3	24.827	1-Dodecanethiol	$C_{12}H_{22}SH$	202.4 g/mol	1.65
4	28.378	Phenol,2.4-bis 1,1-dimethylethyl	$C_{14}H_{22}O$	206.3 g/mol	3.38
5	30.821	Cyclodecane	$C_{10}H_{20}O$	140.3 g/mol	3.08
6	36.500	1-Eicosanol	$C_{20}H_{42}O$	298.6 g/mol	4.67
7	37.364	11,14-Eiocosadienoic acid, methyl ester	$C_{12}H_{24}$	168.3 g/mol	0.98
8	37.639	Benzaldehyde, 3-2,4,6-trichlorophenoxymethyl-4-methoxy	$C_{20}H_{42}O$	298.6 g/mol	1.08
9	38.063	Cyclopropanebutanoic acid, 2-2-2-2-pentylcycloprophyl	$C_{12}H_{38}O_2$	322.4 g/mol	1.01
10	32.263	Hexadecanoic acid, methyl ester	$C_{17}H_{42}O$	270.5 g/mol	0.70
11	34.409	Benzenepropanoic acid, 3-5 bis-4-hydroxyl-methyl ester	$C_{18}H_{28}O_3$	292.4 g/mol	1.01
12	38.590	Phen-1,4-diol, 2-3-dimethyl-5-trifluoromeththyl	$C_{17}H_{34}O_2$	270.5 g/mol	0.89
13	38.833	2-Hexadecanol	$C_{16}H_{34}O$	242.5 g/mol	3.27
14	15.456	Decane, 3,6-dimethyl	$C_{12}H_{26}$	170.3 g/mol	2.74
15	39.572	9-Octadecanoic acid, 2-methyl ester	$C_{16}H_{34}O$	242.5 g/mol	22.71
16	39.477	Octadecanol, 2-bromo	$C_{18}H_{37}BrO$	349.4 g/mol	2.58
17	39.729	Heptadecanoic acid, 16-methyl, methyl ester	$C_{19}H_{38}O_2$	298.5 g/mol	4.69
18	42.022	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.6 g/mol	7.95



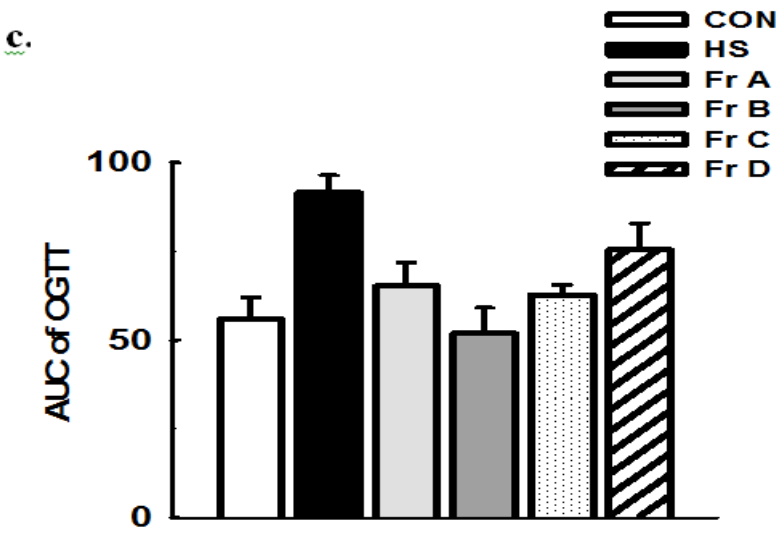

c.
CON
HS
Fr A
Fr B
Fr C
Fr D
AUC of OGTT
100
50
0

Fig. 1

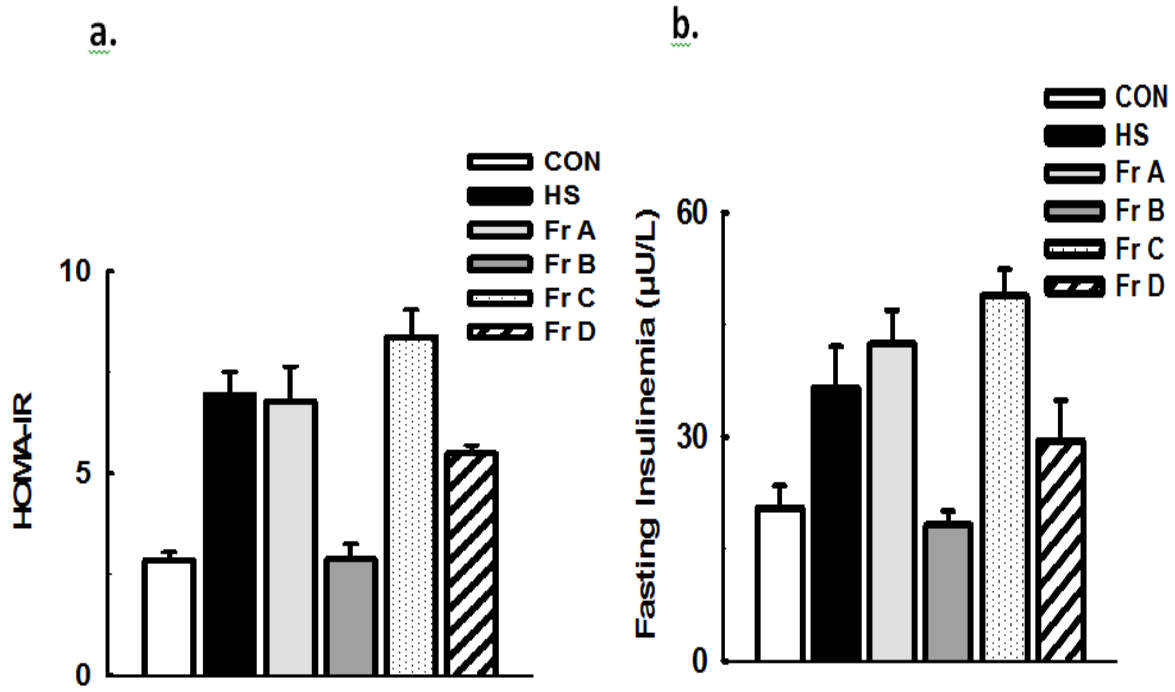

a.
b.
CON
HS
Fr A
Fr B
Fr C
Fr D
HOMA-IR
10
5
0
Fasting Insulinemia (μU/L)
60
30
0

Fig. 2

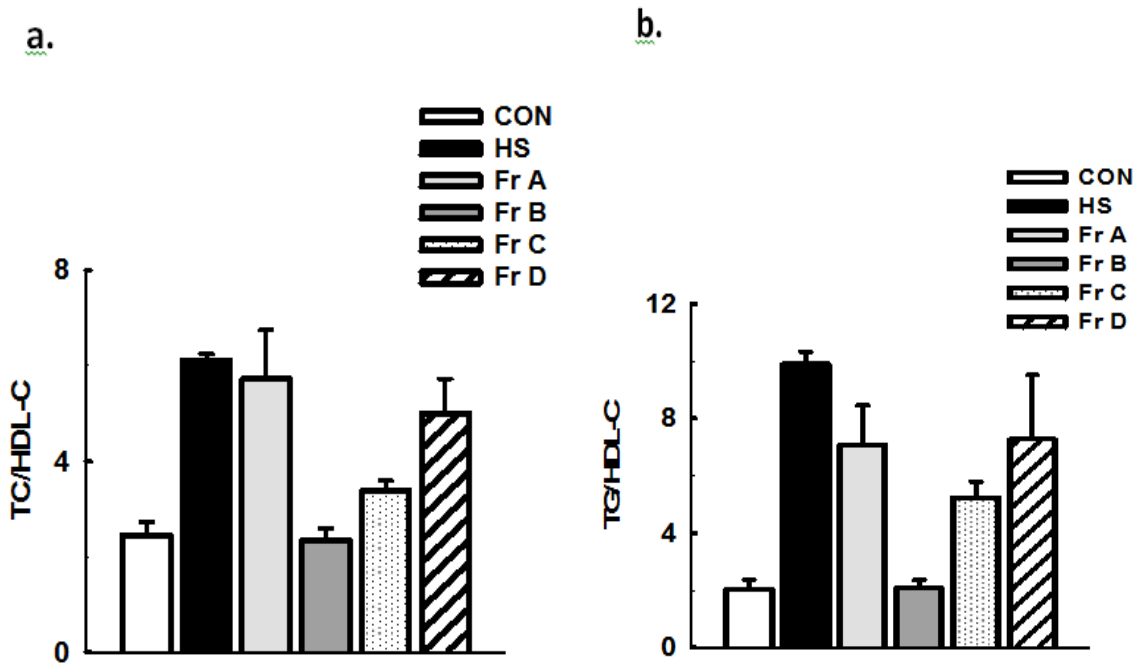

a.
b.
CON
HS
Fr A
Fr B
Fr C
Fr D
TC/HDL-C
TG/HDL-C
0
4
8
12

Fig. 3

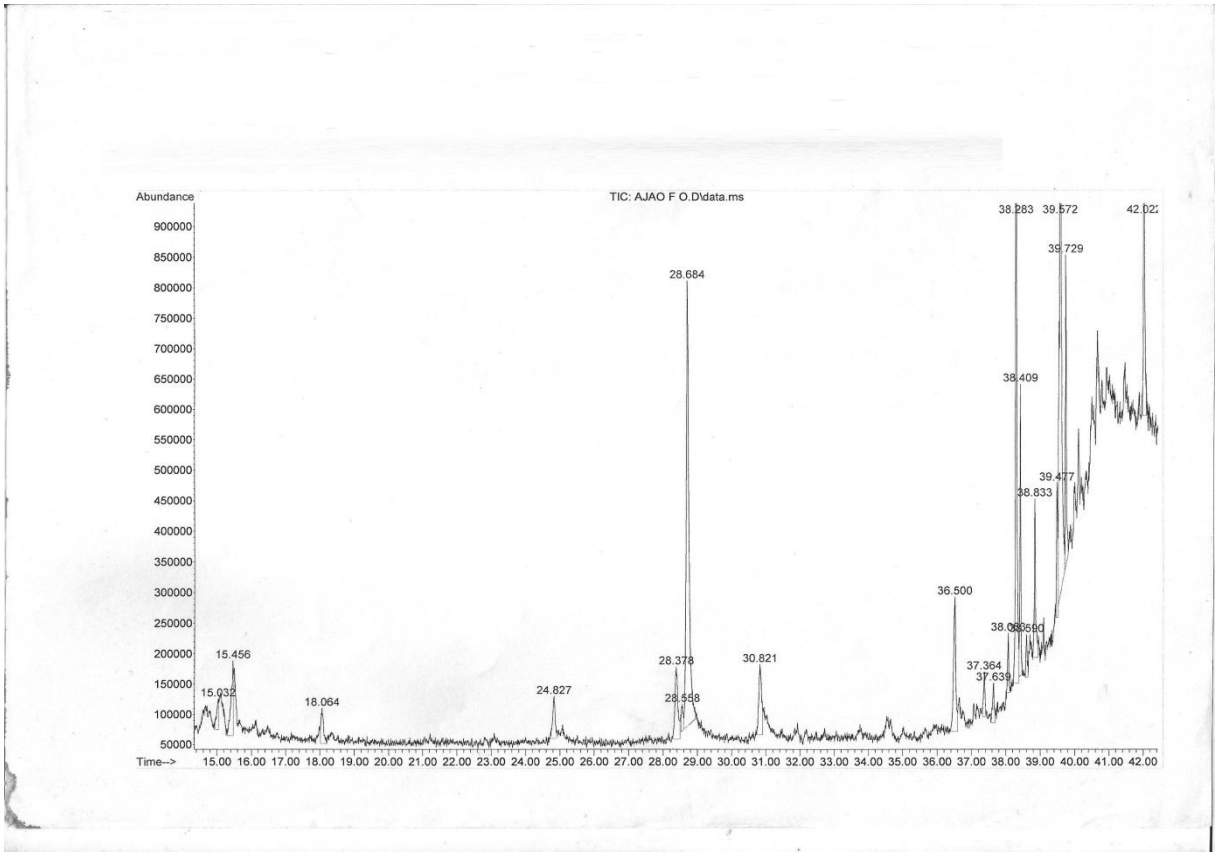

Abundance
TIC: AJAO F O.D\data.ms
Time-->
15.032
15.456
18.064
24.827
28.378
28.558
28.684
30.821
36.500
37.364
37.639
38.283
38.409
38.833
39.572
39.729
39.477

Fig. 4

DISCUSSION

The complexities of crude extracts as a result of numerous compounds they contain may interfere with their efficacy in treating diseases. There are also possibilities of unwanted side effects or toxicity as a result of the presence of some components of the whole plant extracts. This explains why bioassay guided fractionation of crude extracts is an important step in optimizing the therapeutic effectiveness of medicinal plants and in overcoming herbal toxicity which is a major concern in the medicinal application of herbs or plant extracts. In the process of fractionation, phytoconstituents which are not relevant to the medicinal potential of given crude extract in treating a particular ailment are removed.

Bridelia micrantha leaf extract has been reported to have several beneficial metabolic effects in animal models, including blood glucose lowering and antioxidant effects.[24] Also in our previous study, we established that crude methanolic extract of *Bridelia micrantha* leaf attenuate insulin resistance and hyperlipidaemia in rats fed high salt diet. In this present study however, an attempt was made to identify the component responsible for the above-mentioned pharmacological action in the active crude methanolic extract of *Bridelia micrantha* leaf. The identification of the constituent responsible for attenuation of insulin resistance and hypolipidaemic activities was performed through the column chromatography technique and GC-MS analysis. Fractionation of *Bridelia micrantha* crude extract using column chromatography yielded 48 fractions. Pooling together of the fractions with similar TLC mobility profile; afforded four (4) major fractions (A, B, C and D).

The findings in the present animal model provide further evidence that high salt intake would cause insulin resistance. Studies in both humans and animals have reported the link between high-salt diet intake and insulin resistance.[16,17] Fraction B of methanolic extract of *Bridelia micrantha* leaf (Fr B) was the most effective in ameliorating the increased fasting insulin level and HOMA-IR, insulin resistance markers in high salt-induced IR rats. This suggests an improvement of insulin sensitivity in this experimental model of insulin resistance.

Salt intake has also been shown to cause dyslipidaemia.[32] Insulin resistance a key component of metabolic syndrome involved a series of risk factors such as abdominal obesity, hypertension and dyslipidaemia.[33] In this study, feeding the rats with high salt diet resulted in significant increases ($p<0.05$) in TC, LDL-C, and TG and a significant decrease ($p<0.01$) in HDL. On administration of fractions of methanolic extract of *Bridelia micrantha* leaf, fraction B caused significant reductions ($p<0.01$) in the elevated TC, TG, LDL-C and

lipid ratios with a significant increase ($p<0.01$) in the reduced HDL-C. Increase in TG/HDL-C ratio, has been suggested to be a stronger independent risk factor for the development and/or progression of atherosclerotic cardiovascular events than elevated TG, decreased HDL-C or TC/HDL-C ratio.[34] The heightened risk of cardiovascular event as a result of increase in TG/HDL-C ratio was attenuated on administration of fractions B and C with fraction B being the most effective.

Fraction B therefore showed positive insulin sensitizing and hypolipidaemic effects against insulin resistance and hyperlipidaemia in this experimental model. To identify the compounds responsible for insulin sensitizing and antihyperlipidaemic activities associated with 'B' respectively, a GC-MS analysis was carried out. Eighteen peaks were noted that indicated the presence of 18 compounds: Ethanol 2-[octadecyloxy]-; Cyclooctane, methyl-; 1-Dodecanethiol; Phenol, 2.4-bis 1,[1-dimethylethyl]-; Cyclodecane; 1-Eicosanol, 11,14-Eiocosadienoic acid, methyl ester; Benzaldehyde, 3-[2,4,6-trichlorophenoxymethyl]-4-methoxy-; Cyclopropanebutanoic acid, [2-2-2-2-pentylcycloprophyl]; Hexadecanoic acid, methyl ester; Benzenepropanoic acid, 3-5 bis-4-hydroxyl-methyl ester; Phen-1,4-diol, 2-3-dimethyl-5-trifluoromeththyl-; 2-Hexadecanol-; Decane, 3,6-dimethyl-; 9-Octadecanoic acid, 2-methyl ester; Octadecanol, 2-bromo-; Heptadecanoic acid, 16-methyl, methyl ester; Diisooctyl phthalate.

Out of these compounds, hexadecanoic acid, octadecanoic acid and eicosanoic acid have been proven to have antidiabetic activity by possessing insulin secretion, insulin stimulation, α-glucosidase inhibitors properties.[35,37] The above GCMS studies also showed the presence of phenolic compounds in the active fraction of *Bridelia micrantha* leaves which could be responsible for its insulin sensitizing effect. Phenolic compounds have been reported to have insulin sensitizing, hypoglycaemic mechanisms predicted in the management of diabetes.[38] Presently, the exact compound isolated from the active fraction responsible for the above activities is unknown. The exact compound and mechanism of action of compounds from the active fraction will be the subject of further studies.

CONCLUSION

The present research work reported the use of fractions of *Bridelia micrantha* leaves in the attenuating insulin resistance in rats fed high salt diet. This research work also identified the presence of compounds in active fraction of *Bridelia micrantha* leaves responsible for their insulin sensitizing and hypolipidaemic actions. Further comprehensive studies are needed to

determine the exact compounds and elucidate the exact mechanism of the insulin sensitizing and hypolipidaemic effects of *Bridelia micrantha* leaves.

ACKNOWLEDGEMENTS

The authors are appreciative of the contributions from the HOPE Cardiometabolic Research team, University of Ilorin, Ilorin, Nigeria.

Conflict of interest

The authors have no conflict of interest to declare.

REFERENCES

1. International Diabetes Federation. Diabetes Atlas Seventh Edition, 2015. International Diabetes Federation. 2016; Seventh Edition, 9-12.
2. Fuller JH, Shipley MJ, Rose GJ, Jarrett RJ, Keen H. Coronary heart disease risk and impaired glucose tolerance: The Whitehall study. Lancet, 1980; 1: 1373-1376.
3. Kim SH, Hyun SH, Choung SY. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. J. Ethnopharmacol., 2006; 104: 119-123.
4. Said O, Fulder S, Khalil K, Azaizeh H, Kassis E, Saad B. Maintaining a physiological blood glucose level with 'glucolevel', a combination of four anti-diabetes plants used in the traditional arab herbal medicine. Evid Based Complement Alternat Med., 2008; 5: 421–428.
5. Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH. *et al.* Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr, 2007; 86: 899-906.
6. Popkin BM. Global nutrition dynamics: the world is shifting rapidly toward a diet linked with non-communicable diseases. Am J Clin Nutr, 2006; 84: 289-298.
7. Delahaye F. Should we eat less salt? Arch Cardiovasc Dis, 2013; 106: 324-332.
8. Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intake around the world; implications for public health. Int J Epidemiol, 2009; 38: 791-813.
9. Gray C, Long S, Green C, Gardiner SM, Craigon J, Gardner DS. Maternal fructose and/or salt intake and reproductive outcome in the rat: effect on growth, fertility, sex ratio, and birth order. Biol Reprod, 2013; 89: 51-58.
10. WHO/FAO Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO expert consultation. Technical Report Series 916, World Health Organization, Geneva (2003).

11. Chan TF, Lin WT, Huang HL, Lee CY, Wu PW, Chiu YW, *et al.*Consumption of sugar-sweetened beverages is associated with components of the metabolic syndrome in adolescents. Nutrients, 2014; 23: 2088-2103.
12. Bermudez OI, Gao X. Greater consumption of sweetened beverages and added sugars is associated with obesity among US young adults. Ann Nutr Metab, 2010; 57: 211-218.
13. Lutsey PL, Steffen LM, Stevens J. Dietary intake and the development of the metabolic syndrome; The Atherosclerosis Risk in Communities Study. Circulation, 2008; 117: 754-761.
14. Bibbins-Domingo K, Chertow GM, Coxson PG, Moran AE, Lightwood JM, Pletcher M.J., *et al.* Reductions in cardiovascular disease projected from modest reductions in dietary salt. N Engl J Med, 2010; 362: 590-599.
15. Tappy L, Lê KA. Metabolic effects of fructose and the worldwide increase in obesity Physiol Rev, 2010; 90: 23-46.
16. Midori SY, Junichi Y, Minoru Y, Tsuyoshi W, Makoto O, Robin AF. et al. Salt sensitivity is associated with insulin resistance sympathetic overactivity, and decreased suppression of circulating renin activity in lean patients with essential hypertension. Am. J. Clin. Nutr., 2010; 92: 77–82.
17. Takehide O, Tomoichiro A, Katsuyuki A, Hideyuki S, Motonobu A, Nobuhiro S. et al. High-salt diet enhances insulin signaling and induces insulin resistance in dahl salt-sensitive rats. Hypertension, 2002; 40: 83–89.
18. Zeyda M, Stulnig, T.M. Obesity, inflammation, and insulin resistance −a mini review. Gerontology, 2009; 55: 379–386.
19. Adewunmi CO, Ojewole, JAO. Safety of traditional, medicines complementary and alternatives medicines in Africa. Afr. J. Trad. CAM, 2004; 1: 1-3.
20. Aliyu R, Adebayo AH, Gatsing D, Garba IH. The effects of ethanolic leaf extract of Commiphora Africana (Burseraceae) on rat liver and kidney functions. J. Pharmacol. Toxicol., 2007; 2: 373-379.
21. Kafaru E. Immense Help from Natures Workshop. Health Services Ltd. Academic Press Plc, Lagos, Nigeria., 1994; 1-27.
22. Tanko Y, Haldu A, Mohammed A, Ahmed MK, Musa KY. Effect of ethanol extract of *Caralluma dalzielii*. E.Br.(Asclepiadaceae) on blood glucose levels of fructose-induced insulin resistance in laboratory animals. Annals of Biological Research, 2012; 3(11): 4980-4984.

23. Abo KA, Jaiyesimi F. Ethnological studies of medicinal plants used in management of diabetes mellitus. J. Ethnopharm, 2008; 115: 67-71
24. Adika OA, Madubunyi II, Asuzu IU. Antidiabetic and antioxidant effects of the methanol extract of *Bridelia micrantha* (Hochst) Baill. (Euphorbiaceae) leaves on alloxan-induced diabetic albino mice. Comparative Clinical Pathology, 10: 1205-1208.
25. Agidew E, Reneela P, Deyou, T. Phytochemical investigation of Sapium ellipticum. Journal of Natural Products and Plant Resources, 2013; 3(5): 1 – 6.
26. Olatunji LA, Omolekulo TE, Usman TO, Kim I. Improvement of oral contraceptive-induced glucose dysregulation and dyslipidemia by valproic acid is independent of circulating corticosterone. Archives of Physiology and Biochemistry. The Journal of Metabolic Diseases, 2016; 122(3): 123 –129.
27. Succurro E, Arturi F, Lugara M, Grembiale A, Fiorentino TV, Caruso V, et al. One-Hour Postload Plasma Glucose Levels Are Associated with Kidney Dysfunction. Clin J Am Soc Nephrol, 2010; 5: 1922–1927.
28. Sciacqua A, Miceli S, Carullo G, Greco L, Succurro E, Arturi F, et al. One-Hour Postload Plasma Glucose Levels and Left Ventricular Mass in Hypertensive Patients. Diabetes Care, 2011; 34: 1406–1411.
29. Bianchi C, Miccoli R, Trombetta M, Giorgino F, Frontoni S, Faloia E, et al. Elevated 1-hour postload plasma glucose levels identify subjects with normal glucose tolerance but impaired beta-cell function, insulin resistance, and worse cardiovascular risk profile: the GENFIEV study. The Journal of clinical endocrinology and metabolism, *2013;* 98(5): 2100–2105.
30. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density ipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. Clin Chem, 1972; 18: 499–502.
31. Ogihara T, Asano T, Ando K, Chiba Y, Sekine N, Sakoda, H. et al. Insulin resistance with enhanced insulin signaling in high-salt diet fed rats. Diabetes. 2001; 50: 573–583.
32. Hekmatdoost A, Mirmiran P, Hosseini-Esfahani F, Azizi F. Dietary fatty acid composition and metabolic syndrome in Tehranian adults. Nutrition, 2011; 27(10): 1002–7.
33. Gaziano JM, Hennekens CH, O'Donnell CJ, Breslow JL, Buring JE. Fasting triglycerides, high density lipoprotein, and risk of myocardial infarction. Circulation, 1997; 96: 2520–2525.

34. Wuttke A, Idevall-Hagren O, Tengholm A. $P2Y_1$ receptor dependent diacylglycerol signaling microdomains in β cells promote insulin secretion. FASEB J., 2013; 27(4): 1610-20.
35. Parker SM, Moore PC, Johnson LM, Poitout V. Palmitate potentiation of glucose-induced insulin release: a study using 2- bromopalmitate. Metabolism, 2003; 52(10): 1367-71.
36. Balogun OS, Oladosu IA, Akinnusi A, Zhiqiang L. Fatty acids composition, α-glucosidase inhibitory potential and cytotoxicity activity of Oncoba spinosa Forssk. Elixir Appl. Chem., 2013; 59: 15637-15641.
37. Channabasava, Govindappa M, Chandrappa CP, Sadananda TS. *In Vitro* Antidiabetic Activity of Three Fractions of Methanol Extracts of *Loranthus Micranthus,* Identification of Phytoconstituents by GC-MS and Possible Mechanism Identified by GEMDOCK Method. Asian Journal of Biomedical and Pharmaceutical Sciences, 2014; 4(34): 34-41.