(RESEARCH ARTICLE)

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Two antihyperglycaemic compounds from *Globimetula braunii* (Engl.) Van Tiegh (Loranthaceae)

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Abstract

Large numbers of medicinal plants are constantly being screened for possible pharmacological values, especially for chronic diseases such as diabetes, with the view of discovering new compounds that may serve as templates for synthesis of more active/less toxic drugs. Therefore, the antihyperglycaemic activity of the leaf of *Globimetula braunii* with antidiabetic ethnomedical usage in Nigeria was investigated is this study to justify this folkloric claim. The median lethal dose, LD_{50} of the ethanol leaf extract of *G. braunii* was determined using Lorke's method and its antihyperglycaemic effect at 100, 200 and 400 mg/kg was evaluated using glucose-induced hyperglycaemic rats while glibenclamide (5 mg/kg) and 1 % Tween 80 in normal saline served as positive and negative controls, respectively. Anti-hyperglycaemic activity-directed purification of the extract of the plant in glucose-loaded rats, led to the isolation and characterisation of phyllanthone and methyl 2, 6-dihydroxy-4-methoxybenzoate from the dichloromethane partitioned fraction. The findings showed that the LD_{50} of the ethanol leaf extract of *G. braunii* was greater than 5,000 mg/kg while its 100 mg/kg was the most active dose with comparable activity (p>0.05) to the standard drug, glibenclamide. The dichloromethane and aqueous partitioned fractions of the extract were the most promising fractions. Chromatographic separations of the dichloromethane fraction yielded phyllanthone, and methyl 2, 6-dihydroxy-4-methoxybenzoate that elicited comparable activity to glibenclamide (5 mg/kg) at 10 and 20 mg/kg at all time-points. The study justified the antidiabetic folkloric use of *G. braunii* leaf and confirmed phyllanthone and methyl 2,6-dihydroxy-4-methoxybenzoate as two of its antihyperglycaemic constituents.

Keywords: *Globimetula braunii*; Antihyperglycaemic activity; Phyllanthone; methyl 2,6-dihydroxy-4-methoxybenzoate

1. Introduction

Medicinal plants are plants which, in one or more of their organs, contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. They have the ability to synthesis a wide variety of chemical compounds that possess pharmacological properties, and defend against attack from predators such as insects, fungi and herbivores [2, 3]. These compounds also known as phytochemicals, have been found to exert their effects on the human body through mechanisms that are similar to those already established in conventional drugs revealing that herbal medicines may be as effective as the latter [2, 4]. About 25 % of the drugs prescribed worldwide come from medicinal plants and out of the 252 drugs considered as basic and essential by the World Health Organisation, 11 % are exclusively of plant origin and a significant number are synthetic drugs obtained from natural

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precursors [5]. The plant kingdom is believed to hold many new drug templates; hence, the continued investigation into ethno-medicinal plants [6].

Globimetula braunii, commonly known as African Mistletoe and called, ''Àfòmóonísànó'' among the Yoruba tribe in Nigeria is a hemi-parasitic shrub that grows on dicotyledonous trees such as *Albizzialebbeck, Terminalia mantaly, Terminalia catappa, Khaya senegalensis, Citrus grandis*, *Cola acuminata* and *Theobroma cacao* [7]. It attaches itself to the host by modified roots otherwise known as "haustorium" [8]. Ethno medicinally, its various parts (leaves, stems, berries and flowers) are majorly used in herbal medicine for the treatment of headache, rheumatic pain, hypertension, ulcer, pulmonary problems, and cancer [8,9,10]. It has been reported for various biological activities including lipid lowering [10], antioxidant [11], antibacterial [7], laxative [12] oxytocic [13] hypoglycaemic [14], anticonvulsant property [15], anti-inflammatory and analgesic activities [16]. Two lactones namely 6-[2-hydroxy-4-(4-hydroxyphenyl)butyl]-5,6-dihydropyran-2-one and 2-[(4-hydroxyphenyl)ethyl-2,6-dioxabicyclo-1]-nonan-3-one and five flavonoids namely, quercetin, catechin, qercitrin, rutin and avicularin have been isolated from its leaf [17]. This study was carried out to scientifically justify the antidiabetic ethnomedical usage of the leaf of *Globimetula braunii* in Nigeria.

2. Methods

2.1. Chemicals, equipment and instrumentation

Finetest™ Glucometer (model IGM-0005A) with Finetest™ strips, Infopia Co.Ltd., Korea), column chromatographic (dimension: 80 × 4 cm) apparatuses were used. Others were aluminium plates, thin-layer chromatographic, silica gel (60 F_{254}, 0.25 mm) and silica gel (80-400 mesh, Merck & Co., Inc., U.S.A.). Nuclear magnetic resonance (NMR) spectra (300 MHz) were obtained with Bruker AMX 300 spectrometer. All solvents used were of analytical grade.

2.2. Animals

Male and female healthy Wistar albino rats, average weight of 150 g and bred at 27±3° C, 65 % relative humidity, 12 h day–night and housed in different cages in the animal house, Department of Pharmacology, Faculty of Pharmacy, O.A.U., Ile-Ife, Nigeria, were used for the study. They were fed on a standard pellet diet from 'Bendel Feeds', Benin, Nigeria, with water given as required. Groups of five rats were fasted for 24 h before administration of either glucose, extract, fractions, drugs or vehicle. All animal experiments conformed to the *Guide for the Care and Use of Laboratory Animals* published by the National Academies Press [18].

2.3. Plant material and Extraction

The leaf of *Globimetula braunii* was collected on *Leucena leucocephala* (Fabaceae) at Obafemi Awolowo University, Ile-Ife, Osun State and authenticated at the Ife Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, with the Voucher number IFE 17229. It was then air-dried and milled into powder. A 1.5 kg of the leaf was extracted with 7.5 Litres of 80 % ethanol by maceration at room temperature. It was then filtered and concentrated using rotary evaporator to afford a yield of 105 g of ethanol extract.

2.4. Partitioning of the extract

The ethanol leaf extract (100 g), coded, **A** was suspended in 200 mL of distilled water and successively partitioned with dichloromethane (500 mL x 3), ethylacetate (500 mL x 5) and *n*-butanol (200 mL x 2). Each partitioned fraction was concentrated to dryness at 50°C using rotary evaporator to give, dichloromethane (**B₁**, 23.7 g), ethylacetate (**B₂**, 17.4 g), *n*-butanol (**B₃**, 24.0 g) and aqueous (**B4**, 26.5 g) partition fractions.

2.5. Acute toxicity study of the extract

The biological safety of *Globimetula braunii* was evaluated by determining the LD_{50} of its ethanol extract, using the 1983 Lorke's method [19]. Animals weighing between 120-150 g were fasted overnight before doses of 10 – 5,000 mg/kg of the extract were administered orally. The study was carried out in two phases. Phase 1 of the study involved nine (9) rats that were divided into 3 groups of 3 rats each and were given the extract, with each group receiving 10, 100 and 1000 mg/kg, respectively. These animals were observed for mortality and or toxicity signs within each group over a 24-hour period. Due to the results obtained from phase 1, phase 2 study was carried out, using eight (8) rats that were divided into 4 groups of 2 rats each, which were given 1000, 1600, 2900 and 5000 mg/kg of the extract, respectively. The animals were also observed for mortality and/or toxicity signs for 24 hours. The LD_{50} was calculated as the geometric mean of the dose that resulted in100 % lethality and that which caused no lethality at all [19].

$$LD_{50} = \sqrt{D_0 \times D_1}$$

Where, D_0 = highest dose that gave no mortality: D_1 = lowest dose that produced mortality.

2.6. Antihyperglycaemic assay of the extract fractions

The antihyperglycaemic assay of the extract, **A** and its partition fractions, **B₁–B₄** were carried out in glucose-induced hyperglycaemic normal rats as described by Adebajo, Ayoola and their co-workers [20-23].

2.7. Isolation of Compounds from the Active DCM Fraction

A 20.0 g of active dichloromethane partition fraction **(B₁)** was adsorbed on silica gel and subjected to column chromatography using gradient solvent systems of increasing polarity comprising of *n*-Hex, DCM, EtOAc and MeOH. A total of four bulked column fractions based on TLC analysis were obtained and coded, **C₁**– **C₄**. Column fractions **C₁** and **C₂** formed solid deposits within 12 hrs of standing. Therefore, **C₁** was washed with 20 % DCM in MeOH, which yielded a whitish amorphous powder, coded, **G₁** (30 mg). Also, **C₂** was washed with DCM (100 %) which yielded an ash amorphous powder, coded, **G₂** (746 mg).

2.8. Antihyperglycaemic Assay of Isolated Compounds

The two isolated compounds, **G₁** and **G₂** were tested for anti-hyperglycaemic assay as described earlier.

2.9. Statistical analysis

The data obtained from the study were expressed as the mean ± SEM for the number (n=5) of animals in the groups. They were analysed with One Way Analysis of Variance (ANOVA), followed by Bonferroni t-test or Student-Newman-Keuls post hoc tests, using GraphPad® Instat, version 5.0 (GraphPad Software Inc., San Diego, USA). $P < 0.05$ was considered significant.

2.10. Structure Elucidation of Isolated Compounds

G1: m.p 151-152 ^{0}C; **ESI-MS (rel. int. %):** [M]$^+$ at *m/z* 424.9 (9.5 %) consistent with the molecular formula $C_{30}H_{48}O$, [M – O$^-$] $^+$ at *m/z* 410.0 (43.6 %), [M – $C_{23}H_{29}$]$^+$ at *m/z* 105.3 (10.0 %); **UV** (CHCl₃) λ_{max}: 270.50 nm; **IR** (KBr) υ_{max}cm^{-1}: 2948.3 (Sp₃ C – H), 2836.5 (Sp₃ C – H), 1681.0 (C=O of ketone), 1325.1 – 1121.9 (C – O, overtone); **¹H-NMR** (300 MHz, CDCl₃) δ $_{ppm}$: 0.75 (3H, *s*, H-25), 0.89 (3H, *d*, *J* = 6.0 Hz, H-29), 0.98 (3H, *s*, H-26), 1.03 (3H, *d*, *J* = 3.0 Hz, H-30), 1.07 (3H, *s*, H-24), 1.20 (3H, *s*, H-23), 1.28 (3H, *s*, H-28).**¹³C-NMR** (75 MHz, CDCl₃) δ $_{ppm}$: 39.27 (C-1), 32.80 (C-2), 213.18 (C-3), 42.16 (C-4), 59.51 (C-5), 36.03 (C-6), 22.30 (C-7), 37,47 (C-8), 58.25 (C-9), 38.32 (C-10), 35.65 (C-11), 35.36 (C-12), 28.19 (C-13), 30.02 (C-14), 30.52 (C-15), 32.46 (C-16), 39.72 (C-17), 53.12 (C-18), 42.82 (C-19), 35.04 (C-20), 41.32 (C-21), 41.54 (C-22), 31.80 (C-23), 20.27 (C-24), 14.68 (C-25), 17.96 (C-26), 18.26 (C-27), 32.11 (C-28), 6.84 (C-29), 18.68 (C-30).
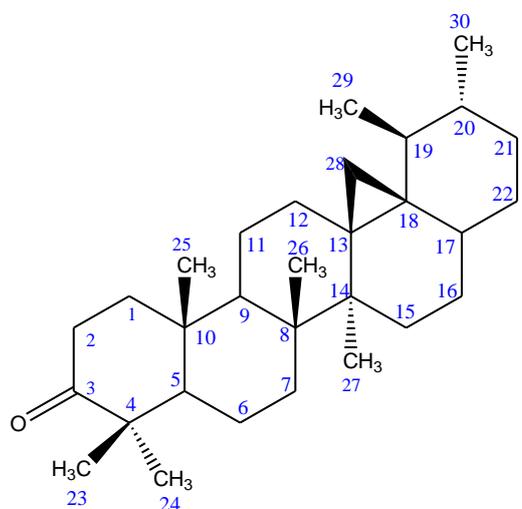
Chemical structure of (G1)
13,28-cycloursan-3-one
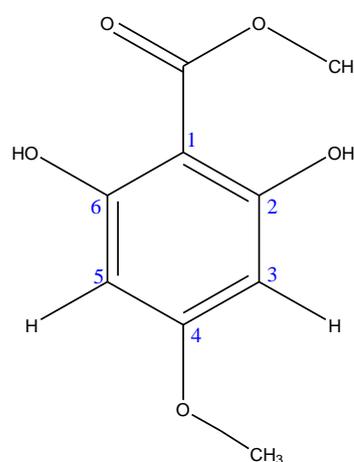
Chemical structure of (G2)
methyl 2,6-dihydroxy-4-methoxybenzoate

G2: m.p 160-161 °C; **ESI-MS** (rel. int. %): [M + 3] $^+$ at (50.8 %) *m/z* 198.0 consistent with the molecular formula $C_9H_{10}O_5$,loss of –OCH₃ [M – 31] $^+$ at *m/z* 167.3, [M-34] $^+$ at *m/z* 154.3 (13.5 %), loss of methyl ethanoate [M – 60] $^+$ at *m/z* 135.2 (3.5 %), loss of both –OCH₃ and methyl ethanoate substituents [$C_3H_6O_3$] $^+$ at *m/z* 107.1 [M – 91]$^+$; **UV-Vis (MeOH) λ$_{max}$:**

210 nm, 232 nm, 253.0 nm; **1H-NMR**: (300 MHz, MeOD) δ ppm: 3.85 (3H, s, H-1$_a$), 3.91 (3H, d, J = 3.0 Hz, H-4$_a$), 4.89 (1H, s, H-2, H-6), 7.36 (1H, s, H-3, H-5). **13C-NMR**: (75 MHz, MeOD) δ ppm: 55.26 (C-4$_a$), 59.72 (C-1$_b$), 106.80 (C-3, C-5), 125.72 (C-1), 142.41 (C-4), 152.90 (C-2, C-6), 168.05 (C-1$_a$).

3. Results and discussion

3.1. Safety profile of *G. braunii*

Acute toxicity studies that were carried out through oral administration of the ethanol leaf extract of *G. braunii* at 10, 100, 1,000, 1,600, 2,900 and 5,000 mg/kg did not produce death in the animals used. Also, there were no significant changes in their behaviours such as breathing, cutaneous effect, sensory and nervous system responses or on gastrointestinal effects. This showed that the median lethal dose (LD$_{50}$) of the ethanol leaf extract of *G. braunii* is above 5,000 mg/kg and that the chosen doses of 100, 200 and 400 mg/kg used in the evaluation of the antihyperglycaemic activity of the extract were therapeutically safe.

3.2. Antihyperglycaemic effects of the extract

In glucose-induced antihyperglycaemic experiments of medicinal plants or orthodox drugs involving the use of glibenclamide and other insulinotropic drugs as positive controls, it has been established that results obtained from such experiments can be extrapolated on the Type 2 diabetic state in humans [20, 21, 24]. Furthermore, the use of glibenclamide in antidiabetic experiments as the standard drug [25] could be used to determine the early extra-pancreatic and late insulin stimulating effects in terms of the mechanisms of action of the extract being investigated [22, 23, 26].

Table 1 Antihyperglycaemic effects of *Globimetula braunii* extract

Dose of extract/Drug (mg/kg)	Blood glucose level as percentage of T$_0$ (reduction in blood glucose relative to negative control at T$_t$)				
	0 h	0.5 h	1 h	2 h	4 h
GLU (10g/kg)	100.0	83.79 ± 3.8[b]	85.89 ± 0.50[b]	76.45 ± 1.71 [b]	74.18 ± 1.97[c]
A (100)	100.0	60.84 ± 4.80[a] 27.39%	56.59 ± 5.24[a] 34.11%	47.70 ±3.26[a] 37.61%	40.24 ± 5.11[a] 45.75%
A (200)	100.0	83.06 ± 4.82[b] 0.87%	75.52 ± 5.16[b] 12.07%	70.35 ± 5.59 [b] 7.98%	63.83 ± 6.28[b,c] 13.95%
A (400)	100.0	93.01 ± 3.77[b] -11.0%	87.62 ± 3.42[b] -2.01%	68.06 ± 3.04[b] 10.97%	63.44 ± 1.89[b,c] 14.47%
GLI (5)	100	75.60 ± 6.70[a,b] 9.77%	70.70 ± 6.90[a,b] 17.69%	58.30 ± 6.40[a,b] 23.74%	45.30 ± 6.90[a,b] 38.93%

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T$_0$),n = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$). **GLU**: Glucose in < 1 % of Tween 80 in normal saline administered at 10 g/kg (hyperglycaemic negative control); **A**: *Globimetula braunii* extract; **GLI**: Glibenclamide (5 mg/kg, positive control).

A significant ($p < 0.05$) time-dependent hyperglycaemia-lowering effects up to the fourth hour was observed in the negative control group of rats that were given normal saline. This activity could be attributed to the normal homeostatic regulatory mechanism in normal animals which confirmed the healthy state of the pancreases of the animals used in the study [20-23]. Also, the positive control group of rats that were administered with glibenclamide(5 mg/kg) gave a significant time-dependent antihyperglycaemic activity up to fourth hour (Table 1). This observation confirmed the early minor extra pancreatic and late major insulin releasing (insulinotropic) mechanisms of action of glibenclamide [25]. The extract of *G. braunii* at 100 mg/kg elicited a time-dependent glucose lowering activity up to the fourth hour

that was significantly higher ($p<0.05$) than the 200 and 400 mg/kg doses indicating a non-dose dependent activity of the extract (Table 1). Also, the extract at this dose gave similar profile ofactivity at 0.5-4 h to glibenclamide (5 mg/kg) which suggested that the extract had similar early extra pancreatic and late major insulin stimulating mechanisms ofaction with glibenclamide at this dose [25]. The high blood glucose level reductions of 27, 34 and 38 % elicited by 100 mg/kg of the extract at 0.5-2 h further suggested additional extrapancreatic effect of the extract at this dose (Table 1). The ethanol leaf extract of *G. braunii* had previously been reported to show hypoglycaemic effect in alloxan-induced diabetic rats at 250 and 500 mg/kg after 2 and 4 h [27]. This result further supported insulin stimulation as the main mechanism of action of *G. braunii* leaf extract that was suggested by the results of this present work (Table 1). The extracts of *Carica papaya, Xylopia aethiopica, Parquetina nigrescens, Chrysophylum albidum, Senecio biafrae* have similarly been suggested to work through insulin stimulation [28-32].

3.3. Antihyperglycaemic Activity of Partition Fractions of *G. braunii* Extract

Table 2 Antihyperglycaemic effects of partition fractions (100 mg/kg) of *Globimetula braunii* ethanol extract

Doses of extract/Drug(mg/kg)	Blood glucose level as percentage of T_0 (reduction in blood glucose relative to negative control at T_t)				
	0 h	0.5 h	1 h	2 h	4 h
GLU(10 g/kg)	100.0	83.79 ± 3.8^b	85.89 ± 0.50^b	76.45 ± 1.71^b	74.18 ± 1.97^c
A (100)	100.0	60.84 ± 4.80^a 27.39%	56.59 ± 5.24^a 34.11%	47.70 ± 3.26^a 37.61%	40.24 ± 5.11^a 45.75%
B_1(100)	100.0	$79.04 \pm 3.59^{a,b}$ 5.67%	$68.30 \pm 5.85^{a,b}$ 20.48%	$59.87 \pm 4.45^{a,b}$ 21.69%	$44.79 \pm 3.56^{a,b}$ 39.62%
B_2 (100)	100.0	83.76 ± 6.24^b 0.04%	83.55 ± 6.23^b 2.73%	82.01 ± 6.27^b 7.27%	$51.51 \pm 4.7^{a,b}$ 30.56%
B_3 (100)	100.0	86.23 ± 10.62^b -2.91%	95.34 ± 14.48^b -11.00%	79.58 ± 8.09^b -4.09%	$65.78 \pm 7.54^{b,c}$ 11.32%
B_4 (100)	100.0	$69.19 \pm 3.86^{a,b}$ 17.43%	$65.29 \pm 4.72^{a,b}$ 23.98%	57.11 ± 4.40^a 25.3%	40.55 ± 4.19^a 45.34%
GLI (5)	100.0	$75.60 \pm 6.70^{a,b}$ 9.77%	70.70 ± 6.90^b 17.69%	$58.30 \pm 6.40^{a,b}$ 23.74%	$45.30 \pm 6.90^{a,b}$ 38.93%

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T_0),*n* = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$).**GLU**: Glucose in < 1 % of Tween 80 in normal saline administered at 10 g/kg (hyperglycaemic negative control); A: *Globimetula braunii* extract; **B_1=** dichloromethane fraction; **B_2**= Ethylacetate fraction; **B_3=** Butanol fraction; **B_4=** Aqueous fraction**; GLI**: Glibenclamide (5 mg/kg, positive control).

Based on the results of the antihyperglycaemic effects of *G. braunii* ethanol leaf extract (Table 1), the active extract was subjected to solvent partitioning and the resulting partitioned fractions of the extract were tested for antihyperglycaemic activity at the most effective dose of the extract (100 mg/kg). Ethylacetate **(B_2)** and Butanol **(B_3)** fractions were devoid of antihyperglycaemic activity at 0.5- 2 h but gave 31 and 11 % blood glucose levels reduction at 4 h, respectively which indicated that they lacked extrapancreatic activity but had insulin stimulating effect with **B_2** showing higher activity (Table 2). Fractions **B_1** and **B_4** gave comparable ($p>0.05$) and time-dependent activity at all-time points that were similar in profile to glibenclamide which suggested early extrapancreatic and late insulin stimulation mechanism of action [25]. It also indicated that the antihyperglycaemic constituents of the extract were more concentrated in **B_1** and **B_4** which were non polar and very polar in nature, respectively. Furthermore, none of the partitioned fractions, **B_1-B_4** was significantly more active than the extract indicating that the antihyperglycaemic constituents of **A** were working in synergism (Table 2).

3.4. Antihyperglycaemic activity of the bulked column fractions of *G. braunii*

Table 3 Antihyperglycaemic effects of bulked column C_1-C_4 fractions (100 mg/kg) of *Globimetula braunii* extract

Dose of extract/Drug (mg/kg)	Blood glucose level as percentage of T_0 (reduction in blood glucose relative to negative control at T_t)				
	0 h	0.5 h	1 h	2 h	4 h
GLU (10g/kg)	100.0	83.79±3.8[b]	85.89±0.50[b]	76.45±1.71[c]	74.18±1.97[b]
A(100)	100.0	60.84±4.80[a] 27.39%	56.59±5.24[a] 34.11%	47.70±3.26[a] 37.61%	40.24±5.11[a] 45.75%
B_1(100)	100.0	69.19±3.86[a,b] 17.43%	65.29±4.72[a,b] 23.98%	57.11±4.40[a,b] 25.3%	40.55±4.19[a] 45.34%
C_1(100)	100.0	74.03±6.72[a,b] 11.65%	56.67±6.69[a] 34.02%	54.82±6.11[a,b] 28.29%	41.18±5.51[a] 44.49%
C_2(100)	100.0	81.34±7.06[b] 2.92%	60.21±4.34[a] 29.90%	54.40±7.75[a,b] 28.84%	37.83±2.72[a] 49.00%
C_3(100)	100.0	83.35±6.47[b] 0.53%	69.86±8.85[a,b] 18.66%	61.96±4.06[b] 18.95%	49.26±6.72[a] 33.59%
C_4(100)	100.0	82.89±3.26[b] 1.07%	72.59±7.84[a,b] 15.48%	56.44±8.51[a,b] 26.17%	44.51±4.71[a] 39.99%
GLI (5)	100.0	75.60±6.70[a,b] 9.77%	70.70±6.90[a,b] 17.69%	58.30±6.40[a,b] 23.74%	45.30±6.90[a] 38.93%

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T_0), *n* = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$). **GLU** (10 g/kg): Glucose in < 1 % of Tween 80 in normal saline administered at 10 g/kg (hyperglycaemic negative control); **A**: *Globimetula braunii* extract; **B_1**: Dichloromethane fraction of *Globimetula braunii* extract; **C_1-C_4:** bulked column fractions of dichloromethane fraction of *Globimetula braunii* extract; **GLI** (5 mg/kg): Glibenclamide (5 mg/kg, positive control).

Active **B_1** was subjected to column chromatography as described above and it afforded four bulked column fractions, **C_1- C_4** that were thereafter tested for antihyperglycaemic activity at 100 mg/kg (the most effective dose of the extract). The findings showed that **C_1- C_4** elicited comparable (p>0.05) blood glucose level reduction effect with glibenclamide at 4 h suggesting insulin release as their major mechanism of action (Table 3). While **C_1** and **C_2** gave a non time-dependent antihyperglycaemic effect, the activity of **C_3** and **C_4** were time dependent. The additional extrapancreatic effect that was observed in the extract was found in **C_1** and **C_2**. This showed that chromatographic purification of the extract in this work has successfully separated the extrapancreatic constituents. Furthermore; **C_1- C_4** gave comparable effect both to **B_1** and the **A** at 4 h which showed synergism in the antihyperglycaemic constituents of the bulked column fractions (Table 3).

3.5. Antihyperglycaemic effects of the isolated compounds from *G. braunii*

Isolated compound **G_1,** phyllanthone demonstrated a time-dependent and comparable antihyperglycaemic activity at 10 and 20 mg/kg suggesting insulin release as its major mechanism of action and that its activity was not dose dependent (Fig.1). However, the activity of **G_2,** methyl 2, 6-dihydroxy-4-methoxybenzoate was not time-dependent while the 10 mg/kg dose gave a significantly better blood glucose lowering effect than the 20 mg/kg at 4 h. Both phyllanthone, and methyl 2, 6-dihydroxy-4-methoxybenzoate demonstrated comparable and similar profile of antihyperglycaemic effects to glibenclamide which suggested minor extrapancreatic and major insulin stimulating mechanism of action for the compounds [21-24]. These results thereforeidentified phyllanthone, and methyl 2,6-dihydroxy-4-methoxybenzoate as two of the antihyperglycaemic constituents of *Globimetula braunni*.
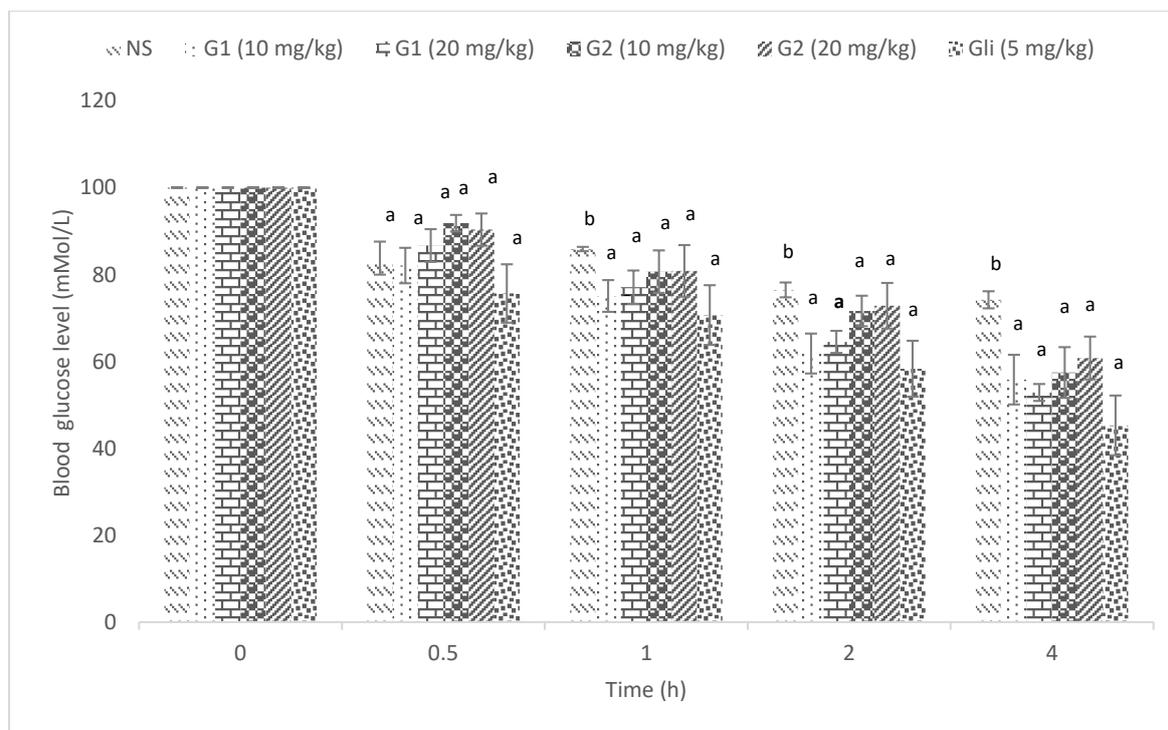
Figure 1 Antihyperglycaemic effects of the isolated compounds from *Globimetula braunii*

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T_0),*n* = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$). **NS**: Glucose in < 1 % of Tween 80 in normal saline; **G_1:** phyllanthone; **G_2**: methyl 2, 6-dihydroxy-4-methoxybenzoate; **Gli**: Glibenclamide (5 mg/kg).

The spectra data of the isolated compounds, **G_1** and **G_2** were compared with those from the literature. Compound **G_1** has been characterized as a 13, 27-cycloursan-3-one reported from the stem bark of *Phyllanthus polyanthus* as "phyllanthone" [33]. Also **G_2** was characterized as methyl 2, 6-dihydroxy-4-methoxybenzoate [34]. The two compounds were isolated for the first time from the leaf of *Globimetula braunii* and reported in this work.

4. Conclusion

Lack of death in the animals when administered with high dose of the ethanol leaf extract of *G. braunii* in acute toxicity study confirmed its safety. Also the antihyperglycaemic activity of the extract at the lowest tested dose, 100 mg/kg indicated its efficacy while phyllanthone and methyl 2, 6-dihydroxy-4-methoxybenzoate with comparable antihyperglycaemic effect to glibenclamide were two of its active constituents. These results justified the antidiabetic ethno-medicinal use of the plant.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared that there is no conflict of interest with regard to this work.

Statement of ethical approval

All animal experiments in this study conformed to the Guide for the Care and Use of Laboratory Animals published by the National Academies Press.

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