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Identification of Potential Antioxidant and Hypoglycemic Compounds in Aqueous-Methanol Fraction of Methanolic Extract of *Ocimum canum* Leaves

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Ocimum canum belongs to a genus of aromatic annual and perennial herbs and shrubs in the family of Lamiaceae. It is native to Africa and cultivated commonly for its culinary and medicinal value. In a previous study, we reported the antioxidant and hypoglycemic activity of the crude methanol extract and the solvent fractions of Ocimum canum leaves. This present study was aimed to partially isolate and identify some bioactive compounds in the aqueous-methanol solvent fraction of methanol extract of Ocimum canum leaves. The sample was fractionated *via* silica gel-column and solvent system to yield one hundred and ninety seven fractions (197) which was pooled using analytical TLC. The pooled fractions were screened for antioxidant activity (DPPH scavenging assay and Ferric Reducing Power) and ability to reduce fasting blood glucose (FBG). The most active sub fraction was analysed by LC-MS and GC-MS to identify the active compounds. A total of 197 fractions was collected and pooled to seven (7) subfractions. Sub fraction OC4 showed the best activity with IC_{50}s 48.6 ± 6.6 µg ml^{-1} and 15.2 ± 3.9 µg ml^{-1} for DPPH scavenging and Ferric Reducing Power assays, respectively, a 37% reduction in FBG and a concomitant increase in insulin level in treated diabetic rats. The LC/GC-MS analysis revealed tentatively the following compounds: Diosmetin, Dihydroquercetin, Digalloyl glucose isomer, Ananaflavoside isomer, Kaempferol glucoside, embinin isomers, eugenol, hexadecanoic acid, 9,12 octadecanoic acid, and β-sitosterol. The study corroborates the previously reported antioxidant activity and hypoglycemic potentials of the leaves of Ocimum canum and further more identified bioactive compounds that can be isolated and further characterised.

Keywords: Ocimum canum, Hypoglycemic, Antioxidant, LCMS, GCMS

INTRODUCTION

Diabetes mellitus is a major public health problem in the developing as well as the developed world. It is possibly the world's largest growing metabolic disorder [1,2,3]. It is ranked seventh among the leading causes of death and third when all fatal complications are considered [4]. A more recent estimates, according to IDF Diabetes Atlas, reported that in 2017 there were 451 million (age 18-99 years) people with diabetes worldwide and 16 million of this population were from the South/Western Africa.

These figures were predicted to increase to 41 million and 693 million by 2045 in South/West Africa and globally respectively. It was also reported that almost half of all the people (49.7%) living with diabetes are undiagnosed and an estimated 374 million people are currently living with impaired glucose tolerance (IGT) [2].

The disease is a heterogeneous group of metabolic disorders of carbohydrate, fat and proteins characterized by chronic hyperglycemia and glucosuria with secondary disturbance of protein and fat metabolism [5]. It results from either the lack of or defect in insulin secreting action

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or both. Type II diabetes is the most common type found in which there is decreased plasma level and activity of insulin. It is reported that about 95% of diabetic cases are type II [6,7]. A lot of chronic complications are associated with diabetes. These include; retinopathy, neuropathy and atherosclerotic coronary vascular diseases, dyslipidemia, and peripheral atherosclerotic vascular diseases [4].

Management of diabetes has received huge research efforts in recent time. Most of the antidiabetic drugs currently available act *via* one known mechanism and may also present with some undesirable effect when used. As alternatives, research focus has turned to herbal remedy with a premise that they hold strong potential to provide a comparative advantage because of the presence or possession of diverse bioactive secondary metabolites which could exert their effect *via* different mechanisms [8,9].

Plants have been the basis of traditional medicine systems around the world for thousands of years. Even in modern medicine systems, ethnobotanical treatments continue to play an important role in health care. About 80% of the population in developing countries rely on traditional medicine for their health care [9].

In Nigeria, *Ocimum* genus spices form a significant part of the folk medicine system for management of diabetes mellitus. However, this practice still suffers from lack of scientific validation of their efficacy, dosage, safety, and mechanisms of action. Furthermore, they represent a potential major source of lead compounds for treatment of diabetes mellitus. In our previous study, we evaluated the antioxidant and antidiabetic activity of the crude methanol extract/solvent fractions of *Ocimum canum* leaves [27]. As a sequel to that, this present research is intended to further fractionate and identify the potential active compounds in the active aqueous-methanol solvent fraction of the leaves.

EXPERIMENTALS

Chemicals

All chemicals used are of analytical grade.

Preparation of Plant Extracts

The extract and solvent fractions were prepared as described by Teke *et al.* [10] with little modifications. The plant leaves were shade dried and coarsely powdered. A mass of 500 g of the ground leaves was macerated in 2.5 l of methanol for 72 h. It was then filtered and the solvent was evaporated under reduced pressure in a rotary evaporator at 50 °C to afford 32 g of methanol extract of *Ocimum canum*. The extracts were labelled and preserved for further use. A mass of 20.14 g of this extract was pre-dissolved in 100 cm^3 of methanol and then partitioned into n-hexane (350 $cm^3 \times 3$) and the upper (n-hexane) phase was collected and concentrated as n-hexane solvent fraction. The residual methanol phase was collected, concentrated and then re-dissolved in 100 cm^3 of aqueous-methanol (55:45 v/v) and partioned into ethyl acetate (350 ml × 3). The resulting upper (ethyl acetate) phase was collected and concentrated as the ethyl acetate solvent fraction while the lower (aqueous-methanol) phase was collected and concentrated as the aqueous-methanol solvent fraction. All extract and fractions were labelled and preserved for further use.

Column Fractionation of the Aqueous-methanol Solvent Fraction of Crude Extract of *Ocimum Canum Leaves*

The aqueous-methanol solvent fraction of the crude methanol extract *Ocimum canum* (8.5 g) was subjected to column chromatography to separate the fractions into its component sub fractions according to the method of Odeh *et al.,* [11]. Briefly, A gel-slurry (stationary phase) was prepared by mixing 250 g of silica gel, 60G (Merk, F254, 100-200 mesh size) in 600 ml of chloroform. The slurry was poured down carefully into a glass column with the aid of a glass funnel. The tap of the column was left open to allow free flow of solvent into a conical flask below. The set-up was seen to be in order when the solvent drained freely without letting either the silica gel or glass wool through the tap. At the end of the packing process, the tap was locked. The column was allowed 24 h to stabilize, after which, the clear solvent on top of the silica gel was allowed to drain down to the silica gel meniscus.

The wet packing method was used in preparing the silica gel column. The sample was prepared in a ceramic mortar by adsorbing 8.5 g of the dried aqueous-methanol solvent fraction to 16 g of silica gel 60G in chloroform and allowed to dry in air. The dry powder was allowed to cool

and then gently layered on top of the column. The column tap was opened to allow the eluent to flow at the rate of 40 drops per minute. Elution of the extract was done with solvent systems (mobile phase) of gradually increasing polarity using chloroform, methanol and distil water. The following solvent combinations were sequentially used in the elution process; Chloroform-100%, Chloroform: methanol- 70:30, 50:50 and 30:70; Methanol -100%, Methanol:Water -80:20, 60:40. A measured volume (200 ml) of each solvent combination was gradually added to the column system until the eluents are apparently clear, then the next solvent combination is introduced. The eluted fractions were collected in aliquots of 40 ml in beakers [11].

Analytical Thin Layer Chromatography (TLC) and Pooling of Fractions

Analytical TLC was carried out on pre-coated (silica gel F254) aluminium plates in a small chromatography tank to separate the different fractions based on their relative mobilities in solvent systems. A strip of the precoated silica gel was cut out (0.6 cm × 8.5 cm). With the aid of a 5ul-micro pipette, a spot of the sample was applied on the plate about 1.0 cm from the edge. It was allowed to dry in air. The strip was carefully lowered into a small chromatography jar containing the solvent system MeOH: $CHCl_3$ (7:3) 15% acetic acid. The jar was covered with a glass lid. The solvent was allowed to ascend until the solvent front was about ¾ of the length of the strip. The strip was removed and dried in a hot air dryer and viewed under a UV lamp at 254 nm to identify any fluorescing spot. The strip was subsequently transferred into an airtight iodine tank (55 s) to develop the spots/bands visibly. The relative Retentive factor (Rf) value was calculated based on the formula Rf = Distance travelled by the spot or band from the starting point/Distance travelled by the solvent from the starting point to the solvent front. Sub fractions with similar RF and TLC patterns were pooled together and labelled in the order they were eluted [11].

Bioactivity of the Sub Fractions of Aqueous-methanol Solvent Fraction *Ocimum Canum* Extract

Determination of *in vitro* antioxidant activity of the extract. The *in vitro* antioxidant activity of the subfractions were determined according to the methods described in Saeed *et al*. [12].

DPPH assay (2,2-diphenyl-1-picrylhydrazyl). The free radical scavenging activity of the fractions was measured *in vitro* by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) with slight modifications. Briefly, DPPH stock solution (0.1 mM) was prepared by dissolving 4 mg of DPPH in 100 ml methanol and stored at 20 °C until required. The working solution was obtained by diluting the DPPH solution with methanol to attain an absorbance of about 1.2 ± 0.09 at 517 nm using the spectrophotometer. A 3 cm^3 aliquot of this solution was mixed with 100 µl of the various concentrations (0-100 µg cm^{-3}) of the subfractions. The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. The absorbance was taken at 517 nm. The control was prepared as described above with methanol substituted for the sample. The %scavenging activity was determined by the following equation:

DPPH scavenging activity (%) = [(control absorbance-sample absorbance)/(control absorbance)] × 100

Reducing Power Assay

The reducing power was based on Fe(III) to Fe(II) reductive transformation in the presence of the test samples that can be monitored by measuring the formation of Perl's Prussian blue at 700 nm as described in [12]. Briefly, various concentrations (0-200 µg ml^{-1}) of the sub fractions (2 cm^3) were mixed with 2 cm^3 of phosphate buffer (0.2 M, pH 6.6) and 2 cm^3 of potassium ferricyanide (10 mg cm^{-3}). The mixture was incubated at 50 °C for 20 min followed by addition of 2 cm^3 of trichloroacetic acid (100 mg l^{-1}). The mixture was centrifuged at 3000 rpm for 10 min to collect the supernatant of the solution. A volume of 2 cm^3 from each of the mixture earlier mentioned was mixed with 2 cm^3 of distilled water and 0.8 cm^3 of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. The higher absorbance of the reaction mixture indicates a higher reducing power. A standard curve of Ferric sulphate was used to quantify the ferric reduction potential of the sub fractions. IC_{50} for FRAP was determined as the concentration of sub fraction that gave a reduction equivalence of 50 mM of Fe^{2+}.

Antididiabetic Activity of the Pooled Subfractions

Twenty seven healthy wistar rats weighing 120-150 g were used for this study. The wistar rats were randomly divided into ten groups of three (3) rats each. Each group was denoted by the fraction/drug administered. Experimental diabetes was induced as described by Juarez-Rojop *et al.* [13] with modifications. Following an overnight fast, a single intraperitoneal injection of 60 mg kg^{-1} streptozotocin dissolved in sterile water for injection was administered. This was followed by an oral administration of 5% glucose solution two hours after administration. The control animals received the sterile water as placebo. The animals were checked for successful induction of diabetes after 48 h. Animals with blood glucose above 300 mg/dl were classified as diabetic. The groups were randomly assigned treatment as follows:

Positive Control (NDBC): Normoglcemic rat + vehicle (DMSO 10%v/v)
Negative Control (DBC): Diabetes-induced wistar rats + vehicle (DMSO 10%v/v)
Standard Drugs (GLMD): Diabetes-induced wistar rats + glibenclimide (5 mg/kgbw)
OC1: Diabetes-induced wistar rats + subfraction OC1 (100 mg/kgbw)
OC2: Diabetes-induced wistar rats + subfraction OC2 (100 mg/kgbw)
OC3: Diabetes-induced wistar rats + subfraction OC3 (100 mg/kgbw)
OC4: Diabetes-induced wistar rats + subfraction OC4 (100mg/kgbw)
OC5: Diabetes-induced wistar rats + subfraction OC5 (100 mgkgbw)
OC6: Diabetes-induced wistar rats + subfraction OC6 (100 mg/kgbw)
OC7: Diabetes-induced wistar rats + subfraction OC7 (100 mg/kgbw)

The treatment was administered orally for 7days. Blood glucose was determined on days 1, 3, 5, and 7 using blood samples collected from tail vein and accucheck glucometer as described by Ezekwe *et al.* [14]. The wistar rats were sacrificed on the 8th day, under light anaesthesia following a 12-hour fast [14]. Blood samples were collected *via* cardiac puncture into nonheparinized tubes, centrifuged and the serum stored for determination of biochemical parameters.

Determination of Serum Insulin

Serum insulin was determined using Accu Bind ELISA Microwell Insulin Test System (product code: 2425-300) according to the manufacturer's manual.

Liquid chromatography-mass spectrometry (LC/MS) analysis of most the active sub fraction. The liquid chromatography-mass spectrometry separation was performed with Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source. The LC and MS Parameters were as follows: The mobile phase containing solvent A and B (ran in gradient), where A was 0.1% (v/v) formic acid in water and B was 0.1% (v/v) formic acid in acetonitrile for the following gradient: 5% B for 0 min, 5% B for 5 min and 5-100% B in 20 min and 100% B for 25 min at a flow rate of 0.5 ml min^{-1}. The column was Agilent Zorbax Eclipse XDB-C18 (2.1 × 150 mm × 3.5 µm) (P/N: 930990-902), column temperature 25 °C and the injection volume was 1 µl and sample concentration was 0.1 mg ml^{-1} in Chloroform:methanol (3:7). The eluent was monitored by electrospray ion mass spectrometer (ESI-MS) under negative ion mode and scanned from 200 to 1000 m/z. ESI was conducted by using a fragmentor voltage of 125 V, skimmer 65 V. High-purity nitrogen (99.999%) was used as drying gas and at a flow rate of 10 l min^{-1}, nebulizer at 45 psi and capillary temperature at 350 °C. As a blank, Chloroform:methanol (3:7) was used. Data analysis was processed with Agilent Mass Hunter Qualitative Analysis B.05.00 software and compounds were identified by comparing the retention times and mass spectra of the analytes in the sample to the data from METLIN_AM_PC & RESPECT database.

Gas chromatography-mass spectrometry (GC/MS) analysis of the most active sub fraction. The analyses were performed using a Shimadzu GC-MS QP 2010 Ultra equipped with MSD, fused silica capillary column Rtx-5MS (30 m × 0.25 mm × 0.25 µm), and an autosampler. The initial temperature programme was 90 °C, held for 2 min. The temperature was then ramped up to 260 °C at 5 °C min^{-1} and held for 5 min. The injector temperature was

250 °C. The carrier gas was helium at the flow rate of 2.17 ml min^{-1} with average velocity of 54.6 cm s^{-1}. The GC-MS was operated in splitless mode with a purge flow of 3 ml min^{-1}; the injection volume was 1 µl and the pressure was set at 150 kPa. The GC interface temperature was 300 °C. The mass spectrometer was operated in electron impact (EI) ionization at 0.2 V with ion source temperature of 230 °C and in full scan mode with the range of 45-500 m/z. The identification of the compounds were accomplished by comparing the retention times and mass spectra of analytes in samples to data from NIST 14 mass hunter spectral library (US National Institute of Standards and Technology).

RESULTS AND DISCUSSION

The result of the column fractionation of the aqueous-methanol fraction of methanol extract of *Ocimum canum* is presented in Table 1. The result showed that a total of 197 fractions were collected and pooled into seven (7) subfractions designated (OC1-OC7). The highest yield was observed in OC6 and OC7.

The antioxidant (DPPH scavenging assay (DSA) and Ferric reducing power (FRAP)) and hypoglycemic activity of the pooled subfractions are presented in Table 2 and Fig. 1. The results revealed that subfractions OC3, OC4 and OC5 exhibited a significantly ($p < 0.05$) higher radical scavenging activity as well as ferric reducing power relative to the other subfractions as depicted by their IC_{50}s values (Table 2, column 2 and 3). The best activity was observed in OC4.

The result of hypoglycemic activity of the subfractions showed that STZ-induction of diabetes significantly ($p < 0.05$) increased the fasting blood glucose (FBG) in the wistar rats but on treatment, subfraction OC4 and the standard drug, Glibenclimide significantly ($p < 0.05$) reduced the high fasting blood glucose by 37.00% and 48.48%, respectively with a concomitant, significant ($p < 0.05$) increase in serum insulin level.

Tables 3 and 4 present the proposed compounds in the most active subfraction, OC4 as determined by LC-MS and GC-MS analysis. Identification was done tentatively based on sample spectral data showing over 90% match with that of the database. The LC-MS analysis tentatively revealed the following compounds- Diosmetin, Dihydrquercetin, Digalloyl glucose isomer, Ananaflavoside isomer, Kaempferol glucoside and embinin isomers. The GC-MS, on the other hand, identified eugenol, hexadecanoic acid, 9,12-octadecanoic acid, and β-sitosterol

Plant materials are complex blend of vast array of bioactive principles of which their isolation, identification and characteristics are important in pharmacological evaluation and this requires expertise and cutting edge analytical protocols and instrumentation- Thin Layer Chromatography (TLC), High performance liquid chromatography (HPLC), Mass Spectroscopy (MS) Nuclear magnetic resonance spectroscopy and their hyphenated techniques [15]. In this present study, we used a combination of chromatographic and spectroscopic techniques to identify potential active compounds in the most active solvent fraction (aqueous-methanol) of methanol extract of *Ocimum canum* leaves. The overall result of the bioactivity screening of the sub fractions revealed a varying degrees of radical scavenging activity, reducing power and potential to control blood glucose with sub fraction, OC4 showing the best activity. The disparity in the observed activities of the sub fractions may have been due to the different classes of compounds they contain, which may either act in synergy or antagonistically. Electron transfer (ET) and hydrogen transfer (HT) pathways have been proposed for most antioxidant activity [12], thus, the findings of this study suggest that OC4 may contain compounds that have high reductive power, *i.e.* is compounds that can readily receive electrons or transfer protons fro/to oxidants respectively. Streptozotocin has been shown to cause direct irreversible damage (necrosis) to β-cells of pancreatic islets of Langerhans, resulting in the loss (of) or impaired insulin secretions and consequently, hyperglycemia. Hence, it is used as a model for studying diabetes mellitus [16]. The induction of diabetes significantly reduced ($p < 0.05$) insulin level and consequently the increased blood glucose level. OC4 just like the standard drug reversed this trend significantly ($p < 0.05$) giving a percentage reduction of 37% relative to the blood glucose level on nduction of diabetes. This is an indication that OC4 may contain compounds that may mimic the action of glibenclimide and act by protecting the beta cells from further damages *via* their antioxidative

Table 1. Subfractions Obtained from Column Fractionation of Aqueous-methanol Solvent Fraction of *Ocimum Canum*

Subfractions	Solvent combination	Fractions pooled	Rf	Yield (g)	Colour of subfraction
OC1	C (100%)	1-29	0.87	0.51	light green
OC2	C/M (7:3)	29-52	0.73, 0.71	1.13	light green
OC3	C/M (5:5)	53-78	0.50	0.94	Light greenish yellow
OC4	C/M (3:7)	79-121	0.43, 0.39	0.81	light maroon
OC5	M (100%)	122-146	0.21	0.94	Dark maroon
OC6	M/W (8:2)	147-163	0.75	2.08	Dark maroon
OC7	M/W (6:4)	164-197	0.64	2.21	dirty reddish brown

M: Methanol, C: Chloroform, W: Water, Fractions: OC1-OC7.

Table 2. Bioactivity of Subfractions of Aqueous-methanol Solvent Fraction of *Ocimum Canum*

Subfractions	DSA	FRAP	Reduction in FBG (%)	Insulin (IU/ml)
OC1	87.20 ± 5.80^{c}	27.00 ± 1.00^{b}	10.63	9.10 ± 0.97^{a}
OC2	89.40 ± 9.80^{c}	29.20 ± 2.70^{b}	9.98	9.80 ± 1.10^{a}
OC3	53.40 ± 1.90^{ab}	18.10 ± 1.60^{a}	9.80	11.79 ± 1.91^{ab}
OC4	48.60 ± 6.60^{a}	15.20 ± 3.90^{a}	66.01	14.87 ± 1.30^{b}
OC5	68.40 ± 8.10^{b}	31.30 ± 2.80^{b}	6.20	10.20 ± 1.24^{ab}
OC6	71.70 ± 4.20^{bc}	30.30 ± 1.90^{b}	3.00	8.10 ± 0.56^{a}
OC7	69.80 ± 4.30^{bc}	28.30 ± 2.80^{b}	4.71	8.87 ± 0.86^{a}
Ascorbic acid	41.80 ± 3.30^{a}	11.10 ± 1.20^{c}	-	-
GLMD	-	-	48.48	14.91 ± 1.00^{b}
NDBC	-	-	-	19.20 ± 0.94^{c}
DBC	-	-	10.63	7.6 ± 1.56^{a}

OC: *Ocimum canum*, AMOC: Aqueous-methanol solvent fraction of OC, OC1-OC7: Diabetic wistar rats administerd 100 mg/kgbw of each subfractions of AMOC, GLMD: administered with Glibenclimide (5 mg/Kgbw), DBC: Diabetic control administered 10% DMSO, NDBC: Non-diabetic control administered 10% DMSO. DSA: DPPH Scavenging assay, FRAP: Ferric reducing power assay, FBG: Fasting blood glucose, Values are mean ± SEM, n = 3. Superscripts: Bars bearing different superscripts are statistically different ($p > 0.05$).

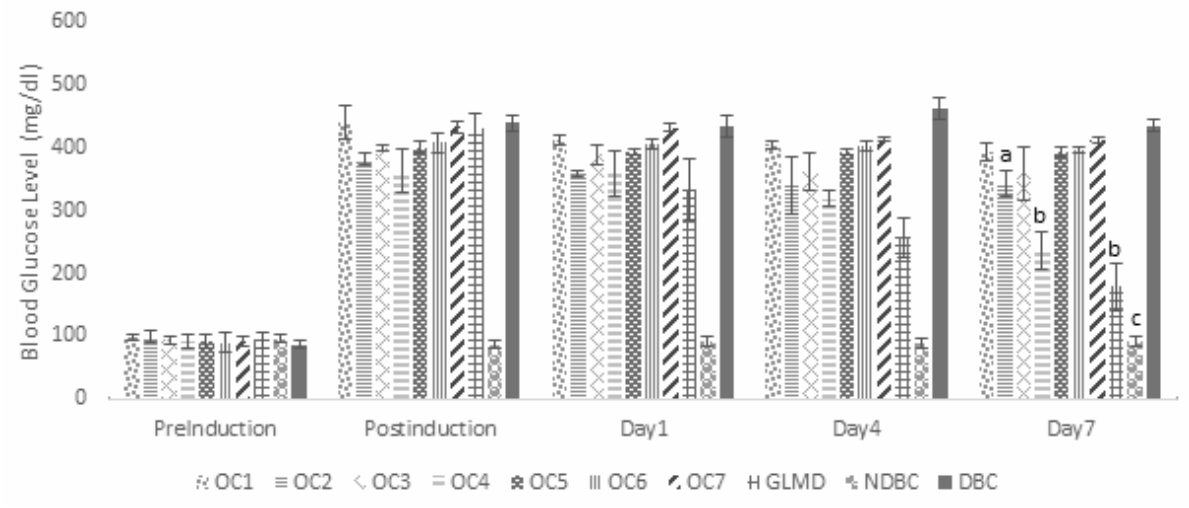

Fig. 1. Effect of the sub fractions of aqueous-methanol solvent fraction of *Ocimum canum* leaves on blood glucose level of treated diabetic wistar rats. OC: *Ocimum canum*, AMOC: Aqueous-methanol solvent fraction of OC, OC1- OC7: Diabetic wistar rats administerd 100 mg/kgbw of each subfractions of AMOC, GLMD: administered with Glibenclimide (5 mg/Kgbw), DBC: Diabetic Control administered 10% DMSO, NDBC: Non-diabetic control administered 10% DMSO. FBG: Fasting blood glucose, Values are mean ± SEM, n = 3. Superscripts: Bars bearing different superscripts are statistically different ($p > 0.05$).

potential, stimulate or potentiate the secretion of insulin from residual beta cells and possibly mimic insulin actions [17]. The study further identified tentatively the following compounds- Diosmetin, Dihydroquercetin, Digalloyl glucose, Ananaflavoside isomer, Kaempferol glucoside, Embinim isomers, eugenol, hexadecanoic acid, 9,12-octadecanoic acid, and β-sitosterol. These are majorly flavonoids/phenolics - secondary metabolites that constitute the most abundant groups of antioxidant compounds. These compounds may have been responsible for the observed activities with OC4 which could have been solely or synergistic. To the best of our knowledge, this is the first study that has identified potentially active polar compounds from methanol extract of leaves of Ocimum canum. However, previous studies have reported structurally related compounds in the other species of Ocimum genus - Ocimum basilicum L [18] and Ocimum tenuiflorum [19]. Abd El-Azim *et al*. [18] reported *p*-hydroxy benzoic acid, Ferulic acid, Gallic acid, *p*-qumaric acid, benzoic acid, kaempferol, catechin, quercetin, chlorogenic acid, caffeic acid, cinnamic acid and ellagic acid from methanol extract of Ocimum basilicum while Mousavi *et al.* [19] in a more recent study reported identifying 3,4-dimethoxycinnamic acid, caffeic acid, diosmetin, luteolin, kaempferol, rosmarinic acid, apigenin and genistein in methanol extract and fractions of Ocimum tenuiflorum leaves. Some of these compounds and their derivatives have been reported or isolated as antioxidant and hypoglycemic agents. Some previous studies reported that flavonoids, including flavanols, flavones, isoflavones and anthocyanidins have been proposed to be effective supplements for the management of diabetes and cardiovascular disease [20]. Kaempferol (flavanol) isolated from Chinese medicinal herbs according Zhang *et al.* [21] proffers cryptoprotective effect on β-cells. Apigenin and luteolin was previously isolated from an ethanol extract of Martynia annua Linn which showed a wound healing effect in STZ-induced diabetic rats [22]. Diosmetin [19], Ananaflavoside and orietin [23], Eugenols [24] and β-Sitosterol [25] have previously been reported to have both antioxidant and hypoglycemic activity. This corroborates the suggestion that this class of compounds may have been responsible for the observed activity in OC4. In conclusion, in order to further characterize the most active solvent fraction of methanol

Table 3. Compounds Proposed from the LC-MS Analysis of Subfraction OC4

R.t.	$[M+H]^+$	M.wt.	Formular	Tentative identity	Biological activity	Ref.
11.834	301.20	300.06	$C_{16}H1_2O_6$	Diosmetin	Hypoglycemic /antioxidant	[19]
14.185	593.25	592.16		unidentified		
14.313	409.25	408.09		unidentified		
15.381	305.10	304.06	$C1_5H_{12}O_7$	Dihydroquercetin	Antioxidative	[26]
16.012	485.30	484.10	$C_{20}H_{20}O_{14}$	Digalloyl glucose		
17.688	523.30	522.14	$C_{24}H_{26}O_{13}$	Ananaflavoside isomer	Hypoglycemic /antioxidant	[23],
19.648	485.35	484.10	$C_{20}H_{20}O_{14}$	Digalloylglucose isomer		
20.070	449.40	448.37	$C_{21}H2_0O_{11}$	Kaempferol glucoside	Hypoglycemic /antioxidant	[21]
20.317	607.35	606.57	$C_{29}H_{34}O_{14}$	Embinin isomers	Antioxidative	
21.037	510.40	509.17		unidentified		
22.520	454.30	453.28		unidentified		

Rt: Retention time, Mwt: Molecular weight.

Table 4. Compounds Proposed from the GC-MS Analysis of Subfraction OC4

R.t.	M.wt.	Formular	Tentative identity	Biological ativity	Ref.
18.96	164.20	$C_{10}H_{12}O_2$	Eugenol	Hypoglycemic/antioxidant	[24]
21.29	256.42	$C_{16}H_{32}O_2$	Hexadecanoic acid		
23.11	280.44	$C_{18}H_{32}O_2$	9,12-Octadecanoic acid		
24.16	414.71	$C_{29}H_{50O}$	β-Sitosterol	Hypoglycemic/antioxidant	[25]

R.t.: Retention time, M.wt.: Molecular weight.

extract of *Ocimum canum* leaves, an activity guided systematic fractionation, high pressure liquid chromatography-Mass spectroscopy and Gas chromatography-Mass analysis was carried out. The result tentatively identified mostly flavonoids, phenolics, sterols, fatty acids, and other unidentified compounds. These compounds may have been responsible for the observed activity of the solvent fraction solely or synergistically. In further study, effort should be devoted to carry out activity guided isolation of these compounds and subsequent screening (of the compounds) individually for activity. Pure promising isolates could be effectively characterized using NMR.

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