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Antioxidant capacities, antidiabetic potentials, and mineral compositions of pap aqua and aqueous extracts from *Ocimum gratissimum* L.

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ABSTRACT

Introduction: This study appraised the antioxidant potentials, mineral compositions, and antidiabetic activities of pap water (aqua) extract (PWE) and aqueous extract (AE) from *Ocimum gratissimum*.

Methods: The total phenolic contents (TPC), total flavonoid contents (TFC), ferric reducing antioxidant powers (FRAP), Fe^{2+}-chelating abilities, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging abilities, and enzymes inhibition potentials of the PWE and AE from *O. gratissimum* were evaluated. Additionally, mineral contents were determined using absorptive absorbance spectroscopy (AAS).

Results: The PWE of *O. gratissimum* exhibited higher TPC of 42.61± 0.04 mg gallic acid equivalent per gram of dried sample (GAE/g) and TFC of 85.7 ± 0.02 µg quercetin equivalent per gram of dried sample (QE/g). In contrast, AE had lower TPC (21.52 ± 0.01 mg GAE/g) and TFC (55.0 ± 0.01 µg QE/g). PWE also displayed a lower FRAP of 2.86 ± 0.01 mg AAE/g, while AE had a higher FRAP of 2.94 ± 0.03 mg AAE/g. PWE of *O. gratissimum* had IC_{50} for DPPH: 100.00 µg/mL, Fe^{2+}-chelating ability: 4.41 µg/mL, while AE had IC_{50} for DPPH: 140.00 µg/mL and Fe^{2+}-chelating ability: 4.90 µg/mL. Similarly, the PWE of *O. gratissimum* showed a higher α-amylase inhibition (IC_{50}: 0.47 mg/mL) than AE (IC_{50}: 0.78 mg/mL); however, AE (IC_{50} =3.09 µg/mL) demonstrated a higher α-glucosidase inhibition than PWE (IC_{50}: 9.09 µg/mL). AAS analyses indicated the presence of Ca, Fe, Mg, Cu, Zn, and Mn in different proportions in both extracts.

Conclusion: Therefore, PWE could be a better alternative in the management of diabetes melitus if properly annexed.

Implication for health policy/practice/research/medical education:
Pap water and aqueous extracts of *Ocimum gratissimum* demonstrate significant antioxidant and antidiabetic potentials, as well as mineral compositions, which might be useful as clinical therapy in hyperglycemia.

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Introduction

Diabetes mellitus (DM) is a multifaceted cellular impairment that prompts an increased serum glucose values initiated by abnormal insulin production or function (1). There are two main categories of DM: type I diabetes (insulin-dependent DM, IDDM), which is an autoimmune disease accounting for 10-15% of cases, and type II diabetes (non-insulin dependent DM, NIDDM), which is influenced by environmental and lifestyle factors and accounts for 90% of the global diabetic population (2).

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Numerous experimental findings have provided evidence for the significance of reactive oxygen species (ROS) participation in the pathophysiology of DM, particularly in the development of complications associated with the condition (3). The production of ROS has been observed in β-cell dysfunction and death in both types of DM. Moreover, several studies have demonstrated that persistent hyperglycemia in the diabetic state leads to the continuous production of superoxide, resulting in redox imbalance (4). This redox imbalance occurs during the higher production of free radicals than the endogenous antioxidant capacity to neutralize them (5). In DM, oxidative stress intensifies due to various factors, including glucose autoxidation, which is a primary contributor to free radical production, cellular redox imbalances, and a low level of cellular antioxidant systems (5). The alteration in antioxidant enzyme level causes the tissues to be vulnerable to the activities of ROS and ultimately trigger DM and associated problems.

Furthermore, postprandial hyperglycemia has been considered a significant risk factor for acute and chronic complications in DM, and so managing postprandial plasma glucose level is crucial in the diagnosis and clinical treatment of DM (6). Targeting postprandial hyperglycemia has been reported helpful with conventional diabetes therapy. Recent studies have identified the inhibition of α-amylase and α-glucosidase as an efficient approach for the management of hyperglycemia in NIDDM. According to several studies, pancreatic α-amylase has been found to hydrolyze α (1→4) glycosidic linkages of amylose in a random manner, resulting in the production of dextrin, maltose, or maltotriose, which contain a non-reducing terminal (7). This enzymatic process follows a double displacement mechanism while retaining the anomeric configuration. However, α-glucosidase, the enzyme found in small intestine whose activities liberate a single α-glucose entity (8). Inhibition of these enzymes activities causes a declined in the hydrolysis of starch, which is an underlying mechanism of most drugs used in managing DM (9). Starch blockers and inhibitors such as acarbose, miglitol, voglibose, etc, are currently available for the therapeutic management of DM (9).

The prevalence of macro- and micro-nutrient deficiencies poses significant public health challenges in numerous developing nations, putting both children and adults at risk. Extensive research has focused on examining the role of macro- and micro-nutrients in the pathophysiology and progression of DM (10). Studies have substantiated that lifestyle interventions, such as dietary modifications, can effectively decrease the likelihood of progressing from impaired glucose tolerance to fully manifested DM. Micro-nutrients are essential nutrients that are required in trace amounts by the body on daily basis for normal metabolic activities (11). Metals play a role in the body's physiology. Some trace elements have been reported to mediate insulin actions (12). Also, some of these elements play crucial beneficial activities aiding cytoprotection against oxidative damage (12).

Recently, considerations have emerged towards replacing synthetic drugs in the management of DM with natural antioxidants from plants (13). Data from scientific reports show that plants are richly endowed with varieties of secondary metabolites that possess the ability to reduce the generation of ROS or scavenge free radicals (14).

Natural antioxidants are present in various parts of higher plants, including wood, bark, stems, pods, leaves, fruits, and seeds (15). *Ocimum gratissimum* is an herbaceous plant belonging to the *Labiatae* family (16). It is native to tropical regions, particularly India and West Africa (17), and can be found in Nigeria's savannah and coastal areas. Traditional medicine has utilized *O. gratissimum* for the treatment of various ailments (18). Numerous phytochemicals found in *O. gratissimum* (Figure 1) have been associated with diverse biological activities (19,20). Previous studies have primarily focused on the solvent extraction of phytonutrient components from crude plant extracts, neglecting the examination of pap water extraction. However, the water layer of pap, a local Nigerian dish made from red grain sorghum, is often overlooked as insignificant. Thus, our report compared the antioxidative effect, antidiabetic capacity (via α-amylase and α-glucosidase inhibitory activities), and mineral compositions of two different extracts of *O. gratissimum*: pap water extract (PWE) and the commonly used aqueous extract (AE) known for its efficacy.

Materials and Methods

Chemicals used

The chemicals and reagents such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), p-nitrophenyl-α-D-glucopyranose (NPG), ascorbic acid (AA), $FeSO_4$, and $AlCl_3$ used in this study were obtained from Sigma-Aldrich, Inc. (Saint Louis, MO, USA). All other chemicals utilized were of analytical grade and prepared using sterilized distilled water in an all-glass apparatus.

Collection and preparation of sample

Sample collection

Fresh samples of *O. gratissimum* leaves were collected from a farmland located in Ado-Ekiti, Nigeria. The plant material was authenticated at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria, and a specimen was deposited there with the herbarium number UHAE 15. The *O. gratissimum* leaves were then dried and ground into powdery form using a laboratory blender.

Preparation of pap water leaves extract of O. gratissimum

Red grain sorghum was obtained from a vendor at Oja Oba in Ado-Ekiti, Ekiti State, Nigeria. The sample was thoroughly washed and subsequently soaked in distilled

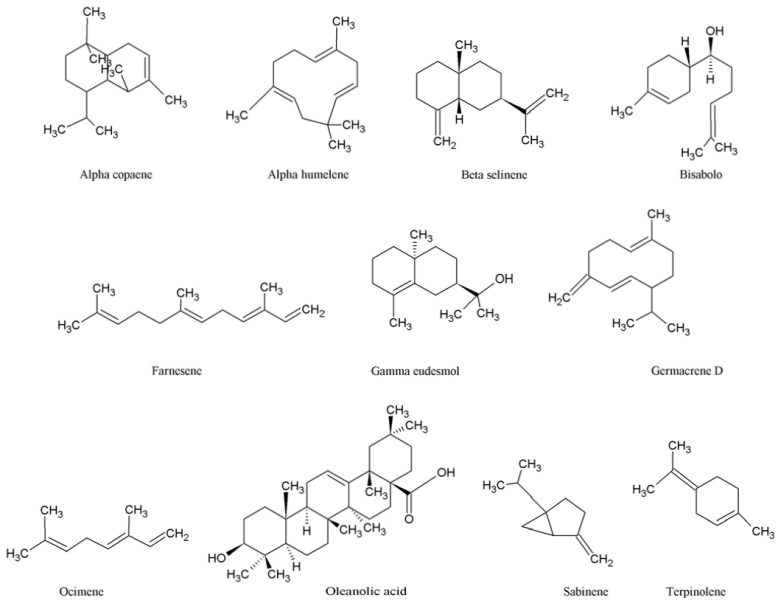

Figure 1. Structures of some biologically active compounds identified in *Ocimum gratissimum* (19,20).

water at 25 °C for 24 hours. Afterward, the sample was filtered and pulverized using an automated blender while intermittently adding 200 mL of distilled water. The resulting solution was allowed to settle, and the water level was collected. This water layer was then utilized to soak the powdered sample of *O. gratissimum* leaves at a ratio of 1:10 (w/v) at 25 °C for 24 hours. Subsequently, the mixture was filtered to obtain the pap water extract of *O. gratissimum*, which was stored in the refrigerator at 8 °C until further use (12).

Preparation of aqueous leaves extract of O. gratissimum

The pulverized leaves specimen was soaked in distilled water (1:10; w/v) at 25 °C for 24 hours. Thereafter, the mixture was filtered and kept in refrigerator (8 °C) for the different bioassays (21).

Quantification for total phenolic content (TPC)

The TPC of PWE and AE from *O. gratissimum* were quantified using the method described by Singleton et al (22). Gallic acid (GA) was used as the standard, and the TPC value was measured in milligram of GA equivalents (E) per gram of dried sample.

Quantification for total flavonoid content (TFC)

The TFC of PWE and AE from *O. gratissimum* were quantified using a spectrophotometric method of Bao et al (23). Quercetin was used as the standard, and the TFC value was measured in milligrams of quercetin equivalent (QE) per gram of dried sample.

Determination of antioxidant activity

Ferric-reducing power (FRAP) assay

The FRAP of PWE and AE from *O. gratissimum* were determined as described by Pulido et al (24). AA was used as a standard, and the reducing power was measured in milligrams of AA equivalent per gram of dried sample.

DPPH radical scavenging ability assay

The DPPH free radical scavenging ability of the PWE and AE from *O. gratissimum* were assessed following the method described by Gyamfi et al (25). The inhibitory activity was expressed as the % inhibition against the control.

Fe^{2+}-chelating ability assay

The chelating ability of the PWE and AE from *O. gratissimum* to Fe^{2+} ions was determined using the method described by Puntel et al (26). The inhibitory activity was expressed as the % inhibition against the control.

In vitro carbohydrate-hydrolyzing enzymes inhibitory assays

α-Amylase inhibitory activity assay

The inhibitory activity of the PWE and AE from *O. gratissimum* against α-amylase was determined spectrophotometrically using a modified version of the method developed by Shai et al (27). The inhibitory activity was expressed as the % of inhibition against the control.

α-Glucosidase inhibitory activity assay

The inhibitory activity of the PWE and AE from *O. gratissimum* against α-glucosidase was assessed using a modified version of the method developed by Ademiluyi and Oboh (28). The inhibitory activity was expressed as the % of inhibition against the control.

Calculation of IC_{50} values

The IC_{50} (mg/mL) of *in vitro* assays were calculated by plotting the curve of the percentage inhibitions against various concentrations of PWE and AE of *O. gratissimum*. The regression curve was used to calculate the concentration at which 50% inhibition occurred.

Mineral composition analyses

Mineral compositions of the PWE and AE of *O. gratissimum* were carried out using absorptive absorbance spectroscopy (AAS) technique according to the methods used by Akintayo (29).

Data analyses

The collected data were statistically analyzed with one-way analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS Inc., USA). Post hoc comparisons were conducted using the Duncan multiple range test whenever necessary. The levels of significance were measured at a *P* value of less than 0.05. Graphical representations of the results were generated using the GraphPad Prism 8.5 software (GraphPad Software, USA).

Results

The DPPH-free radical inhibitory activities of PWE and AE of *O. gratissimum* are presented in Figure 2. PWE (IC_{50} = 100.00 µg/mL) demonstrated a higher inhibitory activity ($P<0.05$) against DPPH-generated free radical compared to AE (IC_{50} = 140.00 µg/mL), in a concentration-dependent manner at different concentrations (0.00-0.30 mg/mL) that were considered.

The Fe^{2+}-chelating power of PWE and AE of *O. gratissimum* are represented in Figure 3. The PWE of *O. gratissimum* (IC_{50} = 4.41 µg/mL) had a higher chelating ability against Fe^{2+} ($P<0.05$) compared to AE of *O. gratissimum* (IC_{50} = 4.90 µg/mL) in some of the concentrations (0.00-8.30 mg/mL) that were considered. Figure 4 represents the antioxidant potentials of PWE and AE of *O. gratissimum*. PWE of *O. gratissimum* had a higher TFC (85.7± 0.02 µg QE/g) and TPC (42.61± 0.04 mg GAE/g) ($P<0.05$) compared to AE of *O. gratissimum* with TFC: 55.0±0.01 µg QE/g and TPC: 21.52± 0.01 mg GAE/g, respectively. However, the AE of *O. gratissimum* demonstrated a slightly higher FRAP (2.94±0.03

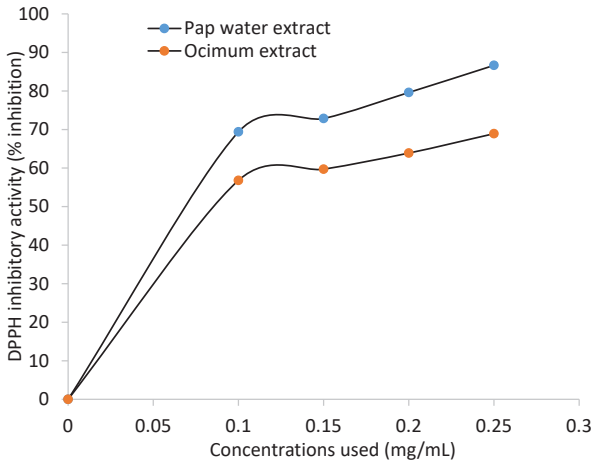

Figure 2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH)-free radical scavenging activities of pap water extract (PWE) and aqueous extract (AE) of *Ocimum gratissimum*. Results represent mean ± SD of three trials (n= 3). PWE revealed a significant ($P<0.05$) inhibitory activity against DPPH-free radical compared to AE of *O. gratissimum* in a concentration-dependent manner.

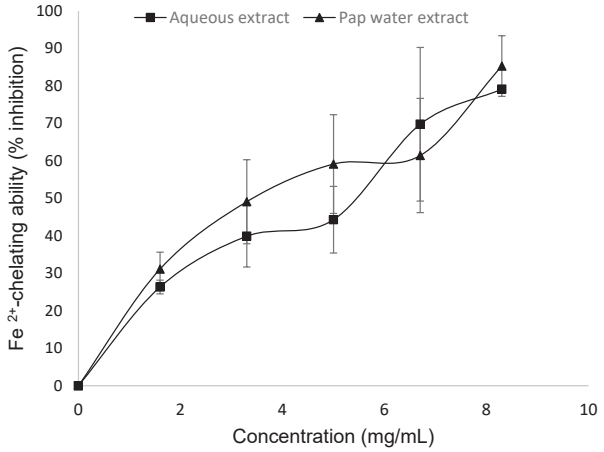

Figure 3. Fe^{2+}-chelating activities of pap water extract (PWE) and aqueous extract (AE) of *Ocimum gratissimum*. Results represent mean± SD of three trials (n= 3). PWE revealed a significant ($P<0.05$) Fe^{2+}-chelating activity compared to AE of O. *gratissimum* in some of the concentrations considered.

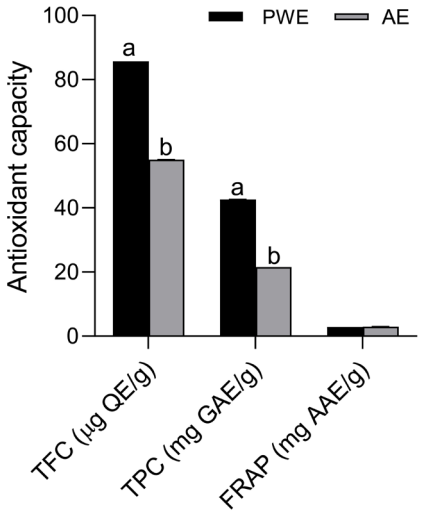

Figure 4. Antioxidant potentials of pap water extract (PWE) and aqueous extract (AE) of *Ocimum gratissimum*. Results represent mean ± SD of three trials (n = 3). a&b indicate significant difference at $P<0.05$ when compared within each parameter in a grouped bar. TFC: total flavonoid content; TPC: total phenolic content; FRAP: ferric reducing ability property; QE/g: quercetin equivalent per gram; GAE/g: gallic acid equivalent per gram; AAE/g: ascorbic acid equivalent per gram.

mg AAE/g) compared to the PWE of *O. gratissimum* (2.86 ± 0.01 mg AAE/g) ($P < 0.05$).

Also, the α-amylase inhibitory activities of PWE and AE of *O. gratissimum* are shown in Figure 5. The PWE of *O. gratissimum* (IC_{50} = 0.47 mg/mL) showed a higher ($P < 0.05$) inhibitory activity against α-amylase enzymatic activity compared to AE of *O. gratissimum* (IC_{50} = 4.90 µg/mL) in a concentration-dependent trend at different concentrations (0.00-0.36 mg/mL) that were considered.

Figure 6 represents the α-glucosidase inhibitory activities of PWE and AE of *O. gratissimum*. AE of *O. gratissimum* (IC_{50} = 3.09 µg/mL) demonstrated a higher α-glucosidase inhibitory activity ($P < 0.05$) compared to PWE of *O. gratissimum* (IC_{50} = 9.09 µg/mL) in a concentration-dependent trend at different concentrations (0.00-0.25 mg/mL) that were considered.

Table 1 shows the AAS analyses of the mineral composition of PWE and AE of *O. gratissimum* leaf extracts. Presence of different elemental compositions were shown in PWE of *O. gratissimum*, such as Ca (65.3 ± 0.3), Fe (0.37 ± 0.004), Mg (8.12 ± 0.010), Cu (0.12 ± 0.004), Zn (0.65 ± 0.002), and Mn (0.41 ± 0.001), whereas in AE of *O. gratissimum* there were Ca (67.0 ± 0.3), Fe (0.25 ± 0.004), Mg (9.4 ± 0.003), Cu (0.177 ± 0.002), Zn (0.66 ± 0.004), and Mn (0.39 ± 0.004).

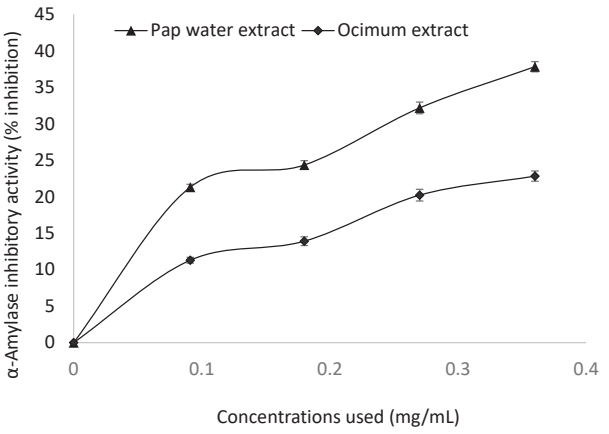

Figure 5. α-Amylase inhibitory activities of pap water extract (PWE) and aqueous extract (AE) of Ocimum gratissimum. Results represent mean± SD of three trials (n = 3). PWE revealed a significant ($P < 0.05$) inhibitory activity against α-amylase activity compared to AE of *O. gratissimum* in a concentration-dependent manner.

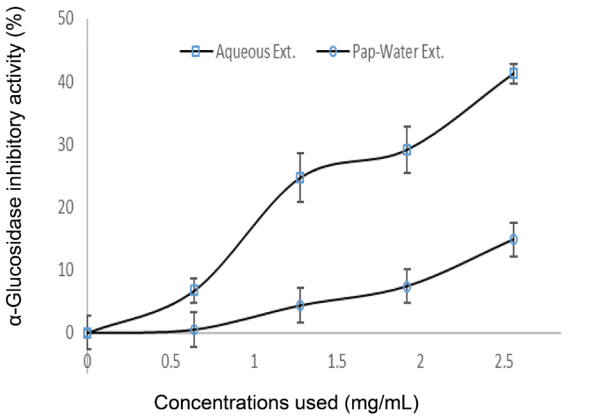

Figure 6. α-Glucosidase inhibitory activities of pap water extract (PWE) and aqueous extract (AE) of *Ocimum gratissimum*. Results represent mean± SD of three trials (n= 3). The AE revealed a significant ($P<0.05$) inhibitory activity against α-glucosidase activity compared to PWE of *O. gratissimum* in a concentration-dependent manner.

Discussion

DM is a significant and ongoing challenge to global healthcare, as emphasized in a report by the World Health Organization (WHO) (30). To address this concern, our study aimed to investigate and compare the antioxidant and antidiabetic properties of the PWE and AE of *O. gratissimum* using in vitro assays. Additionally, we evaluated the mineral compositions of the extracts. It is important to note that oxidative stress plays a crucial role in the development of DM (31), and ROS have been implicated in causing oxidative damage to the pancreas in diabetic individuals (3). Plant-derived bioactive phytonutrients have shown promise in mitigating the adverse effects of oxidative stress (27). A study indicated that the ability of these compounds to donate H-atom (H^+) is the sole mechanism involved in their antioxidative activities (32). The findings of the present study (Figures 3 & 4) indicate that both PWE and AE demonstrated DPPH inhibitory and Fe^{2+}-chelating abilities, significantly. DPPH is an electrophilic compound with the ability to donate and accept H^+ to become a stable molecule (33). Also, Fe^{2+} is a transition metal ion with the ability to transport one electron and then allow the propagation of free radical reactions. The ability of any substance, however, to chelate/deactivate transition metals *in vitro* via the antioxidative mechanism has also been reported in the prevention of such metals in participating in lipid peroxidative metal-based catalytic chain reaction (33). Nevertheless, PWE

Table 1. Atomic absorption spectroscopy (AAS) analysis of mineral elements composition of pap water and aqueous extracts of *Ocimum gratissimum* leaves

Sample	Mineral elements (µg/mL)					
	Ca	Fe	Mg	Cu	Zn	Mn
PWE	65.30 ± 0.30	0.37 ± 0.04*	8.12 ± 0.10	0.12 ± 0.04	0.65 ± 0.02	0.41 ± 0.01
AE	67.00 ± 0.30*	0.25 ± 0.04	9.40 ± 0.03	0.18 ± 0.02	0.66 ± 0.04	0.39 ± 0.04

Abbreviations: PWE: pap water extract, AE: aqueous extract .
Results represent mean ± standard deviation (SD) of three trials (n= 3). *Significantly ($P<0.05$) different when compared down the column.

had a relatively higher inhibitory ability against pro-oxidants than AE. However, according to a previous report, antioxidant activities of any plant extract have been taken to be direct indication of the endowed polyphenolic components among other available secondary metabolites in the plant (34). Therefore, as observed in this study, the DPPH bleaching ability and Fe^{2+} chelating potential could possibly suggest a credit to different antioxidant contents revealed by the both extracts as shown in Figure 4.

Similarly, studies have indicated that nutritional macromolecules such as carbohydrates are degraded into smaller molecules, which eventually are transformed into reducing sugars by the activities of hydrolyzing enzymes (5,28). However, the postprandial plasma elevation of this reducing sugar is a vital approach in the controlling of DM (35). Plant extracts that are rich in bioactive phytonutrients as a result of their oxido-reduction activities are possible inhibitors of carbohydrate-hydrolyzing α-amylase and α-glucosidase enzymes (36,37). In this report (Figures 5 and 6), both PWE and AE demonstrated inhibitory activities against carbohydrate-hydrolyzing activities of pancreatic α-amylase and intestinal α-glucosidase enzymes. However, a higher α-amylase inhibitory activity was observed in PWE than AE of *O. gratissimum*, whereas, reverse is the case to α-glucosidase inhibition. The reason(s) for this observation was not well elucidated in this study; however, it possibly could be attributed to the available phytochemical constituents/contents in different concentrations in the extracts according to the report of Guglani et al (38).

Macro- and micro-nutrients are essential in the human's body functions and day-to-day activities. The human body cannot biosynthesize these essential elements and, therefore, are required in different quantities from the dietary sources (39). The mineral compositions of PWE and AE of *O. gratissimum* were determined in this study; however, the presence of minerals like Ca, Fe, Mg, Cu, Zn, and Mn was indicated in different concentrations (Table 1). Ca, Mg, Cu, and Zn were relatively higher in AE than in PWE; however, Fe and Mn were higher in PWE than in AE. Ca is an essential element in living organisms and the most abundant inorganic constituent in the human body (40). It is specifically required as ca^{2+}-ion in a number of cellular processes such as cofactor and nerve function and impulses, cell division, blood coagulation, and maintenance of blood pH (41). It is essentially required for bone structure and function. Ca^{2+} participates in a myriad of events in the cytoplasm, where it acts as a second messenger in a host of signaling pathways. Also, a study has implicated the direct binding of Ca^{2+} to prompt structural changes, which inhibits enzymatic activity of α-glucosidase. Hence, Ca^{2+} acts as an effective inhibitor of α-glucosidase for the management of NIDDM (42). Similarly, Fe is the most abundant trace element in humans that oscillates between the Fe^{2+} and Fe^{3+} oxidation states, due to its ability to uptake and donate electrons interchangeably, and serves as an essential component of cytochromes and electron transport system. It also activates some metabolic enzymes (43). The estimated values in both PWE and AE, however, are relatively moderate compared to recommended daily allowance (RDA) value of 8.7 to 14.8 mg/d (44). Mg plays essential roles in glucose homeostasis and serves as a cofactor for vital enzymes in carbohydrate metabolic pathway. Alteration in the metabolism of trace elements like Mg has strongly been associated with DM and its complications (45). Cu is an essential redox-active transition metal that participates in many physiological processes due to its oxidation states. Cu is important in a number of enzymatic reactions e.g., mitochondria cytochrome c oxidase reaction (46). Zn is an essential trace element found in PWE and AE. A study showed that Zn demonstrates a fundamental role in the production of insulin, thereby increasing proper glucose uptake (47). A decrease in plasma Zn level negatively influences insulin secreting ability of islet cells. Zn acts a crucial role in normal functioning of the immune system, protein and carbohydrate metabolism, being a cofactor in many enzymatic processes (48). Similarly, Mn is another mineral element found in all body tissues and is needed in trace amount for many enzymatic reactions involved in the biosynthesis of essential macromolecules (49). Mn is also a cofactor of pyruvate carboxylase and plays a part in the conversion of numerous non-carbohydrate complexes into glucose via gluconeogenesis (50), and essential for normal insulin biosynthesis. In sum, the mineral compositions of the extracts have been well documented for different biological and cellular activities that are essential to human health and wellness.

Conclusion

In our study, PWE and AE of *O. gratissimum* demonstrated considerable antioxidant activities, inhibitory activities against carbohydrate-hydrolysing enzymes, as well as significant mineral compositions, which are vital in the management of diabetes, especially NIDDM. However, PWE revealed higher activities in some biological parameters compared to AE. Therefore, PWE could probably be more effective in the management of DM.

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Authors' contributions

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Validation: All authors.
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Writing–review & editing: All authors.

Conflict of interests

The authors declare no conflict of interest concerning this work.

Ethical considerations

Ethics approval was obtained from the Afe Babalola University ethical committee (ethical code: ABUAD/ACA/126). All experiments carried out on the plant (*Ocimum gratissimum*) were performed in accordance with the international guidelines and regulations for standard practice.

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