Globally, the prevalence of diabetes mellitus (DM) is increasing exponentially. Presently, over 382 million people are diabetic and this figure is likely to rise to 592 million by 2035 (1). Among two major types of diabetes (type 1 and type 2), type 2 is the most prevalent one and about 90-95% of diabetics suffer from type 2 diabetes (T2D) (2). T2D is a heterogeneous disorder characterized by insulin resistance and partially dysfunctional pancreatic β-cell to properly secrete insulin in response to hyperglycemia (3). This hyperglycemia increases the generation of reactive oxygen species (ROS), resulting in oxidative stress (4). Subsequently, ROS bind carbohydrates, proteins, lipids or DNA thereby altering the molecular structures; consequently resulting in cellular and tissue damages as seen in T2D (5). Hence, oxidative stress is considered as one of major causal factors of T2D and so plant extracts with potent antioxidative activities can play a vital role in ameliorating oxidative stress as well as T2D.

Furthermore, a majority of patients use oral hypoglycemic drugs for the management of T2D. However, due to the number of short- and long-term side effects and higher price (6, 7), the popularity of these drugs are decreasing gradually. This paves way for a search of newer alternative agents with the ability to inhibit α-glucosidase and α-amylase activities with minimal side effects. The inhibition of carbohydrate digesting enzymes is used as one of the recent alternative approaches for the treatment of T2D. This is achieved by inhibiting the digestion of carbohydrates as well as reduction of the postprandial blood glucose levels. Hence, the search for natural inhibitors of α-glucosidase and α-amylase activities has gradually increased in addition to ROS scavengers. Additionally, Adefegha and Oboh (8) 403

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have reported that active constituents and extracts of plants are potential agents that could be used in management of metabolic disorders such as T2D.

*Aframomum melegueta* (Rosc.) K. Schum. (Zingiberaceae) commonly known as guinea pepper is abundantly found in some part of Africa (9). The seed or fruit are used as a spice and have a wide range of therapeutic uses such as antimicrobial (10), hepatoprotective (11) and remedies for stomachache, diarrhea, and snakebite (12). A very recent study reported that the dipterpenoids isolated from the organic extracts of *A. melegueta* have potential bactericidal activity in vitro against *E. coli, Listeria monocytogenes* and *Staphylococcus aureus* strains (13). In another recent study, Sugita et al. (14) reported that *A. melegueta* alcohol extract activates brown adipose tissue and increases whole-body energy expenditure in human subjects which is directly linked with the pathogenesis of T2D. Additionally, the fruits/seeds and leaves decoctions were reported to be used in the management of DM traditionally in Nigeria (15). In a number of studies reported previously, various extracts from *A. melegueta* seeds possessed antioxidative as well as antidiabetic effects in vitro (16-19) and in vivo (20, 21) when phytochemicals, antioxidative or antidiabetic effects of fruit, leaf and stem are still unknown. Additionally, to our knowledge, no study is available that investigated and compared antioxidative and antidiabetic effects of *A. melegueta* fruit, leaf and stem in a single study either in vitro or in vivo.

Thus, this study was designed to investigate and compare the antioxidative and inhibition of key enzymes linked to type 2 diabetes by various extracts from fruit, leaf and stem of *A. melegueta* using several in vitro models. Additionally, possible bioactive compounds on most active extracts were analyzed using GC-MS.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Ascorbic acid, quercetin, hemoglobin (human lyophilized powder), gallic acid, aluminum chloride, α-amylase from porcine pancreas, α-glucosidase from *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (Saccharomycetaceae), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), were purchased from Sigma-Aldrich through Capital Lab Supplies, New Germany, South Africa. Folin Ciocalteau reagent was purchased from Merck Chemical Company, Durban, South Africa. Gentamycin was purchased from EMD-chemicals, San Diego, CA, USA.

**Plant material**

The fruit, leaf and stem samples of *A. melegueta* were freshly collected in December, 2012 from Ibadan, Oyo State, Nigeria. The plant was identified and authenticated at the herbarium unit of the Biological Science Department, Ahmadu Bello University, Zaria, Nigeria by Mr. Umar Gallah and a voucher specimen number 1511 was deposited accordingly. The plant samples were immediately washed and shade-dried for two weeks to constant weights. The dried samples were ground to a fine powder, and then stored individually in airtight containers for transport to the University of KwaZulu-Natal, Westville campus, Durban, South Africa for subsequent analysis.

**Preparation of the plant extracts**

The fine powdered plant parts (40 g) were separately defatted with 200 mL of hexane. The defatted materials were sequentially extracted with ethyl acetate, ethanol and water by soaking for 48 h in 200 mL of the relevant solvent followed by a 2 h orbital shaking at 200 rpm. After filtration through Whatman filter paper (No. 1), respective solvents were evaporated under vacuum, using a rotary evaporator (Buchi Rotavapor II, Buchi, Germany) at 40°C under reduced pressure to obtain the different solvent extracts with the exception of the aqueous extracts which were dried on a water bath at 45°C. The extracts in each case were weighed, transferred to micro tubes and stored in a refrigerator at 4°C until further analysis.

**Estimation of total polyphenol content**

The total polyphenol content of each extract was determined (as gallic acid equivalent) according to the method described by McDonald et al. (22) with slight modifications. Briefly, 200 µL of various solvent extract (240 µg/mL) was incubated with 1 mL of 10 times diluted Folin Ciocalteau reagent and 800 µL of 0.7 M Na₂CO₃, for 30 min at room temperature. Then, the absorbance values were determined at 765 nm on a Shimadzu UV mini 1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). All measurements were done in triplicate.

**Determination of total flavonoid content**

The total flavonoid content of the plant extracts was determined using a method reported by Chang et al. (23) with slight modification. Briefly, 500 µL (240 µg/mL) of each sample was mixed with 500 µL of methanol, 50 µL of 10% AlCl₃, 50 µL of 1 mol/L potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. Thereafter,
the absorbance of the reaction mixture was subsequently measured at 415 nm on a Shimadzu UV mini 1240 spectrophotometer. The total flavonoid was calculated as quercetin equivalent (QE) in µg per mg dry extract.

**DPPH radical scavenging activity**

The total free radical scavenging activity of the extracts was determined and compared to that of ascorbic and gallic acids by using a slightly modified method described by Tuba and Gulcin.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Yield (%)</th>
<th>Total polyphenols content (mg/g GAE)</th>
<th>Total flavonoids content (mg/g QE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.73</td>
<td>9.03 ± 0.21d</td>
<td>1.53 ± 0.18b</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.53</td>
<td>12.52 ± 0.13e</td>
<td>4.92 ± 0.12d</td>
</tr>
<tr>
<td>Aqueous</td>
<td>1.63</td>
<td>2.97 ± 0.03b</td>
<td>0.17 ± 0.06a</td>
</tr>
<tr>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1.05</td>
<td>5.49 ± 0.51c</td>
<td>0.69 ± 0.12a</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.63</td>
<td>9.43 ± 0.03d</td>
<td>3.50 ± 0.18c</td>
</tr>
<tr>
<td>Aqueous</td>
<td>3.00</td>
<td>1.84 ± 0.44b</td>
<td>0.42 ± 0.29a</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.25</td>
<td>1.58 ± 0.54a,b</td>
<td>ND</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.13</td>
<td>0.66 ± 0.09a</td>
<td>0.31 ± 0.06a</td>
</tr>
<tr>
<td>Aqueous</td>
<td>2.38</td>
<td>0.18 ± 0.01a</td>
<td>0.11 ± 0.06a</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD values of triplicate determinations. a-e Different superscripted letters for a given value within a column are significantly different from each other (Tukey’s-HSD multiple range post hoc test, p < 0.05).

**Table 1. Percentage yield, total polyphenol and flavonoid contents of various solvent extracts of A. melegueta parts.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>IC50 (mg/mL)</th>
<th>DPPH scavenging activity</th>
<th>Non-enzymatic glycosylation of hemoglobin</th>
<th>α-Amylase inhibitory effect</th>
<th>α-Glucosidase inhibitory effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.16 ± 0.01a</td>
<td>1.55 ± 0.32a</td>
<td>3.59 ± 0.12b</td>
<td>2.73 ± 0.08c</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.04 ± 0.01a</td>
<td>0.72 ± 0.03b</td>
<td>0.62 ± 0.01a</td>
<td>0.06 ± 0.01a</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>0.06 ± 0.01a</td>
<td>3.34 ± 0.32a</td>
<td>3.62 ± 0.03b</td>
<td>0.32 ± 0.01a</td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.17 ± 0.01a</td>
<td>16.75 ± 0.39d</td>
<td>25.99 ± 0.26d</td>
<td>1.05 ± 0.01c</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.07 ± 0.02a</td>
<td>5.12 ± 1.21c</td>
<td>8.79 ± 0.44c</td>
<td>0.06 ± 0.01c</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>1.78 ± 0.10a</td>
<td>57.57 ± 0.82b</td>
<td>15.10 ± 0.35c</td>
<td>2.65 ± 0.40b</td>
<td></td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>4.45 ± 0.16a</td>
<td>ND</td>
<td>ND</td>
<td>2.31 ± 0.18b</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.46 ± 0.01a</td>
<td>ND</td>
<td>ND</td>
<td>1.93 ± 0.02a</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>4.70 ± 0.21a</td>
<td>ND</td>
<td>ND</td>
<td>81.29 ± 0.90a</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.03 ± 0.02a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0.05 ± 0.01a</td>
<td>0.20 ± 0.01a</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Acarbose</td>
<td>ND</td>
<td>4.91 ± 0.80d</td>
<td>0.34 ± 0.02a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD values of triplicate determinations. a-g Different superscript letters presented within a column for a given parameter are significantly different from each other (Tukey’s-HSD multiple range post hoc test, p < 0.05). ND = Not determined.
Figure 1. DPPH radical scavenging activity (%) of fruit (A), leaf (B) and stem (C) extracts of *A. melegueta*. Data are presented as the mean ± SD of triplicate determinations. *a-d* Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey’s HSD multiple range post hoc test, p < 0.05)
A 500 µL of 0.3 mM solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol was added to 1 mL of the extracts at different concentrations (30, 60, 120 and 240 µg/mL). These solutions were mixed and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm against blank samples lacking the free radical scavengers. Percentage inhibition calculated according to the following formula:

\[
\% \text{ Inhibition} = \left( \frac{\text{Abs. control} - \text{Abs. extract}}{\text{Abs. control}} \right) \times 100
\]

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC\text{50}) were calculated from the data as well.

The ferric reducing antioxidant power method of Oyaizu (25) was used with slight modifications to measure the reducing capacity of the extracts. To perform this assay, 1 mL of each extract (30, 60, 120 and 240 µg/mL) was incubated with 1 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide at 50°C for 30 min. After 30 min incubation, the reaction mixtures were acidified with 1 mL of 10% trichloroacetic acid. Thereafter, 1 mL of the acidified sample of this solution was mixed with 1 mL of distilled water and 200 µL of FeCl\text{3} (0.1%). The absorbance of the resulting solution was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture

**Ferric (Fe\text{3+}) reducing antioxidant power assay**

Figure 2. Ferric reducing power (relative to gallic acid) of fruit (A) and leaf (B) extracts of *A. melegueta*. Data are presented as the mean ± SD of triplicate determinations. *a-d* Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey’s-HSD multiple range post hoc test, p < 0.05)
indicated greater reductive capability of the extracts (26).

**Inhibition of hemoglobin glycosylation**

Inhibition of non-enzymatic glycosylation of hemoglobin by various extracts was investigated by the modified method of Pal and Dutta (27). Glucose (2%), hemoglobin (0.06%) and gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. One mL each of above solution was mixed with 1 mL of different concentration of the extracts (30, 60, 120 and 240 µg/mL) in dimethyl sulfoxide (DMSO). These mixtures were incubated in dark at room temperature for 72 h. The percentage inhibition of glycosylation of hemoglobin was calculated from absorbance measured at 520 nm. Gallic acid was used as a standard. Percentage inhibition calculated according to the following formula:

\[
\% \text{ Inhibition} = \left( \frac{\text{Abs. control} - \text{Abs. extract}}{\text{Abs. control}} \right) \times 100
\]

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were calculated from the data as well.

**α-Amylase inhibitory effect**

The α-amylase inhibitory effect of the extracts was carried out using a modified method of McCue and Shetty (28). Briefly, 250 µL of extract at different concentrations (30, 60, 120 and 240 µg/mL) was

---

![Graph A](image1)

**Figure 3.** Inhibition of hemoglobin glycosylation (%) of fruit (A) and leaf (B) extracts of *A. melegueta*. Data are presented as the mean ± SD of triplicate determinations. **a-d** Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey’s HSD multiple range post hoc test, p < 0.05)
Table 3. Identified compounds from the EtOH fruit and leaf extracts of *A. melegueta* by GC-MS.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Compounds</th>
<th>Retention time (min)</th>
<th>Mass/amu</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH fruit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Eugenol</td>
<td>6.68</td>
<td>164 [M]^+</td>
</tr>
<tr>
<td>2</td>
<td>n-Hexadecanoic acid</td>
<td>10.22</td>
<td>256 [M]^+</td>
</tr>
<tr>
<td>3</td>
<td>Hexadecanoic acid, ethyl ester</td>
<td>10.39</td>
<td>284 [M]^+</td>
</tr>
<tr>
<td>4</td>
<td>Oleic acid</td>
<td>11.11^a</td>
<td>282 [M]^+</td>
</tr>
<tr>
<td>5</td>
<td>Z-9-Hexadecen-1-ol acetate</td>
<td>11.24^a</td>
<td>282 [M]^+</td>
</tr>
<tr>
<td>8</td>
<td>Gingerol</td>
<td>12.20</td>
<td>294 [M]^+</td>
</tr>
<tr>
<td>9</td>
<td>Capsaicin</td>
<td>12.49</td>
<td>239 [M]^+</td>
</tr>
<tr>
<td>10</td>
<td>13-Docosenoic acid, methyl ester, (Z)</td>
<td>13.37</td>
<td>352 [M]^+</td>
</tr>
<tr>
<td>EtOH leaf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Oleic acid</td>
<td>11.11</td>
<td>282 [M]^+</td>
</tr>
<tr>
<td>6</td>
<td>3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)</td>
<td>11.63</td>
<td>278 [M]^+</td>
</tr>
<tr>
<td>7</td>
<td>Ethyl homovanillate</td>
<td>12.15</td>
<td>210 [M]^+</td>
</tr>
<tr>
<td>8</td>
<td>Gingerol</td>
<td>12.20</td>
<td>294 [M]^+</td>
</tr>
<tr>
<td>10</td>
<td>13-Docosenoic acid, methyl ester, (Z)</td>
<td>13.37</td>
<td>352 [M]^+</td>
</tr>
</tbody>
</table>

^a isomers with same mass/amu at the different retention time.

placed in tubes and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added to the tube. This solution was pre-incubated at 25°C for 10 min, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at time intervals of 10 s and then further incubated at 25°C for 10 min. The reaction was terminated after incubation by adding 1 mL of dinitrosalicylic acid (DNS) reagent. The tubes were then boiled for 10 min and cooled to room temperature. The reaction mixture was diluted with 5 mL of distilled water and the absorbance was measured at 540 nm using Shimadzu UV mini 1240 spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated according to the following formula:

% Inhibition = [(Abs. control - Abs. extracts)/ Abs. control] × 100.

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC<sub>50</sub>) were calculated from the data as well.

**α-Glucosidase inhibitory effect**

The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim et al. (29) using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. Then, 500 µL of α-glucosidase was pre-incubated with 250 µL of the different concentrations of the extracts (30, 60, 120 and 240 µg/mL) for 10 min. Then, 250 µL of 5.0 mM pNPG was dissolved in 20 mM phosphate buffer (pH 6.9) as a substrate to start the reaction. The reaction mixture was incubated at 37°C for 30 min. The α-glucosidase activity was determined by measuring at 405 nm the yellow colored p-nitrophenol released from pNPG. The results were expressed as percentage of the blank control according to the following formula:

% Inhibition = [(Abs. control - Abs. extract)/ Abs. control] × 100.

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC<sub>50</sub>) were calculated from the data as well.

**Gas chromatography-mass spectroscopic (GC-MS) analysis**

Based on the results of antioxidative and anti-diabetic studies, most active extracts (EtOH fruit and leaf) were subjected to GC-MS analysis. The GC-MS analysis was conducted with an Agilent technology 6890 gas chromatograph coupled with an Agilent 5973 Mass Selective detector and driven by Agilent chemstation software. A HP-5MS capil-
lary column was used (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). The carrier gas was ultra-pure helium at a flow rate of 1.0 mL/min and a linear velocity of 37 cm/s. The injector temperature was set at 250°C. The initial oven temperature of 60°C was programmed to 280°C at the rate of 10°C/min with a hold time of 3 min. Injections of 1 µL were made in splitless mode. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230°C, quadruple temperature 150°C, solvent delay 4 min and scan range 50-700 amu. Compounds were identified by direct comparison of the retention times and mass spectral data with those in the National Institute of Standards and Technology (NIST) library.

Statistical analysis

All data are presented as the mean ± SD of triplicates determination. Data were analyzed by using a statistical software package (SPSS for Windows, version 18, IBM Corporation, NY, USA) using Tukey’s-HSD multiple range post hoc test. Values were considered significantly different at p < 0.05.

Figure 4. α-Amylase inhibition (%) of fruit (A) and leaf (B) extracts of A. melegueta. Data are presented as the mean ± SD of triplicate determinations. a-d Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey’s-HSD multiple range post hoc test, p < 0.05)
Figure 5. α-Glucosidase inhibition (%) of fruit (A), leaf (B) and stem (C) extracts of A. melegueta. Data are presented as the mean ± SD of triplicate determinations. “a-d” Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey’s HSD multiple range post hoc test, p < 0.05)
RESULTS

The yields recovered, total polyphenols and flavonoids contents from different solvent extracts of various parts are presented in Table 1. The aqueous leaf extract showed higher yield compared to other extracts, while ethyl acetate (EtOAC) extract of stem was the least recovered. Moreover, significantly (p < 0.05) higher total polyphenols and flavonoids content were observed in the fruit EtOH extract compared to other solvent extracts of the same or different parts of A. melegueta. Interestingly, leaf EtOH extract had higher polyphenols and flavonoids contents compared to EtOAC and aqueous extracts of this part. The EtOAC stem extract showed significantly (p < 0.05) higher
Figure 7. Structures of identified compounds from fruit and leaf of *A. melegueta* [aromatic phenols (A), long chain aliphatic acids and esters (B)].
polyphenols compared to EtOH and aqueous extracts of stem (Table 1).

Furthermore, the extent of DPPH radical scavenging activity (Fig. 1) by various extracts from different parts of A. melegueta was determined by calculating IC\textsubscript{50} values. The IC\textsubscript{50} values recorded for various solvent extracts from fruit ranged from 0.04 to 0.16 mg/mL (Table 2). The EtOH extract demonstrated significantly (p < 0.05) lower IC\textsubscript{50} value (0.04 ± 0.01 mg/mL) compared to other extracts which is comparable to that of standards used in this study (ascorbic acid: 0.03 ± 0.02 mg/mL; gallic acid: 0.05 ± 0.01 mg/mL). Similarly, leaf EtOH extract exhibited lower IC\textsubscript{50} value (0.07 ± 0.02 mg/mL) compared to other extracts of this part which did not differ significantly with those of the standards. Various extracts from stem showed weak scavenging effect as the calculated IC\textsubscript{50} values were significantly higher compared to other solvent extracts from either fruit or leaf part as well as the standards. In addition, stem EtOH extract demonstrated lower value (2.46 ± 0.01 mg/mL) compared to other solvent extracts of this part (Table 2). It is also evident from the results that EtOH extract from different parts exhibited lower IC\textsubscript{50} values for DPPH scavenging activity.

In this study, ferric reducing power (expressed as gallic acid equivalents) of the extracts was further used to access the antioxidative nature of different parts of A. melegueta and is presented in Figure 2. The result showed that various solvent extracts from fruit and leaf parts of A. melegueta showed considerable reducing abilities, irrespective of the solvent used. The EtOH extracts demonstrated significantly (p < 0.05) higher activities compared to other solvent extracts. Fruit EtOH extract showed higher reducing power compared to other solvent extracts of the same or different parts which is comparable to ascorbic acid. Moreover, various solvent extracts from stem showed no Fe\textsuperscript{3+} to Fe\textsuperscript{2+} reducing activity. The extent of DPPH radical scavenging by the extracts is presented in Figure 3. The effectiveness of the inhibition was also expressed based on IC\textsubscript{50} values (Table 2). From the result, fruit EtOH extract exhibited a significantly (p < 0.05) lower IC\textsubscript{50} value (0.72 ± 0.03 mg/mL) compared to other solvent extracts which is comparable to that of gallic acid (0.20 ± 0.01 mg/mL) used as a standard (Table 2). Moreover, leaf EtOH extract exhibited lower IC\textsubscript{50} value of 5.12 ± 1.21 mg/mL compared to other solvent extracts. Extracts from the stem part showed no inhibitory activities towards hemoglobin glycosylation.

The enzyme inhibitory effect of various solvent extracts from different parts of A. melegueta for α-amylase and α-glucosidase are presented in Figures 4 and 5, respectively. It was clearly observed from the α-amylase inhibitory effect that fruit EtOH extract exhibited significantly (p < 0.05) lower IC\textsubscript{50} value (0.62 ± 0.01 mg/mL) in comparison to other solvent extracts (EtOAC: 3.59 ± 0.12 mg/mL; aqueous: 3.62 ± 0.03 mg/mL) of this part as well as acarbose (4.91 ± 0.80 mg/mL). The degree of inhibition was ranged as in the following increasing order: aqueous < EtOAC < EtOH, with EtOH demonstrating the highest activity. Similar trend was observed in various extracts from leaf part. The calculated IC\textsubscript{50} value of leaf EtOH extract was 8.79 ± 0.44 mg/mL which is significantly higher compared to acarbose (2.46 ± 0.01 mg/mL). Stem EtOH extract which shows no α-amylase inhibitory effect recorded an IC\textsubscript{50} value of 1.93 ± 0.02 mg/mL for α-glucosidase inhibition (Table 2).

Furthermore, Figures 6 and 7 (compounds 1-10) present the chromatograms of fruit and leaf EtOH extracts and the structures of the compounds, respectively. Several peaks appeared in the chromatogram for fruit EtOH extract (Fig. 6A) and were identified and compared with standards from the NIST library. The available constituents (1-8) in fruit EtOH extract were phenolics and other long chain fatty derivatives (Table 3). The phenolics include eugenol (1), gingerol (6) and capsaicin (7). The fatty derivatives detected were oleic acid (4), hexadecanoic derivatives (2, 3, 5) and 13-docosenoic acid, methyl ester (8) (Table 3). Furthermore peaks visible from the chromatogram of leaf EtOH extract (Fig. 6B) lead to identification of some phenol derivatives; gingerol (6), 3-decanone, 1-(4-hydroxy-3-methoxyphenyl) (10), ethyl homovallinate (11), with other fatty acid derivatives as oleic acid (4) and 13-docosenoic acid, methyl ester (8) (Table 3). It is evident from the result that all of the aromatic phenols identified in both extracts are 4-hydroxy-3-methoxyphenyl derivatives.

**DISCUSSION**

Natural products in form of crude or plant preparations have been used in the treatment and control of wide array of diseases. In many of such plants, some parts usually receive much attention compared to others. For instance A. melegueta, where most of the studies previously reported
focused on seed alone, rendering the other parts to almost wastage. In this regard, we intend to explore the antioxidative and inhibition activity of key enzymes linked to type 2 diabetes by various solvent extracts from fruit, leaf and stem of A. melegueta. This is of great importance as little is known on the therapeutic potentials of these parts of A. melegueta.

Polyphenols and other related compounds have been implicated with a number of therapeutic potentials such as antioxidant, anti diabetic, anticancer and antimicrobial effects (30). Impact most of the medicinal importance of plant-based formulations were correlated to their polyphenols present in such plant species. In our current study, fruit EtOH extract possessed higher polyphenols and flavonoids content. In order to support this, previous studies indicated that A. melegueta seed to be rich source of flavonoids and other phenolics (18, 20). Interestingly, leaf EtOH extract was found to be rich in polyphenols and flavonoids content. Similarly, it is important to notice that the amount and distribution of plants products such as polyphenols varies greatly from one species of plants to another or within the parts of the same plants. The production and accumulation of polyphenols are influenced by many factors, such as genetic and environmental factors (nature of the soil, high temperature and rain fall) in addition to growth or maturation stages for most of the parts of the plants (31, 32). Hence, the higher polyphenols and flavonoids contents of fruits compared to other parts could be attributed to one or combination of the above mentioned factors.

Furthermore, in this study, a number of in vitro models were used to investigate antioxidative potentials of various solvent extracts from different parts of A. melegueta. Among such models is the DPPH radical scavenging activity, which is a quick and reliable method for determining the antioxidative nature of extracts/compounds. DPPH is usually delocalized by accepting electrons from the referenced antioxidant, thereby becoming a stable molecule (33). The antioxidative effectiveness of various solvent extracts from different parts of A. melegueta was determined by calculating IC₅₀ values. The lower the IC₅₀ value, the higher the antioxidative potential of the extract. It is evident from the result that fruit EtOH extract exhibited the least IC₅₀ value (0.04 ± 0.01 mg/mL) indicating higher scavenging ability. In a recent study, Kazeem et al. (16) reported an IC₅₀ value of 0.11 ± 0.01 mg/mL for seed aqueous extract. This is by far higher compared to that of EtOH extract as well as aqueous extract (0.06 ± 0.01 mg/mL) observed in this study. It is also evident from the results that EtOH extracts from different parts exhibited lower IC₅₀ values for DPPH scavenging activity. This could be attributed to their rich polyphenols and flavonoids contents. Moreover, leaf EtOH extract showed a very good scavenging ability which is comparable to that of fruit as well as the standards used in this study.

Similarly, reduction of Fe³⁺ to Fe²⁺ by plant extracts or compounds is another reliable index and a good indicator of their electron quenching abilities (34). The reduction of Fe³⁺ to Fe²⁺ by the referenced sample results in the formation of Perl’s prussian blue, which is monitored at 700 nm. Consistent with the present study, Adefegha and Oboh, (35) have earlier reported higher reducing potential of A. melegueta seed compared to A. danielli seed. Although no data is available on the A. melegueta leaf, our findings showed that leaf could be a good source of antioxidant in addition to seed and fruit that are popularly utilized.

Hemoglobin glycosylation is a non-enzymatic reaction that contributes to formation of advanced glycation end products (36, 37). In accordance with other antioxidant parameters, EtOH extracts of fruit and leaf demonstrated higher inhibitory effect compared to other solvent extracts in their respective parts. This happens to be the first available data on inhibitory effects of various parts of A. melegueta toward hemoglobin glycosylation. Our data is also in line with results published by some previous studies on other plant species (38, 39).

On the other side, the control of postprandial hyperglycemia is crucial for the management of DM and prevention of its complications at early stage (40). This is achieved by delaying the absorption of glucose from the small intestine through the inhibition of carbohydrates metabolizing enzymes such as α-amylase and α-glucosidase, located in the intestinal tract. The α-amylase catalyzes the endo-hydrolysis of α-1,4-glucosidic linkage releasing disaccharides and oligosaccharides which are further hydrolyzed at the small intestinal brush border by α-glucosidases to release glucose (41, 42). Our available result indicated that fruit and leaf EtOH extracts appeared to show best activity as possible antidiabetic agents as evident by the low IC₅₀ values obtained for inhibitory effects toward α-amylase and α-glucosidase actions. Our results are also supported by the previous published studies (19, 43-45). In their study, Adefegha and Oboh (19) recorded IC₅₀ values of 2.14 ± 1.08 mg/mL and 4.83 ± 0.56 mg/mL for α-glucosidase and α-amylase, respectively, of seed aqueous extract, which is far higher, compared to that of fruit obtained in this study. This indicates higher enzyme inhibitory effect of fruit than seed.
Based on the results of this study, fruit and leaf EtOH extracts that exhibited higher activities were subjected to GC-MS analysis to detect possible bioactive compounds that could be responsible for their effects. It is evident that eugenol (1), gingerol (8), ethyl homovallinate (7), capsaicin (9) and 3-decanone, 1-(4-hydroxy-3-methoxyphenyl) (6) detected in fruit and leaf EtOH extracts were 4-hydroxy-3-methoxyphenyl derivatives. The presence of phenolic group in the structure of these compounds is regarded as the key feature that could be responsible for the higher antioxidative and antidiabetic effects observed in this study (Fig. 7). Phenolics and related compounds have the ability to act as antioxidants due to their low reduction potential compared to highly reactive species such as hydroxyl (·OH), superoxide (O₂⁻), nitric oxide (NO⁻) radicals. Their ability to donate electron or proton from hydroxyl moieties result in stabilizing lipid peroxidation, neutralizing ROS and ultimately inhibit the initiation and propagation of chain reaction associated with oxidative damage (46, 47). Consistent with the present study, recently published data reported the abilities of phenolic rich extracts and compounds to interfere and inhibit the activities of α-glucosidase and α-amylase in the brush-border of the small intestine, which could subsequently decreased glucose absorption and subsequently reduced postprandial hyperglycemia (19, 48).

CONCLUSIONS

Conclusively, various solvent extracts from fruit and leaf of A. melegueta possessed antioxidative as well as antidiabetic effects in vitro when stem extracts showed very low or no significant effects in these regards. The fruit EtOH extract exhibited higher effects compared to other extracts of the same or different parts. Thus, it is recommended for further bioassay-guided fractionation in order to fully investigate the in vivo antidiabetic and antioxidative effects of this extract.

Declaration of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This study was supported by a competitive research grant from the Research Office, University of KwaZulu-Natal (UKZN), Durban; an incentive grant for rated researchers and a grant support for women and young researchers from the National Research Foundation (NRF), Pretoria, South Africa. The first author was awarded a Ph.D. study fellowship by the Ahmadu Bello University, Zaria, Nigeria.

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Received: 25. 02. 2015