INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by elevated blood glucose level. Type 2 accounts for over 90% of DM cases, resulting from defective insulin secretion by pancreatic cells and the inability of insulin-sensitive tissues to respond to insulin. Progression of the disease disrupts glucose homeostasis, which gives rise to hyperglycemia. The chronic metabolic imbalance associated with this disease puts patients at high risk for long-term macro and microvascular complications, leading to cardiovascular diseases (Leahy, 2005). In 2019, diabetes was responsible for 4.2 million deaths and 463 million adults were living with the disease, which is expected to reach 700 million by 2045 (International Diabetes Federation (IDF)). Proper glycemic control remains the main basis for managing T2DM (Zimmet, Alberti, & Shaw, 2001). α-amylase secreted by salivary glands and pancreas plays a major role in the digestion of starch and glycogen. Thus, inhibitors of α-amylase delay the breakdown of carbohydrates in the small intestine, thereby diminishing postprandial blood glucose in the diabetic patient. In this study, a DPPH radical scavenging activity and computational approaches were employed to uncover the potential of *Aframomum Melegueta* phytochemicals against type two diabetes mellitus mainly by molecular docking, molecular dynamic simulation, MMPBSA and ADMET analysis. The results show that it has high antioxidant properties. Molecular docking indicates that laurifolin, genkwanin and galanolactone have good binding scores of -9.9 kcal/mol, -8.9 kcal/mol and -8.2 kcal/mol, respectively. And have interacted with at least two of the catalytic triads of α-amylase: Asp 300, Glu 233 and Asp 197. Molecular dynamic simulation results show that all the compounds are stable at the active site of the enzyme. Furthermore, MMPBSA analysis revealed they bind strongly with the binding energy of -21.77±1.03 Kcal/mol, -17.82±0.84 Kcal/mol and 15.07±0.26 Kcal/mol for laurifolin, genkwanin and galanolactone. ADMET analysis indicated that all the ligands are water-soluble, drug-like and safe. This study shows that A. Melegueta extract has antioxidant properties and possess phytochemicals that can be exploited for further anti-diabetic drug development.

Keywords: Diabetes mellitus, α-amylase, *Aframomum Melegueta*, phytochemicals, Molecular docking, drugs discovery, molecular dynamic simulations and MMPBSA.

Abstract: Diabetes is a chronic metabolic disease characterized by raised levels of blood glucose, which over time leads to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. α-amylase plays a major role in the digestion of starch and glycogen. Thus, inhibitors of this enzyme delay the breakdown of carbohydrates in the small intestine, thereby diminishing postprandial blood glucose in the diabetic patient. In this study, a DPPH radical scavenging activity and computational approaches were employed to uncover the potential of *Aframomum Melegueta* phytochemicals against type two diabetes mellitus mainly by molecular docking, molecular dynamic simulation, MMPBSA and ADMET analysis. The results show that it has high antioxidant properties. Molecular docking indicates that laurifolin, genkwanin and galanolactone have good binding scores of -9.9 kcal/mol, -8.9 kcal/mol and -8.2 kcal/mol, respectively. And have interacted with at least two of the catalytic triads of α-amylase: Asp 300, Glu 233 and Asp 197. Molecular dynamic simulation results show that all the compounds are stable at the active site of the enzyme. Furthermore, MMPBSA analysis revealed they bind strongly with the binding energy of -21.77±1.03 Kcal/mol, -17.82±0.84 Kcal/mol and 15.07±0.26 Kcal/mol for laurifolin, genkwanin and galanolactone. ADMET analysis indicated that all the ligands are water-soluble, drug-like and safe. This study shows that A. Melegueta extract has antioxidant properties and possess phytochemicals that can be exploited for further anti-diabetic drug development.

**Keywords:** Diabetes mellitus, α-amylase, *Aframomum Melegueta*, phytochemicals, Molecular docking, drugs discovery, molecular dynamic simulations and MMPBSA.

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stress has been demonstrated in many studies to play a significant role in diabetes mellitus (DM) pathogenesis. Antioxidants have already been shown to be prospective in the treatment of diabetes (Oyedemi et al., 2017). Alligator Pepper (also known as mbongo spice or hepper pepper) is a West African spice made from the seeds and seed pods of Aframomum melegueta; its seeds have been reportedly used in folkloric medicine in the management of hypercholesterolemia, anti-diabetes and hypertension with the limited scientific basis for their action (Kazeem, Akanji, Hafizur, & Choudhary, 2012).

As such, this study aims to determine the antioxidant properties of alligator pepper (A. Melegueta) extract and the in silico identification of α-amylase inhibitors with the potential to be used in the development of anti-diabetic drugs.

**EXPERIMENTAL SECTION**

**Sample Collection and Extraction**

A. Melegueta was collected from Wudil and identified at the Biology Department of Kano University of Science and Technology, Wudil. Methanolic extracts were prepared using 25g of ground sample soaked in 100 mL of methanol solvent and shaken continuously for 7 d at 25°C in a shaker. The extracts were then filtered using Whatman No. 1 filter paper and allowed to evaporate at room temperature (Haruna, Abdulmumin, Abdulmumin, Murtala, & Dalhatu; Murtala et al.,

**Determination of Antioxidant Activity of Alligator Pepper**

The antioxidant activity was estimated using DPPH (2,2-Diphenyl-1-Picryl-Hydrayl-Hydrate) free radical scavenging assay was conducted according to the standard procedure. 2ml of various concentrations (0.2-1.0ml) of the plant extract was added separately to 2ml of 0.1mmol/L methanolic solution of DPPH and incubated for 30 minutes in the dark at room temperature in triplicate (Murtala et al.,

The absorbance was measured against a control 517nm. The scavenging rate (1%) on the DPPH radical was calculated using the expressions:

\[
\text{DPPH radical Scavenging rate} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100
\]

The concentration with 50% inhibition (IC50) was determined from the calibration curve.

**GC-MS Analysis**

The bioactive compounds extracted from A. melegueta were analyzed by gas chromatography (GC-MS, Shimadzu, Japan) coupled with a mass spectrometer (Shimadzu, Kyoto, Japan) according to the standard protocol. The names, structures, and molecular weights of the bioactive components in each extract were determined by matching their mass spectra with available data from the NIST and Wiley libraries (Mariswamy, Gnanaraj, Antonisamy, Adaikalam, & Jamesraj, 2013).

**Molecular docking**

Three-dimensional structures of the identified phytochemicals were retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov). The PDB structure of alpha-amylase (PDB ID: 1PIF) was downloaded from the Protein Data Bank (https://www.rcsb.org). And prepared using Discovery studio software.

Molecular docking study was performed by Site-directed docking around the alpha-amylase active site residues with all the identified compounds of A. melegueta using Pyrx-AutoDock Vina software package. The receptor molecule remains rigid and the ligands are flexible. Binding interactions were analyzed using Discovery Studio visualize (version 20) (Trott & Olson, 2010).

**Molecular dynamic simulations**

Molecular dynamic (MD) simulations of the protein and protein-ligand complex were performed with a GROMACS 5.0 package using CHARMM 36 force field (Pronk et al., 2013; Vanommeslaeghe et al., 2010). The complexes were solved with a TIP3P water molecule and neutralized with the 0.154 moles/liter NaCl. The initial energy minimization process was conducted by applying a simulated annealing method with a corresponding equilibration of 1 ns. The production of MD simulation was performed for 50 ns per system at a constant temperature and pressure of 300 K and 1 atm, respectively, with a time step of 2 fs. Root mean square deviation (RMSD) and Root mean square fluctuation (RMSF) were calculated to determine the stability of the protein-ligand complex.

**Binding free-energy calculations using MMPBSA**

The binding free-energies (ΔGbind) of the candidates were computed using the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) algorithm. The total binding free-energy (ΔGtotal) is determined as a total energy released from the ligand-protein complex which is contributed by molecular mechanics binding energy (ΔEMM) and solvation free energy (ΔGSolv) using the following equations:

\[
\Delta G_{\text{bind}}(\text{MM-PBSA}) = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} - \Delta T \Delta S
\]

Where ΔE_{int} stands for internal energy, ΔE_{ele} electrostatic energy, ΔE_{vdw} for vander Waals energy, ΔG_{polar} for polar energy, ΔG_{non-polar} for non-polar energy components and ΔG_{solv} is the contribution to total solvation free energy while ΔG_{bind} stands for the free
energy of binding evaluated after entropic calculations –TΔS (Miller III et al., 2012).

**ADMET analysis**

SwissAdme (www.swissadme.ch) server was used to evaluate the metabolic and toxicological properties of the phytochemicals. The canonical format of the chemical compounds was used as the entry system for ADMET (absorption, distribution, metabolism and toxicity) calculations (Ntie-Kang, 2013).

**Data Analysis**

The data obtained was analyzed at P-value (p<0.05). Using one way analysis of variance (ANOVA). Data was expressed as the mean ± Standard deviation. Statistical significance was accepted at a level of P<0.05.

**RESULTS**

**DPPH Radical Scavenging Capacity of Alligator Pepper**

*A. melegueta* extract exhibited a considerable DPPH radical scavenging capacity in a concentration-dependent manner with ascorbic acid used as control (Figure 1).

![Figure 1: DPPH Radical Scavenging Capacity of Alligator Pepper](image)

**Molecular Docking**

The result shows that *A. melegueta* has phytoc hemicals that can interact with the catalytic residues of α-amylase (Figure 2). Laurifolin has the highest binding scores of -9.9 kcal/mol followed by Genkwanin with the binding scores of -8.9 kcal/mol and galanolactone with binding scores of - 8.2 kcal/mol (Table 1).

**Table 1: Binding scores and interactions between A. melegueta phytochemicals and α-amylase**

<table>
<thead>
<tr>
<th>Compound</th>
<th>PubChem ID</th>
<th>Binding Scores (kcal/mol)</th>
<th>Hydrogen Bond</th>
<th>Other interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurifolin</td>
<td>44257868</td>
<td>-9.9</td>
<td>Gln 63</td>
<td>Glu 333, Asp300, His 305 and Asp 197</td>
</tr>
<tr>
<td>Genkwanin</td>
<td>5281617</td>
<td>-8.9</td>
<td>Gln 63</td>
<td>Glu233,Asp17,His 299 and Tyr 58</td>
</tr>
<tr>
<td>Galanolactone</td>
<td>146156530</td>
<td>-8.2</td>
<td>-</td>
<td>Asp 300,Asp197lu 233,Arg195 and His 305</td>
</tr>
</tbody>
</table>

**Analysis of the phytochemicals interactions**

Alligator pepper phytochemicals interactions with the α-amylase were explored using discovery studio. As shown in Figure 2a, laurifolin forms two hydrogen bonds with Glu 63 and hydrophobic interactions with Asp 300, Asp197 and Glu 233 catalytic triad of α-amylase genkwanin also forms hydrogen bonds with Glu 63 and hydrophobic interactions with Glu 233 and Asp 197 of the α-amylase catalytic residues (Figure 1b. Similarly, galanolactone also interacted with the active site Asp 300, Glu 233, Asp193 and His 299 (Figure 2c).
Molecular dynamics (MD) simulation
Dynamic behavior and stability of the protein-ligand complex were determined by MD simulation of 50ns and investigated by RMSD and RMSF analysis.

Root mean square deviation (RMSD)
RMSD results shown in Fig 3 revealed that α-amylose-laurifolin and α-amylose-Genkwanin complexes have RMSD values lower than the Apo-protein, indicating an increase in the stability of the complexes, which remain stable throughout the simulations. On the other hand, α-amylose-Galanolactone has RMSD values higher than the Apo-protein but remains stable throughout the simulation. Laurifolin RMSD value fluctuates between 0.6Å and 1.2 Å, with the average RMSD value of 0.8 Å. However, genkwanin fluctuates between RMSD values of 0.2 Å and 1.2 Å, with an average RMSD value of 0.8 Å. Similarly, galanolactone RMSD fluctuates between 1.0 Å and 2.0 Å, with the average RMSD value of 1.5 Å. Two major fluctuations observed in the Apo-protein around 10ns to 15ns and 35ns to 40 ns were stabilized upon binding of the ligands.
Figure 3: Root mean square deviation (RMSD) of C–Ca–N backbone for solvated α-amylose in Apo-state and in complex with the ligands over the time of 50 ns simulation

Root mean square fluctuation (RMSF)

RMSF analysis was conducted to determine the residual fluctuations of the system. The result shows that the stability of the enzyme was not significantly affected by the ligands binding, especially around the catalytic residues, Asp 300, Asp197 and Glu 233 (Figure 4). Major fluctuations were observed between residue 235 and 240 (2.19Å) upon binding of laurifolin and between residue 290 and 305 (1.70 Å) upon binding of Galanolactone. Largest fluctuation was observed with Genkwanin at residue between 290 and 305 (3.48Å).

Figure 4: Root mean square fluctuation (RMSF) of α-amylose and in complex with alligator pepper ligands

MMGBSA study

All the 3 ligands were subjected to a ligand-receptor binding energy study using the MMPBSA approach. The binding energy of the docked ligand complex confirms the stability of the ligand after binding to the enzyme's active site.

Table 2: Binding energy of α-amylose in complex with the ligands using MMPSA

<table>
<thead>
<tr>
<th>Complex structures</th>
<th>Vander Waals Energy (±SD) (Kcal/mol)</th>
<th>Electrostatic Energy (±SD) (Kcal/mol)</th>
<th>Polar Solvation Energy(±SD) (Kcal/mol)</th>
<th>Apolar Energy (±SD) (Kcal/mol)</th>
<th>Total Binding Energy (±SD) (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Amylase-Genkwanin</td>
<td>-28.17 ± 0.04</td>
<td>-28.15 ± 0.68</td>
<td>37.39 ± 0.29</td>
<td>-2.85 ± 0.02</td>
<td>-21.77± 1.03</td>
</tr>
<tr>
<td>α-Amylase-Laurifolin</td>
<td>-37.54± 0.56</td>
<td>-23.95 ± 1.49</td>
<td>47.34 ± 0.07</td>
<td>-3.66 ± 0.02</td>
<td>-17.82 ± 0.84</td>
</tr>
<tr>
<td>α-Amylase-Galanolactone</td>
<td>-26.83± 0.26</td>
<td>-5.17± 1.41</td>
<td>20.09± 1.39</td>
<td>-3.17± 0.02</td>
<td>-15.07± 0.26</td>
</tr>
</tbody>
</table>

The result shows that vander waals interaction mainly drives the binding (Table 2). Genkwanin (-21.77± 1.03 Kcal/mol) has higher total binding energy compared to laurifolin (-17.82 ± 0.84 Kcal/mol) and galanolactone (-15.07± 0.26 Kcal/mol). Unfavorable contributions from polar solvation energy
are relatively higher in laurifolin (47.34 ± 0.07 Kcal/mol), followed by genkwanin (37.39 ± 0.29 Kcal/mol) and least in galanolactone (20.09 ± 1.39 Kcal/mol).

Drug-likeliness properties and ADMET Screening
ADMET properties of the ligands were analyzed with a Swisssadme server. All the compounds have obeyed the Lipinski rule of 5 for drug-likeliness (Benet, Hosey, Ursu, & Oprea, 2016); they are found to be water-soluble and highly absorbed through GIT. Laurifolin has a molecular weight of 356.37g/mol, 6 hydrogen bond acceptors and 3 hydrogen bond donors, while Genkwanin has a molecular weight of 284.26g/mol, 5 hydrogen bond acceptors and 2 hydrogen donors. Galanolactone has a molecular weight of 318.45g/mol, 3 hydrogen bond acceptors and 0 hydrogen donors. Furthermore, they are found to be relatively safe with minimal side effects.

### Table 3: ADMET Properties of the identified compounds

<table>
<thead>
<tr>
<th></th>
<th>Genkwanin</th>
<th>Laurifolin</th>
<th>Galanolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water solubility</td>
<td>Moderately soluble</td>
<td>Moderately soluble</td>
<td>Moderately soluble</td>
</tr>
<tr>
<td>Blood-brain barrier</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human intestinal absorption</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Drugs likeness (Lipinski)</td>
<td>Yes</td>
<td>yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lead likeness</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>P-gp substrate</td>
<td>No</td>
<td>yes</td>
<td>No</td>
</tr>
<tr>
<td>CYP1A2 inhibitors</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CYP2C19 inhibitors</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CYP2C9 substrate</td>
<td>yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>CYP2D6 substrate</td>
<td>Yes</td>
<td>yes</td>
<td>No</td>
</tr>
<tr>
<td>CYP3A4 inhibitors</td>
<td>yes</td>
<td>yes</td>
<td>No</td>
</tr>
</tbody>
</table>

DISCUSSIONS

α-amylase is an enzyme that is present in pancreatic juice and saliva. It digests carbohydrates and increases the postprandial glucose level in diabetic patients. Inhibiting the activity of the enzyme can control postprandial hypoglycemia (Oyedemi et al., 2017). Several inhibitors of this enzyme with varying efficacy but limited information on their interaction with the enzyme have been reported (Derosa & Maffioli, 2012). In silico molecular docking can provide more insights into understanding their mode of action. Our study revealed that all of the phytochemicals have interacted with the enzyme's active site, highlighting their potential to inhibit α-amylase. They form strong bonds with at least two α-amylase catalytic triads necessary for its catalysis (Pinto et al., 2015). We also found by MM-PBSA studies that they have very high binding energy at the active site; among them, genkwanin (-21.77 ± 1.03 Kcal/mol) tends to bind much stronger, followed by laurifolin (-17.82 ± 0.84 Kcal/mol) and galanolactone (-15.07 ± 0.26 Kcal/mol). ADMET analysis shows that Genkwanin, laurifolin and Galanolactone have obeyed Lipinski rule of 5; water-soluble, highly absorbed through GIT, and not toxic to humans. Furthermore molecular dynamic studies show that the ligands are highly stable at the active site of the enzyme. Antioxidant studies revealed high radical scavenging activity of the A. melegueta extract (0.393mg/ml), which is comparable to the findings of Kazeem et al., (IC50 0.125 mg/mL, using 80% acetone extract) (Kazeem et al., 2012). Numerous studies have revealed the therapeutic potential of genkwanin which is a flavonoid with anti-inflammatory activity, anti-bacterial, anti-plasmodia and radical scavenging activity (Porras, Bacs, Tang, & Quave, 2019), laurifolin is a flavonoid which was previously found in flourensia laurifolia with broad range of bioactivities such as anti-tumor (Kurkin, Sentsov, Zapesochnaya, Braslavskii, & Tolkachev, 1994). While galanolactone is a di-terpenoid lactone that was reported to have an anti-obesity effect through down-regulating adipogenic transcription factors and adipogenic marker genes (Ahn & Oh, 2012). Our findings and the above clarifications indicated that alligator pepper phytochemicals possess significant broad-spectrum medicinal activities and could be explored further by in-vitro studies to develop new effective drugs against diabetes mellitus.

CONCLUSION

Diabetes mellitus remains among the leading health issues around the globe. One of its therapeutic approaches is by inhibiting the digestion of dietary carbohydrates which α-amylase plays an important role. Based on molecular docking, molecular dynamic simulation, MMPBSA and ADMET analysis, we can conclude that Alligator pepper’s laurifolin, genkwanin and galanolactone could strongly bind and inhibit α-amylase activity and thereby control diabetic complications. This study will accelerate the design of new drugs with high clinical value and should be explored further by in-vitro studies to confirm their efficacy.

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Competing Interests: The authors declare no conflict of interest.

REFERENCE