Trop J Nat Prod Res, October 2023; 7(10):4937-4944
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Prediction of Antidiabetic Compounds in *Curcuma longa – In vitro* and *In silico* Investigations

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ARTICLE INFO

Article history:
Received 10 March 2023
Revised 28 September 2023
Accepted 02 October2023
Published online 01 November 2023

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ABSTRACT

Mitigation of postprandial blood glucose and inhibition of carbohydrate-digesting enzymes is an indispensable measure for the treatment or management of type II diabetes mellitus. Medicinal plants due to their diverse bioactive compounds have been reported umpteen times in the management and treatment of diabetes. Hence, the research exploits both *in vitro* and *in silico* methodologies to investigate the antidiabetic capacity of *Curcuma longa* aqueous extract. Its phytochemical components were deduced and quantified in conjunction with its antioxidant potential and inhibitory potential against alpha-amylase and alpha-glucosidase (enzymes indispensable to carbohydrate metabolism) through *in vitro* assay. GC-MS revealed bioactive compounds from Aqueous *Curcuma longa* extract were subjected to ADMET profile, Lipinski rule, and Molecular docking studies. *Curcuma longa* aqueous extract had enormous phenol, flavonoid, and tannin. The extract scavenged DPPH and NO in addition to its inhibitory capacity against alpha-amylase and alpha-glucosidase with IC_{50} values of 93.34ug/ml and 45.23ug/ml respectively. Consensus molecular docking studies revealed stigmasterol and 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid as top-rank hits against alpha-glucosidase. They also proclaimed promising ADMET and bioactive properties in comparison to the standard, acarbose. Consequently, they could be prospective compounds that contribute highly to alpha-glucosidase inhibition as observed in the enzyme assay result. The inhibitory potential of *Curcuma longa* might be due to the strong binding affinity of its bioactive compounds to alpha-glucosidase. Therefore, this research establishes *Curcuma longa* as a functional food for the management of type-2 diabetes while the bioactive compounds especially stigmasterol and 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid could be a nutraceutical for the management of type 2 diabetes.

***Keywords*:**Type 2 diabetes; Curcuma longa; Molecular docking; Consensus scoring

Introduction

Pancreatic alpha-amylase breaks down dietary carbohydrates like starch into disaccharides and simple monosaccharides which are further degraded by the brush border intestinal alpha-glucosidase to glucose which is absorbed into the bloodstream.[1, 2]This results in postprandial hyperglycemia, a condition that is seen in type 2 diabetes. Inhibiting these two enzymes is one of the most powerful anti-diabetic treatment/management approaches. Oral anti-diabetics are powerful alpha-glucosidase blockers that restrict carbohydrate digestion, hence decreasing the impact of carbohydrate breakdown on blood sugar. Acarbose is an example of an alpha-glucosidase inhibitor with adverse effects that make it unpopular among patients and clinicians.[3]However, natural plant bioactive inhibitors found in functional foods such as green tea and touch have been reported to be free of side effects.[4]

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Citation: Adelusi TI, Adeyemi RO, Ashiru MA, Divine UC, Boyenle ID, Oyedele AK, Adewoye IM. Prediction of Antidiabetic Bioactive Compounds in *Curcuma longa – In vitro* and *Insilico* Investigations. Trop J Nat Prod Res. 2023; 7(10):4937-4944. http://www.doi.org/10.26538/tjnpr/v7i10.33.

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Furthermore, the antioxidant properties of several antidiabetic functional foods, as well as their alpha-amylase and alpha-glucosidase inhibitory potential, have been reported, and it has been shown that the majority of these foods are important in the management/treatment of type 2 diabetes.[5-10]However, the bioactive components in these functional meals responsible for their anti-diabetic properties are yet to be identified. These bioactive substances can halt starch absorption, hence preventing postprandial hyperglycemia in type 2 diabetes mellitus.
The general application of *Curcuma longa*, commonly known as Turmeric includes spice in curries (food additive), natural dyes, cosmetics, the rhizome of turmeric is used as a valuable cash crop,[11] and traditional medicine. It has been found to possess countless therapeutic activities ranging from an anti-inflammatory, anti-diabetic, anti-oxidant, anti-hepatotoxic, anti-microbial, anti-depressant to the more recent chemo-preventive, anti-fertility, neuroprotective, HIV-1 and HIV-2 protease inhibitor.[12]It has a long history of traditional use dating back around 4000 years to the Vedic culture in India. During this time, it was utilized both as a culinary spice in Indian cuisine and as a remedy for various ailments. These included enhancing digestion and promoting a healthy intestinal environment, relieving gas and eliminating intestinal worms, supporting liver health, reducing swelling, for local application on sprains, burns, cuts, bruises, insect bites, and itching, for soothing action in cough and asthma and addressing weakness when used internally and externally.[13]
Therefore, in this research, we present a unique contribution by using both *in vitro* enzyme inhibition assays and *in silico* simulation to

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 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

uncover the bioactive phytochemical constituents of *Curcuma longa* (turmeric) that exhibit formidable affinity for the active pockets of the key enzyme associated with diabetes type 2 as this might serve as the rationale underlying the *Curcuma longa*'s antidiabetic potential.

Materials and Methods

Experimental methodologies
Plant source
Curcuma longa (Turmeric) fresh plant materials were bought on August 2, 2022, from the market for traditional medicine items in Ogbomoso, Oyo, Nigeria. The rhizome was validated by a taxonomist (Dr. Nalza George) at the University of Lagos with the voucher specimen number LUH/8967.

Extraction
The turmeric rhizome that was collected was air-dried. 5g of pulverized rhizome was weighed and dissolved in 200ml of distilled water (the mixture was vigorously stirred for 24h). The mixture was decanted after 24 hours, and the supernatant was filtered using Whatman filter paper No.1. The extract's remaining solvent was dried in a 40°C oven (Gallenhamp, England). The extract was put in sterile sample vials and stored at 4^{0}C for subsequent analysis.

Phytochemical Analysis
The presence or lack of certain phytochemicals in the rhizome aqueous extract was investigated using conventional phytochemical screening techniques.[14]

DPPH scavenging activity
The rhizome aqueous extract's free radical scavenging capability was measured using a modified approach of Manzocco and his colleagues.[15]

Nitric oxide scavenging activity
The rhizome extract's nitric oxide scavenging capacity was measured using the Garrat technique (1964).[16]

Alpha-amylase inhibition assay
The Bernfield approach was used to assess alpha-amylase inhibitory activity (1951).[17]The phenolic extracts (0-200µL) and (0-250µL) of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing porcine pancreatic alpha-amylase (EC 3.2.1.1) (0.5mg/mL) were incubated at 25°C for 10 minutes. After that, 50µL of 1% starch solution in 0.02M sodium phosphate buffer was added to the reaction mixture. The reaction mixture was then incubated for 10 minutes in a water bath at 25°C before being cooled to 23ºC. A total of 200µL of dinitrosalicylic acid (DNSA) was added. The process was then stopped by incubating it for 5 minutes in a 100°C water bath before cooling to room temperature (23ºC). After diluting the reaction mixture with 2mL of distilled water, absorbance at 540nm was measured with a spectrophotometer. The reference sample comprised all other reagents and the enzyme, except the test sample.
The alpha-amylase inhibitory effect was expressed as a percentage of inhibition.

$$Inhibition\ (\%) = \left[\frac{ABSref - ABSsample}{ABSref}\right] * 100$$

Where ABS_{ref} = absorbance of the reference; ABS_{sample} = absorbance of the test sample.

Alpha-glucosidase Inhibition Assay
Dahlqvist's standard technique was used to determine alpha-glucosidase inhibitory activity (1964).[18] Appropriate dilution of phenolic extracts (20 – 100µL), alpha-glucosidase solution (20 µL), (130 - 250µL) 0.1M phosphate buffer at pH 7.0 and 50µL at 37mM were incubated for 10 minutes at 25^{0}C before boiling for 5 minutes at 100°C in a water bath. Each test tube received 20µL of the combination (sample, buffer, enzyme, and maltose), and 2mL of GOD-PAP (glucose oxidase-phenol and 4-aminophenazone) was added. At 450nm, the absorbance was measured. Except the test sample, the reference sample contained all other reagents and the enzyme.
The inhibitory activity of alpha-glucosidase was represented as a percentage of inhibition.

$$Inhibition\ (\%) = \left[\frac{ABSref - ABSsample}{ABSref}\right] * 100$$

Where ABS_{ref}= absorbance of the reference; ABS_{sample}= absorbance of the test sample.

GC-MS Analysis
The rhizome combination's GC-MS analysis was performed using a slightly modified technique published by Hadiand his co-authors (2016).[19]

Computational methodologies
Preparation and Active Site Identification of alpha-*glucosidase and molecular docking protocol*
The target protein for this investigation was alpha-glucosidase protein (PDB ID: 2QMJ). The target protein's X-ray crystallographic Protein Data Bank (PDB) (https://www.rcsb.org/) structure (PDB ID: 2QMJ) was downloaded and treated appropriately utilizing BIOVIA Discovery Studio Software version 19.1 (http://www.accelrys.com) to circumvent unintended molecular interactions in the course of virtual screening. The active sites of the target receptors were identified using the Computed Atlas for Surface Topology of Proteins (CASTp) webserver platform.[20]Using the technique used in previous research; we docked into the active region of this protein with the aid of IGEMDOCK, PyRx, and Autodock Vina.[21-24]

Preparation of Ligands
Bioactive compounds of the aqueous extract detected using GC-MS were gotten from a public database: their SMILES (Simplified Molecular-Input Line-Entry System) format was obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/); an open chemistry database of compounds, substances, and biological assays.[25]The canonical SMILES were translated to 3D coordinates in PDB format utilizing the cactus online SMILES translator website https://cactus.nci.nih.gov/translate/.
2.2.3 Pharmacokinetics and Drug-likeness Prediction
The pharmacokinetic properties of these bioactive compounds which include Adsorption, Distribution, Metabolism, Excretion, and Toxicity were executed, and drug-likeness characteristics of these compounds were evaluated through the Molinspiration platform (https://www.molinspiration.com) and admetSAR web server (http://lmmd.ecust.edu.cn/admetsar2/).

Consensus Scoring
Easydock, AutodockVina, and iGEM DOCK were used to perform molecular docking studies on the sixteen compounds derived from the GC-MS results of the rhizome combination. The acquired result was renormalized to eliminate the influence of a too-big and too-small number, the resultant values were stratified for each software, and compounds that scored high in all three software were labeled as hits and subjected to additional computational investigations.

Statistical analysis
All statistical analyses were performed using SPSS v. 27.

Results and Discussion

For the very first time, we might have discovered the antidiabetic bioactive compounds in turmeric (*Curcuma longa*) using both *in vitro* and *in silico* methodologies. Firstly, we used the aqueous extract of turmeric to evaluate its total phenolic and flavonoid contents after which we estimated its antioxidant potential using different protocols. We further evaluated its type 2 antidiabetic potential by inhibiting the key enzymes related to carbohydrate digestion (alpha-amylase and alpha-glucosidase) after which the GC/MS analysis was executed to access the library of bioactive ingredients in this functional food. These bioactive compounds were subjected to various computational analyses which include physicochemical properties determination using the

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Lipinski rule of 5 (RO5), pharmacokinetic properties assessment using ADMET (Adsorption, Distribution, Metabolism, Excretion, and Toxicity) profiling methodologies on ADMETSar2 web server, consensus molecular docking approaches using various software (Autodockvina, Samson and IGEMdock) and Fragment Based Drug Interaction Analysis (FBDIA) coupled with amino acid residues analysis using Discovery Studio visualizing tool. Our utmost aim here spans beyond the assessment of the antidiabetic potential of *Curcuma longa* but, to critically identify the specific bioactive compounds with the best binding affinity to alpha-glucosidase in *Curcuma longa* responsible for this antidiabetic capacity to suggest or propose their development as supplements or nutraceuticals for the treatment/management of type II diabetes. Furthermore, we delve to detect the fragments of these bioactive compounds interacting with the active pockets of these key enzymes to propose the characters or nature of other compounds that might emerge as better antidiabetic therapeutic measures.

Phytochemical screening

Phytochemical screening involves the identification and quantification of medicinally active compounds present in plants that can contribute to the discovery of new pharmacological agents for drug discovery.[26]Table 1 indicates that the aqueous extract of *Curcuma longa* is rich in phenol, flavonoids, alkaloids, saponin, terpenoids, tannins, and reducing sugar. Although, its phenolic content was high, accompanied by flavonoid then tannin with (110.5 ± 6.13 mg/100g), (53.18 ± 2.37 mg/100g), and (80.38 ± 4.45 mg/100g) respectively. A phytochemical screening investigation conducted on *Curcuma longa* was portrayed to have high phenolic content (Table 1). Phytochemical components have been acclaimed with antidiabetic potential.[27-28]

vitro inhibition of carbohydrate metabolizing enzymes

The effect of turmeric aqueous extract on alpha-glucosidase and alpha-amylase is depicted in Figure 1. Inhibition of these enzymes occurs dose-dependently. Turmeric aqueous extract inhibited alpha-glucosidase with an IC_{50} value of 45.23ug/ml. In contrast, the standard had an IC_{50} value of 53.76ug/ml, as a consequence, the extract was revealed to be as potent as the standard acarbose in inhibiting alpha-glucosidase. Contrastingly, the aqueous extract inhibited alpha-amylase with an IC_{50} value of 93.34ug/ml while that of the standard was 93.65ug/ml, indicating that the extract is as potent as the standard in inhibiting the enzyme. Summarily, the aqueous extract of *Curcuma longa's* efficacy in inhibiting carbohydrate metabolizing enzymes might be an indication that it can be a substitute in glucose-lowering therapy for type II DM. Postprandial blood glucose can be mitigated by the inhibition of carbohydrate metabolizing enzymes.[29]

Table 1: Qualitative and Quantitative phytochemical screening of *Curcuma longa* aqueous extract

Phyto-chemicals	Qualitative	Quantitative (mg/100g)
Flavonoid	+	53.18 ± 2.37
Phenol	+	110.5 ± 6.13
Saponin	+	40.93 ± 0.47
Tannin	+	80.38 ± 4.45
Steroid	+	34.51 ± 1.97
Terpernoid	+	20.22 ± 0.24
Alkaloid	+	35.44 ± 0.31
Reducing sugar	+	30.94 ± 0.71
Phlobatanin	-	-

Key: "+" = Present, "-" = Absent

In vitro Antioxidant Assay

DPPH radical scavenging activity

Medicinal plants possess bioactive chemicals that have several medicinal qualities, including the ability to neutralize free radicals.[30] Oxidative stress is an essential upstream event in the development of diabetic complications.[31] As a result, in the therapy of diabetes mellitus, plants with antidiabetic and antioxidant characteristics should be targeted to halt the progression of diabetes mellitus. Turmeric aqueous extract scavenged free radicals in a dose-dependent manner (Figure 2) which can be attributed back to its high phenolic content as revealed by phytochemical screening (Table 1). The aqueous extract had an $IC_{50}$of 65.28 µg/ml and a maximum scavenging activity of 100ug/ml, whereas ascorbic acid had an IC_{50} of 55.75µg/ml and a maximum scavenging activity of 100µg/ml (Table 2). The DPPH test is used to determine an extract's antioxidant activity.[32] Phenols are molecules that consist of an aromatic cyclic nucleus coupled to a hydroxyl group and are essential in the removal of free radicals.[33]The aqueous extract's extraordinary scavenging effect can be attributed to its high phenolic content, as revealed by phytochemical screening (Table 1).

Nitric oxide (NO) activity

NO radical is associated with a variety of biological activities, including neuronal messenger, vasodilator, and effector molecule.[34] However, it combines with O_2 radical to produce peroxynitrite radicals (ONOO-), a hazardous chemical to biomolecules like lipids, nucleic acids, and proteins.[35] The IC_{50} of turmeric aqueous extract was 55.47g/ml, while the standard (ascorbic acid) was 62.85g/ml. Figure 3 shows that the extract is more effective in lowering NO activity.

Gas Chromatography-Mass Spectroscopy (GC/MS)

Innumerable biological research has used gas chromatography-mass spectrometry as an analytical tool for qualitative study of volatile and semi-volatile substances.[36-37]. The presence of sixteen bioactive chemicals identified by GC-MS analysis may be responsible for the plant extract's biological activities. Table 4 shows the bioactive chemicals in ascending order of peak area.

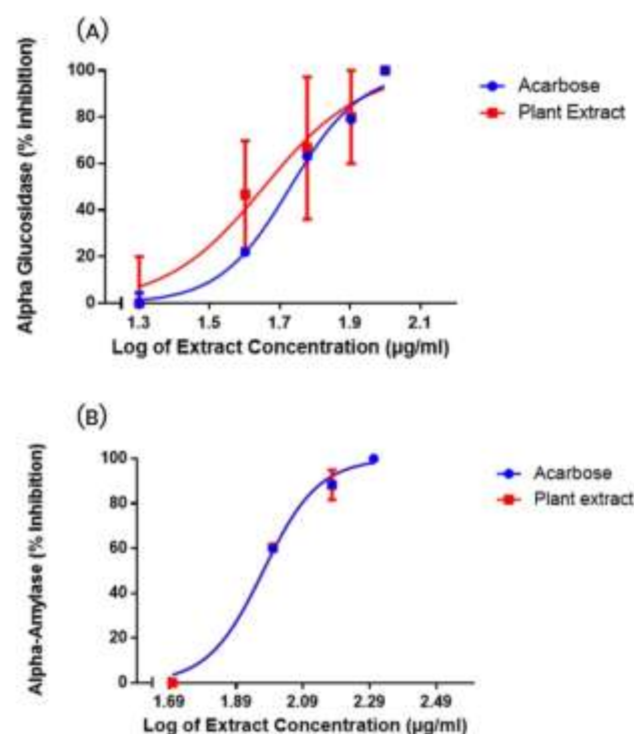

Figure 1: Inhibition of carbohydrate metabolizing enzymes

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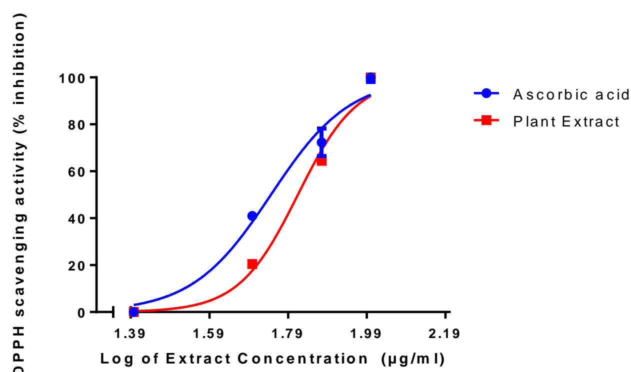

Figure 2: DPPH free radical scavenging activity of the aqueous extract of *Curcuma longa*

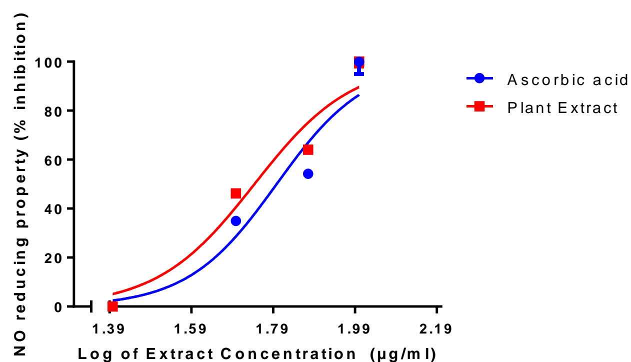

Figure 3: Reducing power of the aqueous extract of *Curcuma longa*

Table 2: IC_{50} values of DPPH scavenging activity of aqueous turmeric extract

Sample (µg/ml)	IC_{50}
Aqueous plant extract	65.28
Ascorbic acid (standard)	55.75

Table 3: IC50 values of NO reducing property of aqueous turmeric extract

Sample (µg/ml)	IC_{50}
Aqueous plant extract	55.47
Ascorbic acid (standard)	62.85

Molecular Docking Study

Bioactive compounds from *Curcuma longa* GC/MS fingerprint were docked to the active site of alpha-glucosidase to deduce the bioactive component accountable for its antidiabetic potential. Molecular docking enhances the prediction of molecular interaction that keeps ligands and proteins of known three-dimensional structure in the bound state.[38]However, a major complication in computational-based drug discovery is excessive false positive errors which are inclined towards utilizing a single docking model.[39] In lieu, researchers discovered that the amalgamation of software algorithms has subjugated this fizzle.[40]iGEMDOCK, AutodockVina, and Easydock were employed in this study. The results from the three software were compared based on their rank (as shown in Figure 4) rather than raw scores since the former enhances performance more than the.[41].

Table 4: Prime phytochemical compounds identified in the aqueous extract of the *Curcuma longa*

S/N	Compound	Area %	Molecular Weight (g/mol)	Molecular Formula
1	PIPERIDIN-4-OL	0.010	101.15	$C_5H_{11}NO$
2	STIGMASTEROL	0.018	412.7	$C_{29}H_{48}O$
3	PHENTOLAMINE	0.034	381.35	$C_{17}H_{19}N_3O$
4	2-BROMO-4,5-DIMETHOXYCINNAMIC ACID	0.035	287.11	$C_{11}H_{11}BrO_4$
5	2,1-BENZOXAZOLE-4-CARBOXYLIC ACID	0.038	163.13	$C_8H_5NO_3$
6	2-[4-(1-ETHYL-3-METHYL-1H-PYRAZOL- 4-YL)-4-OXOBUT-2-ENAMIDO]BENZOIC ACID	0.100	327.33	$C_{17}H_{17}N_3O_4$
7	4-AMINO BENZOIC ACID	0.102	137.14	$C_7H_7NO_2$
8	4-FLUORO-BENZYL ALCOHOL	0.122	206.26	$C_{13}H_{15}FO$
9	2-FURANCARBOXYLIC ACID	0.124	112.08	$C_5H_4O_3$
10	CHOLESTANE-3,6,7,8,15,16,26-HEPTOL	0.190	484.7	$C_{27}H_{48}O_7$
11	BENZENEACETALALDEHYDE	0.213	134.17	$C_9H_{10}O$
12	BENZOIC ACID	0.215	122.12	$C_7H_6O_2$
13	3-BROMO-4-HYDROXY-2,3'-DIMETHYL-5,5',8,8'-TETRAMETHOXY-1,2'-BINAPHTHALENE-1',4'-DIONE	0.222	527.4	$C_{26}H_{23}BrO_7$
14	P-CRESOL	0.381	108.14	C_7H_8O
15	P-TOLUIC ACID	0.488	136.15	$C_8H_8O_2$
16	2,5-DIETHYLPHENOL	0.722	150.22	$C_{10}H_{14}O$

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

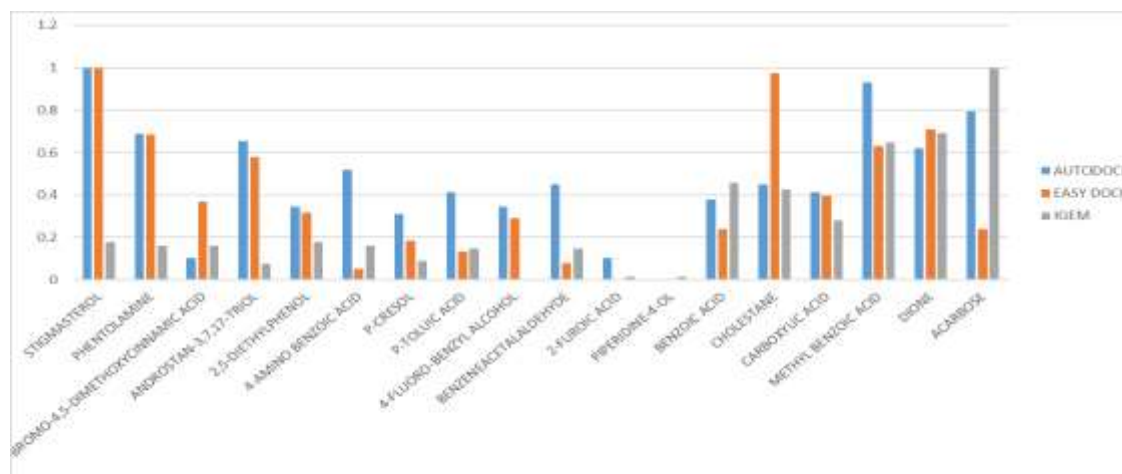

Figure 4: The Bar chart showing the scored 16 ligands and the standard acarbose in iGEMDOCK, AutodockVina, and Easydock.

Table 5: Drug-likeness Evaluation of the selected hits and standard Acarbose

Ligand	Molecular weight	MiLogP	nHBA	nHBD	nViolation
Stigmasterol	412.70	7.87	1	1	1
2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid	291.37	1.80	7	2	0
Acarbose	645.61	-8.56	14	19	4

Note: miLogP: Octanol-water partition coefficient nHBA: number of Hydrogen bond acceptor; nHBD: number of Hydrogen bond donors; nViolation: number of Violation.

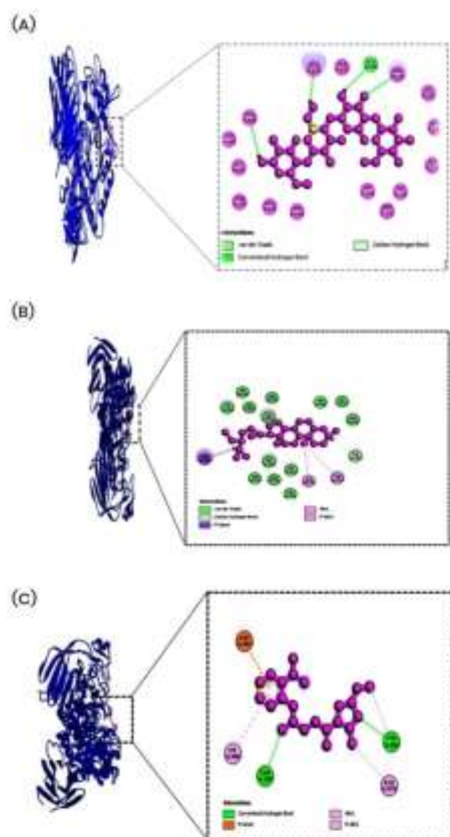

Figure 5: Molecular interaction between the three selected hits, the standard at the active site of alpha-glucosidase (A) Acarbose (B) Stigmasterol (C) 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid. The structures were rendered using Discovery Studio 2019.

Based on this criteria, stigmasterol and 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid were selected as prospective hits that instigate antidiabetic prowess of *Curcuma longa*. Stigmasterol emerged first in both Autodockvina and Easydock but sixth in IGEMdock, thus regarded as the top hit in this study. 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid emerged second in both IGEMdock and AutodockVina but fifth in Easydock. Remarkably, stigmasterol and 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid emerged before the standard acarbose, portraying that they both might be top binders of alpha-glucosidase in comparison to acarbose. These compounds had hydrogen interaction, pi interaction, and Vander Waal force with amino acids in the active pocket of alpha-glucosidase (Figure 5). Relevant amino acid residues are indispensable for co-crystallized ligand binding should be greatly considered during virtual screening.[42] Asp 203, Phe450, Trp406, and Phe575 are the residues the hits made interactions with the enzyme. As a consequence, these hits might alter the hydrolysis of disaccharides by accurately binding to the active site of alpha-glucosidase. Stigmasterol, an unsaturated phytosterol in combination with omega 3 fatty acid has blood sugar-reducing properties in addition to amelioration of insulin resistance in IGR (Impaired Glucose Regulation) patients.[43]

ADMETox Profiling and Drug Likeness

For a bioactive compound to be considered as a prospective clinical candidate, it must meet almost all the drug-likeness and pharmacokinetics properties.[44]Stigmasterol and 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido] benzoic acid were subjected to Lipinski rule and admetSAR. The former is for the evaluation of drug likeness and the latter for the determination of pharmacokinetic and toxicological endpoints. An orally bioavailable drug candidate should not violate more than one of the rules of five (molecular weight < 500, HBA < 10, HBD < 5, Logp< 5).[45] Both stigmasterol and 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid passed Lipinski although stigmasterol violated

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one Lipinski rule which is also a pass (Table 5). They both manifest better efficacy in comparison to acarbose which violated three of the rules. Both hits exhibit excellent ADME properties as revealed by admetSAR 2.0 (Table 6). Inhibitors of cytochrome P450 family enzymes engender numerous drug-to-drug interactions.[46]Admiringly, both hits portray not be inhibitors and substrates of the cytochrome P450 families. Drug candidate for alpha-glucosidase, an intestinal enzyme should not exhibit human intestinal absorption (HIA) property to prevent it from eluding its target. This study proclaimed that stigmasterol lacked HIA potential, although 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid appeared to be intestinally absorbed by humans. Both hits in addition to acarbose appeared non-carcinogenic. Although 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid and the standard acarbose are liable to be hepatotoxic, stigmasterol manifested to be non-hepatotoxic. Inhibition of hERG is potentiated towards QT prolongation and peradventure fatal cardiac arrhythmia.[47]Stigmasterol and acarbose revealed an affinity for hERG channel while 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid lacked affinity for it. Ames toxic candidates can induce mutations in DNA.[48]Both hits lacked ames toxicity, although acarbose was unveiled to be ames toxic. In lieu of these parameters, stigmasterol and 2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido] benzoic acid divulge to be better candidates considered for pharmacological study in comparison to the standard acarbose.

Conclusion

Aqueous extract of *Curcuma longa* inhibited pancreatic alpha-amylase and intestinal alpha-glucosidase, the key enzymes required for postprandial blood glucose management, which can be attributed to its extraordinary phenolic compound combined with terpenoid, alkaloid, and free radical scavenging activities. Molecular docking experiments demonstrated that stigmasterol and 2-[4-(1-Ethyl-3-methyl-1H-pyrazol-4-yl)-4-oxobut-2-enamido]benzoic acid exhibited a great inhibitory capacity against alpha-glucosidase when compared to ordinary acarbose. This study discovered that *Curcuma longa* possesses antidiabetic effects as well as the ability to alleviate oxidative stress-induced illness. As a consequence, they might be promising compounds that contribute significantly to alpha-glucosidase inhibition, as demonstrated by the enzyme assay results. *Curcuma longa*'s inhibitory effect may be owing to the high binding affinity of its bioactive components for alpha-glucosidase. Further clinical trials for the treatment/management of type 2 diabetes mellitus might be conducted with stigmasterol, a phytosterol, and 2-[4-(1-Ethyl-3-methyl-1H-pyrazol-4-yl)-4-oxobut-2-enamido]benzoic acid.

Table 6: ADMETox profiling of selected hits in comparison with standard acarbose

ADMET properties	**Stigmasterol**	**2-[4-(1-Ethyl-3-methyl-1H-pyrazol- 4-yl)-4-oxobut-2-enamido]benzoic acid**	**Acarbose**
Blood-brain-barrier	+	+	-
Human Intestinal Absorption	-	+	-
Caco-2	+	-	-
CYP2C19 Inhibition	-	-	-
CYP1A2 Inhibition	-	-	-
CYP3A4 Inhibition	-	-	-
CYP3A4 substrate	+	-	-
CYP2C9 Inhibition	-	-	-
CYP2C9 substrate	-	-	-
CYP2D6 Inhibition	-	-	-
CYP2D6 substrate	-	-	-
AMES toxicity	-	-	+
Acute Oral Toxicity	I	III	IV
CYP inhibitory promiscuity	-	-	-
Human either-a-go-go inhibition	+	-	+
Human oral bioavailability	-	+	-
Carcinogenicity (binary)	-	-	-
Hepatotoxicity	-	+	+

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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