Informatics in Medicine Unlocked 36 (2023) 101148

Contents lists available at ScienceDirect

Informatics in Medicine Unlocked

journal homepage: www.elsevier.com/locate/imu

In vitro and in-silico inhibitory validation of *Tapinanthus cordifolius* leaf extract on alpha-amylase in the management of type 2 diabetes

Amarachi Chike-Ekwughe [a,*], Abayomi Emmanuel Adegboyega [b,c], Titilayo Omolara Johnson [b,c], Abiodun Humphrey Adebayo [a], Olubanke Olujoke Ogunlana [a]

[a] Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria
[b] Department of Biochemistry, Faculty of Basic Medical Science, College of Health Sciences, University of Jos, Jos, Nigeria
[c] Bioinformatics Unit, Jaris Computational Biology Centre, Jos, Nigeria

ARTICLE INFO

Keywords:
Tapinanthus cordifolius
Hyperglycemia
Alpha amylase
Computational methods
Molecular docking
Benzaldehyde
4-(Ethylthio)-2
5-Dimethoxy

ABSTRACT

Tapinanthus cordifolius an African mistletoe is an important medicinal plant that has been shown to lower postprandial hyperglycemia. It has been proposed that mistletoe's hypoglycemic effects are related to its ability to lower blood glucose levels and that the anti-diabetic activity of some of these parasitic plants might be due to their ability to block the action of alpha-amylase. To identify prospective alpha-amylase inhibitors for anti-diabetic drug discovery, the current study used *in vitro* experiments and computational methods such as molecular docking, pharmacophore modelling, and ADMET profiling to evaluate the alpha-amylase inhibitory ability of *Tapinanthus cordifolius* leaf extracts and its bioactive components. The crude extract of the plant showed the highest inhibiting activity with an IC_{50} value of 26.88 μg/ml. Gas chromatography-mass spectroscopic analysis of the extract gave 43 phytochemicals, including sesquiterpenes, diterpenes, triterpenes and their derivatives, phytosterols, and tocopherols. The molecular docking analysis of these compounds with alpha-amylase identified benzaldehyde, 4-(Ethylthio)-2,5-dimethoxy, alpha-Tocopherol-Beta-D-Mannoside, 5-ergosterol, 3,4,5-trimethoxybenzoic acid, and acetosyringone as the five top-scoring compounds. Their binding energies which ranged from −4.944 to −4.365 kcal/mol, were close to that of the reference compound, which was −5.67 kcal/mol. Like the reference ligand, these compounds interacted with crucial active site amino acid residues of alpha-amylase. They also possess favorable drug-like properties as well as a low-risk profile. Hence, these compounds could be subjected to lead optimization and experimental studies for further development into novel drugs for managing type 2 diabetes mellitus.

1. Introduction

Diabetes mellitus is a collection of metabolic diseases characterized by hyperglycemia caused by insufficient insulin secretion, insulin function, or both. It is distinguished by hyperglycemia and persistent vascular dysfunction [1]. Damage to the cardiovascular system, blood vessels, eyes, kidneys, and nervous system can develop slowly over time [2]. Sustained hyperglycaemia can lead to stupor, coma and perhaps death, often due to ketoacidosis or in rare cases, nonketotic hyperosmolar syndrome [3]. Diabetes is one of the leading causes of mortality across the world [4], and ranked ninth on the list [5]. Type 2 diabetes is the most prevalent form, and it is caused by either insulin resistance or an insufficient insulin supply. Type 2 diabetes has shown a dramatic increase in frequency over the past three decades in countries of all income levels. Formerly known as juvenile diabetes or insulin-dependent diabetes, type 1 diabetes is a chronic illness in which the pancreas generates little or no insulin on its own. In order to survive, persons with diabetes need ready access to affordable treatment options and there is a global agreement to prevent the increase of diabetes and obesity by 2025 [2]. Diabetes affects about 422 million people worldwide, mostly in low- and middle-income countries, and is directly responsible for 1.5 million deaths annually. The incidence and prevalence of diabetes have both skyrocketed in recent decades [2].

Diabetes is currently treated with insulin and other oral antidiabetic medications such as sulfonylureas, biguanides, and glinides. Many existing antidiabetic medicines however have a wide range of serious adverse effects, making the quest for alternatives a crucial area of study [6,7]. Reducing postprandial hyperglycemia is one of the most

* Corresponding author.
E-mail address: Amarachi.agbafor@mouau.edu.ng (A. Chike-Ekwughe).

https://doi.org/10.1016/j.imu.2022.101148
Received 10 October 2022; Received in revised form 23 November 2022; Accepted 7 December 2022
Available online 22 December 2022

important treatment techniques for managing diabetes. By inhibiting carbohydrate-hydrolyzing enzymes, such as alpha-amylase, glucose absorption can be restricted. The α-amylase, which may be found in microbes, plants, and higher animals, is one of the principal secretions of the salivary glands and pancreas and aids in the digestion of starch and glycogen. This enzyme catalyzes the first stage in starch hydrolysis, resulting in a combination of oligosaccharides that includes maltose, malt triose and oligosaccharides with branched glucose units at both the 1,4 and 1,6 levels [1]. Therefore, the amylase enzyme breaks down the huge, complicated starch molecule into simpler sugar molecules. When starch begins to break down into simpler sugar units after a carbohydrate meal, blood glucose levels rise. The α-amylase activity therefore contributes to an increase in glucose levels after meals [8]. The inhibition of α-amylase can considerably minimize the post-prandial rise in blood glucose and is therefore an effective strategy for controlling blood glucose levels in type 2 diabetic and borderline individuals [8,9].

Many drugs for managing type 2 diabetes work by blocking the action of alpha-amylase. Such medications include acarbose and miglitol. However, they may be accompanied with adverse effects such as flatulence, diarrhea, bloating, and abdominal discomfort [10]. As a result, the search for a therapeutic intervention against type 2 diabetes has persisted, with a particular emphasis on plant-based remedies due to their comparative affordability. There is a resurgence of interest in plant-based medications and functional foods that modulate physiological effects for the treatment and prevention of diabetes and obesity. Various researches have shown the anti-hyperglycaemic abilities of medicinal plants, making them a rich source of anti-diabetic agents [11]. One of such plant is *Tapinanthus cordifolius*.

Tapinanthus cordifolius is a plant belonging to the family loranthaceae, which have been shown to have activity against various ailments, including diabetes [12]. *Tapinanthus cordifolius* is a species of African mistletoe of the genus Tapinanthus [13,14]. Mistletoes are parasitic, evergreen tropical plants characterized by their woody shoots [15,16]. They grow on a range of native trees in West Africa, as well as economically significant tree crops such as shea butter, neem, cocoa, and rubber. Mistletoe is common in the south-western region of Nigeria, especially on tree crops including orange, guava, cocoa, kola, coffee, bush mango, and others. This medicinal plant is used to cure a wide range of illnesses, including stomach aches, diarrhea, dysentery, wounds, and cancer (6, 7). It has been established that mistletoe has hypoglycemic effects due to its capacity to reduce blood glucose levels and regulate weight loss in diabetics [12,17]. Oboh et al. (2018) investigated the mechanism of anti-diabetic activity of extracts of mistletoe (Loranthus begwensis L.) and discovered an in vitro inhibiting effect of the plant on some carbohydrate-metabolizing enzymes, including alpha amylase. They suggested that the plants' antidiabetic action is due to their suppression of these enzymes [18]. The inhibitory effects of antidiabetic plants on alpha amylase and other therapeutic targets is said to be due to the presence of some bioactive compounds, which could be employed as potential agents towards the development of effective drugs for the treatment of type II diabetes mellites [19]. In order to explore the hypoglycemic potential of this plant in the development to drugs for the treatment of diabetes mellitus, it is necessary to identify possible inhibitors of alpha amylase in the mistletoes. This study was initiated to assess the inhibitory potency of *Tapinanthus cordifolius* leaf extracts and its bioactive compounds against alpha amylase through in vitro and *in silico* studies towards the identification of therapeutic agents for the management of type 2 diabetes mellitus.

2. Materials and methods

2.1. Plant collection and preparation of crude extract

Tapinanthus cordifolius plants were gathered from a *citrus sinensis* (Orange) tree in Nibo, Awka South, Anambra State, Nigeria. After being thoroughly cleaned and air-dried at room temperature (25 °C) for four weeks, they were pulverized using a mortar and pestle. Subsequently, 2.8 kg of *Tapinanthus cordifolius* powder was macerated in 22 L of ethanol (85%) for 72 h. The extract was filtered using mucilin cloth and concentrated at 78 °C with a rotary evaporator. The extracts were stored in an airtight glass jar at a temperature of 4 °C in the refrigerator [20].

2.2. Differential solvent fractionation process

The ethanolic crude extract of TC (275.3 g) was suspended in I L of distilled water and partitioned in sequence with n-hexane, ethyl acetate and n-butanol using a separating funnel. A rotary evaporator was used to concentrate the different portions to obtain the fractions.

2.3. In vitro determination of alpha-amylase inhibition activity

In 100 mM phosphate buffer (pH 6.8), 250 L of each fraction or acarbose at varied doses (0.2–1.0 mg/ml) were incubated with 500 L of porcine pancreatic amylase (2 U/mL) for 20 min at 37 °C. The reaction mixture was supplemented with 250 L of a 1% starch solution (dissolved in 100 mM phosphate buffer (pH 6.8)) and then incubated at 37 °C for 1 h. The mixture was brought to a boil for 10 min after 1 mL of DNS color reagent was added. The resultant solution's inhibitory activity was quantified as a percentage of the control without inhibitors, and its absorbance was measured at 540 nm.

2.4. Gas chromatography-mass spectroscopic (GC-MS) analysis

The chemical components of TC were analyzed by GCMS utilizing a Shimadzu QP-2010 GC equipped with a QP-2010 Mass Selective Detector [MSD, operating in the EI mode (electron energy = 70 eV), a scan range of 45–700 amu, and a scan rate of 3.99 scans/sec]. The GC column was an Optima-5 ms fused silica capillary, 30 m in length, 0.25 mm in internal diameter, and 0.01 μm in film thickness, and it included a 5% phenyl-methyl polysiloxane stationary phase. Helium was used as the carrier gas, and the rate of flow was 1.61 ml/min. The gas chromatography oven was set to 60° Celsius and maintained there for 1 min. The temperature was then raised from 60° to 260° Celsius at a rate of 14° Celsius per minute. Detector temperatures were 290° Celsius, whereas the injection port temperature was 250° Celsius. The sample was prepared by dissolving 1 g (g) in 100 mL (mL) of ethanol; then, 1.0 L of this solution was injected into an auto sampler operating in splitless mode. The mass spectra of the individual components were compared to those of recognized chemicals in the NIST Mass Spectral Library, allowing for their identification (NIST 11). Without standardization, the percentages of each component were presented as raw percentages depending on the total ion current [21].

2.5. In silico analysis of Alpha-amylase inhibition potential

2.5.1. Molecular docking

As previously described by Johnson et al. [22], the molecular docking analysis was carried out on Schrodinger Maestro 12.8. The x-ray diffraction crystal structure of pancreatic alpha-amylase with PDB ID: 2QV4 (Resolution: 1.97 Å, R-Value Free: 0.224 and R-Value Work: 0.176) was retrieved from the repository of Protein Data Bank (PDB). The protein preparation wizard panel of Glide was used to prepare the protein for docking. The co-crystallized ligand (QV4) was used in defining the receptor grid. The 43 bioactive compounds obtained from TC during the GCMS analysis and the co-crystallized ligand were further prepared for docking using the ligprep module. The Glide tool was used in carrying out the docking study.

2.5.2. Receptor-ligand complex pharmacophore modelling

PHASE was utilized in the generation of a pharmacophore model of the receptor-ligand complex [22]. Pharmacophore models were generated for the protein in complex with the top five compounds chosen by

the criteria of their binding affinity to the protein. The auto (E-pharmacophore) tool was employed, leaving the maximum number of features capable of forming set at 7. Also, 2 and 4 were set for the minimum distance between features and the minimum distance between features of the same kind respectively. In this process donors were employed as vectors.

2.5.3. Induced fit docking (IFD)

Following the Schrödinger methodology as previously described [22], the molecular interaction between the α-amylase and the top-scoring compound was explored using Maestro 12.5's induced fit docking panel. By assuming the receptor would change significantly in response to the ligand's presence, the Induced Fit Docking approach seeks to optimize ligand docking. First, the receptor is minimized under constraints, and then the softening potential is used to dock the ligands with a glide. The docked poses were divided into 20 groups and sent on to Prime for further refining. After prime side-chain prediction and minimization, the optimal receptor structures for each ligand were sent back to Glide for redocking. Using information on solvent-accessible surface areas, salt bridges, B-factors, and rotamer searches, the extended sampling method automatically constructs receptors by choosing which residues to trim and determining van der Waals scaling factors for individual atoms. The first docking stage provides a massive number of poses, which are subsequently collected and filtered to yield up to 80 postures per ligand, which are then sent on to the prime step. Scores for the last docked poses were computed using the Glide SP.

2.5.4. Pharmacology parameters

Integrated model predictions at the Swiss ADME and PROTOX-II servers were used to analyze the ADMET characteristics of the test compounds. ADMET is an acronym that stands for absorption, distribution, metabolism, and toxicity [23,24].

3. Results

3.1. Alpha-amylase inhibiting activity

The percentage α-amylase inhibiting activity and IC_{50} values of the extract and standard drug are displayed in Figs. 1 and 2 respectively. Acarbose exhibited inhibitory effects ranging from 80.55 to 80.73% at 0.2 mg/ml to 1.0 mg/ml respectively, with an IC_{50} value of 15.38 μg/ml. Among the test samples, the crude extract showed the highest inhibiting activity which was between 39.26% and 47.63% (IC_{50}: 26.88 μg/ml), that of the butanol fraction was between 24.50% and 48.76% (IC_{50}: 31.72 μg/ml), that of ethyl acetate was between 23.62% and 29.50% (IC_{50}: 50.75 μg/ml), that of n-hexane between 33.42% and 34.85% (IC_{50}: 46.95 μg/ml), and that of the aqueous extract between 29.68% and 43.04% (IC_{50}: 43.35 μg/ml).

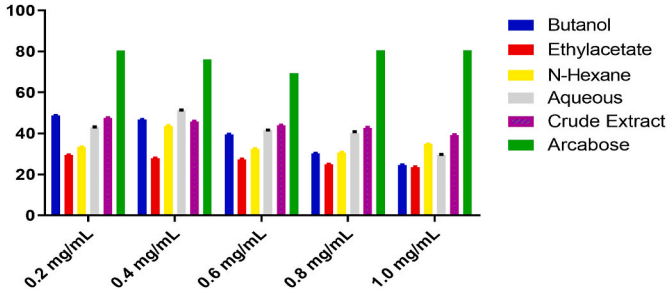

Fig. 1. Percentage inhibition of alpha-amylase activity by the extract and fractions of *Tapinanthus cordifolius*.

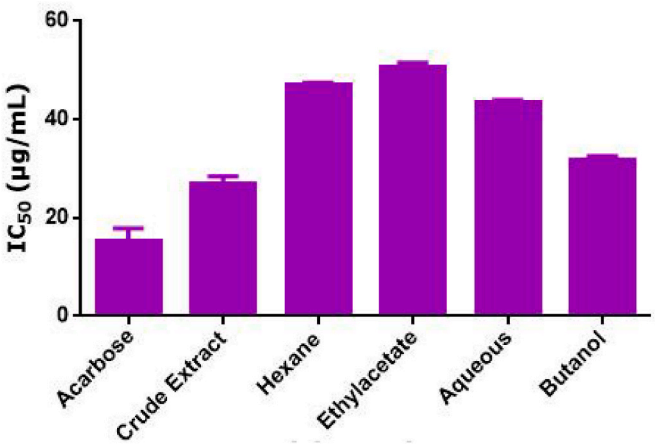

Fig. 2. IC_{50} values of extract and fractions of *Tapinanthus cordifolius* in the inhibition of alpha-amylase activity.

3.2. Gas chromatography-mass spectroscopic (GC-MS) analysis

The GC-MS chromatogram of *T. cordifolius* compounds is shown in Fig. 3. 48 peaks corresponding to 43 different chemicals were detected during the analysis. The chemical profile of the bioactive compounds is shown in Table 1. The phytochemicals include aromatic and aliphatic carboxylic acids, carboxylic acid esters, fatty acids, fatty acid esters, sesquiterpenes, diterpenes, triterpenes and their derivatives, phytosterols, tocopherols, and so on.

3.3. In silico analysis

3.3.1. Molecular docking

The docking scores of selected hit compounds are shown in Table 2 with Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy (−4.944 kcal/mol) having the highest score. The 2D and 3D analysis of interactions between the compounds with alpha amylase showed that the five top scoring compounds interacted with the active site amino acid residues of the protein (Figs. 4–9). Among other important molecular contacts, benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy interacted by forming a hydrogen bond with ILE 235 and hydrophobic interactions with LEU 162, ALA 198, TYR 62, VAL 234, ILE 235 and TYR 151 as well as a Pi-Pi stacking interaction with HIS 201. α-tocopherol-β-D-mannoside interacted by forming hydrogen bonds with ASP 300, ASP 197, GLU 233, HIS 201 and hydrophobic interactions with LEU 165, LEU 162, ALA 198, TYR 62, ILE 235, TYR 151, TRP 58 and TRP 59. 5-Ergosterol also interacted similarly with α-tocopherol-β-D-mannoside but has only one hydrogen bond with THR 163 and an additional hydrophobic interaction with VAL 107.3,4,5-trimethoxybenzoic acid interacted by forming hydrogen bonds with LYS 200, ILE 235 and hydrophobic interactions

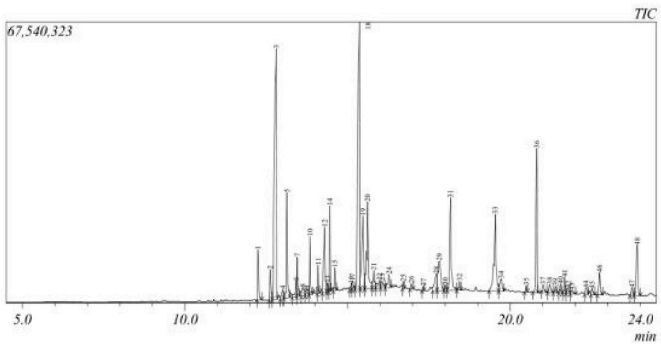

Fig. 3. Gas chromatography-mass spectrometry (GC-MS) chromatogram of ethanol extract of *T. cordifolius*.

Table 1
Chemical profile of ethanol extract of *T. cordifolius*.

Peak	IUPAC Name	Retention Time (minute)	Peak Area (%)	Peak Height (%)	Molecular formula	Chemical Classification
1.	Benzoic acid, 3,4,5-trimethoxy-, methyl ester	12.243	1.76	2.74	$C_{11}H_{14}O_5$	Aromatic carboxylic acid methyl ester
2.	3,4,5-Trimethoxyphenylacetic acid	12.620	1.31	1.68	$C_{11}H_{14}O_5$	Methoxybenzene
3.	3-(3,4,5-Trimethoxyphenyl)propionic acid	12.805	17.95	13.70	$C_{12}H_{16}O_5$	Monocarboxylic acid
4.	Undecanoic acid, 11-bromo-ethylester	13.000	0.57	0.50	$C_{13}H_{25}BrO_2$	Fatty acid, methyl ester
5.	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	13.133	3.60	5.75	$C_{10}H_{12}O_4$	Acetophenone
6.	2-Pentadecanone, 6,10,14-trimethyl-	13.411	0.69	0.87	$C_{18}H_{36}O$	Sesquiterpene
7.	Benzoic acid, 3,4,5-trimethoxy-	13.453	1.22	2.16	$C_{10}H_{12}O_5$	Aromatic carboxylic acid
8.	Campesterol	13.622	0.67	0.46	$C_{28}H_{48}O$	Phytosterol
9.	Ergost-5-en-3-ol, (3.beta.)-	13.750	0.55	0.33	$C_{28}H_{48}O$	Phytosterol
10.	Benzoic acid, 3,4,5-trimethoxy-	13.843	1.68	3.19	$C_{10}H_{12}O_5$	Aromatic carboxylic acid
11.	Dibutyl phthalate	14.092	0.72	1.53	$C_{16}H_{22}O_4$	Phthalate ester
12.	*n*-Hexadecanoic acid	14.297	3.64	3.57	$C_{16}H_{32}O_2$	Fatty acid
13.	Ethyl 9-hexadecenoate	14.383	0.39	0.49	$C_{18}H_{34}O_2$	Fatty acid ethyl ester
14.	Hexadecanoic acid, ethyl ester	14.456	1.99	4.68	$C_{18}H_{36}O_2$	Fatty acid ethyl ester
15.	Benzoic acid, 3,4,5-trimethoxy-	14.610	0.71	1.25	$C_{10}H_{12}O_5$	Aromatic carboxylic acid
16.	n-Propyl 14-methyl-hexadecanoate	15.108	0.28	0.34	$C_{20}H_{40}O_2$	Fatty acid methyl ester
17.	11,14,17-Eicosatrienoic acid, methyl ester	15.149	0.44	0.46	$C_{21}H_{36}O_2$	Fatty acid methyl ester
18.	Phytol	15.363	17.54	14.60	$C_{20}H_{40}O$	Diterpenoid
19.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	15.476	4.96	4.00	$C_{18}H_{30}O_2$	Fatty acid
20.	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,	15.612	5.09	4.76	$C_{27}H_{52}O_4Si_2$	Fatty acid ethyl ester
21.	Octadecanoic acid, 17-methyl-, methyl ester	15.808	0.87	0.99	$C_{20}H_{40}O_2$	Fatty acid methyl ester
22.	Phytol, acetate	15.984	0.72	0.43	$C_{22}H_{42}O_2$	Diterpenoid
23.	bis[(3E)-Hex-3-en-1-yloxy](dimethyl)silane	16.060	0.57	0.39	$C_{14}H_{28}O_2Si$	Silane
24.	7-Hexadecenal, (Z)-	16.273	0.86	0.72	$C_{16}H_{30}O$	Fatty aldehyde
25.	Butyl 9,12,15-octadecatrienoate	16.712	0.23	0.36	$C_{22}H_{38}O_2$	Fatty acid butyl ester
26.	4,8,12,16-Tetramethylheptadecan-4-olide	16.958	0.19	0.29	$C_{21}H_{40}O_2$	Diterpene lactones
27.	Methyl 19-methyl-eicosanoate	17.330	0.14	0.25	$C_{22}H_{44}O_2$	Fatty acid methyl ester
28.	beta.-Sitosterol	17.725	1.38	0.92	$C_{31}H_{52}O_2$	Phytosterol
29.	beta.-Sitosterol	17.816	2.88	1.62	$C_{31}H_{52}O_2$	Phytosterol
30.	Cycloheptadecanol	18.003	0.32	0.29	$C_{17}H3_4O$	Cyclic fatty alcohol
31.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	18.164	4.95	5.05	$C_{19}H_{38}O_4$	Fatty acid methyl ester
32.	Bis(2-ethylhexyl) phthalate	18.442	0.32	0.49	$C_{24}H_{38}O_4$	Phthalate ester
33.	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	19.541	5.24	4.19	$C_{21}H_{36}O_4$	1-monoglyceride
34.	Octadecanoic acid, 2,3-dihydroxypropyl ester	19.723	1.09	0.68	$C_{21}H_{42}O_4$	1-monoglyceride
35.	Ethyl tetracosanoate	20.489	0.23	0.35	$C_{26}H_{52}O_2$	Ethyl Ester
36.	Squalene	20.815	6.19	7.90	$C_{30}H_{50}$	Triterpene
37.	*tert*-butyl 1-[4-(2,6-ditert-butyl-4-methoxyphenoxy)-3-nitro-4-oxobutyl]pyrrolidine-2-carboxylate	21.011	0.36	0.36	$C_{28}H_{44}N_2O_7$	Alpha amino acid ester
38.	*tert*-butyl 1-[4-(2,6-ditert-butyl-4-methoxyphenoxy)-3-nitro-4-oxobutyl]pyrrolidine-2-carboxylate	21.213	0.51	0.43	$C_{28}H_{44}N_2O_7$	Alpha amino acid ester
39.	1-Heptacosanol	21.392	0.28	0.37	$C_{27}H_{56}O$	Fatty alcohol
40.	Tetracontane	21.546	0.34	0.49	$C_{40}H_{82}$	Alkane
41.	6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)-	21.673	0.66	0.88	$C_{30}H_{51}BrO$	Triterpene
42.	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	21.792	0.45	0.30	$C_{27}H_{46}O_2$	Tocopherol
43.	Urs-12-ene	21.892	0.26	0.19	$C_{30}H_{50}$	Triterpene
44.	trans-Geranylgeraniol	22.329	0.50	0.34	$C_{20}H_{34}O$	Diterpenoid
45.	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	22.513	0.32	0.32	$C_{30}H_{52}O$	Triterpenoid
46.	.beta.-Tocopherol	22.748	1.06	1.22	$C_{28}H_{48}O_2$	Tocopherol
47.	Tetratetracontane	23.745	0.40	0.42	$C_{44}H_{90}$	Alkane
48.	Vitamin E	23.903	2.95	2.74	$C_{29}H_{50}O_2$	Tocopherol

IUPAC: International union of pure and applied chemistry.

Table 2
The binding affinity (kcal/mol) of the top ten ranked bioactive compounds of *Tapinanthus cordifolius* against alpha amylase.

Compounds name	PubChem CID	Docking score
QV4 (Standard inhibitor)	24755467	−5.67
Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy	12006734	−4.944
Alpha. -Tocopherol-. Beta. -D-Mannoside	597057	−4.854
5-Ergosterol	68924503	−4.785
3,4,5-trimethoxybenzoic acid	8357	−4.688
Acetosyringone	17198	−4.365
Clionasterol	457801	−4.26
Beta-tocopherol	6857447	−3.968
Methyl 3,4,5-Trimethoxybenzoate	15956	−3.803
3-(3,4,5-Trimethoxyphenyl) Propionic Acid	64860	−3.794
Campesterol	173183	−3.785

with LEU 162, ALA 198, ILE 235, TYR 151 and VAL 234 as well as a pi-pi stacking interaction with HIS 201 while Acetosyringone interacted by forming a hydrogen bond with HIE 305 and hydrophobic interactions with LEU 165, LEU 162, ALA 198, TYR 62, TRP 58 and TRP 59. Reference compound (QV4) formed hydrogen bonds with HIE 305, ASP 300, THR 163, GLN 63, and HIS 201, and made hydrophobic contacts with ILE 51, TRP 58, TYP 59, TYR 62, VAL 107, TYR 151, LEU 162, LEU 165, ILE 235 and ALA 307.

QV4 (Standard inhibitor): 4,6-dideoxy-4-{[(1S,4R,5R,6S)-4-{[alpha-D-glucopyranosyl-(1->4)-alpha-D-glucopyranosyl-(1->4)-alpha-D-glucopyranosyl]oxy}-5,6-dihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino}-alpha-D-glucopyranose.

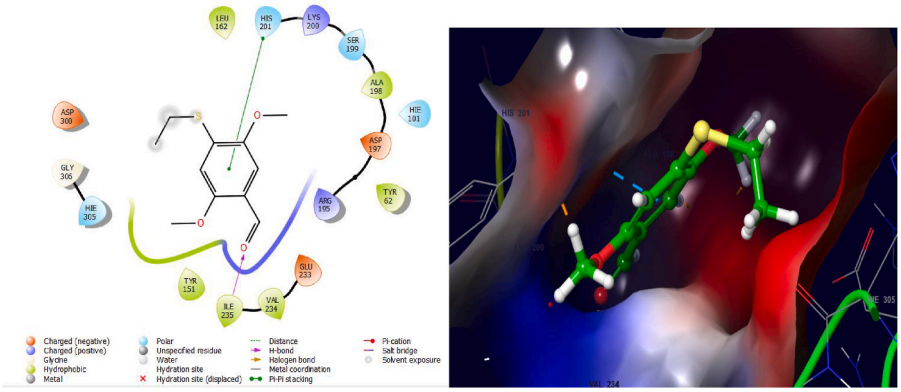

Fig. 4. 2D and 3D representations of the molecular interactions of Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy with alpha-amylase.

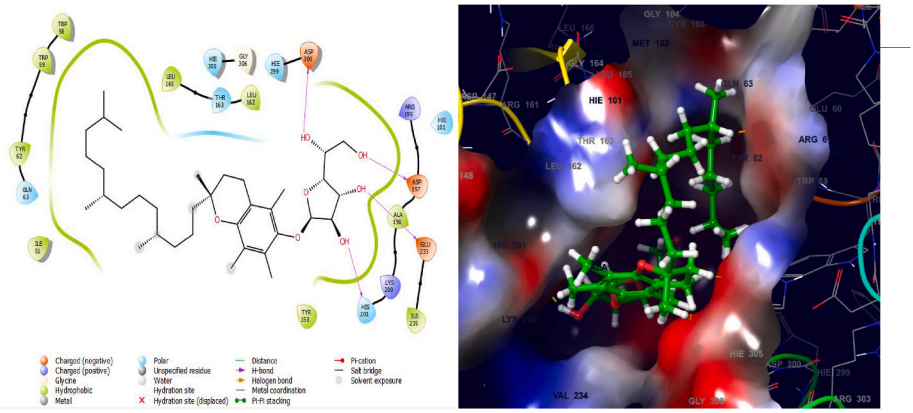

Fig. 5. 2D and 3D representations of the molecular interactions of Alpha-Tocopherol-Beta-D-Mannoside with alpha-amylase.

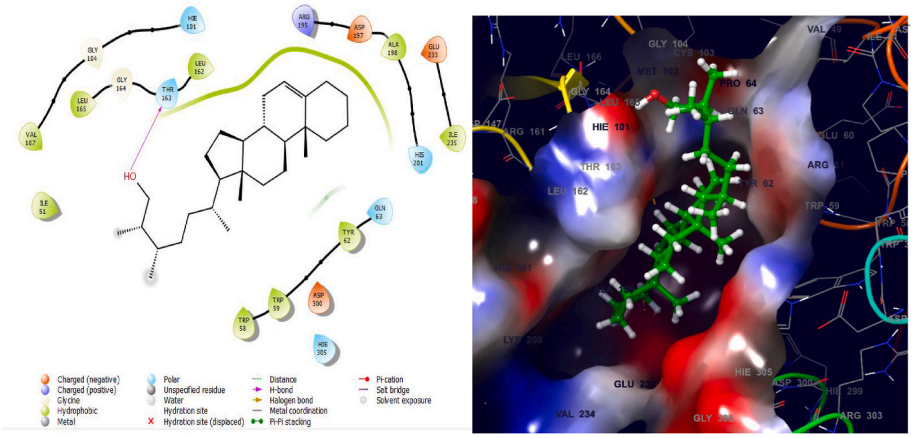

Fig. 6. 2D and 3D representations of the molecular interactions of 5-Ergostenol with alpha-amylase.

3.3.2. *Pharmacophore modelling*

The pharmacophore models are shown in Figs. 10–12. The models show interactions such as A: hydrogen bond acceptor, D: hydrogen bond donor, H: hydrophobic interaction, N: negative ionic and R: aromatic ring. Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy shows one aromatic ring and two hydrogen bond acceptors while α-tocopherol-β-D-mannoside shows one aromatic ring and one hydrogen bond donor. The properties involved in that of 5-Ergostenol are two hydrophobic interactions and one hydrogen bond donor while that of 3,4,5-trimethoxybenzoic acid are one aromatic ring and one negative ionic bond. Acetosyringone shows one aromatic ring responsible for its interaction with the target.

3.3.3. *Induced fit docking*

The molecular interactions of the best three poses of Benzaldehyde, 4-(Ethylthio)-2, 5-Dimethoxy (the most promising TC compound) in the flexible binding site of α-amylase are depicted in Fig. 13. Among other key interactions, it was found that this molecule formed a pi-pi stacking interaction with HIS 201 and a hydrogen bond with ILE 235. Fig. 14 is a fingerprint depiction of the 20 best poses of Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy interacting with α-amylase amino acid residues during the IFD. Some protein amino acid residues, most notably HIS 201

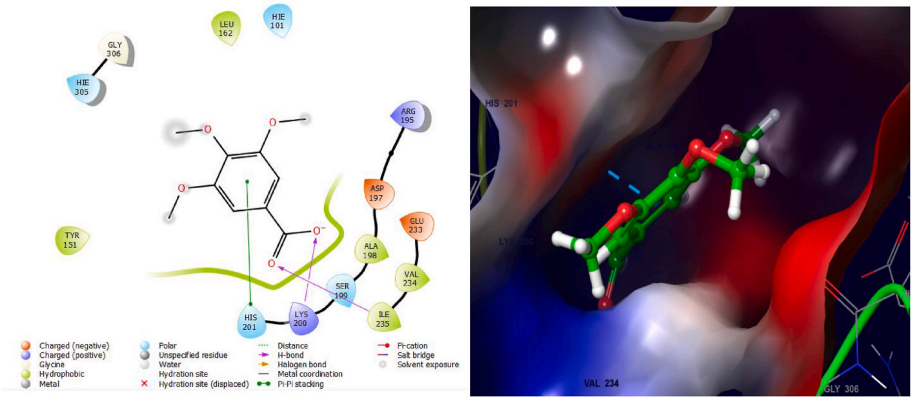

Fig. 7. 2D and 3D representations of the molecular interactions of 3,4,5-trimethoxybenzioc acid with alpha-amylase.

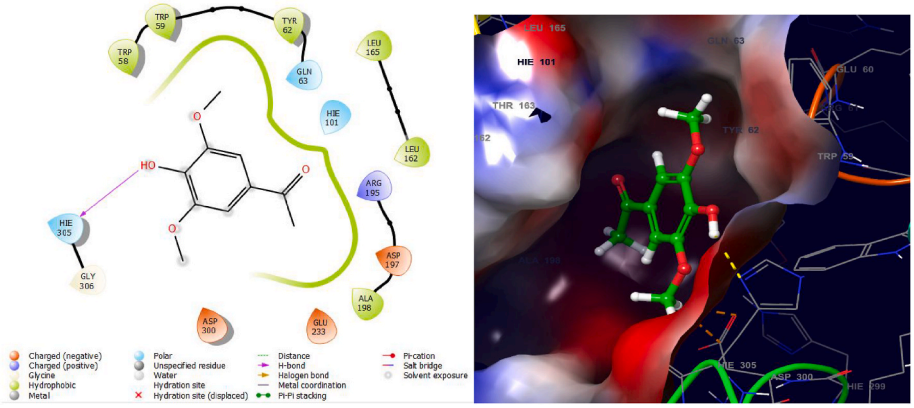

Fig. 8. 2D and 3D representations of the molecular interactions of Acetosyringone with alpha-amylase.

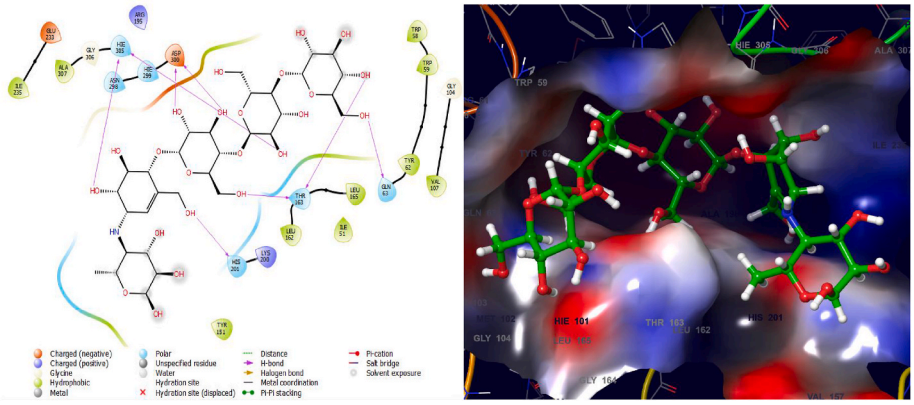

Fig. 9. 2D and 3D representations of the molecular interactions of QV4 (Standard inhibitor) with alpha-amylase.

and ILE 235, were in constant interaction with the ligand as shown by the almost continuous coloured lines in the figure.

3.3.4. ADMET predictions

Tables 3–7 shows the ADMET properties of five selected hit compounds, demonstrating their lipophilicity, water solubility, drug likeness bioavailability, toxicity as well as other parameters. The consensus Log P values of the compounds range from 1.21 (Acetosyringone) to 6.97 (5-ergosterol). Acetosyringone, 3,4,5-trimethoxybenzioc acid and Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy have their consensus log P values less than five while the rest do not, however, all the compounds obey Lipinski's rule. Acetosyringone, 3,4,5-trimethoxybenzoic acid and Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy are soluble, BBB permeant and have high GI absorption however none of the compounds are pgp substrates. Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy is an inhibitor of CYP1A2 and CYP2C19 while α-tocopherol-β-D-mannoside is an inhibitor of CYP3A4. Alpha-Tocopherol-Beta-D-Mannoside and 5-ergosterol are immunotoxic while 3,4,5-trimethoxybenzioc acid is hepatotoxic, however, the rest are inactive for the other toxicity parameters as shown on Table 6.

4. Discussion

One therapeutic approach for treating type 2 diabetes in its first stage

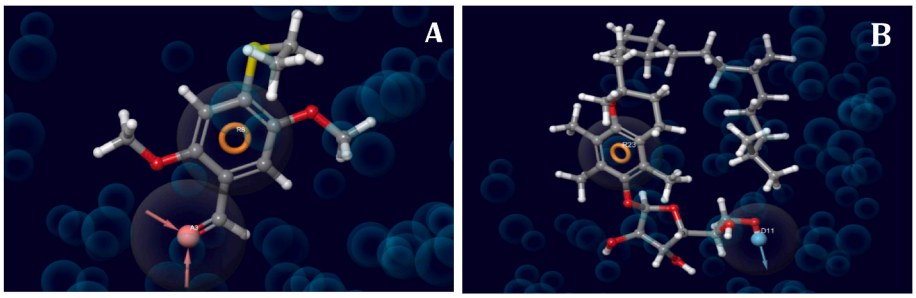

Fig. 10. The receptor-ligand complex pharmacophore models of A = Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy and B = α-tocopherol-β-D-mannoside on alpha-amylase.

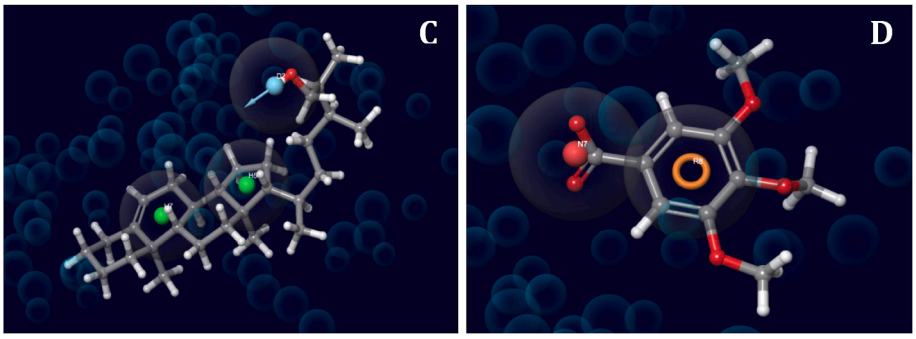

Fig. 11. The receptor-ligand complex pharmacophore models of C = 5-Ergostenol and D = 3,4,5-trimethoxybenzioc acid on alpha-amylase.

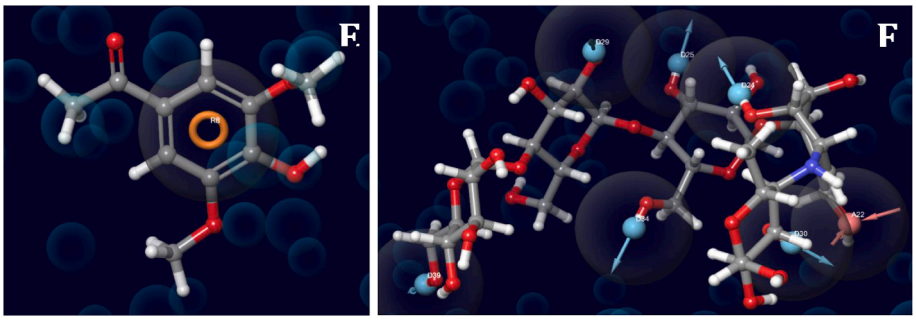

Fig. 12. The receptor-ligand complex pharmacophore models of E = Acetosyringone, F = QV4 (Standard inhibitor) on alpha-amylase.

is lowering postprandial hyperglycemia. This is achieved by decreasing the rate at which glucose is absorbed, through the inhibition of the digestive enzymes responsible for breaking down carbohydrates, α-amylase and α-glucosidase. Inhibitors of these enzymes, thus, result in a reduced glucose absorption, thereby attenuating the post-prandial rise in plasma glucose [19,25]. Some of the unwanted side effects of current medications, which include hypoglycemia at higher doses, liver problems, lactic acidosis, and diarrhea, has however necessitated the need for increased research into new therapeutic options for the treatment and management of type 2 Diabetes. In addition to currently existing therapies, numerous medicinal plants have been proposed for the treatment of diabetes. Traditional medicines are commonly given in many parts of the world due to their efficacy, lower risk of side effects, and lower cost [26]. Therefore, it is very crucial to study the bioactive compounds of medicinal plants as possible therapeutic agents for anti-diabetic drug development. *Tapinanthus cordifolius*, an African mistletoe, is an important medicinal plant with reported ability to reduce postprandial hyperglycemia. It has been suggested that the hypoglycemic effects of mistletoe is due to its capacity to reduce blood glucose levels [12,17] and the anti-diabetic activity of some African mistletoes have been linked to its ability to inhibit the activity of α-amylase [18]. However, there appear to be little or no information on the bioactive compounds of *Tapinanthus cordifolius* with alpha amylase inhibiting activity. The current study employed *in vitro* study and computational techniques such as molecular docking, pharmacophore modelling and ADMET profiling to assess the alpha amylase inhibiting activity of *Tapinanthus cordifolius* leaf extracts and its bioactive compounds in order to identify potential α-amylase inhibitors for anti-diabetic drug discovery.

The α-amylase inhibitory studies performed demonstrated that the extracts and fractions of *Tapinanthus cordifolius* has some inhibitory potentials. The crude extract demonstrated the highest inhibiting potential among the test samples as revealed by the IC_{50} value (26.88 μg/ml). This was followed by the aqueous extract, butanol, n-hexane and ethyl-acetate fractions. The higher activity demonstrated by the crude extract could be connected with the components of the extract as shown by the GCMS analysis. The extract was found to contain important phytochemicals like aromatic and aliphatic carboxylic acids, carboxylic acid esters, fatty acids, fatty acid esters, sesquiterpenes, diterpenes, triterpenes, and their derivatives, phytosterols, tocopherols, etc. Many of these compounds have been linked to the therapeutic activity of medicinal plants. For example, terpenes have been shown to play

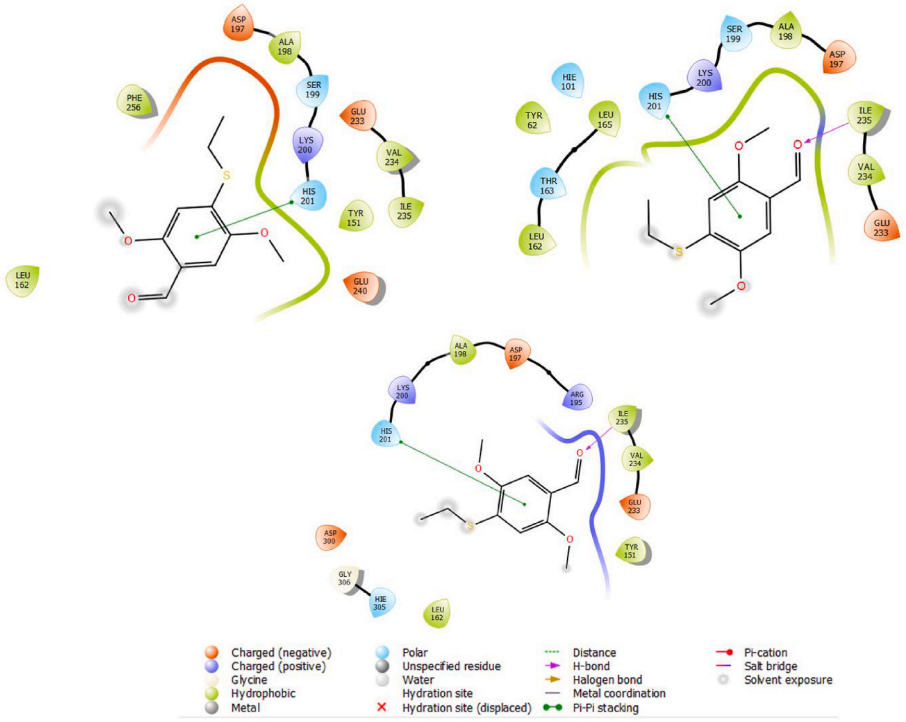

Fig. 13. Best three poses of Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy in the binding site of alpha-amylase following induced fit docking.

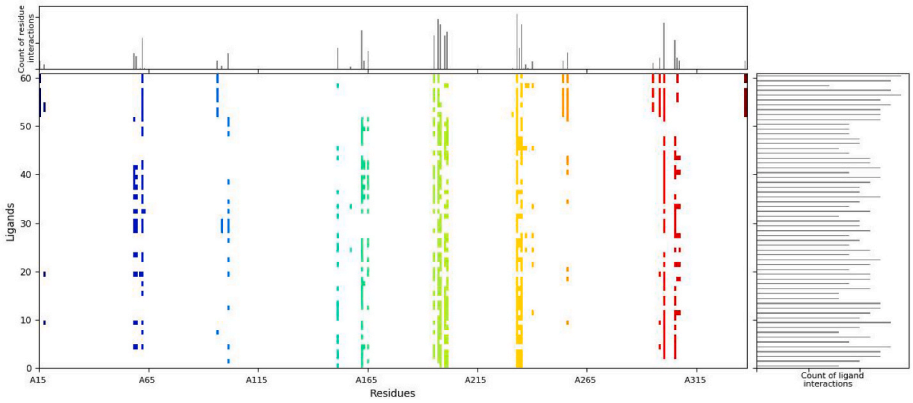

Fig. 14. A fingerprint representation of the interactions of the best 20 poses of Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy with amino acid residuesin the binding site of alpha-amylase following induced fit docking.

Table 3
The lipophilicity profile of the five top-ranking phytochemical constituents of *Tapinanthus cordifolius*.

Molecule	MW	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log P	Consensus Log P
Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy-;	226.29	2.53	2.2	2.63	1.69	2.96	2.4
Alpha.-Tocopherol-.Beta.-D-Mannoside	592.85	6.14	8.89	6.31	3.49	8.12	6.59
5-Ergosterol	400.68	4.68	9.1	7.64	6.54	6.92	6.97
3,4,5-Trimethoxybenzoic acid	212.2	1.85	1.45	1.41	0.8	1.28	1.36
Acetosyringone	196.2	1.98	0.21	1.61	0.55	1.72	1.21

significant roles among the natural products that offer therapeutic advantages to an organism. The *in vitro* inhibiting activity of some terpenes against alpha-amylase were demonstrated by Jelenković et al. [27]. Phytosterols are a type of plant-derived molecule that is structurally similar to cholesterol. They are useful to human health because of their blood sugar and cholesterol-lowering, anti-inflammatory, and anti-cancer activities [28,29]. Some phytosterols were shown by Sheng et al. to inhibit the activities of α-amylase and α-glucosidase [30]. Also of interest is the presence of tocopherols, which are phenolic antioxidants that can prevent lipid peroxidation by scavenging free radicals and interacting with singlet oxygen. The most essential of them is alpha-tocopherol (vitamin E), a powerful, lipid-soluble, and chain-breaking antioxidant [31]. This compound is known to be deficient in diabetic patients. The antioxidant effects of α-tocopherol on lipid oxidation, protein glycation, and insulin sensitivity have led to the proposition that it improves metabolic control in diabetes [32]. The

Table 4
The water solubility profile of the five top-ranking phytochemical constituents of *Tapinanthus cordifolius*.

Molecule	ESOL Log S	ESOL Solubility (mg/ml)	ESOL Solubility (mol/l)	ESOL Class
Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy	−2.6	5.75E-01	2.54E-03	Soluble
Alpha.-Tocopherol-.Beta.-D-Mannoside	−8.17	4.04E-06	6.82E-09	Poorly soluble
5-Ergostenol	−7.66	8.74E-06	2.18E-08	Poorly soluble
3,4,5-trimethoxybenzoic acid	−2.1	1.68E+00	7.92E-03	Soluble
Acetosyringone	−1.31	9.66E+00	4.92E-02	Very soluble

Table 5
The drug-likeness properties of the five top-ranking phytochemical constituents of *Tapinanthus cordifolius*

Molecule	Bioavailability Score	Lipinski violations
Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy-;	0.55	0
Alpha.-Tocopherol-.Beta.-D-Mannoside	0.55	1
5-Ergosterol	0.55	1
3,4,5-trimethoxybenzoic acid	0.85	0
Acetosyringone	0.55	0

presence of these compounds might be contributing to the anti-diabetic property of African mistletoes. Yet, the molecular docking results further revealed the α-amylase inhibiting potentials of *Tapinanthus cordifolius*.

According to the results of the molecular docking analysis, *Tapinanthus cordifolius* compounds displayed varying levels of binding affinities for alpha-amylase, with Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy (−4.944 kcal/mol) having the highest docking score, followed by Alpha.-Tocopherol-.Beta.-D-Mannoside (−4.854 kcal/mol), and 5-Ergostenol (−4.785 kcal/mol). The binding affinity of these *Tapinanthus cordifolius* bioactive compounds can be linked to their interactions with alpha-amylase amino acid residues. The amino acid residues to which the compounds and standard ligand interacted with are known be crucial for the catalytic activity of the alpha amylase. Three distinct regions (domains) make up the structure of α-amylases, with the catalytically active residues located in domain A comprising of residues 1–99 and 169–404. Active site residues ASP 197, GLU 233, and ASP 300 are clustered at one end of a central eight-stranded parallel beta-barrel that comprises the catalytic area. Also present in this region is a chloride ion that interacts with ARG 195, ASN 298 and ARG 337. The smallest domain, B (residues 100–168), forms a calcium binding site against the beta-barrel wall in Domain A. Residues ASN 100, ARG 158, ASP 167, and HIS 201 all interact with this calcium [33]. When the glycosidic bonds of carbohydrates are broken by α-amylases, the stereochemistry of the resulting molecule is preserved. Specifically, ASP 197 and GLU 233 facilitate an acid hydrolysis pathway that breaks down the glycosidic link [34]. X-ray crystallography of a complex generated by a similar glucosyltransferase revealed that the aspartate residue created a covalent link with the C1 site of the substrate while the glutamate residue functions as an acidic catalyst [35]. Molecular interactions with these crucial amino acid residues, which prevent substrates from reaching the active site, are reported to be the target of some alpha amylase inhibitors [36,37] and the overall interactions are consistent with those observed in this study.

Using in-silico pharmacophore modelling, the structural features of the test molecules responsible for their affinity for the target protein were identified. This was done to further confirm the molecules' alpha-amylase inhibitory activity. Hydrogen bond acceptors and donors, hydrophobic contacts, negative ionic, and aromatic rings are some of the interactions depicted in the models. Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy has one aromatic ring and two hydrogen bond acceptors in its interaction with the target, whereas Alpha-Tocopherol-Beta-D-Mannoside has one aromatic ring and one hydrogen bond donor. Hydrophobic amino acids with one hydrogen bond donor are engaged by 5-Ergostenol, whereas one aromatic ring and one negative ionic bond are involved in 3,4,5-trimethoxybenzoic acid binding. Acetosyringone has one aromatic ring that is responsible for the target's interaction. The capacity of the chemicals to block alpha amylase is evident from the fact that they are able to form hydrogen bonds and hydrophobic interactions in addition to other types of bonds, which are crucial molecular interactions. Enhancement of ligand-protein binding is known to occur in the presence of hydrogen bond donors and acceptors [38,39]. Aromatic rings are key residues in many molecular interactions, including protein–ligand and protein–protein interactions. Due to their natural occurrence in amino acid residues such as histidine, tryptophan, phenylalanine, and tyrosine, they are thought to be particularly important for protein stability and molecular recognition processes. In addition, aromatic rings are commonly employed in drug design due to their ability to increase the binding affinity and specificity of drug-like compounds [40]. The result of the IFD further shows the involvement of an aromatic interaction (pi-pi stacking) in the binding of Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy to α-amylase.

IFD is a technique for simulating the conformational changes caused by ligand interaction. Many proteins undergo modifications on ligand binding, which are not accounted for in standard docking analyses due to the receptor being maintained rigid and the ligand being allowed to move freely. IFD provides the protein side chains with flexibility, which makes it possible for the ligand to adjust and improve the binding

Table 7
The toxicity profile of the five top-ranking phytochemical constituents of *Tapinanthus cordifolius* against alpha amylase.

Target	A	B	C	D	E
Hepatotoxicity	–	–	–	+	–
Carcinogenicity	–	–	–	–	–
Immunotoxicity	–	+	+	–	–
Mutagenicity	–	–	–	–	–
Cytotoxicity	–	–	–	–	–
Predicted LD50 mg/kg	1410 mg/kg	3000 mg/kg	667 mg/kg	2000 mg/kg	10000 mg/kg
Predicted Toxicity Class	4	5	4	4	6

Table 6
The pharmacokinetic profile of the five top-ranking phytochemical constituents of *Tapinanthus cordifolius*.

Molecule	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
A	High	Yes	No	Yes	Yes	No	No	No
B	Low	No	No	No	No	No	No	Yes
C	Low	No	No	No	No	No	No	No
D	High	Yes	No	No	No	No	No	No
E	High	Yes	No	No	No	No	No	No

A = Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy; B = α-tocopherol-β-D-mannoside; C = 5-Ergostenol; D = 3,4,5-trimethoxybenzoic acid; E = Acetosyringone.

interactions that occur within the active site. It also gives an indication of the best poses and stable interactions of the ligand in the flexible binding site of the protein [22]. The IFD docking of Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy against α-amylase showed that this molecule formed a consistent hydrogen bond with ILE 235 and a pi-pi stacking interaction with HIS 201 in the binding pocket of the enzyme. The 20 most active poses of Benzaldehyde, 4-(Ethylthio)-2, 5-Dimethoxy in its interaction with α-amylase amino acid residues maintained a constant interaction with certain amino acid residues in the protein, notably HIS 201 and ILE 235. This supports the molecular docking result and provides some evidence for the stability of the complex, which is an indication of the alpha-amylase inhibitory potential of the *Tapinanthus cordifolius* compound.

Despite the alpha-amylase inhibitory capabilities of the *Tapinanthus cordifolius* compounds, their ADMET characteristics are a critical factor in determining their pharmacological efficacy against the target. In silico ADMET prediction is a low-cost and quick technique to assess if a molecule is swiftly absorbable, well-dispersed to its target site of action, well metabolized, and quickly eliminated from the body without generating harmful side effects [41]. New drug candidates can be quickly screened for oral drug-likeness using the Lipinski filter, which takes into account the candidates' lipophilicity, hydrogen bond donors and acceptors, and molecular weights [42]. To be considered orally active, a medicine must adhere to the criteria laid out by Lipinski Rule, which include having a molecular weight of less than 500 g/mol, a log P less than or equal to 5, having no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors. If a molecule breaks two or more of these guidelines, it cannot be taken orally. As a result, compounds with zero or one Lipinski violations, such as Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy, Alpha.-Tocopherol-.Beta.-D-Mannoside, 5-Ergosterol, 3,4,5-trimethoxybenzoic acid, acetosyringone have the tendency to be orally active, as seen by their oral bioavailability scores. In rat or human colon cancer absorptivity [41], the 0.55% bioavailability score indicates that these drugs have a 55% chance of a minimum of 10% oral absorption.

Furthermore, the inhibitory potentials of Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy and Alpha.-Tocopherol-.Beta.-D-Mannoside, on cytochrome P450 gives an indication of their possible interaction with other drugs. This is because certain CYP isoforms metabolize more than half of all drugs, and their blockage, which could prevent these pharmaceuticals from being digested, is a major source of pharmacokinetics-related drug-drug interactions [23]. It's worth mentioning that the toxicity predictions for the compounds appear to be moderate (Table 7). The compounds have LD_{50} values ranging from 667 mg/kg to 10000 mg/kg, indicating that when taken within these dose levels, they might be safe. Furthermore, none of the compounds are likely to be cytotoxic, hepatotoxic, mutagenic or carcinogenic. However, these compounds could be subjected to further optimization and experimental studies for further development into novel drugs for the management of type 2 diabetes mellitus.

5. Conclusion

The current work employed *in vitro* study and computational techniques such as molecular docking, pharmacophore modelling and ADMET profiling to assess the alpha amylase inhibiting activity of *Tapinanthus cordifolius* leaf extracts and its bioactive compounds in order to identify potential α-amylase inhibitors for anti-diabetic drug discovery. The plant showed some *in vitro* inhibiting activity for α-amylase with the crude extract demonstrating the highest activity. Of the forty-three bioactive compounds from the *Tapinanthus cordifolius* leaf extract subjected to molecular docking analysis against alpha-amylase, Benzaldehyde, 4-(Ethylthio)-2,5-Dimethoxy exhibited the highest binding affinity, followed by α-tocopherol-β-D-mannoside and 5-Ergosterol. The compounds occupied alpha-amylase's binding site, where they interacted with critical amino acid residues as the reference ligand. The molecular interactions of the three test compounds with the enzyme are mediated by aromatic rings and hydrogen bond donors. No indication of cytotoxicity, hepatotoxicity, mutagenicity, or carcinogenicity exists in any of the substances, according to the toxicity prediction. As a result, these *Tapinanthus cordifolius* compounds could be examined for further research and development as diabetic medications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The study described in this publication was carried out entirely by the authors, without the funding from any source.

References

[1] Bilal Shah S, Sartaj L, Ali F, Ali Shah SI, Tahir Khan M. Plant extracts are the potential inhibitors of α-amylase: a review. MOJ Bioequivalence Bioavailab 2018; 5. https://doi.org/10.15406/mojbb.2018.05.00113.
[2] Graham TE, Abel ED. Autophagy in Diabetes and the Metabolic Syndrome. Autophagy Heal. Dis. Elsevier; 2013. p. 117–39. https://doi.org/10.1016/B978-0-12-385101-7.00008-5.
[3] Kharroubi AT. Diabetes mellitus: the epidemic of the century. World J Diabetes 2015;6:850. https://doi.org/10.4239/wjd.v6.i6.850.
[4] Gerensea H, Moges A, Shumiyee B. Type one diabetic paitents knowledge and atittude on insulin self administration in mekele tigray, Ethiopia. J Diabet Complicat Med 2016;1:1–6. https://doi.org/10.4172/2475-3211.1000104.
[5] WHO. The top 10 causes of death. World Heal Organ; 2020. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. [Accessed 10 October 2022].
[6] Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed 2012; 2:320–30. https://doi.org/10.1016/S2221-1691(12)60032-X.
[7] Saxena A, Vikram NK. Role of selected Indian plants in management of type 2 diabetes: a review. J Alternative Compl Med 2004;10:369–78. https://doi.org/10.1089/107555304323062365.
[8] Kaur N, Kumar V, Nayak SK, Wadhwa P, Kaur P, Sahu SK. Alpha-amylase as molecular target for treatment of diabetes mellitus: a comprehensive review. Chem Biol Drug Des 2021;98:539–60. https://doi.org/10.1111/cbdd.13909.
[9] Kazeem M, Ogunbiyi J, Ashafa A. In vitro studies on the inhibition of α-amylase and α- glucosidase by leaf extracts of picralima nitida (stapf). Trop J Pharmaceut Res 2013;12. https://doi.org/10.4314/tjpr.v12i5.9.
[10] Hemlata B, Pornima G, Tukaram K, Pankaj B. In vitro anti-amylase activity of some Indian dietary spices. J Appl Biol Biotechnol 2019;7:70–4. https://doi.org/10.7324/JABB.2019.704011.
[11] Mentreddy SR. Medicinal plant species with potential antidiabetic properties. J Sci Food Agric 2007;87:743–50. https://doi.org/10.1002/jsfa.2811.
[12] Obatomi DK, Bikomo EO, Temple VJ. Anti-diabetic properties of the African mistletoe in streptozotocin-induced diabetic rats. J Ethnopharmacol 1994;43:13–7. https://doi.org/10.1016/0378-8741(94)90111-2.
[13] Polhill RM, Wiens D. Tapinanthus cordifolius polhill & wiens. Plants world online kew sci. 1998. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:1003132-1. [Accessed 23 June 2022].
[14] JSTOR. Tapinanthus cordifolius (P beauv. Glob plants. https://plants.jstor.org/stable/10.5555/al.ap.upwta.3_952. [Accessed 23 June 2022].
[15] Adesina SK, Illoh HC, Johnny II, Jacobs IE. African mistletoes (Loranthaceae); ethnopharmacology, chemistry and medicinal values: an update. African J Tradit Complement Altern Med AJTCAM 2013;10:161–70. https://doi.org/10.4314/ajtcam.v10i4.26.
[16] Ogunmefun OT, Fasola TR, Saba AB, Oridupa OA. The ethnobotanical, phytochemical and mineral analyses of Phragmanthera Incana (Klotzsch), a species of mistletoe growing on three plant hosts in South-western Nigeria. Int J Biomed Sci 2013;9:37–44.
[17] Saleh I, Maigandi SA, Hudu MI, Abubakar MI, Shehu AU. Uses and chemical composition of mistletoe (viscum album) obtained from different species of trees. Dutse J Agric Food Secur 2015;2:8–12.
[18] Oboh G, Babatola LJ, Ademiluyi AO. In vitro inhibitory effects of mistletoes (Loranthus begwensis L.) phenolic-rich extracts on α-amylase, α-glucosidase, and angiotensin converting enzyme activities. J Food Biochem 2018;42:1–7. https://doi.org/10.1111/jfbc.12504.
[19] Tundis R, Loizzo MR, Menichini F. Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini-Rev Med Chem 2010;10. https://doi.org/10.2174/138955710791331007. 315–31.

[20] Adebayo AH, Zeng GZ, Fan JT, Ji CJ, He WJ, Xu JJ, et al. Biochemical, haematological and histopathological studies of extract of ageratum conyzoides L. in Sprague Dawley rats. J Med Plants Res 2010;4:2264–72.
[21] Se O, Ge U, Po O, Ja I, Ho E, Of K, et al. Chemical constituents and antimicrobial activity of the leaf essential oil of Ixora coccinea L (Rubiaceae) collected from North Central. Int J Bioassays 2018;5:5630–7.
[22] Johnson TO, Adegboyega AE, Ojo OA, Yusuf AJ, Iwaloye O, Ugwah-Oguejiofor CJ, et al. A computational approach to elucidate the interactions of chemicals from artemisia annua targeted toward SARS-CoV-2 main protease inhibition for COVID-19 treatment. Front Med 2022;9:907583. https://doi.org/10.3389/fmed.2022.907583.
[23] Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:1–13. https://doi.org/10.1038/srep42717.
[24] Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Res 2018;46:W257–63. https://doi.org/10.1093/nar/gky318.
[25] Chiasson J-L. Acarbose for the prevention of diabetes, hypertension, and cardiovascular disease in subjects with impaired glucose tolerance: the study to prevent non-insulin-dependent diabetes mellitus (Stop-Niddm) trial. Endocr Pract 2006;12:25–30. https://doi.org/10.4158/EP.12.S1.25.
[26] Venkatesh S, Reddy GD, Reddy BM, Ramesh M, Rao AVNA. Antihyperglycemic activity of Caralluma attenuata. Fitoterapia 2003;74:274–9. https://doi.org/10.1016/S0367-326X(03)00021-2.
[27] Jelenkovic L, Jovanovic V, Palic I, Mitic V, Radulovic M. In vitro screening of α-amylase inhibition by selected terpenes from essential oils. Trop J Pharmaceut Res 2014;13:1421. https://doi.org/10.4314/tjpr.v13i9.7.
[28] Miras-Moreno B, Sabater-Jara AB, Pedreno MA, Almagro L. Bioactivity of phytosterols and their production in plant in vitro cultures. J Agric Food Chem 2016;64:7049–58. https://doi.org/10.1021/acs.jafc.6b02345.
[29] Kritchevsky D, Chen SC. Phytosterols-health benefits and potential concerns: a review. Nutr Res 2005;25:413–28. https://doi.org/10.1016/j.nutres.2005.02.003.
[30] Sheng Z, Dai H, Pan S, Ai B, Zheng L, Zheng X, et al. Phytosterols in banana (Musa spp) flower inhibit α-glucosidase and α-amylase hydrolysations and glycation reaction. Int J Food Sci Technol 2017;52:171–9. https://doi.org/10.1111/ijfs.13263.
[31] Frankel EN. The antioxidant and nutritional effects of tocopherols, ascorbic acid and beta-carotene in relation to processing of edible oils. Bibl Nutr Dieta 1989:297–312. https://doi.org/10.1159/000416714.
[32] Ble-Castillo JL, Carmona-Díaz E, Méndez JD, Larios-Medina FJ, Medina-Santillán R, Cleva-Villanueva G, et al. Effect of α-tocopherol on the metabolic control and oxidative stress in female type 2 diabetics. Biomed Pharmacother 2005;59:290–5. https://doi.org/10.1016/j.biopha.2005.05.002.
[33] Brayer GD, Luo Y, Withers SG. The structure of human pancreatic α -amylase at 1.8 Å resolution and comparisons with related enzymes. Protein Sci 1995;4:1730–42. https://doi.org/10.1002/pro.5560040908.
[34] Qian M, Nahoum V, Bonicel J, Bischoff H, Henrissat B, Payan F. Enzyme-catalyzed condensation reaction in a mammalian α-amylase. High-resolution structural analysis of an Enzyme−Inhibitor complex. Biochemistry 2001;40:7700–9. https://doi.org/10.1021/bi0102050.
[35] Rydberg EH, Li C, Maurus R, Overall CM, Brayer GD, Withers SG. Mechanistic analyses of catalysis in human pancreatic α-amylase: detailed kinetic and structural studies of mutants of three conserved carboxylic acids. Biochemistry 2002;41:4492–502. https://doi.org/10.1021/bi011821z.
[36] Westermann J-C, Craik DJ. Plant peptide toxins from nonmarine environments. Compr. Nat. Prod. II. Elsevier; 2010. p. 257–85. https://doi.org/10.1016/B978-008045382-8.00115-5.
[37] Pereira PJB, Lozanov V, Patthy A, Huber R, Bode W, Pongor S, et al. Specific inhibition of insect α-amylases: yellow meal worm α-amylase in complex with the Amaranth α-amylase inhibitor at 2.0 Å resolution. Structure 1999;7:1079–88. https://doi.org/10.1016/S0969-2126(99)80175-0.
[38] Samuel BB, Oluyemi WM, Johnson TO, Adegboyega AE. High-throughput virtual screening with molecular docking, pharmacophore modelling and adme prediction to discover potential inhibitors of plasmodium falciparum lactate dehydrogenase (Pfldh) from compounds of combretaceae family. Trop J Nat Prod Res 2021;5:1665–72. https://doi.org/10.26538/tjnpr/v5i9.22.
[39] Johnson TO, Adegboyega AE, Iwaloye O, Eseola OA, Plass W, Afolabi B, et al. Computational study of the therapeutic potentials of a new series of imidazole derivatives against SARS-CoV-2. J Pharmacol Sci 2021:1–10. https://doi.org/10.1016/j.jphs.2021.05.004.
[40] Lanzarotti E, Defelipe LA, Marti MA, Turjanski AG. Aromatic clusters in protein-protein and protein-drug complexes. J Cheminf 2020;12:1–9. https://doi.org/10.1186/s13321-020-00437-4.
[41] Ntie-Kang F. An in silico evaluation of the ADMET profile of the StreptomeDB database. SpringerPlus 2013;2:1–11. https://doi.org/10.1186/2193-1801-2-353.
[42] Lipinski AC, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 1997;23:3–25.