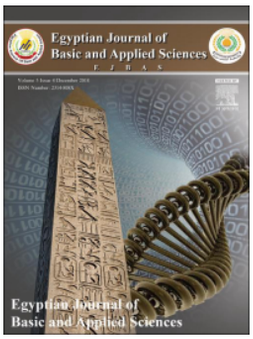

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Daniellia oliveri as a type 2 diabetes remedy: evidence from *in-silico* evaluation

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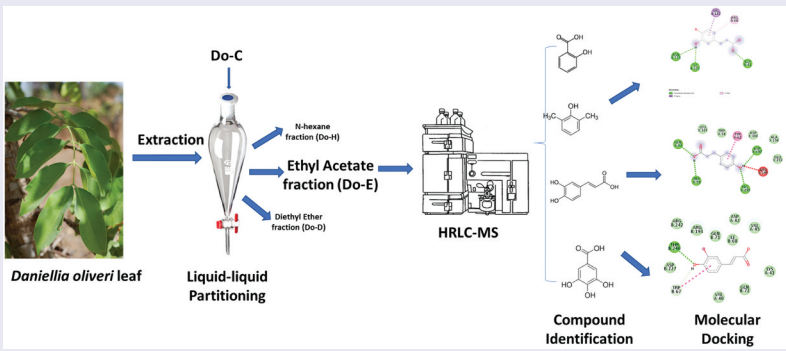
ABSTRACT

The prevalence of diabetes mellitus is increasing, and well-known conventional medications are associated with harmful effects. Therefore, this study aimed to identify the compounds present in the young leaves of *Daniellia oliveri* and assess their antidiabetic potential using an *in-silico* model. The best column chromatographic fraction obtained from ethyl acetate fraction was analyzed using HRLC-MS to identify the prominent compounds. The identified compounds were subjected to ADMET prediction using online servers to confirm their draggability. In addition, the compounds were screened for activity against type 2 diabetes using molecular docking. Eight compounds with prominent peaks were identified, viz; gallic acid, 2,6-dihydroxybenzoic acid, salicylic acid, caffeic acid, phenylacetaldehyde, 2,5-dimethylphenol, 3-(−4-Hydroxyphenyl) propionic acid, and 16-hydroxyhexadecanoic acid. Most of the identified compounds were phenolics and had little toxic effects. The molecular docking results revealed the potentials of the identified compounds in inhibiting the therapeutic targets viz; 11β-hydroxysteroid dehydrogenase type 1, human salivary alpha-amylase, glycogen phosphorylase, human protein tyrosine phosphatase 1B, glutamine fructose-6-phosphate amidotransferase, human sirtuin 6, and dipeptidyl peptidase IV, responsible for the development of type 2 diabetes. This study has successfully revalidated and provided scientific insight into the usage of *D. oliveri* in managing type 2 diabetes by Nigerians. However, the limitation of this study remains the purification of the lead compounds that can serve as lead for type 2 diabetes treatment in conventional medicinal practices.



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Introduction

Diabetes is a serious, chronic condition that emerges when the body either does not produce enough insulin or cannot effectively utilize the insulin it produces, leading to a persistent metabolic imbalance [1]. It is estimated that approximately 537 million adults aged 20 to 79 worldwide, comprising 10.5% of all individuals in this age group, are affected by diabetes. Projections suggest that by 2030, the number of global diabetes cases will reach 643 million, and this figure is expected to rise to 783 million by 2045 [2]. As a result, if diabetes is not managed appropriately over time, it can negatively affect various physiological systems, including neurons, blood vessels, kidney and eyes. A well-controlled blood sugar level is necessary to avoid significant diabetic consequences [3]. Despite this, diabetic problems are exacerbated by a lack of regular exercise, smoking, high blood pressure, and obesity [4]. Untreated diabetes complications usually result in morbidity and mortality [5].

Although strict control of hyperglycemia can reduce the risk of diabetic complications, these are frequently unavoidable, necessitating management strategies that increase the cost of diabetes treatment by more than 50% [6]. On the market, oral hypoglycemic medications to treat type 2 diabetes include insulin secretagogues (sulfonylureas), insulin sensitizers (biguanides and thiazolidinediones), glucose absorption inhibitors (alpha-glucosidase inhibitors, guar gum), and repaglinide and nateglinide, which are early-phase insulin secretagogues that target postprandial hyperglycemic peaks [7]. These oral anti-hyperglycemic medications have many drawbacks, ranging from gastrointestinal disturbances such as diarrhea, nausea, and dyspepsia to Urinary Tract Infections (UTIs) resulting in urethritis, pyelonephritis, and genital mycosis.

Medicinal plants use dates to ancient times, and they are still widely utilized today to treat various conditions [8,9]. Bioactive constituents such glycosides, alkaloids, terpenoids, flavonoids, carotenoids, peptidoglycans and hypoglycans, and guanidine and amino acids are found in many plant species and have been linked to hypoglycemic action [10]. Mounting evidence demonstrates the use of therapeutic plant supplements to prevent and control type 2 diabetes [11,12]. Plant-derived phytochemicals like curcumin, isolated from *Curcuma longa* root, are becoming increasingly popular in the scientific community [13].

The modern pharmaceutical industry's recognition of medicinal plants as therapeutic agents has produced precious drugs extensively used in clinical therapy. Natural phyto-compounds and analogues have not yet developed viable treatments for complex human diseases with complex pathophysiologies, such as diabetes, autoimmune disorders, and degenerative diseases [14]. Numerous early pharmaceuticals were developed because of extensive research on numerous traditional herbal treatments, including Digoxin (from *Digitalis purpurea*), Morphine (from the *Opium poppy*), and Quinine (from *Cinchona* barks). However, it is estimated that more than 50% of all medications currently available are derived from medicinal plants [15,16].

Previously, drug development from plant materials and the process of identifying the structures of active compounds in extracts were both complex and time-consuming, taking weeks, months, or even years depending on the complexity of the compounds [17]. However, the introduction of high-performance liquid chromatography/mass spectrometry (HPLC/MS), liquid chromatography-mass spectrometry (LC/MS), and nuclear magnetic resonance (NMR) has considerably boosted the rate of bioassay-guided fractionation in recent years [18].

The computer-based method, commonly known as the '*in-silico*' approach, facilitates the

exploration of disease-related targets through bioinformatics. This approach encompasses the design of molecules for potential therapeutic applications, the optimization of molecular compatibility, and the simulation of interactions [19,20]. *In-silico* studies employing computer-aided drug design (CADD) tools, which include structure-based drug design (SBDD) and ligand-based drug design (LBDD) methodologies [21], have facilitated the identification and development of novel drugs. The Target-Based Virtual Screening method is extensively used in drug development, often incorporating docking or molecular dynamics techniques for this purpose [22]. The advantages of the *in-silico* method include environmental sustainability, as it reduces solvent usage, minimizes electronic waste, and decreases gas emissions. Moreover, it adheres to the core principles of the 3Rs (replacement, reduction, and refinement) by lessening the reliance on animal testing. Additionally, it saves time, lowers financial costs, and enables the analysis of chemical and physical properties [23,24].

Daniellia oliveri is the lone member of *Daniellia* subgenera *Paradaniellia*, and it belongs to the family Fabaceae [25]. *Daniellia* consists of 10 species, nearly all restricted to woodland habitats in West and Central Africa, except *D. oliveri*, extending to Sudan and Uganda [26]. As reported by Onefeli [27], the species of *Daniellia* are indigenously distributed to Nigeria, Mali, Senegal, Sudan, Sierra Leone, Niger, Togo, Uganda, Liberia, Ivory Coast, Zambia, Cameroon, Cabinda, Gulf of Guinea Is., Guinea-Bissau, Angola, Ghana, Gabon, Burkina, Benin, Guinea, Equatorial Guinea, Chad, Gambia, Congo, Congo, and the Central African Republic. In Nigeria, *D. oliveri* is pervasive in savannah environments such as Adamawa, Jalingo, Shaki-Iseyin, Jebba, and Olokemeji forests [25,27]. It is found in abundance at the University of Ilorin, Ilorin, Nigeria. The usage of *D. oliveri* leaves in the management of diabetes is popular in Nigeria local communities and tribes. However, limited empirical data are reporting its efficacy in the literature. Meanwhile, several ethnobotanical studies have documented the usage of the roots, leaves, and stem barks in diabetes management [28]. The plant has been reported as an antihyperglycaemic and glycolytic enzymes inhibitors [29,30]. Furthermore, the leaves' aqueous extract is a safe and effective treatment for type 2 diabetes, as reported by Shauibu et al. [31]. However, we have reported in previous studies the efficacy of tender leaves of *D. oliveri* in the management of type 2 diabetes [32,33].

The use of *in-silico* model in this study not only allows for a more efficient and safer identification of potential antidiabetic compounds from the young leaves of *Daniellia oliveri*, but it also addresses limitations present in traditional laboratory experiments. Conventional laboratory methods often require extensive time and resources, and they may involve the use of live animal models or complex *in vitro* systems that can introduce ethical concerns and variability in results [34]. In contrast, the *in-silico* model provides a rapid means of assessing the biological activity and safety of compounds without the need for initial physical testing [35]. By utilizing computer-aided drug design (CADD) tools, including structure-based and ligand-based drug design approaches, this research facilitates the identification of compounds with promising antidiabetic properties while predicting their absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles.

Furthermore, the use of molecular docking in this study allows for a detailed understanding of how identified compounds interact with key therapeutic targets involved in the development of type 2 diabetes. This computational assessment streamlines the drug discovery process, and also enhances the reliability of findings by minimizing the potential for experimental errors associated with conventional techniques.

The high incidence and increasing prevalence of type 2 diabetes, as well as the limitations and side effects of currently available hypoglycemic agents, clearly justify the search for new antidiabetic agents. Despite the growing use of medicinal plants as hypolipidemic [36,37], antihypertensive [38,39], and hypoglycemic [40–42] agents in the management of diabetes and its associated complications, the reserves of plant-derived antidiabetic therapeutics remain untapped. *Daniellia oliveri*, though reported for its antidiabetic efficacy using the leaf crude extracts [32] and solvent–solvent fractions [33], is yet to be explored using its identified compounds for its role in the management of type 2 diabetes. Although various phytochemicals from *Daniellia oliveri* have been identified [43], their direct engagement with key proteins like alpha-glucosidase, alpha-amylase, or dipeptidyl peptidase-4 (DPP-4), which are critical in glucose metabolism and insulin regulation, is not well-documented. Also, the prediction of drug-likeness, bioavailability, and toxicity profiles of these compounds, are vital for the potential development of therapeutics derived from *D. oliveri*.

Methodology

Chemicals

Folin-Ciocaltue reagent (FC Reagent), 1,1-diphenyl-2-picraylhydrazine (DPPH), α-glucosidase (From *Saccharomyces cerevisiae*), Acarbose, Ethanol, Diethyl ether, ethyl acetate, and n-hexane were products of SRL Pvt limited, India; p-Nitrophenyl-α-D-glucopyranoside was a product of Hi-media. All buffers were prepared using standard procedures. All reagents were supplied locally by Dualife Science PVT limited, Kaveri Hebitet, Surat, India.

Collection of plant materials

Daniellia oliveri's fresh young leaves were obtained in their native habitat at the University of Ilori, Nigeria. In the Department of Plant Biology Herbarium, the collected plant was authenticated to the species level and a voucher specimen (UILH/001/1291/2021) was submitted.

Extraction procedure

A total of 2 kg of dried *D. oliveri* young leaves was extracted using the cold maceration method for 72 h in 70% Ethanol to obtain 182 g of extract. 103 g of the crude extract suspended in water was placed in separating funnel and fractionated with the same volume of n-hexane, diethyl ether, and ethyl acetate, successively, in order of polarity, to obtain 5 g of ethyl acetate fraction (Do-E). The Do-E was further separated by silica gel column-chromatography using the mobile phase, petroleum Ether/Ethyl Acetate/Methanol (2:8:1) to obtain 8 pulled fractions (DoEF1 – DoEF8) using their Rf values. Thin-layer chromatography technique was used to arrive at the mobile phase used.

Total flavonoid content of the chromatographic fractions (DoEF1-DoEF8)

1 ml of extract solution/Quercetin (different concentrations) was taken in a test tube. 3 ml methanol, 200 µl of 10% $AlCl_3$, 200 µl of 1 M potassium acetate solution were added. 5.6 ml of distilled water was added to the reaction, after which the test tubes were incubated at room temperature to complete the reaction. The absorbance of the solution was measured at 420 nm using a UV-visible spectrophotometer (Shimadzu UV-1800) against the blank.

The total content of flavonoid compounds in the plant extract in quercetin equivalent was calculated as follows:

$$C = \frac{(c \times V)}{M}$$

Where:

C = Total content of flavonoid compound, mg/g plant extract in Quercetin equivalent (QE)

c = Concentration of Quercetin established from the calibration curve, mg/ml

V = Volume of extract, ml

M = The weight of the plant extract used, gm.

Total phenolic content of the chromatographic fractions (DoEF1-DoEF8)

The modified method of Singleton et al., [44] was used. 0.5 ml of methanolic solution of the extracts/aallic acid (10, 25, 50, 100 and 200 µg/ml) was mixed with 2.5 ml 10% Folin–Ciocalteu reagent (FCR) and 2.5 ml 7.5% $NaHCO_3$. The mixture was incubated at 45°C for 45 min. The absorbance was determined at 765 nm using a UV Spectrophotometer (Shimadzu UV-1800). The gallic acid calibration curve was constructed by plotting the OD against the concentrations.

The total phenolic compounds in the plant extract in gallic acid equivalent was calculated using as follows;

$$C = \frac{(c \times V)}{M}$$

Where;

C = Total content of Phenolic compound, mg/g plant extract in gallic acid equivalent (GAE)

c = Concentration of gallic acid established from the calibration curve, mg/ml

V = Volume of extract, ml

M = The weight of the plant extract used, gm.

In-vitro *α-glucosidase inhibition assay (IC_{50}) of chromatographic fractions (DoEF1-DoEF8)*

Based on the size of the chromatographic fractions, the standard protocols of Kim *et al.*, [45] and Telagari & Hullatti [46] with modifications were used to ascertain the IC_{50} of the subfractions. 36 µl of phosphate buffer (6.8 pH), 30 µl sample solution with various concentrations (2, 4, 8, 10 µg/ml) and 17 µl *p*NPG substrate at a concentration of 5 mm were put in a 96-well microplate. The mixture was incubated at 37°C for 5 min, and 17 µl of α-glucosidase solution (0.15 U/ml) was added to each well to obtain a total volume of 100 µl. The mixture was incubated for 15 min to get the complete hydrolysis reaction. After 15 min, the reaction was stopped by adding 100 µl of Na_2Co_3 (200 mm). Absorbance was read at 405 nm using a microplate reader. Each test was repeated thrice.

The OD obtained from the test was processed using the formula:

$$\%\, Inhibition = \frac{(A1 - A2)}{A1} \times 100$$

Where:

A1 = Blank absorbance (B*)- Control of blank absorbance (KB**)

A2 = Sample absorbance (S) – Control of sample absorbance (KS***)

*Blank contained substrate and enzyme, without extracts

**Control of blank contained substrate and buffer, without enzyme and extracts

***Control of the sample contained substrate and extract with the addition of the enzyme after incubation.

The IC_{50} (concentration needed to inhibit 50% of enzyme activity) was estimated using a regression equation derived by plotting concentrations in the range of 2–10 µg/mL (x-axis) against percentage inhibition (y-axis) for the extract and various fractions.

Metabolite profiling of putative lead from chromatographic fraction

TLC fingerprinting of DoEF3

The stationary phase used was silica gel 60 F_{254} pre-coated aluminum plates (3 × 10 cm, 200 µm thick). Before sample application, the plates were pre-washed with methanol and activated at 110°C for 10 min. A CAMAG Linomat V applicator was used to apply 250 µg/ml of the chromatographic fraction (DoEF3) and standard compound, gallic acid in methanol as 5 mm wide bands. The mobile

phase consisted of toluene, ethyl acetate, formic acid, and methanol (3:3:0.8:0.2 v/v). The development occurred in a twin trough glass chamber saturated with the mobile phase at room temperature (25 ± 2°C) for 30 min. The bands were scanned at 200–700 nm, with the plate imaged at 254 nm and scanned at 500 nm using a TLC scanner 3. The developed method was validated as per the ICH guidelines for quality control of herbal drugs and botanicals [47,48].

HRLC-MS fingerprint of bioactive fraction

The LC-MS analysis was performed using Agilent HIP Sampler equipped with a binary pump. The HPLC was interfaced with a Q-TOF mass spectrometry fitted with an ESI source (Dual AJS ESI). Full scan mode from m/z 100 to 1100 was performed with a source temperature of 30°C. HPLC column Hypersil GOLD 3µ, C18 (100 × 2.1 mm i.d.) was used for analysis. The mobile phase comprises solvent A (0.1% Formic Acid in water) and solvent B (90% Acetonitrile + 10% H_2O + 0.1% Formic Acid) for 1 min. The column was held as presented in Table 1. The flow rate was 0.300 mL/min, and the injection volume was 3 µL. For the electrospray ionization with positive ion polarity, the capillary voltage was set to 3500 V, the capillary temperature to 250°C, the nebulizer pressure to 35psi, and the drying gas flow rate to 13 L/min.

In-silico *pharmacokinetics properties and antidiabetic evaluations*

In-silico *ADME properties*

SwissADME (http://www.swissadme.ch/) and ADMETLab 2.0 (https://admetmesh.scbdd.com/service/evaluation/cal) were utilized in predicting the ADME properties of the identified compounds. These web servers calculate the pharmacokinetics, ADME, drug-likeness, and medicinal chemistry compatibility of small molecules [49,50]. Phytochemicals identified using LC-MS were retrieved in SMILES formatted structure from the PubChem database.

Table 1. Mobile phase timetable for LC-MS.

S/N	Time (minutes)	Solvent A	Solvent B
1	1.00	95%	5%
2	20.00	0%	100%
3	25.00	0%	100%
4	26.00	95%	5%
5	30.00	95%	5%

In-silico *toxicity risk assessment and drug likeliness*

The chemical scaffolds of the identified phytochemicals were evaluated using OSIRIS Property Explorer, an open-source program (http://www.organic-chemistry.org/prog/peo/), SwissADME (http://www.swissadme.ch/index.php), and ADMETLab 2.0 (https://admetmesh.scbdd.com/service/evaluation/cal) which calculates the toxicity risks and drug-relevant properties of compounds [49,50].

Molecular docking studies

Ligand preparation. Structures of the compounds identified using LC-MS were downloaded from the PubChem database in 3D SDF format for docking. Using Open Babel in PyRx (Version 0.9.8), all the ligand structures were relaxed by performing energy minimization by conjugate gradient algorithm for 200 steps using uff force field with RMS gradient of 0.1 and converted into pdbqt format for docking.

Macromolecule preparation. The three-dimensional structures of various protein targets were retrieved from the RCSB protein database. For docking studies, any bound ligands were removed. And receptor protein was dehydrated, hydrogenated and Gasteiger charges were added along with the atomic type set to Assign AD4 type, which was saved as pdbqt format using PyRx software (Version 0.9.8). Protein used in the study are depicted with the PDB IDs in Table 2.

Virtual screening. The atomic level interactions between protein and phytochemical

Table 2. Diabetic proteins target along with PDB ID.

S/No	PDB ID	Proteins
1	1XU7	11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1)
2	1Z32	Human Salivary alpha-amylase (HSA)
3	2AT1	Glycogen Phosphorylase (GP)
4	2HNP	Human Protein Tyrosine Phosphatase 1B (PTP1B)
5	2ZJ4	Glutamine Fructose-6-phosphate amidotransferase (GFAT)
6	3K35	Human sirtuin 6 (SIRT6)
7	4N8D	Dipeptidyl peptidase IV (DPP4)

ligands were investigated using molecular docking studies using PyRx 0.9.8 software which uses the AutoDock Vina algorithm to predict protein–ligand interactions. The high exhaustiveness of 27 with 20 binding modes was considered for all docking to increase the prediction accuracy. The dimension of the grid box encloses the site of ligand interaction and active sites of the protein receptor, while the position of the grid box was centered on enclosing the residues of the protein are represented in Table 3. In this virtual screening technique, 20 conformations for the ligand were generated, and the binding energies of the ligand conformations were analyzed. The Dock score of the best poses docked into the target protein was calculated for all examined compounds and proteins.

Table 3. Binding pockets configuration of various receptors used in the study.

		Binding pocket configuration	
S/No	PDB ID	Size	Centre
1	1XU7	x = 35.981	x = −57.468
		y = 40.288	y = −80.721
		z = 34.402	z = −29.054
2	1Z32	x = 48.873	x = 5.968
		y = 43.456	y = 49.204
		z = 43.845	z = 18.492
3	2AT1	x = 37.002	x = 20.390
		y = 37.404	y = 86.367
		z = 37.379	z = 107.408
4	2HNP	x = 66.766	x = 43.417
		y = 49.036	y = 15.893
		z = 40.190	z = 14.725
5	2ZJ4	x = 46.889	x = 68.037
		y = 46.699	y = 33.592
		z = 48.840	z = 7.601
6	3K35	x = 34.881	x = 18.844
		y = 40.481	y = 4.520
		z = 42.303	z = 5.909
7	4N8D	x = 31.892	x = 14.833
		y = 41.224	y = 30.612
		z = 38.939	z = 52.827

Results

This is the first time the ethyl acetate fraction obtained from the ethanolic extract of *D. oliveri* is being separated using column chromatography. However, after several trials, the mixture of Petroleum ether, Ethyl Acetate, and Methanol (2:8:1) gave satisfactory separation. Eight sub-fractions, DoEF1, DoEF2. DoEF3, DoEF4, DoEF5, DoEF6, DoEF7 and DoEF8 resulted from the column chromatographic separation.

Total flavonoid and total phenolic contents

The total flavonoids and phenolic contents of the chromatographic fractions are presented in Figure 1 (a,b). Out of the eight sub-fractions, the DoEF7 had the highest flavonoid contents (250.61 ± 4.01), while DoEF6 contained the least total flavonoids (44.21 ± 2.14). DoEF1 also contained significant flavonoid contents (221.82 ± 7.16). However, no significant differences were observed in the total flavonoid contents of DoEF2 (125.79 ± 3.30) and DoEF3 (121.58 ± 2.33), as well as those of DoEF4 (99.31 ± 2.55) and DoEF6 (101.22 ± 2.20) at $p \leq 0.05$. The total phenolic content (TPC) of DoEF3 (205.56 ± 0.00) was the highest estimated in all the chromatographic fractions and was significantly different ($p \leq 0.05$) from other chromatographic fractions. DoEF1 contained the lowest estimate of total phenolics. Meanwhile, there was no significant difference ($p \leq 0.05$) in the TPC of DoEF2 and DoEF6.

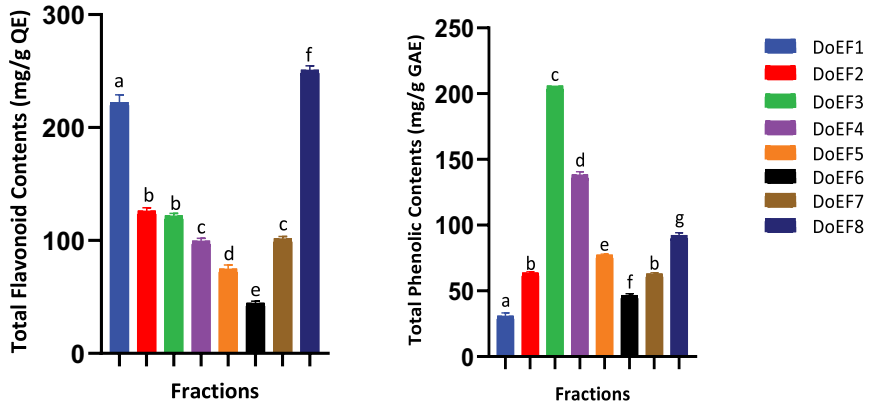

Figure 1. Total Flavonoid Content (a) and Total Phenolic Contents (b) of chromatographic fractions of Ethyl acetate fraction obtained from D. oliveri. *mg/g QE: mg/g Quercetin Equivalent; mg/g GAE: mg/g Gallic Acid Equivalent. **Values are mean of three replicates ± Standard Error of Mean (SEM). Different superscripts denote significant differences (P≤0.05).

In vitro *α-glucosidase inhibitory assay of chromatographic fractions of ethyl acetate fraction*

To select the most active chromatographic fraction obtained from the ethyl acetate fraction of *D. oliveri*, the *in vitro* alpha-glucosidase inhibitory efficacy in the form of inhibitory concentrations (IC_{50}) was evaluated. In our study, the IC_{50} was used to measure the performance of the chromatographic fractions. Our finding revealed that DoEF3 ($IC_{50} = 0.66 \pm 0.03$) was desirable because it had a lower IC_{50} after Acarbose (0.65 ± 0.01), a standard drug (Table 4). The alpha-glucosidase inhibitory potential and its phenolic content made it a sort-after fraction for further analysis.

Table 4. Alpha glucosidase inhibitory activity IC_{50} (µg/ml) of chromatographic fractions of ethyl acetate fraction obtained from D. oliveri.

Fractions	Alpha glucosidase IC_{50} (µg/ml)
Acarbose	0.65 ± 0.01*
DoEF1	1.25 ± 0.23
DoEF2	0.68 ± 0.06
DoEF3	0.66 ± 0.03
DoEF4	0.85 ± 0.19
DoEF5	0.67 ± 0.05
DoEF6	0.78 ± 0.09
DoEF7	0.75 ± 0.13
DoEF8	0.76 ± 0.07

Values are the mean of three replicates ± Standard Error of Mean (SEM).

TLC fingerprinting of DoEF3

HPTLC fingerprinting studies of DoEF3 and the standard, gallic acid, showed distinct band patterns at 254 nm. Gallic acid, which appeared to be absent under the 254 nm wavelength, was observed using the TLC scanner (Figures 2 (a,b)). A good correlation was obtained between the gallic acid and sample overlay spectra (Figures 2c).

HRLC-MS fingerprint of the bioactive subfraction

Based on the outlook in the LC-MS chromatogram of DoEF3 (Figure 3), eight (8) compounds, viz; gallic acid, 2,6-dihydroxybenzoic acid, salicylic acid, caffeic acid, phenylacetaldehyde, 2,5-dimethylphenol, 3-(-4-Hydroxyphenyl) propionic acid, and 16-hydroxyhexadecanoic acid were prominently present in DoEF3 (Table 5).

In-silico *pharmacokinetics properties and antidiabetic evaluations*

The physicochemical properties of the prominent compounds identified in the chromatographic fraction, DoEF3 are presented in Table 6. The properties such as the number of rotatable bonds, number of hydrogen

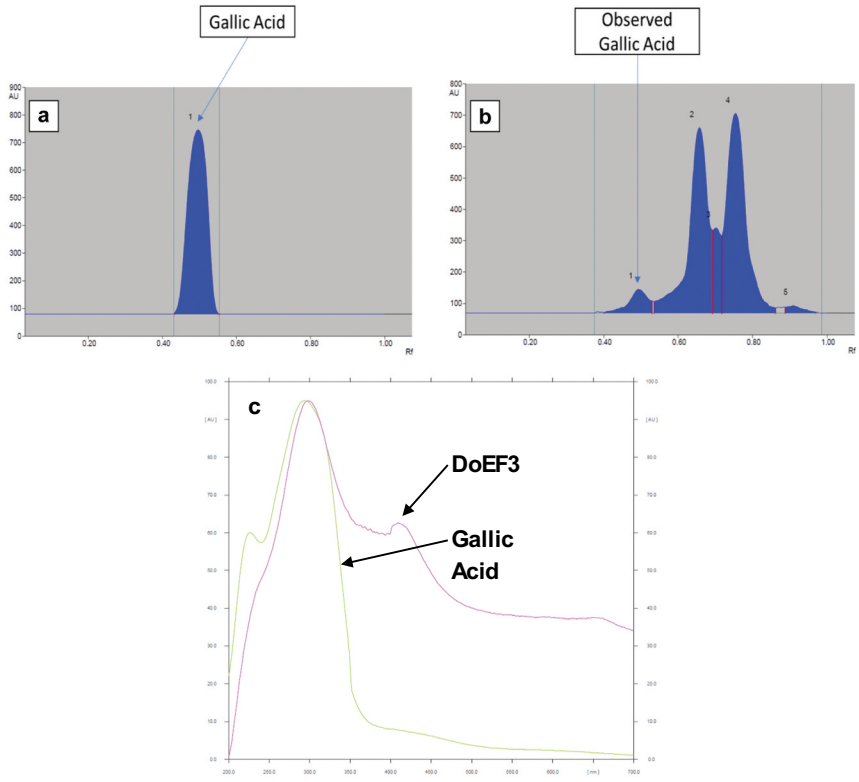

Figure 2. High-performance thin-layer chromatography (HPTLC). HPTLC chromatogram at 500 nm showing different peaks and bands for gallic acid standard (a), and sample (b); spectra comparison of gallic acid and DoEF3 (c).

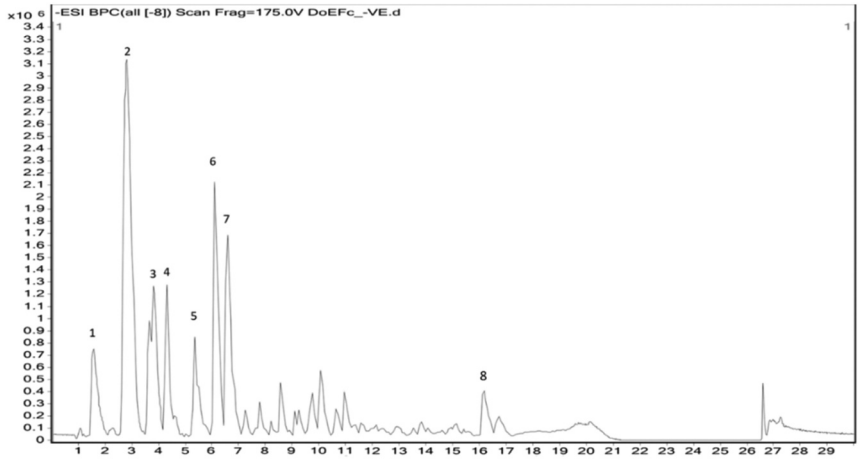

Figure 3. LC-MS chromatogram of DoEF3 subfraction from the ethyl acetate fraction of *D. oliveri* leaves. 1- Gallic acid; 2- 2,6-dihydroxybenzoic acid; 3- salicylic acid; 4- caffeic acid; 5- phenylacetaldehyde; 6- 2,5-dimethylphenol; 7- 3-(4-hydroxyphenyl) propionic acid; 8- 16-hydroxyhexadecanoic acid.

Table 5. LC-MS fingerprint of prominent compounds present in the chromatographic fraction DoEF3.

S/No	Compound	Retention time (mins)	Molecular formula	Molecular weight	m/z
1	Gallic acid	1.864	$C_7H_6O_5$	170.12	169.0145
2	2,6-Dihydroxybenzoic acid	2.906	$C_7H_6O_4$	154.12	153.0199
3	Salicylic acid	4.032	$C_7H_6O_3$	138.12	137.0247
4	Caffeic acid	4.480	$C_9H_8O_4$	180.16	177.0196
5	Phenylacetaldehyde	5.686	C_8H_8O	120.15	119.0501
6	2,5-Dimethylphenol	6.372	$C_8H_{10}O$	122.16	121.0660
7	3-(4-Hydroxyphenyl) propionic acid	6.372	$C_9H_{10}O_3$	166.17	165.0559
8	16-Hydroxy hexadecanoic acid	16.661	$C_{16}H_{32}O_3$	272.42	271.2293

Table 6. Physicochemical properties of compounds prominently present in DoEF3.

S/N	Compound name	Formula	Molecular weight (g/mol)	NRBs	NHBAs	NHBDs	TPSA (A^2)	Consensus Log P	Water solubility Class
1	Gallic acid	$C_7H_6O_5$	170.12	1	5	4	97.99	0.21	Very soluble
2	2,6-Dihydroxybenzoic acid	$C_7H_6O_4$	154.12	1	4	3	77.76	0.89	Soluble
3	Salicylic acid	$C_7H_6O_3$	138.12	1	3	2	57.53	1.24	Soluble
4	Caffeic acid	$C_9H_8O_4$	180.16	2	4	3	77.76	0.93	Very soluble
5	Phenylacetaldehyde	C_8H_8O	120.15	2	1	0	17.07	1.73	Soluble
6	2,5-Dimethylphenol	$C_8H_{10}O$	122.16	0	1	1	20.23	2.12	Soluble
7	3-(4-Hydroxyphenyl) propionic acid	$C_9H_{10}O_3$	166.17	3	3	2	57.53	1.31	Very soluble
8	16-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	272.42	15	3	2	57.53	4.30	Moderately soluble

NRBs: Number of Rotatable Bonds; NHBAs: Number of Hydrogen Bond acceptors; NHBDs: Number of Hydrogen bond donors; TPSA: Topological Polar Surface Area.

acceptors, number of hydrogen donors, topological polar surface area, and water solubility class are essential to predict the nature of drugs to determine their druggability.

To assess the molecules' drug-likeness, we have considered six physicochemical viz; saturation, lipophilicity, polarity, dimensional stability, solubility, and flexibility which is represented in Figure 4. The lipophilicity, polarity and size of all the compounds are within the confine of the radar. The saturation levels of the compounds like gallic acid, 2,6-hydroxybenzoic acid, salicylic acid, caffeic acid, and phenylacetaldehyde fall outside the recommended radar, whereas other compounds are within the confine of recommended saturation levels (Figure 4).

The predicted absorption, distribution, excretion, and toxicological analysis of the compounds of prominence in DoEF3 as presented (Tables 7–11) are essential in drug discovery. CaCo-2 permeability, MDCK permeability, and Human Intestinal Absorption were predicted (Table 7). The predicted CaCo-2 permeability in the compounds screened revealed that only phenylacetaldehyde (−4.291 log cm/s) and 2.5-dimethylphenol (−4.356) are accepted as they are higher than the −5.15-log cm/s. Optimally, the CaCo-2 permeability coefficient must be higher than the −5.15-log unit. The predicted MDCK-permeability, Pgp-inhibitory values, and human intestinal absorption are all within the accepted range for all the compounds. The 20% bioavailability (F20%) was not accepted for gallic acid as the probability of being F20% (bioavailability <20%) is 0.964.

The compounds were subjected to a set of analyses to ascertain their drug-likeness, and how natural product-like they were, among other tests to ascertain their medicinal chemistry (Table 8). A quantitative estimate of drug-likeness (QED), synthetic accessibility score (SAscore), and natural product (NPscore) score were determined.

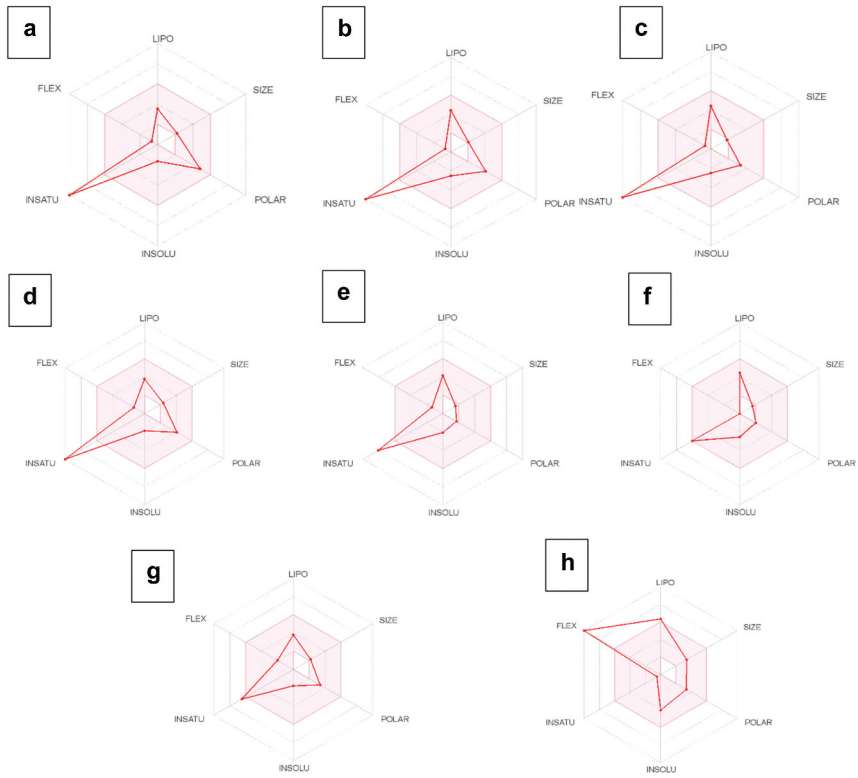

Figure 4. Bioavailability radar of identified compounds based on physicochemical indices ideal for oral bioavailability. Gallic acid (a); 2,6-hydroxybenzoic acid (b); salicylic acid (c); caffeic acid (d); phenylacetaldehyde (e); 2,5-dimethylphenol (f); 3-(4-hydroxyphenyl) propionic acid (g); 16-hydroxyhexadecanoic acid (h).

Table 7. Absorption of compounds prominently present in DoEF3 as predicted.

		Absorption					
S/N	COMPOUNDS	CaCo-2 Permeability	MDCK Permeability	Pgp-Inhibitor	Pgp-Substrate	HIA	F20%
1	Salicylic acid	−5.179	1.1×10^{-5}	0.000	0.001	0.017	0.008
2	Gallic acid	−5.728	5.0×10^{-6}	0.001	0.003	0.085	0.964
3	Phenylacetaldehyde	−4.291	3.3×10^{-5}	0.000	0.007	0.005	0.056
4	2,5-Dimethylphenol	−4.356	2.7×10^{-5}	0.002	0.120	0.003	0.321
5	2,6-Dihydroxybenzoic acid	−5.626	6.0×10^{-6}	0.000	0.001	0.079	0.579
6	3-(4-Hydroxyphenyl) propionic acid	−5.163	1.1×10^{-5}	0.001	0.005	0.020	0.051
7	16-Hydroxy hexadecanoic Acid	−5.250	3.5×10^{-5}	0.017	0.000	0.012	0.346
8	Caffeic acid	−5.220	1.1×10^{-5}	0.000	0.024	0.009	0.009

Human epithelial colorectal adenocarcinoma cells` (CaCo-2) Permeability (Optimal Higher than −5.15 Log cm/s); Madin-Darby canine kidney (MDCK): low permeability: $< 2 \times 10^{-6}$ cm/s; medium permeability: $2–20 \times 10^{-6}$ cm/s; high passive permeability: $> 20 \times 10^{-6}$ cm/s; Pg-P Inhibitor: Category 1: Inhibitor; Category 0: Non-inhibitor; The output value is the probability of being Pgp-inhibitor, within the range of 0 to 1. Human Intestinal Absorption: Category 1: HIA+(HIA <30%); Category 0: HIA-(HIA <30%); The output value is the probability of being HIA+; F20% = 20% Bioavailability. Category 1: F20%+ (bioavailability <20%); Category 0: F20%- (bioavailability ≥20%); The output value is the probability of being F20%+.

In addition, the compounds were subjected to Lipinski's rule of 5 and Pfizer's rule to determine their drug-likeness. The predicted distribution and excretion of the drugs in the human system are presented in Table 9. Most compounds show limited BBB penetration, with the highest being

Table 8. Medicinal chemistry analysis of compounds prominently present in DoEF3 using ADMETLab 2.0.

		Medicinal chemistry				
S/N	Compounds	QED	SAscore	NPscore	Lipinski rule	Pfizer rule
1	Salicylic acid	0.61	1.425	0.139	Accepted	Accepted
2	Gallic acid	0.46	2.095	0.981	Accepted	Accepted
3	Phenylacetaldehyde	0.539	1.706	0.798	Accepted	Accepted
4	2,5-Dimethylphenol	0.558	1.586	0.026	Accepted	Accepted
5	2,6-Dihydroxybenzoic acid	0.559	1.972	0.622	Accepted	Accepted
6	3-(4-Hydroxyphenyl) propionic acid	0.713	1.521	0.602	Accepted	Accepted
7	16-Hydroxy hexadecanoic a cid	0.431	1.748	0.515	Accepted	Rejected
8	Caffeic acid	0.472	2.035	1.124	Accepted	Accepted

QED: Quantitative estimate of drug-likeness; Attractive: > 0.67; unattractive: 0.49 ~ 0.67; too complex: < 0.34; SAscore: Synthetic accessibility score is designed to estimate ease of synthesis of drug-like molecules. SAscore ≥ 6, difficult to synthesize; SAscore < 6, easy to synthesize; NPscore: Natural product-likeness score. This score is typically in the range from −5 to 5. The higher the score is, the higher the probability is that the molecule is a NP; Lipinski rule: MW ≤ 500; logP ≤ 5; H-acceptors ≤10; H-donors ≤5, If two properties are out of range, a poor absorption or permeability is possible, one is acceptable; Pfizer Rule: logP > 3; TPSA < 75 Compounds with a high log P (>3) and low TPSA (<75) are likely to be toxic.

Table 9. Distribution and excretion of compounds prominently present in DoEF3.

		Distribution				Excretion	
S/N	Compounds	PPB (%)	VD (L/Kg)	BBB Penetration	Fu (%)	CL (mL/min/Kg)	$T_{1/2}$
1	Salicylic acid	67.27	0.251	0.456	26.12	6.39	0.913
2	Gallic acid	53.49	0.466	0.099	33.59	10.108	0.947
3	Phenylacetaldehyde	36.94	1.575	0.933	67.03	9.592	0.784
4	2,5-Dimethylphenol	84.00	1.406	0.744	12.74	15.302	0.853
5	2,6-Dihydroxybenzoic acid	81.44	0.325	0.139	21.56	5.018	0.915
6	3-(4-Hydroxyphenyl) propionic acid	68.09	0.213	0.093	21.67	13.199	0.894
7	16-Hydroxy hexadecanoic acid	96.65	0.496	0.523	1.405	2.877	0.757
8	Caffeic acid	87.70	0.37	0.119	11.07	10.108	0.947

PPB = Plasma Protein Binding (Optimal: < 90%). Drugs with high protein-bound may have a low therapeutic index; VD = Volume Distribution (Optimal: 0.04-20 L/kg); Blood-Brain Barrier (BBB) Penetration Category 1: BBB+; Category 0: BBB- (The output value is the probability of being BBB+); The fraction unbound in plasma (Fu) (Low: <5%; Middle: 5 ~ 20%; High: > 20%); CL= Clearance (High: >15 mL/min/kg; moderate: 5–15 mL/min/kg; low: <5 mL/min/kg); T1/2 Category 1: long half-life; Category 0: short half-life; long half-life: >3 h; short half-life: <3 h. The output value is the probability of having long half-life.

Table 10. Toxicity of compounds prominently present in DoEF3.

		Toxicity							
S/N	Compounds	hERG Blockers	H-HT	DILI	AMES Toxicity	ROAT	Carcinogenicity	Skin sensitizers	DL
1	SalicylicaAcid	0.048	0.594	0.856	0.018	0.516	0.046	0.288	0.12
2	Gallic acid	0.017	0.433	0.852	0.053	0.03	0.024	0.871	−3.27
3	Phenylacetaldehyde	0.097	0.059	0.313	0.395	0.019	0.319	0.957	−1.44
4	2,5-Dimethylphenol	0.017	0.042	0.057	0.033	0.284	0.539	0.534	1.62
5	2,6-Dihydroxybenzoic acid	0.032	0.505	0.869	0.118	0.123	0.152	0.409	−3.82
6	3-(4-Hydroxyphenyl) Propionic acid	0.035	0.153	0.049	0.029	0.565	0.458	0.285	−3.84
7	16-Hydroxy hexadecanoic acid	0.043	0.035	0.034	0.005	0.019	0.093	0.913	−1.56
8	Caffeic acid	0.018	0.73	0.365	0.183	0.833	0.233	0.942	−24.60

hERG Blockers Category 1: active; Category 0: inactive; The output value is the probability of being active; H-HT: Human Hepatotoxicity. Category 1: H-HT positive (+); Category 0: H-HT negative (-); The output value is the probability of being toxic; Drug-Induced Liver Injury (DILI) Category 1: drugs with a high risk of DILI; Category 0: drugs with no risk of DILI. The output value is the probability of being toxic; AMES Toxicity. Category 1: Ames positive (+); Category 0: Ames negative (-); The output value is the probability of being toxic; ROAT = Rat Oral Acute Toxicity. Category 0: low-toxicity; Category 1: high-toxicity. The output value is the probability of being highly toxic; Carcinogenicity Category 1: carcinogens; Category 0: non-carcinogens; The output value is the probability of being toxic; Skin Sensitizer; Category 1: Sensitizer; Category 0: Non-sensitizer; The output value is the probability of being sensitizer; DL= Drug Likeness.

Table 11. Predicted ADME properties – metabolism.

S/N	Compound name	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
1	Gallic acid	No	No	No	No	Yes	−6.84
2	2,6-Dihydroxybenzoic acid	No	No	No	No	Yes	−5.68
3	Salicylic acid	No	No	No	No	No	−5.54
4	Caffeic acid	No	No	No	No	No	−6.58
5	Phenylacetaldehyde	Yes	No	No	No	No	−5.77
6	2,5-Dimethylphenol	Yes	No	No	No	No	−5.39
7	3-(4-Hydroxyphenyl) propionic acid	No	No	No	No	No	−6.49
8	16-Hydroxyhexadecanoic acid	No	No	No	Yes	No	−3.99

CYP1A2: Enzyme Cytochrome P450 1A2; CYP2C19: Enzyme Cytochrome P450 2C19; CYP2C9: Enzyme Cytochrome P450 2C9; CYP2D6: Enzyme Cytochrome P450 2D6; CYP3A4: Enzyme Cytochrome P450 3A4; log Kp: Skin permeation coefficient.

Phenylacetaldehyde (0.933), while others like gallic Acid (0.099) and 3-(4-Hydroxyphenyl) Propionic Acid (0.093) showed very low penetration. The predicted toxicity properties covered a range of essential endpoints, including AMES, hERG, H-HT, AMES, ROAT, carcinogenicity, and skin sensitizer (Table 10). The human hepatotoxicity prediction revealed that all compounds are well tolerated except caffeic acid, with a 0.73 (73%) probability of being H-HT toxic. Salicylic acid, gallic acid, and 2,6-dihydroxybenzoic acid are mildly toxic with a probability value of 0.594, 0.433 and 0.505, respectively, of being H-HT toxic. A glance into the rat oral acute toxicity test (ROAT) revealed that caffeic acid is highly toxic, with a 0.833 probability of being highly toxic (Table 10). In this study, most identified compounds are non-inhibitors of CYP enzymes isoforms (Table 11). Gallic acid and 2,6-dihydroxybenzoic acid are inhibitors of CYP3A4, while phenylacetaldehyde and 2,5-dimethylphenol are inhibitors of CYP1A2. 16-hydroxyhexadecanoic Acid is an inhibitor of CYP2D6. In contrast, salicylic acid, caffeic acid, and 3-(4-hydroxyphenyl) propionic acid inhibit none of the isoforms of the CYP enzyme evaluated *in-silico*.

The binding energies of all the eight compounds identified in DoEF3, and metformin, a standard drug on seven proteins related to type 2 diabetes are presented in Table 12. Among the docking results, the values of binding energy ranged from −7.3 to −4.5 Kcal/mol. The binding affinity of caffeic acid, −7.3 Kcal/mol, was the highest against 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (PDB ID: 1XU7) and Glutamine Fructose-6-phosphate amidotransferase (GFAT) (PDB ID: 2ZJ4), which was the lowest recorded binding energy, even when compared with Metformin (−5.0 and −5.5 Kcal/mol, respectively). Also, some compounds such as gallic acid and 3-(4-Hydroxyphenyl) Propionic Acid had low energy against their various protein targets. Caffeic acid produced the most potent inhibition against 11β-HSD1, with its phenyl group interacting with VAL 124 and ARG 66 residues of the protein via pi-sigma and pi-alkyl bonds (Figure 5).

Table 12. Binding energy (kcal/mol) of identified compounds in DoEF3 of *Daniellia oliveri* to target proteins.

S/No.	Protein PDB ID	MET	GA	2,6-D	SA	CA	PAH	2,5-D	3,4-HPA	16-HHA
1	1XU7	−5.0	−6.3	−6.0	−5.7	−7.3	−4.9	−5.4	−6.3	−5.5
2	1Z32	−5.1	−6.1	−6.1	−5.9	−6.7	−5.2	−5.6	−6.2	−5.3
3	2ATI	−5.0	−6.3	−5.8	−5.8	−6.3	−5.3	−5.9	−6.0	−6.0
4	2HNP	−5.8	−5.6	−5.6	−5.3	−5.9	−4.8	−5.1	−5.4	−4.5
5	2ZJ4	−5.5	−6.5	−6.5	−6.1	−7.3	−4.9	−5.5	−6.4	−5.0
6	3K35	−5.1	−6.6	−6.5	−6.3	−7.1	−6.1	−6.0	−6.6	−6.1
7	4N8D	−4.9	−6.5	−5.8	−5.9	−6.3	−5.3	−5.7	−6.0	−4.9

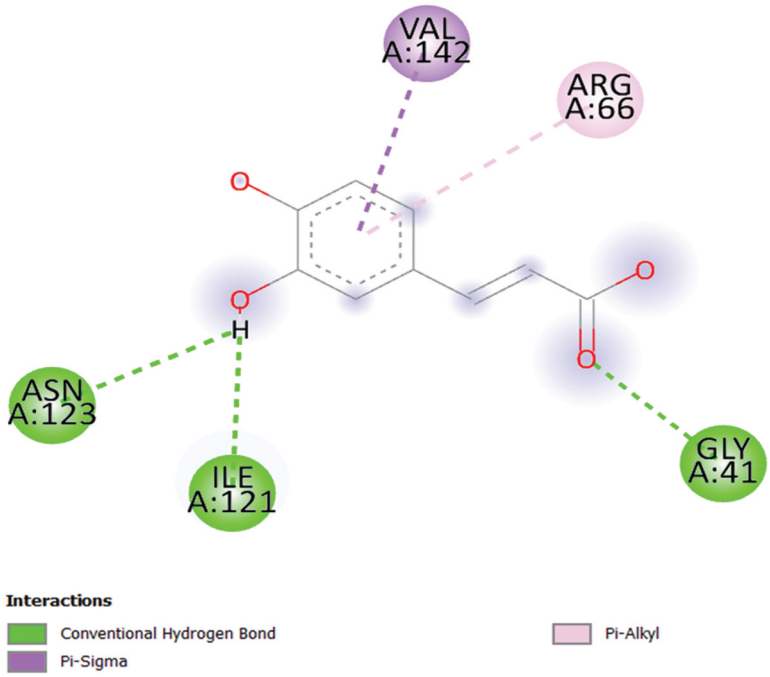

Figure 5. 2-D interaction of caffeic acid with 11β- hydroxysteroid dehydrogenase type I (11β-HSD1) (PDB ID: 1XU7).

Similarly, caffeic acid's interaction with GFAT was also remarkable. The caffeic acid's hydroxyl group interacted with the LYS 675, GLN 421, and SER 422 via hydrogen bond, while the ligand's carboxyl group interacted with THR 375 and SER 473 via conventional hydrogen bonding and unfavorable donor–donor bonds, respectively (Figure 6).

Discussions

Nature has endowed humanity with a wealth of medicinal plants worldwide. Natural products have long been a valuable resource for drug design and discovery, with their use tracing back to ancient time [51]. However, several tools are needed to identify the bioactive principles responsible for the acclaimed activity of medicinal plants. Fractionating plant extracts affords researchers the opportunity of obtaining the bioactive molecules responsible for therapeutic outcome. The *in-vitro* α-glucosidase recorded as IC_{50} was used early in the discovery process to evaluate the suitability and performance of the fractions. IC_{50}, also called half-maximal inhibitory concentration, is the most frequently used and informative parameter for determining a drug's efficacy. It represents the amount of drug required to stop a biological process completely, hence providing a measure of an antagonist's potency in pharmacological research [52]. The lower IC_{50} recorded for DoEF3 among others made it the best performing fractions for further studies.

The IC_{50} recorded for DoEF3 could be as a result of its higher total phenolic content, significantly higher than other subfractions. It has been suggested that specific plant phenolics, due to their structural properties, can inhibit risk factors of Type 2 diabetes [53,54]. Dietary polyphenols have been reported as inhibitors of diabetes mellitus by several researcher [55–57].

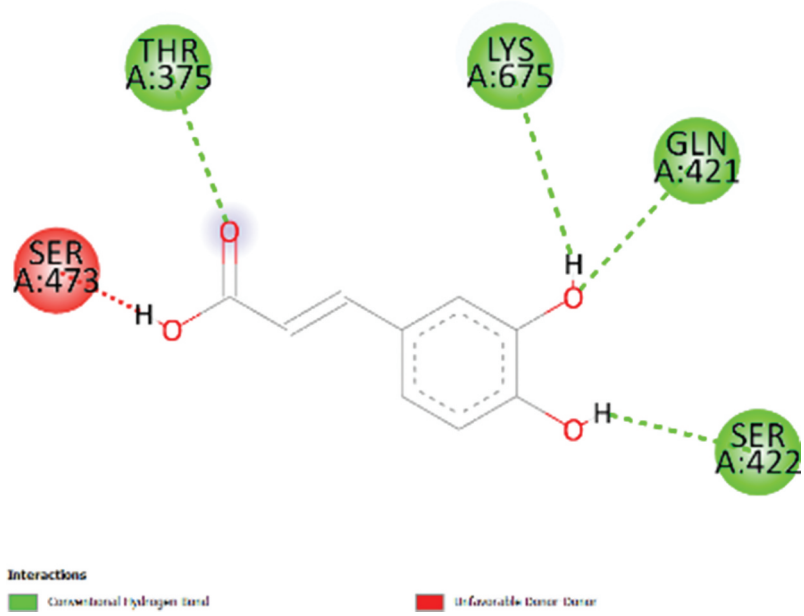

Figure 6. 2-D interaction of caffeic acid with glutamine fructose-6-phosphate amidotransferase (GFAT) (PDB ID: 2ZJ4).

High-performance thin-layer chromatography (HPTLC) fingerprint analysis has evolved into a highly effective and powerful tool for estimating chemical and biological markers [58]. In our study, the HPTLC laid bare the presence of a known drug gallic acid, the chromatographic fraction, DoEF3. Quality control is a key consideration in the analysis of herbal formulations. TLC fingerprinting is commonly employed to establish the metabolite profile of an extract, enabling its identification in the future. To further identify the metabolites present in the chromatographic fraction, HRLC-MS was used. In this study, gallic acid, salicylic acid and caffeic acid were among the prominent compounds identified.

Gallic acid (GA) which is a naturally occurring phenolic chemical is found in plants in abundance, especially in tea leaves, grapes, various berries and fruits, and wine [59,60]. GA has been reported to have anti-diabetic activity in animal models with insulin deficiency and insulin resistance [61]. As reported, When GA extracted from *Terminalia bellerica* is administered orally, it causes a dose-dependent reduction in blood glucose levels. In another research, GA (20 mg/kg BW), reportedly decreased blood glucose (81.8 mg/dL), total cholesterol, triglycerides, low-density lipoprotein cholesterol, urea, uric acid, and creatinine levels while significantly boosting plasma insulin (16.3 U/mL), C-peptide, and glucose tolerance [62]. Additionally, the presence of GA in the sub-fraction when checked using HPLC is a testament of its marked antidiabetic efficacy. Several other researchers have reported gallic acid as a remarkable antidiabetic agent, in addition to its anti-oxidative potential [63–65].

Salicylic acid (SA), also known as ortho-hydroxybenzoic acid, and its derivatives are a class of phenolic compounds found in plants. Salicylates generated from plants have been used in medicine since antiquity [66]. Salicylic acid is a potent inhibitor of oxidative stress. SA interacted with hydroxyl radicals in granulocytes, as demonstrated by Sagone and Husney

[67]. Additionally, SA reportedly reduced superoxide anion radicals by inhibiting NADPH activity, which reduced reactive oxygen species (ROS) in human endothelial cells *in vitro* [68]. Caffeic acid (CA) is a polyphenol formed during the secondary metabolism of vegetables such as olives, coffee beans, fruits, potatoes, carrots, and propolis. It is the primary source of hydroxycinnamic acid in the human diet [69–71]. Various *in vitro* and *in vivo* studies have demonstrated the numerous physiological benefits of caffeic acid and its derivatives as an antidiabetic, antioxidant, and cardioprotective [72,73]. Dietary polyphenols, especially CA, may inhibit α-amylase and β-glucosidase, sodium-dependent glucose transporter 1 (SGLT1) absorption in the gut, promote insulin secretion, and decrease hepatic glucose output [74,75].

Medicinal chemists must access absorption, metabolism, distribution, and excretion (ADME) and physiochemical data early in the discovery stage to avoid failure at the later stage of drug discovery process including preclinical trials. By utilizing rational drug design concepts, information can be used to manufacture novel structural analogues that improve those features. While it would be ideal for screening many drug candidates using *in vivo* pharmacokinetic tests, but it is unrealistic due to its fundamentally slow, labor-intensive, and non-automatable nature [76]. Therefore, the *in-silico* model is needed to achieve much in a short period.

Solubility determination of a potential drug is an initial step, as poor solubility can affect the subsequent ADME, hence the quality of pharmacokinetic predictions. Physicochemical property such as drug's solubility is strongly related to absorption, as only soluble drugs may be absorbed via the gut. A drug's solubility is determined by its intrinsic properties (lipophilic or hydrophilic nature). While hydrophilic drug molecules are easily soluble in bodily fluids, their penetration into the bloodstream is limited due to the lipophilic character of the biological membrane barrier, which restricts penetration, and vice versa for lipophilic drug molecules [77,78]. The predicted absorption, distribution, excretion, and toxicological analysis of the compounds of prominence in DoEF3 are essential in drug discovery which helps to make a rational decision on whether inhibitors can be administered to a biological system or not [79,80]. Membrane permeability is a critical driver of drug absorption efficiency and is the rate-limiting stage in oral bioavailability, according to Lipinski et al. [81]. Even in preclinical research, attention is being paid to how membrane permeability affects therapeutic efficacy [82].

Lipinski's rule of 5 is a rule of thumb for identifying an inhibitor's drug-likeness and whether an inhibitor with specified biological and pharmacological features would be an orally active medication in the human body [81]. When there are more than 5 h-bond donors, 10 h-bond acceptors, the molecular weight (MWT) is greater than 500 g/mol, and the estimated Log P (CLogP) is greater than 5 (or MlogP > 4.15), 'the rule of 5' indicates that poor absorption or permeability is more likely [81].

Several drugs are considerably restricted from entering the fluid environment of central nervous system (CNS) due to their inability to pass the blood–brain barrier (BBB). The BBB is usually assumed to be composed of a layer of CNS capillary endothelial cells that is selectively permeable [83]. The ability of a tiny molecule to traverse lipid bilayers, such as those that make up the GIT membrane, the BBB, and all cell membranes, is also critical for medicine and food distribution and elimination [84,85]. If the hERG potassium channels are blocked, the QT interval may be prolonged, leading to severe cardiac adverse effects in clinical trials of potential drug candidates [86]. In the early stages of drug discovery, AMES mutagenicity is employed to assess possible teratogenicity and genotoxicity.

Additionally, acute oral toxicity and carcinogenicity are the most concerning toxicological endpoints for human health. In particular, carcinogenicity has led to the withdrawal of numerous drugs from the market, including canrenone and hexestrol [87]. Around 90% of oxidative metabolic processes were catalyzed by CYP enzymes, specifically isoforms 1A2, 2C9, 2C19, 2D6, and 3A4 [88]. According to Cheng et al. [89], the more CYP isoforms inhibited by a particular small molecule, the more probable it will be implicated in drug–drug interaction (DDI). The ADMET property of the compounds predicts their druggability. The ADMET property of the studied compoumds, especially gallic acid [90], salicylic acid [91] and caffeic acid [92] comforms with report of other researchers. In our study, caffeic acid, gallic acid, and salicylic acid performed positively to all the ADMET tests, hence, potential antidiabetic drugs.

The *in-silico* method allows for exhibiting interactions and binding energies between small molecules and proteins at the atomic level. This enables the analysis of the behavior of metabolites within the target protein's binding site and provides insight into the potential mechanism of action of the molecules [93]. The 11β-HSD1 enzyme catalyzes the conversion of cortisone to cortisol in humans, available in the brain, liver, and adipose tissues [94], leading to the production of insulin resistance [95,96]. Hence, inhibition of 11β-HSD1 is a viable therapeutic target for the treatment of T2DM [97]. Hassan et al., [98] similarly highlighted the docking potential of caffeic acid, among other compounds identified in *Carica papaya* against 11β-HSD1. Several reported ligand–protein interaction studies have been carried out on 11β-HSD1 protein [99,99–101].

Amylase is found in human saliva and pancreatic secretions; the pancreatic and salivary α-amylases have highly comparable basic structures and demonstrate a high degree of structural similarity [102,103]. Inhibiting the human salivary α-amylase (HAS) is a therapeutic strategy to slow down the rate of glucose metabolism, thereby inhibiting hyperglycemia [104]. Due to its prominent role in the oral cavity, HSA has been used as a target for the structure-assisted design of compounds capable of preventing the production of undesirable dental plaque and the following process of dental caries formation and progression. Understanding the mechanism by which these inhibitors bind to the enzyme should provide a rationale for developing novel compounds with greater affinity and specificity for HSA [104]. Our study on HAS is corroborated by the study carried out by Lolok et al., [105], who reported docking potentials of some ligands against HAS proteins as a promising target of type 2 diabetes.

Similarly, the inhibition of glycogen phosphorylase is a therapeutic strategy to control the hallmark of type 2 diabetes [106,107]. Glycogenolysis, the process by which monomeric glucose is released from its polymeric storage form, glycogen, is a significant contributor to hepatic glucose output. The enzyme that catalyzes this reaction is glycogen phosphorylase [106]. Hence, the inhibition of the enzyme will slow down the release of monomeric glucose, which reduces the glucose output. Protein tyrosine phosphatase-1B (PTP1B), also acts as a negative regulator of the insulin signaling pathways by dephosphorylating the insulin receptor's tyrosine and inhibiting the insulin signaling cascade. Compounds or substances that inhibit PTP1B's negative regulation can induce the insulin pathway, hence aiding in controlling diabetes mellitus [108]. The expression of specific PTPs in muscle and adipose tissue is associated with the development of type 2 diabetes [109]. The results from our *in-silico* study suggest that the ligands, especially caffeic acid, could improve glucose levels, insulin metabolism, and fat accumulation by binding to this protein. As reported, molecular docking of PTP protein with epigallocatechin and caffeic acid extracted from *Geranium collinum* showed good binding energy [110]. The same efficacy is being repeated in our study.

Glutamine fructose-6-phosphate amidotransferase (GFAT) is the rate-limiting enzyme in the hexosamine biosynthesis pathway and is involved in the pathogenesis of type 2 diabetes [111]. It controls glucose uptake and catalyses the synthesis of glucosamine 6-phosphate. Thus, GFAT has been highlighted as a potential therapeutic target for T2DM treatment. Sirtuin 6 (SIRT6) is one of seven members of the mammalian sirtuin family found in the nucleus and is involved mainly in chromatin signaling and genomic integrity regulation [112]. SIRT6 is a worthwhile therapeutic target in the treatment of type 2 diabetes. The molecular reason for claiming that inhibiting SIRT6 may be a promising strategy for treating T2DM is that SIRT6 can restrict the expression of glucose transporters GLUT-1, −4, and glycolytic enzymes [113–115]. In the present study, all the ligands docked against SIRT6 yielded low energy, implying that they are potent inhibitors of SIRT6 enzymes.

Regarding insulin secretion stimulation, one of the targets of drugs for antidiabetic action is the serine protease dipeptidyl peptidase-IV (DPP-IV), as DPP-IV inhibition has been demonstrated to be an effective treatment for T2DM [116]. Natural DPP-IV inhibitors such as berberine, a class of isoquinoline alkaloids, have also been shown to be efficient in inhibiting the DPP-IV enzyme [117]. The interactions between our ligands and DPP-IV were remarkable. Salicylic acid, caffeic acid, phenylacetaldehyde, and 2,5-dimethylphenol have been found to interact with the residues reportedly found in the lipophilic S1 binding pocket by Kuhn et al. [118]. This implies that the compounds prominently present in *D. oliveri* subfraction are potential DPP-IV inhibitors.

Conclusion

Natural products remain an invaluable source for drug discovery, with bioactive principles playing a crucial role in therapy. Fractionation and chromatographic analyses, such as HPTLC and HRLC-MS, enabled the identification of potent compounds like gallic acid, salicylic acid, and caffeic acid, which exhibit significant antidiabetic properties, *in-silico*. The in-silico studies provided insights into the interaction mechanisms of these compounds with key enzymes such as 11β-HSD1, PTP1B, and DPP-IV, which are crucial for managing type 2 diabetes. Furthermore, ADMET profiling and Lipinski's rule of 5 reinforced the drug-likeness of the studied compounds, supporting their potential as therapeutic agents. Rational drug design strategies offer promising avenues for developing novel diabetes treatments based on these bioactive compounds. However, further studies on the purification and clinical trials of the lead compounds present in the young leaves are still needed.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Conceptualization: SBA and RK; Methodology: SBA; Investigation: SBA, RK, and BPJ; Writing-review & Editing: SBA, AAS, BPJ, and RK; Funding Acquisition: SBA and RK.

References

[1] American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2020. Diabetes Care. 2020;43(Supplement_1):S14–S31. doi: 10.2337/dc20-S002
[2] Kumar A, Gangwar R, Ahmad Zargar A, et al. Prevalence of diabetes in India: a review of IDF diabetes atlas 10th edition. Curr Diabetes Rev. 2024;20(1):105–114. doi: 10.2174/1573399819666230413094200
[3] Balaji R, Duraisamy R, Kumar MP. Complications of diabetes mellitus: a review. Drug Invent Today. 2019;12:98–103.
[4] Pittenger GL, Liuy D, Vinik AI. The toxic effects of serum from patients with type 1 diabetes mellitus on mouse neuroblastoma cells: a new mechanism for development of diabetic autonomic neuropathy. Diabet Med. 1993;10(10):925–932. doi: 10.1111/j.1464-5491.1993.tb00008.x
[5] Papatheodorou K, Banach M, Bekiari E, et al. Complications of diabetes 2017. J Diabet Res. 2018;2018:1–4. doi: 10.1155/2018/3086167
[6] Bloomgarden ZT. Cardiovascular disease in type 2 diabetes. Diabetes Care. 1999;22(10):1739–1744. doi: 10.2337/diacare.22.10.1739
[7] Pirie F. Selection of oral agents in the management of type 2 diabetes. Contin Med Educ. 2003;21:574–582.
[8] Asadi-Samani M, Bagheri N, Rafieian-Kopaei M, et al. Inhibition of Th1 and Th17 cells by medicinal plants and their derivatives: a systematic review. Phytother Res. 2017;31(8):1128–1139. doi: 10.1002/ptr.5837
[9] Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: past history and future perspective. J HerbMed Pharmacol. 2017;7(1):1–7. doi: 10.15171/jhp.2018.01
[10] Unuofin JO, Lebelo SL. Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: an updated review. Oxidative Med Cell Longev. 2020. doi: 10.1155/2020/1356893
[11] Demmers A, Korthout H, van Etten-Jamaludin FS, et al. Effects of medicinal food plants on impaired glucose tolerance: a systematic review of randomized controlled trials. diabetes res. Clin. Pract. 2017;131:91–106. doi: 10.1016/j.diabres.2017.05.024
[12] Suksomboon N, Poolsup N, Boonkaew S, et al. Meta-analysis of the effect of herbal supplement on glycemic control in type 2 diabetes. J Ethnopharmacol. 2011;137(3):1328–1333. doi: 10.1016/j.jep.2011.07.059
[13] Pivari F, Mingione A, Brasacchio C, et al. Curcumin and type 2 diabetes mellitus: prevention and treatment. Nutrients. 2019;11(8):11. doi: 10.3390/nu11081837
[14] Li F-S, Weng J-K. Demystifying traditional herbal medicine with modern approach. Nat Plants. 2017;3(8):1–7. doi: 10.1038/nplants.2017.109
[15] Harvey AL. Natural products in drug discovery. Drug Discov Today. 2008;13:894–901. doi: 10.1016/j.drudis.2008.07.004
[16] Yarnell E, Abascal K. Dilemmas of traditional botanical research. HerbalGram. 2002;55:54–56.
[17] Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: past history and future perspective. J HerbMed Pharmacol. 2018;7(1):1–7. doi: 10.15171/jhp.2018.01
[18] Schroeder FC, Gronquist M. Extending the scope of NMR spectroscopy with microcoil probes. Angew Chem Int Ed. 2006;45(43):7122–7131. doi: 10.1002/anie.200601789
[19] Mitra D, Mitra D, Bensaad MS, et al. Evolution of bioinformatics and its impact on modern bio-science in the twenty-first century: special attention to pharmacology, plant science and drug discovery. Comput Toxicol. 2022;24:100248. doi: 10.1016/j.comtox.2022.100248
[20] Pappalardo F, Russo G, Tshinanu FM, et al. In silico clinical trials: concepts and early adoptions. Brief Bioinform. 2019;20(5):1699–1708. doi: 10.1093/bib/bby043

[21] Vemula D, Jayasurya P, Sushmitha V, et al. CADD, AI and ML in drug discovery: a comprehensive review. Eur J Pharm Sci. 2023;181:106324. doi: 10.1016/j.ejps.2022.106324

[22] Gomes AFT, de Medeiros WF, Medeiros I, et al. In silico screening of therapeutic targets as a tool to optimize the development of drugs and nutraceuticals in the treatment of diabetes mellitus: a systematic review. Int J Mol Sci. 2024;25(17):9213. doi: 10.3390/ijms25179213

[23] Sabe VT, Ntombela T, Jhamba LA, et al. Current trends in computer aided drug design and a highlight of drugs discovered via computational techniques: a review. Eur J Med Chem. 2021;224:113705. doi: 10.1016/j.ejmech.2021.113705

[24] Wiesmeth H, Häckl D. Integrated environmental policy: a review of economic analysis. Waste Manag Res. 2017;35(4):332–345. doi: 10.1177/0734242X16672319

[25] de la Estrella M, Aedo C, Mackinder B, et al. Taxonomic revision of Daniellia (Leguminosae: Caesalpinioideae). Syst Bot. 2010;35(2):296–324. doi: 10.1600/036364410791638414

[26] Schmelzer GH, Louppe D. Daniella oliveri (Rolfe) Hutch. and Dalziel [WWW Document]. Rec. From PROTA4U. URL 2012. Available from: https://uses.plantnet-project.org/en/Daniellia_oliveri_(PROTA)

[27] Onefeli AO. Effectiveness of DNA barcoding in discriminating daniellia ogea (harms) Rolfe ex Holland and daniellia oliveri (Rolfe) Hutch. & Dalziel Trees, For People. 2021;4:100067. doi: 10.1016/J.TFP.2021.100067

[28] Mohammed A, Ibrahim MA, Islam MS. African medicinal plants with antidiabetic potentials: a review. Planta Med. 2014;80(5):354–377. doi: 10.1055/s-0033-1360335

[29] Iwueke AV, Nwodo OFC. Antihyperglycaemic effect of aqueous extract of Daniella oliveri and sarcocephalus latifolius roots on key carbohydrate metabolic enzymes and glycogen in experimental diabetes. Biokemistri. 2008;20:63–70.

[30] Iwueke AV, Nwodo OFC. Effect of combined root extract of sarcocephalus latifolius and Daniella oliveri on blood glucose and serum lipids in alloxan-diabetic rats. Niger J Biochem Mol Biol. 2008;23:37–41.

[31] Shauibu G, Abu AH, Agwu EO, et al. Effects of Daniella Oliveri aqueous leave extract on blood glucose, haematological and biochemical values in streptozotocin-induced type 2 diabetes in male albino rats. Vom J Vet Sci. 2018;13:81–87.

[32] Adeyemi SB, Quadri LA, Krishnamurthy R. Ameliorative effects of Daniellia oliveri leaf extracts on streptozotocin-nicotinamide-induced type II diabetes in Wistar rats. Trop J Nat Prod Res. 2021;5:1158–1165. doi: 10.26538/tjnpr/v5i6.30

[33] Adeyemi SB, Surana V, Krishnamurthy R. In-vitro inhibition of type 2 diabetes key enzymes; effect of extracts and solvent-solvent fractions of daniellia oliveri (Rolfe) Hutch. & Dalziel Bull Pharm Sci Assiut Univ. 2021;44(2):415–426. doi: 10.21608/bfsa.2021.207160

[34] Vashishat A, Patel P, Das Gupta G, et al. Alternatives of animal models for biomedical research: a comprehensive review of modern approaches. STEM Cell Rev And Rep. 2024;20(4):881–899. doi: 10.1007/s12015-024-10701-x

[35] Shaker B, Ahmad S, Lee J, et al. In silico methods and tools for drug discovery. Comput Biol Med. 2021;137:104851. doi: 10.1016/j.compbiomed.2021.104851

[36] Nechchadi H, Nadir Y, Benhssaine K, et al. Hypolipidemic activity of phytochemical combinations: a mechanistic review of preclinical and clinical studies. Food Chem. 2024;459:140264. doi: 10.1016/j.foodchem.2024.140264

[37] Nematalla KH, Arafa SA, Khalil EM. Effect of different plant parts, as hypolipidemic agents on weight management and their mechanisms of action: a review. Curr Sci Int. 2024;8:595–603.

[38] Amtaghri S, Slaoui M, Eddouks M. Moroccan antihypertensive plants and their mechanisms of action. Endocrine, Metab. Immune disord. Targets (formerly curr. Drug Targets-Immune, Endocr Metab Disord. 2024;24(11):1254–1279. doi: 10.2174/0118715303272284231126145853

[39] Riaz M, Rasool G, Abbas M, Munir, N, Irshad, S, Tahir, IM. Plants and phytochemicals for prevention and cure of hypertension. In Pullaiah, T, Ojha, S (eds.) Cardioprotective Plants. Singapore, Springer2024:87–105 doi:10.1007/978-981-97-4627-9_4

[40] Andrade C, Gomes NGM, Duangsrisai S, et al. Medicinal plants utilized in Thai traditional medicine for diabetes treatment: ethnobotanical surveys, scientific evidence and phytochemicals. J Ethnopharmacol. 2020;113177:113177. doi: 10.1016/j.jep.2020.113177

[41] Harshitha HS, Kumar R, Shilpa V et al. Comprehensive review on various medicinal plants having potent anti-diabetic activity. Int J Pharm Drug Anal. 2020:8(5) 1–6.
[42] Kavitha R, Rigley CM, Salomy SR, et al. In vitro analysis of antidiabetic and anti-inflammatory activities of selected medicinal plants. J Pharm Sci Res. 2021;13:259–270.
[43] Tittikpina NK, Kirsch G, Duval RE, et al. Daniellia oliveri (Rolfe) hutch and Dalziel: antimicrobial activities, cytotoxicity evaluation, and phytochemical identification by GC-MS. Antibiotics. 2022;11(12):1699. doi: 10.3390/antibiotics11121699
[44] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 1999;299:152–178.
[45] Kim J-S, Hyun TK, Kim M-J. The inhibitory effects of ethanol extracts from sorghum, foxtail millet and proso millet on α-glucosidase and α-amylase activities. Food Chem. 2011;124 (4):1647–1651. doi: 10.1016/j.foodchem.2010.08.020
[46] Telagari M, Hullatti K. In-vitro α-amylase and α-glucosidase inhibitory activity of Adiantum caudatum Linn. and Celosia argentea Linn. extracts and fractions. Indian J Pharmacol. 2015;47(4):425. doi: 10.4103/0253-7613.161270
[47] Mallick MN, Singh M, Parveen R, et al. Research article HPTLC analysis of bioactivity guided anticancer enriched fraction of hydroalcoholic extract of picrorhiza kurroa. Biomed Res Int. 2015;2015:1–18. doi: 10.1155/2015/513875
[48] Singh M, Tamboli ET, Kamal YT, et al. Quality control and in vitro antioxidant potential of Coriandrum sativum Linn. J Pharm Bioallied Sci. 2015;7(4):280–283. doi: 10.4103/0975-7406.168026
[49] 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):1–13. doi: 10.1038/srep42717
[50] Xiong G, Wu Z, Yi J, et al. Admetlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic Acids Res. 2021;49(W1): W5–W14. doi: 10.1093/nar/gkab255
[51] Ahmad Dar A, Sangwan PL, Kumar A. Chromatography: an important tool for drug discovery. J Sep Sci. 2020;43(1):105–119. doi: 10.1002/jssc.201900656
[52] Aykul S, Martinez-Hackert E. Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. Anal Biochem. 2016;508:97–103. doi: 10.1016/j.ab.2016.06.025
[53] Aleixandre A, Gil JV, Sineiro J, et al. Understanding phenolic acids inhibition of α-amylase and α-glucosidase and influence of reaction conditions. Food Chem. 2022;372:131231. doi: 10.1016/j.foodchem.2021.131231
[54] Uyanır E, Šoral M, Seyhan G, et al. Alpha-Glucosidase inhibitory effects of Flavonoids, phenolic acids and iridoids isolated from Vinca Soneri: In Vitro and in silico perspectives. Chem Biodivers. 2024;21:e202401386. doi: 10.1002/cbdv.202401386
[55] Ćorković I, Gašo-Sokač D, Pichler A, et al. Dietary polyphenols as natural inhibitors of α-amylase and α-glucosidase. Life; 2022. doi: 10.3390/life12111692
[56] Liu H, Zheng C, Li Z, et al. Inhibitory mechanism of phenolic compounds in rapeseed oil on α-amylase and α-glucosidase: spectroscopy, molecular docking, and molecular dynamic simulation. Spectrochim Acta Part A Mol Biomol Spectrosc. 2023;289:122251. doi: 10.1016/j.saa.2022.122251
[57] Zheng Y, Tian J, Yang W, et al. Inhibition mechanism of ferulic acid against α-amylase and α-glucosidase. Food Chem. 2020;317:126346. doi: 10.1016/j.foodchem.2020.126346
[58] Patil AG, Koli SP, Patil DA. Pharmcognostical standardization and HPTLC fingerprint of Averrhoa *bilimbi* (L.) fruits. J Pharm Res. 2013;6(1):145–150. doi: 10.1016/j.jopr.2012.11.030
[59] Ma J, Luo X-D, Protiva P, et al. Bioactive novel polyphenols from the fruit of Manilkara *zapota* (sapodilla). J Nat Prod. 2003;66:983–986. doi: 10.1021/np020576x
[60] Singh J, Rai GK, Upadhyay AK, et al. Antioxidant phytochemicals in tomato (Lycopersicon *esculentum*). Indian J Agric Sci. 2004;74:3–5.
[61] Gandhi GR, Jothi G, Antony PJ, et al. Gallic acid attenuates high-fat diet fed-streptozotocin-induced insulin resistance via partial agonism of PPARγ in experimental type 2 diabetic rats and enhances glucose uptake through translocation and activation of GLUT4 in PI3K/p-akt signaling pathway. Eur J Pharmacol.

2014;745:201–216. doi: 10.1016/j.ejphar.2014.10.044

[62] Latha RCR, Daisy P. Insulin-secretagogue, antihyperlipidemic and other protective effects of gallic acid isolated from Terminalia *bellerica* Roxb. In streptozotocin-induced diabetic rats. Chem Biol Interact. 2011;189(1–2):112–118. doi: 10.1016/j.cbi.2010.11.005

[63] Abdel-Moneim A, Abd El-Twab SM, Yousef AI, et al. New insights into the in vitro, in situ and in vivo antihyperglycemic mechanisms of gallic acid and p-coumaric acid. Arch Physiol Biochem. 2022;128(5):1188–1194. doi: 10.1080/13813455.2020.1762659

[64] Uddin SJ, Afroz M, Zihad SMNK, et al. A systematic review on anti-diabetic and cardioprotective potential of gallic acid: a widespread dietary phytoconstituent. Food Rev Int. 2022;38(4):420–439. doi: 10.1080/87559129.2020.1734609

[65] Xu Y, Tang G, Zhang C, et al. Gallic acid and diabetes mellitus: its association with oxidative stress. Molecules. 2021;26(23):7115. doi: 10.3390/molecules26237115

[66] Popova L, Pancheva T, Uzunova A. Salicylic acid: properties, biosynthesis and physiological role. Bulg J Plant Physiol. 1997;23:85–93.

[67] Sagone AL, Husney RM. Oxidation of salicylates by stimulated granulocytes: evidence that these drugs act as free radical scavengers in biological systems. J Immunol. 1987;138(7):2177–2183. doi: 10.4049/jimmunol.138.7.2177

[68] Dragomir E, Manduteanu I, Voinea M, et al. Aspirin rectifies calcium homeostasis, decreases reactive oxygen species, and increases NO production in high glucose-exposed human endothelial cells. J Diabetes Complications. 2004;18(5):289–299. doi: 10.1016/j.jdiacomp.2004.03.003

[69] Genaro-Mattos TC, Maurício ÂQ, Rettori D, et al. Antioxidant activity of caffeic acid against iron-induced free radical generation—A chemical approach. PLoS One. 2015;10(6):e0129963. doi: 10.1371/journal.pone.0129963

[70] Tošović J. Spectroscopic features of caffeic acid: theoretical study. Kragujevac J Sci. 2017;(39):99–108. doi: 10.5937/KgJSci1739099T

[71] Verma RP, Hansch C. An approach towards the quantitative structure–activity relationships of caffeic acid and its derivatives. Chembiochem. 2004;5(9):1188–1195. doi: 10.1002/cbic.200400094

[72] Agunloye OM, Oboh G, Ademiluyi AO, Ademosun AO, Akindahunsi AA, Oyagbemi AA, Omobowale TO, Ajibade TO, Adedapo AA. Cardio-protective and antioxidant properties of caffeic acid and chlorogenic acid: mechanistic role of angiotensin converting enzyme, cholinesterase and arginase activities in cyclosporine induced hypertensive rats. Biomed Pharmacother. 2019;109:450–458. doi: 10.1016/j.biopha.2018.10.044

[73] Chen JH, Ho C-T. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. J Agric Food Chem. 1997;45:2374–2378. doi: 10.1021/jf970055t

[74] Magnani C, Chiari BG, Isaac VLB, et al. In vitro safety evaluation of caffeic acid. Athens J Heal. 2014;1(3):181–188. doi: 10.30958/ajh.1-3-2

[75] Oršolić N, Sirovina D, Odeh D, et al. Efficacy of caffeic acid on diabetes and its complications in the mouse. Molecules. 2021;26(11):3262. doi: 10.3390/molecules26113262

[76] Mandagere AK, Thompson TN, Hwang K-K. Graphical model for estimating oral bioavailability of drugs in humans and other species from their caco-2 permeability and in vitro liver enzyme metabolic stability rates. J Med Chem. 2002;45:304–311. doi: 10.1021/jm010152k

[77] Alavijeh MS, Chishty M, Qaiser MZ, et al. Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. NeuroRx. 2005;2(4):554–571. doi: 10.1602/neurorx.2.4.554

[78] Thapa RK, Choi H-G, Kim JO, et al. Analysis and optimization of drug solubility to improve pharmacokinetics. J Pharm Investig. 2017;47(2):95–110. doi: 10.1007/s40005-016-0299-z

[79] Attique SA, Hassan M, Usman M, et al. A molecular docking approach to evaluate the pharmacological properties of natural and synthetic treatment candidates for use against hypertension. Int J Environ Res Public Health. 2019;16(6):923. doi: 10.3390/ijerph16060923

[80] Umar AB, Uzairu A, Shallangwa GA, et al. Design of potential anti-melanoma agents against SK-MEL-5 cell line using QSAR modeling and molecular docking methods. SN Appl Sci. 2020;2:1–18. doi: 10.1007/s42452-020-2620-8

[81] Lipinski CA, Lombardo F, Dominy BW, et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 1997;23(1–3):3–25. doi: 10.1016/S0169-409X(96)00423-1

[82] Cabrera-Pérez MÁ, Sanz MB, Sanjuan VM et al. Importance and applications of cell-and tissue-based in vitro models for drug permeability screening in early stages of drug development. In: Sarmento, B, Pereira, CL, Neves, JD eds Concepts and models for drug permeability studies 2nd Edition. Elsevier; 2016. p. 5–41.
[83] Crone C, Thompson AM. Permeability of brain capillaries. In: Crone C Lassen NA, editors. Capillary permeability. Alfred Benzon Symp. II. Copenhagen: Munksgaard; 1970. p. 447–453.
[84] Avdeef A. Physicochemical profiling (solubility, permeability and charge state). Curr Top Med Chem. 2001;1(4):277–351. doi: 10.2174/1568026013395100
[85] Castillo-Garit JA, Casanola-Martin GM, Le-Thi-Thu H, et al. A simple method to predict blood-brain barrier permeability of drug-like compounds using classification trees. Med Chem (Los Angeles). 2017;13(7):664–669. doi: 10.2174/1573406413666170209124302
[86] Hansen K, Mika S, Schroeter T, et al. Benchmark data set for in silico prediction of ames mutagenicity. J Chem Inf Model. 2009;49:2077–2081. doi: 10.1021/ci900161g
[87] Siramshetty VB, Nickel J, Omieczynski C, et al. Withdrawn—a resource for withdrawn and discontinued drugs. Nucleic Acids Res. 2016;44:D1080–D1086. doi: 10.1093/nar/gkv1192
[88] Williams JA, Hyland R, Jones BC, et al. Drug-drug interactions for udp-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/auc) ratios. Drug Metab Dispos. 2004;32(11):1201–1208. doi: 10.1124/dmd.104.000794
[89] Cheng F, Yu Y, Zhou Y, et al. Insights into molecular basis of cytochrome p450 inhibitory promiscuity of compounds. J Chem Inf Model. 2011;51:2482–2495. doi: 10.1021/ci200317s
[90] Aras M, Yayıntaş Ö. In silico analysis of quercetin, gallic acid, oleanolic acid, and ursolic acid on diabetes mellitus. Troia Med J. 2022;3:100–110.
[91] Aminu KS, Uzairu A, Umar AB, et al. Salicylic acid derivatives as potential α-glucosidase inhibitors: drug design, molecular docking and pharmacokinetic studies. Bull Natl ResearchCent. 2022;46(1):162. doi: 10.1186/s42269-022-00853-6
[92] Dhiman P, Malik N, Khatkar A. Hybrid caffeic acid derivatives as monoamine oxidases inhibitors: synthesis, radical scavenging activity, molecular docking studies and in silico ADMET analysis. Chem Cent J. 2018;12(1):1–17. doi: 10.1186/s13065-018-0481-7
[93] Parveen S, Ansari MHR, Parveen R, et al. Chromatography based metabolomics and in silico screening of Gymnema sylvestre leaf extract for its antidiabetic potential. Evidence-Based Complement Altern Med. 2019;2019:1–14. doi: 10.1155/2019/7523159
[94] Asensio C, Muzzin P, Rohner-Jeanrenaud F. Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance. Int J Obes. 2004;28(S4):S45–S52. doi: 10.1038/sj.ijo.0802856
[95] Nguyen NDT, Le LT. Targeted proteins for diabetes drug design. Adv Nat Sci: Nanosci Nanotechnol. 2012;3(1):13001. doi: 10.1088/2043-6262/3/1/013001
[96] Rathore PK, Arathy V, Attimarad VS, et al. In-silico analysis of gymnemagenin from Gymnema sylvestre (Retz.) R.Br. With targets related to diabetes. J Theor Biol. 2016;391:95–101. doi: 10.1016/j.jtbi.2015.12.004
[97] Anderson A, Walker BR. 11β-HSD1 inhibitors for the treatment of type 2 diabetes and cardiovascular disease. Drugs. 2013;73(13):1385–1393. doi: 10.1007/s40265-013-0112-5
[98] Hassan AM, Sadiya AH, Jafaru KE, et al. In-silico investigation of potential inhibitors of 11-β-hydroxysteroid dehydrogenase. Asian J Biochem Genet Mol Biol. 2023;15(2):10–23. doi: 10.9734/ajbgmb/2023/v15i2329
[99] Galandarli L, Mollayeva C, Javadzade A, et al. Molecular docking of Glycyrrhizin with 11β-HSD1 protein. Res Agric Vet Sci. 2022:6 (3) 141–147.
[100] Pérez LCC, Padilla-Martínez II, Cruz A, et al. Design, synthesis, molecular docking and in vitro evaluation of benzothiazole derivatives as 11β-hydroxysteroid dehydrogenase type 1 inhibitors. Mol Divers. 2020;24(4):1–14. doi: 10.1007/s11030-019-10006-z
[101] Studzińska R, Kupczyk D, Płaziński W, et al. Novel 2-(adamantan-1-yl amino) thiazol-4 (5 h)-one derivatives and their inhibitory activity towards 11β-HSD1—Synthesis, molecular docking and in vitro studies. Int J Mol Sci. 2021;22(16):8609. doi: 10.3390/ijms22168609
[102] 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;4:1730–1742. doi: 10.1002/pro.5560040908
[103] Ramasubbu N, Paloth V, Luo Y, et al. Structure of human salivary α-amylase at 1.6 Å resolution:

implications for its role in the oral cavity. Acta Crystallogr Sect D Biol Crystallogr. 1996;52 (3):435–446. doi: 10.1107/S0907444995014119

[104] Gyémánt G, Kandra L, Nagy V, et al. Inhibition of human salivary α-amylase by glucopyranosylidene-spiro-thiohydantoin. Biochem Biophys Res Commun. 2003;312(2):334–339. doi: 10.1016/j.bbrc.2003.10.119

[105] Lolok N, Sumiwi SA, Muhtadi A, et al. Molecular docking and molecular dynamics studies of bioactive compounds contained in noni fruit (morinda citrifolia L.) against human pancreatic α-amylase. J Biomol Struct Dyn. 2022;40 (15):7091–7098. doi: 10.1080/07391102.2021.1894981

[106] Henke BR, Sparks SM. Glycogen phosphorylase inhibitors. Mini Rev Med Chem. 2006;6 (8):845–857. doi: 10.2174/138955706777934991

[107] Treadway JL, Mendys P, Hoover DJ. Glycogen phosphorylase inhibitors for treatment of type 2 diabetes mellitus. Expert Opin Investig Drugs. 2001;10(3):439–454. doi: 10.1517/13543784.10.3.439

[108] Prabhakar PK, Sivakumar PM. Protein tyrosine phosphatase 1B inhibitors: a novel therapeutic strategy for the management of type 2 diabetes mellitus. Curr Pharm Des. 2019;25 (23):2526–2539. doi: 10.2174/1381612825666190716102901

[109] Gurzov EN, Stanley WJ, Brodnicki TC, et al. Protein tyrosine phosphatases: molecular switches in metabolism and diabetes. Trends Endocrinol Metab. 2015;26(1):30–39. doi: 10.1016/j.tem.2014.10.004

[110] Tamrakar AK, Maurya CK, Rai AK. PTP1B inhibitors for type 2 diabetes treatment: a patent review (2011–2014). Expert Opin Ther Pat. 2014;24(10):1101–1115. doi: 10.1517/13543776.2014.947268

[111] Nakaishi Y, Bando M, Shimizu H, et al. Structural analysis of human glutamine: fructose-6-phosphate amidotransferase, a key regulator in type 2 diabetes. FEBS Lett. 2009;583(1):163–167. doi: 10.1016/j.febslet.2008.11.041

[112] Bae EJ. Sirtuin 6, a possible therapeutic target for type 2 diabetes. Arch Pharm Res. 2017;40:1380–1389. doi: 10.1007/s12272-017-0989-8

[113] Kugel S, Mostoslavsky R. Chromatin and beyond: the multitasking roles for SIRT6. Trends Biochem Sci. 2014;39(2):72–81. doi: 10.1016/j.tibs.2013.12.002

[114] Mostoslavsky R, Chua KF, Lombard DB, et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell. 2006;124(2):315–329. doi: 10.1016/j.cell.2005.11.044

[115] Zhong L, D'Urso A, Toiber D, et al. The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1α. Cell. 2010;140(2):280–293. doi: 10.1016/j.cell.2009.12.041

[116] Havale SH, Pal M. Medicinal chemistry approaches to the inhibition of dipeptidyl peptidase-4 for the treatment of type 2 diabetes. Bioorg Med Chem. 2009;17 (5):1783–1802. doi: 10.1016/j.bmc.2009.01.061

[117] Chakrabarti R, Bhavtaran S, Narendra P, et al. Dipeptidyl peptidase-iv inhibitory activity of berberis aristata. J Nat Prod. 2011;4:158–163.

[118] Kuhn B, Hennig M, Mattei P. Molecular recognition of ligands in dipeptidyl peptidase IV. Curr Top Med Chem. 2007;7(6):609–620. doi: 10.2174/156802607780091064