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Research Article

In Silico Analysed Diabetic Drug Candidates from GC-MS Screened Phytochemicals and *In-vitro* Antioxidant Activity of *Oldfieldia dactylophylla*, A Medicinal Plant from Zambia

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Abstract

Oldfieldia dactylophylla (OD) is a miniature, semi-deciduous spiny shrub that grows up to 10 meters high. The root part has been used extensively in the management of abdominal pains, general body pain, Sexually transmitted diseases (STD'S), malaria, hernia, diabetes and many others. This study aimed at identifying the secondary metabolites using standard chemical tests and GC-MS phytochemical profiling as well as evaluating the total phenol, total flavonoid content and in vitro antioxidant activity of crude root extract of *Oldfieldia dactylophylla*. The preliminary qualitative phytochemical screening of the crude leaf extract showed presence of phytochemicals such as alkaloids, flavonoids, saponins, phenols, tannins, terpenoids and steroids. GC-MS phytochemical Profiling revealed putative compounds such as Neric acid, Copaene, Beta-bisbolene, Hepetanediamide, N,N'-dibenzoyloxy- and many more. The total flavonoid content was found to be 143.3667 mg/L ± 0.2887, while total phenol content was found to be 53.4667 mg/L ± 0.0577. The IC50 was found to be 35.71 mg/L indicating that the root extract is a very effective antioxidant. The IC50 of the standard antioxidant ascorbic acid was 23.19 µg/mL. ADMET analysis and molecular docking analysis of selected phytochemicals confirmed certain molecules as promising drug candidates. However, these candidates necessitate further consideration through in vitro and *in vivo* studies.

Keywords: Phytochemical Screening; *Oldfieldia dactylophylla*; Antioxidant Activity; Total Flavonoid Content; Total Phenol Content

Introduction

Numerous metabolic and physiological processes are intrinsic to all living organisms, resulting in the regular production of free radicals within cells [1]. These radicals, originating from nitrogen (reactive nitrogen species-RNS) or oxygen (reactive oxygen species-ROS) compounds, exhibit high reactivity owing to the presence of an unpaired electron [2]. Free radicals have the potential to cause oxidative damage to lipids, proteins, and DNA, contributing to a range of chronic conditions in humans, including diabetes, cancer, aging, autoimmune disorders, and other degenerative ailments [3]. Medicinal plants have a long history of being used to answer various health concerns and, more recently, as precursors in the pharmaceutical and cosmetic industries. Beyond providing essential nutrients to humans and animals, medicinal plants also possess therapeutically active compounds crucial for enhancing human and animal well-being and managing various illnesses [4].

Medicinal plants include phytochemicals called alkaloids, flavonoids, saponins, tannins, phenols, and glycosides that are used to treat a range of illnesses and reduce the intensity of pain [5]. It has previously been demonstrated that phytochemicals possess a wide range of pharmacological characteristics, including antifungal, antiulcer, antidiabetic, antibacterial, antioxidant, and antiviral effects [6-8]. To this effect, phytochemical research is often viewed as a successful method of discovering novel bioactive compounds whose therapeutic potential can further be explored [9].

Oldfieldia dactylophylla is a small, partially deciduous shrub with spines, reaching a height of up to 10 meters. It is characterized

by a short trunk and wide-spreading branches covered in dense reddish-brown hairs. This shrub belongs to the Oldfieldia genus within the Picrodendraceae family [10]. It showcases brown female flowers and vibrant yellow male inflorescence, while its fruit is ovoid to subglobose, turning orange and developing a pubescent texture when ripe [11]. This species is endemic to Tanzania and southern tropical Africa [11]. Known by different local names in various regions such as Msamina, Nyamwezi, Mkalanga, Kampangwila, Mubonga, Kalikali or Nakali, Kafutu, Kafumbafumba, Mufutu, Kazonga, muliwanfengi among others, the plant has wide applications. For instance, in an effort to determine the abundance and distribution as well as to investigate the traditional uses of *Oldfieldia dactylophylla* by local communities in Malawi, Manda (2007), reported that the root of *Oldfieldia dactylophylla* was the most used part with a reported plant part value (PPV) of 55%, while the leaf was the least used with a PPV of 1%. The root was reported to have numerous uses, with abdominal pain, general body pain, sexually transmitted diseases (STD'S), and diabetes being mostly cited uses with intra specific use values (IUV) of 25%, 18%, 11% and 10% respectively. It was further noted that from the IUVs of the bark of *Oldfieldia dactylophylla*, abdominal pain, body weakness, malaria, STD's and purging were among the most important uses with values of 22%, 16%, 14%, 12% and 10% respectively [11].

Diabetes, a metabolic disorder, arises when the body stops producing or absorbing insulin adequately, leading to elevated blood sugar levels [12]. The popularity of traditional herbal remedies is growing due to their lower incidence of adverse effects compared to chemical or allopathic treatments. This study also focuses on computationally screening phytochemicals obtained from *Oldfieldia dactylophylla* known for its anti-diabetic properties. The objective is to assess the inhibitory effects of these phytochemicals on the human α-amylase (PDB ID: 1HNY) and α-glucosidase (PDB ID: 3WY2) proteins, which play crucial roles in breaking down various carbohydrates into glucose [13].

Within pharmaceutical development, computational models such as Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) play a crucial role in expediting the preliminary evaluation of these properties for potential drug candidates. This initial screening aims to prioritize compounds for more in-depth *in vitro* and *in vivo* investigations [14-17]. In the current study, an analysis was conducted to assess the ADMET properties of selected phytocompounds and a reference drug. The notable findings from the ADMET evaluation underscore the need for further investigation of these molecules through comprehensive *in vitro* and *in vivo* analyses.

Therefore, in the light of the above statistical evidence and other wider claims of the effectiveness of the root of *Oldfieldia dactylophylla* this study aimed at conducting a preliminary phytochemical screening as well investigating the total phenol, total flavonoid and antioxidant capacity of the root methanolic extract of *Oldfieldia dactylophylla*. In Silico and ADMET analysis of some selected phytochemical was also conducted leading to interesting results.

Experimental

Materials and Methods

Collection and Authentication of plant samples

The root part of *Oldfieldia dactylophylla* was collected from the Lufwanyama district (13°25'60''S and 27°45'0'' E in Degrees Minutes seconds) located in the Copperbelt province of Zambia. Freshly collected plant samples (leaves) were verified and authenticated by the Zambia Forestry Department, after which the roots were collected. Figure 1 shows *Oldfieldia dactylophylla* plant in its natural environment before sample collection for identification.

Figure 1: *Oldfieldia dactylophylla* plant.

Chemicals, reagents and apparatus

The study utilized specific chemicals, namely Folin-Ciocalteu reagent, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 99% methanol, sodium hydroxide (97% extra pure pellets), sodium nitrate (97% extra pure), gallic acid (99.5% extra pure), sodium carbonate anhy-

drous (99.5% extra pure), aluminum chloride hexahydrate (99% AR), and quercetin dehydrate extra pure (98%). These chemicals were procured from Pallav Chemicals and Solvents Pvt, Ltd, India. Additionally, laboratory equipment, including a Cary Agilent 60 UV-VIS Spectrophotometer, and other utensils were provided by the Chemistry Department at The Copperbelt University.

Preparation of extract for preliminary phytochemical screening and GC-MS analysis

The crude root extract of *Oldfieldia dactylophylla* was obtained through Soxhlet extraction method, following a method reported by Chibuye et al. (2023). In brief, 20g of powdered root sample was placed in a thimble, and 300 mL of 98% ethanol served as the solvent. The extraction process continued for 10 hours, followed by filtration. The filtrate was then concentrated under vacuum pressure at 80°C using a rotary evaporator (Büchi Rotovapour 200). The resulting concentrated extracts were utilized for analysis.

Preparation of extract for estimation of total phenol content, total flavonoid content and antioxidant activity

The crude root extract for assessing total flavonoid, total phenols content, and in-vitro antioxidant activities was obtained through maceration, employing procedure reported by Chibuye et al., (2023) [18]. Briefly, 2.0 g of powdered leaves underwent maceration with 30 mL of 99% methanol, with continuous shaking of the mixture for 24 hours at 100 rpm. Subsequently, the mixture was filtered using White Man No 1 filter paper and stored in sterile amber bottles in preparation for further analysis.

Preliminary phytochemical screening

The initial phytochemical screening tests were conducted, employing a method similar to that of Devi N. N. et al. (2012) [19]. These tests were performed to assess the presence of various secondary metabolite classes such as alkaloids, flavonoids, saponins, terpenoids, tannins, phenols, steroids, and anthraquinones in the root extract of *Oldfieldia dactylophylla.*

Test for Alkaloids (Wagner's test)

Wagner's reagent and 1 mL of diluted HCL were added to 3 mL of the crude plant extract in a test tube, and the mixture was well agitated. A positive test resulted from the emergence of a reddish-brown precipitate.

Test for saponins (Foam test)

About 3 mL of ethanoic crude extract was placed in a test tube to which 5 mL of distilled water was added and then shaken vigorously and allowed to stand for 10 minutes. The appearance of a fairly stable emulsion indicated the presence of saponins.

Test for flavonoids (Alkaline reagent test)

Initially, approximately 3 mL of the ethanoic crude extract was placed into a test tube. Subsequently, 3 drops of a diluted 20% NaOH solution were added. The emergence of a vibrant yellow color, which turned colorless upon the addition of a few drops of diluted HCl, signified the presence of flavonoids.

Test for phenols and tannins (Braymer's test)

About 3 mL of the ethanoic crude plant extract was initially placed in a test tube, 3 drops of 10% ferric chloride solution were then added. The appearance of bluish precipitation indicated a positive test.

Test for terpenoid (Salkowski test)

About 3 mL of chloroform was placed initially in a test tube, 5 mL of plant extract was added slowly. Subsequently, 4 drops of concentrated Sulphuric acid was added to this test tube and the solution was allowed to stand for a few minutes. Appearance of a reddish or brownish coloration indicated a positive test for terpenoids.

Test for antraquinone (Borntrager's test)

About 3 mL of chloroform was placed in a test tube, followed by addition of 5 mL of plant extract. 3 mL of 10% ammonia was then immediately to the mixture. A redish, violet colouration indicated presence of Antraquinones.

Test for steroid: (Salkowski Reaction)

About 3 mL of chloroform was placed initially in a test tube followed by the addition of 5 mL of the plant extract. Further, 4 drops of concentrated Sulphuric acid were added to the mixture and the solution was allowed to stand for a few minutes. Formation of red color indicated a positive test.

GC-MS analysis conditions

The study used Gas Chromatography-Mass Spectrometry (GC–MS) with Thermo GC-Trace Ultra Ver 5.0 and Thermo MS DSQ II for chemical identification in *Oldfieldia dactylophylla* root extract. The

GC-MS operation was conducted using the Electron Ionization system with a 70eV ionization energy. The column type utilized was a High-performance GC capillary column (Scion -5MS) consisting of the following features: column length of 30 m, 0.25 mm diameter (narrow bore) and a film of 0.25μm with temperature limits of -60 °C to 325°C. The carrier gas utilized was Helium (99.99%) i.e. with a baseline of N5.0 at constant flow rate of 1 mL/min and an injection volume of 1 μL. The injector type S/SL set at temperature of 250°C, hold time 20 min with a split ratio 10:1 was used. The Auto sampler (8400) was used while a micro syringe of size 10μl, were used. The oven temperature was initially programmed to run from 80°C (Isothermal) for 3 mins followed by a systematic rate increase of 10.0 °C/min to 300°C for a total GC running time of 34.00 mins for a 9 min hold time. The GC–MS chromatogram of ethanoic extracts of plant gave the peaks corresponding to the bioactive compounds that were recognized by relating the peak retention time, peak area (%), height (%) and mass spectral fragmentation patterns to that of known compounds stored in NIST library.

Estimation of flavonoid content using aluminum chloride

The assessment of total flavonoids content in *Oldfieldia dactylophylla* root extracts was conducted using the aluminum chloride colorimetric assay, following the method outlined by Zhishen et al. (1999) [14-20].

Preparation of quercetin and sample extracts

A calibration curve for quercetin was generated using a method reported by Zhishen et al., (1999) [20]. Initially, a quercetin stock solution was prepared by dissolving 100 mg in 100 mL methanol, followed by serial dilution to produce concentrations from 20 to 100 mg/L. Subsequently, 100 μL of each concentration was added to 10 mL volumetric flasks, followed by the addition of 400 μL distilled water. After 5 minutes, 300 μL of 10% $AlCl_3$ was added, and after 6 minutes, 200 μL of 1 M NaOH was introduced. Distilled water was added to reach the mark, and absorbance was measured at 510 nm using a UV-Visible spectrophotometer (Agilent, Cary 60) against the blank (all reagents mixed without quercetin). For the extract, a stock solution was prepared by diluting 1 mL of concentrated extract in 49 mL methanol. From this solution, 200 μL was pipetted into three 10 mL volumetric flasks, and the same conditions as for the standard quercetin were applied to determine the total flavonoid content expressed via the calibration curve.

Determination of total phenolic content (TPC)

The assessment of total phenolic content in *Oldfieldia dactylophylla* root bark extracts was conducted using the Folin–Ciocalteu colorimetric method, following the procedure outlined by Singleton et al. (1999) [21].

Preparation of standard Gallic acid and Sample extracts

The preparation of gallic acid for the calibration curve followed the method reported by Singleton et al., (1999) [21]. Initially, a gallic acid stock solution was prepared by dissolving 100 mg in 100 mL methanol and then serially diluting to concentrations of 20, 40, 60, 80, and 100 μg/mL. For each concentration, 1 mL was combined with 500 μL of 10% Folin–Ciocalteu reagent (FCR) and 400 μL of 7.5% Na_2CO_3, resulting in a final volume of 10 mL. The mixture was shaken and incubated for 90 minutes at room temperature. Absorbance was measured at 765 nm using a UV-Visible spectrophotometer (Agilent, Cary 60) against the blank. Regarding the extract, a stock solution was prepared by diluting 1 mL of concentrated extract in 49 mL methanol. From this solution, 200 μL was pipetted into three 10 mL volumetric flasks and subjected to the same conditions as the standard gallic acid for analysis.

Antioxidant Activities: DPPH radical scavenging assay

The evaluation of DPPH radical scavenging activity was conducted following a procedure outlined by Brand-Williams et al., (1995) [22]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a widely used method for assessing the antioxidant potential of compounds.

Preparation of DPPH and control solution

A solution of DPPH was prepared by dissolving 4 mg in 100 mL of 99% methanol and then stored in a cool, dark place, covered with aluminum foil. A control was produced by mixing 1 mL of DPPH solution with 1 mL of methanol. The resulting mixture was transferred to a cuvette, and the absorbance at 517 nm was measured against the methanol blank.

Preparation of Ascorbic acid standard solution

The calibration curve for ascorbic acid was constructed using a method reported by Brand-Williams et al., (1995) [22]. Initially, a stock solution of ascorbic acid was prepared by dissolving 100 mg in 100 mL distilled water, which was then serially diluted to create concentrations of 20 to 100 mg/L using 99% methanol. Volumes of

1, 2, 3, 4, and 5 mL from these concentrations were pipetted into 10 mL volumetric flasks. To each flask, 3 mL of DPPH solution was added, and the solution was adjusted to 10 mL with methanol. The mixtures were incubated for 30 minutes in the dark at room temperature to complete the reaction, and the absorbance of each solution was measured using a Carey 60 Agilent spectrophotometer at 517 nm against the methanol blank.

Preparation of extract solutions

The preparation of extracts for antioxidant activity assessment followed a procedure similar to Brand-Williams et al. (1995) [22]. In brief, 1, 2, 3, 4, and 5 mL volumes of the extracted solution were pipetted into five 10 mL volumetric flasks, with methanol added to each to achieve a total volume of 10 mL. Subsequently, 1 mL from each flask was transferred to five other volumetric flasks. To these, 3 mL of freshly prepared DPPH solution was added, and the solution was adjusted to 10 mL with methanol. After incubating the mixtures in the dark at room temperature for 30 minutes to complete the reaction, the absorbance of each solution was measured using a Carey 60 Agilent spectrophotometer at 517 nm against the methanol blank. The percentage of inhibitions (I%) was determined using the following formula in accordance with Nirmala et al., (2020) [23].

$$I\% = \frac{AC-AO}{AC} \times 100 \text{ -------(1)}$$

Where, AC = absorbance of the control, AO = absorbance of the sample solution, and I% = percentage of inhibition.

Molecular docking

Preparation of ligands

The SDF file of 3D structures of selected phytomolecules of *Oldfieldia dactylophylla* root such as 6-methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol (L1), 1-O-cyclohexyl 2-O-pentan-2-yl benzene-1,2-dicarboxylate (L2), (2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-ol (L3), [[7-(benzoyloxyamino)-7-oxoheptanoyl]amino] benzoate (L4), identified in the study plant sample and control diabetic drug molecule metformin, 3-(diaminomethylidene)-1,1-dimethylguanidine (C) were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/docs/downloads#section=From-the-PubChem-FTP-Site). They were prepared using Avogadro 1.2.0 software by adding hydrogens and optimizing the geometry to lead to mol2 files which were subsequently processed leading to corresponding pdbqt files by choosing torsions and selecting active bonds using AutoDock 4.2 tools [24].

Receptor preparation

The receptor proteins α-amylase (PDB ID: 1HNY) and α-glucosidase (PDB ID: 3WY2) play critical roles in breaking down various carbohydrates into glucose[n5]. In our study, we focused on the receptors 1HNY and 3WY2 as target proteins. The crystal structure of the protein complex utilized in our study was obtained from the Protein Data Bank (www.rcsb.org/pdb.pdb reference). The processed protein, acquired through ChimeraX software, underwent several modifications, including removing nonstandard atoms and bonds from the selected chain and residue. Subsequently, using autodock 4.2 tools, water molecules were eliminated, hydrogens added, nonpolar hydrogens merged, Collman charges introduced, and AD4 type atoms were assigned to generate the pdbqt file using AutoDock 4.2 tools [24].

Docking procedure

The grid parameters were adjusted by modifying the X, Y, and Z dimensions to 126. Ligand and protein pdbqt files were processed to generate gpf type files using AutoDock 4.2 tools for the autogrid run. Further, dpf files were created from ligand and protein pdbqt files using AutoDock 4.2, with genetic algorithms set to 70 runs and 2500000 energy evaluations for achieving the best conformations. Default docking parameters were accepted, and the Lamarckian genetic algorithm was selected. To attain the desired conformations, a total of 70 runs were set. Subsequently, autogrid and autodock runs were performed, and the docking results were extracted from resultant glg files[24].

ADMET analysis

ADMET (Adsorption, Distribution, Metabolism, Excretion, and Toxicity) analysis is useful role in assessing the pharmacodynamic characteristics of a molecule. The SWISSADME web-based server (www.swissadme.ch/; accessed on 18 January 2024) was used to evaluate these properties for both phytocompounds and known control drug. This online tool facilitated determining ADMET properties by loading ligand and drug smiles, sourced from PubChem [25,26].

Results and Discussion

Qualitative preliminary phytochemical screening

The qualitative phytochemical compositions of the root ethanoic extracts of *Oldfieldia dactylophylla* are shown in Table 1. The results indicated the presence of key phytochemical classes of compounds such as steroids, phenols, tannins, alkaloids, terpenoids, flavonoids, Saponins and Anthraquinones.

#	Phytochemicals	Outcome
1	Alkaloids	+
2	Saponins	+
3	Flavonoids	+
4	Phenols	+
5	Tannins	+
6	Terpenoids	+
7	Steroids	+
8	Anthraquinones	+

Table 1: Results of preliminary phytochemical screening of ethanoic extracts of *Oldfieldia dactylophylla* root

KEY: + = PRESENT, - = ABSENT.

Alkaloids are known for their therapeutic effects as anesthetics, cardioprotective agents, and anti-inflammatory substances. For example, alkaloids used in clinical settings include morphine, strychnine, quinine, ephedrine, and nicotine [27]. Within nutrients and herbal medicines, bioactive compounds include flavonoids and various phenolic components known for their diverse advantages. For instance, flavonoids exhibit potent antioxidant, anticancer, antibacterial, cardioprotective, anti-inflammatory, immune system-enhancing, and UV radiation-protective properties, positioning them as valuable candidates for pharmaceutical and medical uses [28]. The tannins present in the extract also possess multiple medicinal attributes [29].

Epidemiological and experimental studies emphasize the promising role of monoterpenes in the prevention and treatment of various cancers [30]. Furthermore, steroids contribute to their medicinal significance, particularly concerning anti-inflammatory properties [29]. The diverse classes of compounds identified in the root of *Oldfieldia dactylophylla* affirm its suitability for traditional medicinal use in addressing conditions such as venereal disease, dysentery, diarrhea, malaria, diabetes, and pain relief, among other ailments.

GC-MS phytochemical profiling

The chromatogram generated from GC-MS is defiicted in Figure 2 and a total of 62 molecules were annotated using NIST library. The annotated molecules are depicted in Table 2.

As depicted in Table 2, the putative molecules belong to various classes of phytocompounds outlined in Table 1. It is important to note that the list may not be comprehensive, as only the NIST library was accessible for GC analysis. Nevertheless, specific molecules from Table 2 were considered for in silico analysis and molecular docking to assess their potential for anti-diabetic profile.

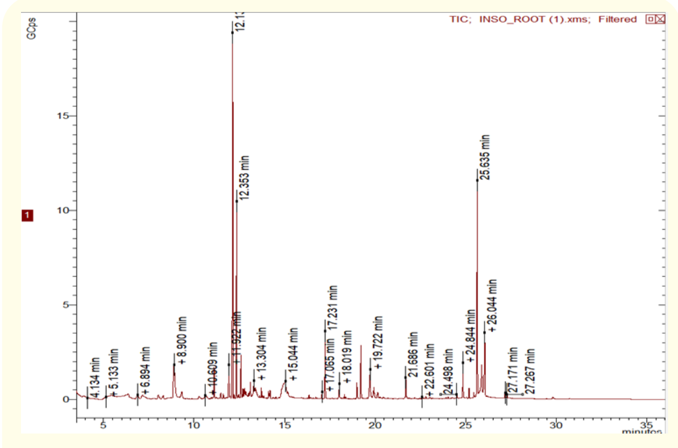


Figure 2: GC-MC chromatogram of ethanolic extract of *Oldfieldia dactylophylla* root.

Evaluation of total flavonoid content (TFC)

In this study, the total flavonoid content of ethanoic root extracts of *Oldfieldia dactylophylla* were determined. The total flavonoid content (Table 3) was determined using the aluminum chloride colorimetric method. The data were presented as mean values ± standard deviation (n = 3). A linear calibration curve (Figure 3) of quercetin ($Y = 0.0007x + 0.1233$, $R^2 = 0.9987$) was used to estimate the total flavonoid content, with results found to be 143.3667 mg/L.

Flavonoids, naturally occurring compounds present in fruits, vegetables, tea, wine, and medicinal plants, exhibit diverse therapeutic properties. These compounds demonstrate anti-inflammatory effects by inhibiting enzymes involved in inflammation. Moreover, flavonoids function as antioxidants, shielding against oxidative damage by neutralizing free radicals. Research indicates that their antioxidant activity contributes to therapeutic effects against chronic diseases. Additionally, flavonoids offer cardiovas-

No	RT	Name	CAS	Molecular Weigh	Molecular Formula
1	6.371	Propanoic acid ,2-mercapto-,methyl ester	53907-46-3	120	C4H8O2S
2	6.889	Thymine	65-71-4	126	C5H6N2O2
3	8.028	4H-Pyran-4-one,2,3-dihydro-3,5-dihydro	28564-83-2	144	C6H8O4
4	8.309	Heptanediamide, N,N'-benzoyloxy	none	398	C21H22N2O6
5	8.903	Catechol	120-80-9	110	C6H6O2
6	9.331	5-hydroxymethylfurfural	67-47-0	126	C6H6O3
7	10.280	1,2-Benzenediol, 4-methyl	452-86-8	124	C7H8O2
8	10.954	Neric acid	4613-38-1	168	C10H16O2
9	11.036	.alpha,-Cubebene	17699-14-8	204	C15H24
10	11.109	Phenol, 2-methoxyl-3-(2-propenyl)-	1941-12-4	164	C10H12O2
11	11.217	6-methyl-2-(4-methylcyclohex-3-en-1-yl) hepta-1,5-dien-4-ol	38142-56-2	220	C15H24O
12	11.477	Copaene	3856-25-5	204	C15H24
13	11.922	3H,3a,7-methanoazulene, 2,4,5,6,7,7,8-hydro-1,4,9,9-tetramethyl-,	2387-78-2	204	C15H24
14	11.988	e-Arachidonic acid methyl ester	13712-70-0	318	C21H34O2
15	12.131	Caryophyllene	87-44-5	204	C15H24
16	12.213	10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane	357414	204	C15H24
17	12.352	(E)- beta-farnesene	18794	204	C15H24
18	12.429	Azulene,1,2,3,5,6,7,8,8a,-octahydro-1,4-dimethyl-7-(1-methylethenyl)-	3691-11-0	204	C15H24
19	12.429	1R,3R,9S-4,11,11-trimethyl-8-methyelenebicyclo[7.2.0]undec-3-ene	None	204	C15H24
20	12.481	Humulene	6753-98	204	C15H24
21	12.696	1,4,6-trimethyl-1,2,3,3a,4,7,8,8a-octahydro-4,7 ethanoazulene	65128-08-7	204	C15H24
22	12.787	1H,cycloprop[e]azulene, 1a,2,3,4,4a,5,6,6,7b-octahydro-1,1,4,7-tetramethyl	489-40-7	204	C15H24
23	12.847	Aromandendrene	489-39-4	204	C15H24
24	13.020	Naphthalene, decahydro-4a-methyl-1-methyl-methylene-7-(1-methylethenyl)-	17066-67-0	204	C15H24
25	13.110	.beta,-bisabolene	495-61-4	204	C15H24
26	13.305	1-isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronapthalene	16729-01-4	204	C15H24
27	13.633	Alpha-Calacorene	2139-99-1	200	C15H20
28	13.716	1,6,10-dodecantrien-3-ol	40716-66-3	222	C15H26O
29	13.781	Aureonitol	71774-51-1	206	C13H18O2
30	13.850	1H,indene, 1-ethylidene octahydro-7a-methyl-, cis	56362-87-9	164	C12H20
31	14.118	Tetracontane,3,5,24-trimethyl-	55162-61-3	604	C43H88
32	14.200	Caryophellene	1139-30-6	220	C15H24 O
33	14.355	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.0.02.7]decane	18252-44-3	204	C15H24
34	14.523	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	19888-34-7	220	C15H24 O
35	14.674	7-epi-cis-sesquisabinene hydrate	none	222	C15H26 O
36	14.937	1,3,5-Benzenetriol	108-73-6	126	C6H6O3
37	15.028	1,3,5-Benzenetriol	108-73-6	126	C6H6O3
38	16.345	Eicosane	112-95-8	282	C20H42
39	16.423	3-Pyridine carboxylic acid, 2-amino-4,6-dihydroxyl ethyl ester	none	198	C8H10N2O4

40	16.729	Neophytadiene	504-96-1	278	C20H38
41	17.063	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)ester	84-69-5	278	C16H22O4
42	17.230	1-Hexadecanol	36653-82-4	242	C16H34O
43	17.641	Methyl 8-methyl-nonaoate	none	180	C11H22O2
44	18.015	n-hexadecanoic acid	57-10-3	256	C16H32O2
45	18.300	Eicosanoic acid, ethyl ester	18281-05-5	340	C22H44O2
46	18.361	Nonadecane	629-92-5	268	C19H40
47	18.933	9-octadecen-1-ol, (Z)-	143-28-2	268	C18H36O
48	19.208	n-Nonadecanol-1	1454-84-8	284	C19H40O
49	19.718	Cis-hexadecanoic acid	2416-19-5	254	C16H30O2
50	20.141	Succinic acid, tridec-2-yl-1-yl, 3-methyl bute-3-ene-1-yl ester	none	364	C22H36O4
51	21.684	9-octadecenamide,(Z)-	301-02-0	281	C18H35NO
52	22.601	2-methyl-3-(3-methyl-bute-2-enyl)-2-(4-methy-pent-3-enyl)Oxetane	none	222	C15H26O
53	22.796	6,11-dimethyl-2,6,10-dodecatrien-1-ol	none	208	C14H24O
54		Pthalic acid, cyclohexyl-2-pentyl ester	none	318	C19H26O4
55	23.916	Isopropyl palmitate	142-91-6	298	C19H38O2
56	25.043	Squalene	111-02-4	410	C30H50
57	25.182	1-methylene-2b-hydromethyl-3,3-dimethyl-4b-(3-methylbut-2enyl) cyclohexane	none	222	C15H26O
58	25.633	1,6,10,14-hexadecatetraen-2-ol, 3,7,11,15-tetramethyl-(E,E)	1113-21-9	290	C20H34O
59	25.876	Epoxyjuanislamin	75628-11-4	448	C23H28O9
60	26.041	1-methylene-2b-hydromethyl-3,3-dimethyl-4b-(3-methylbut-2enyl) cyclohexane	none	222	C15H26O
61	27.171	Trans-geranyl geraniol	24034-73-9	290	C20H34O
62	27.266	(R)-2,8-dimethyl-2-((2E,7E)-4,8,12-trimethyl trideca-3,7,11-trien-1-yl) chroman-6-ol	25612-59-3	396	C27H40O2

Table 2: Tentative bioactive compounds identified in Oldfieldia dactylophylla ethanolic root extract.

S/N	Concentration	Absorbance	TPC (µg/ml)	STD
T1	143.20	0.2270	143.3667	0.2887
T2	143.70	0.2280		
T3	143.20	0.2256		

Table 3: Total Flavonoid content in *Oldfieldia dactylophylla* ethanolic root extract (n = 3).

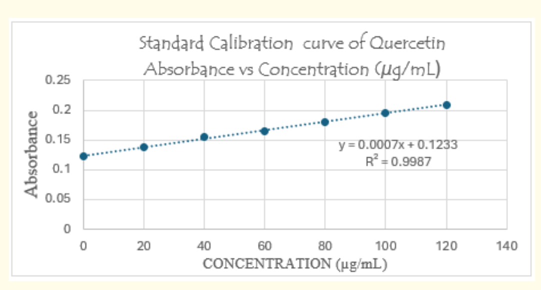


Figure 3: Calibration curve graph for Quercetin.

cular benefits, including antiplatelet, anti-inflammatory, and vasodilatory properties, potentially reducing the risk of cardiovascular diseases. Consuming flavonoid-rich foods is suggested to support heart health [31-33]. The total phenolic content was found to be 79.08 mg/L. The presence of phenols in Paropsia Brazeana Baill leaf sample could be the main reason as to why these plants are ef-

fective in treatment of various ailments as used by the local people since these phytochemicals have previously been cited in literature to be effective in management of various ailments. A similar study in Nepal reported phenolic content reported total phenolic content from72.66 to 292.65 GAE/g [34].

Total phenol content

In this present study, the total phenol content in medicinally used *Oldfieldia dactylophylla* was determined. The total phenol content (TPC) of crude extract of *Oldfieldia dactylophylla* was determined with the aid of a calibration curve (Y = 0.01368x + 0.15211, R2 =0.99188) of gallic acid (Figure 4) and found to be 53.4667 mg/L (Table 4). The effectiveness of these plant in treating various ailments, as observed in local traditional medicine, could be attributed to the presence of phytochemicals such as phenolics.

S/N	Concentration	Absorbance	TPC (µg/ml)	STD
T1	53.5	0.8838	53.4667	0.0577
T2	53.4	0.8829		
T3	53.5	0.8836		

Table 4: Total phenol content in *Oldfieldia dactylophylla* ethanolic root extract (n = 3).

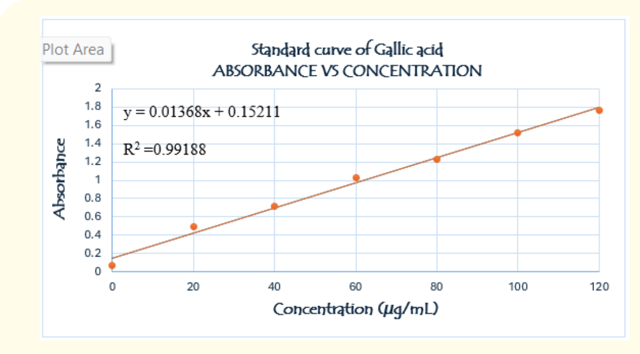


Figure 4: Calibration curve graph for Gallic acid.

Phenolic compounds, abundant in fruits, vegetables, and plant-based foods, have shown various therapeutic properties due to their antioxidant, anti-inflammatory, and antimicrobial activities. These compounds have been extensively studied for their potential in preventing chronic diseases such as cardiovascular disorders, cancer, obesity, and neurodegenerative diseases [35]. Moreover, phenolic compounds have been found to possess anti-aging effects by modulating oxidative stress and reducing inflammation [36]. The consumption of phenolic-rich foods has also been associated with improved gut health, through promoting a healthy gut microbiota and protecting against gastrointestinal disorders [37]. Furthermore, these compounds have shown promising antibacterial and antifungal activities, suggesting their potential for natural food preservation and development of novel antimicrobial agent [38].

Antioxidant activity

In this study, the radical scavenging activity was determined using the free radical DPPH assay. With these tests, the free radical DPPH draws electrons as their number increases, which typically results in a decolorization from deep purple or violet to yellow. The methanoic crude extracts showed strong antioxidant capacity. At concentrations (20-100 mg/L) of the crude root extract, radical scavenging activity (Figure 5) was found to be 39.58, 48.54, 59.78, 68.05 and 79.91%. The IC_{50} was found to be 35.71 mg/L. The IC_{50} of the standard antioxidant was 23.19 µg/mL. The amount of sample required to scavenge 50% of the free radical DPPH is indicated by the IC_{50}.

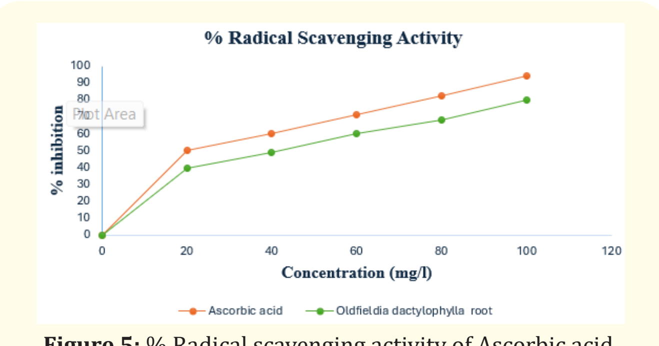


Figure 5: % Radical scavenging activity of Ascorbic acid and *Oldfieldia dactylophylla*.

As per the findings of Jun et al. (2003), antioxidant activity is categorized into five groups, namely: highly active, active, moderate, weak, and inactive as shown in Table 5 [39].

Antioxidants are crucial in preventing oxidative stress and combating free radicals, which are known to cause cellular damage and contribute to various diseases. Medicinal plants have been widely studied for their antioxidant potential, as they are rich sources of bioactive compounds such as phenolics, flavonoids, terpenoids, and tannins. For instance, a study by Li et al. (2019) demonstrated that extracts from plants like Rhodiola rosea and Ginkgo biloba exhibited significant antioxidant activities due to the presence of

No	Intensity	IC_{50} Values
1	Very active	<50 mg/L
2	Active	50-100 mg/L
3	Moderate	101-250 mg/L
4	Weak	250-500 mg/L
5	Inactive	>500 mg/L

Table 5: Classification of antioxidative potential by DPPH method [39].

phenolic and flavonoid compounds [40]. Moreover, another study carried out by Pandey and Rizvi (2009) reported that medicinal plants including Curcuma longa, Allium sativum, and Zingiber officinale possess potent antioxidant properties, attributed to their high content of phenolics and terpenoids [41]. These studies provide evidence of the antioxidant potential of medicinal plants, highlighting their therapeutic value in combating oxidative stress-related disorders. Accordingly, the findings of this study indicate that the crude methanoic root extract of *Oldfieldia dactylophylla* to be a very active antioxidant (IC50 <50 mg/L). This implies the ability of the extract to act as radical scavenger.

Docking results

The molecular docking analysis considered L1, L2, L3, and L4 ligands and metformin (C) as control (Figure 6). The receptor used was human α-amylase (PDB ID: 1HNY) and α-glucosidase (PDB ID: 3WY2). The molecular docking results using Autodock 4.2 are stipulated in the Tables 6 and 7. The interactions between L2, L3, and metformin with 1HNY in two dimensions are depicted in Table 8 while those between L1, L2, L3, L4, metformin and 3WY2 are depicted in Table 9.

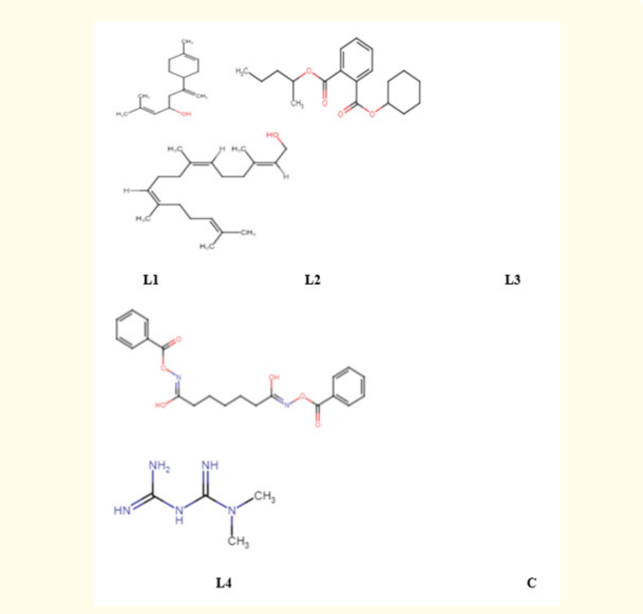


Figure 6: Structure of phytochemicals form *Oldfieldia dactylophylla* and metformin.

Ligand	Binding energy Kcal/mole	Inhibition constant (Ki) (µM)	Total internal energy kcal/mol	Torsional free energy kcal/mol	Unbound energy kcal/mol	Cluster RMSD	Ligand efficiency
L1-CID: 91710334	-6.40	268.01	-0.74	1.49	-0.74	0.00	-0.4
L2-CID: 6423899	-5.47	98.04	-1.86	2.39	-1.86	0.00	-0.24
L3-CID: 5281365	-5.32	125.72	-0.89	3.28	-0.89	0.00	-0.25
L4-CID: 569848	-5.63	75.02	0.00	0.00	0.00	0.00	-0.47
Metformin	-8.03	1.30	0.44	0.00	0.44	0.00	-0.89

Table 6: Docking analysis of selected ligands from *Oldfieldia dactylophylla* with 3WY2 receptor.

Ligand	Binding energy Kcal/mole	Inhibition constant (Ki) (µM)	Total internal energy kcal/mol	Torsional free energy kcal/mol	Unbound energy kcal/mol	Cluster RMSD	Ligand efficiency
L2-CID: 6423899	-5.27	136.15	-1.87	2.39	-1.87	0.00	-0.23
L3-CID: 5281365	-5.48	95.76	-0.77	+3.28	-0.77	0.00	-0.26
Metformin	-7.36	4.05	0.53	0.00	0.53	0.00	-0.82

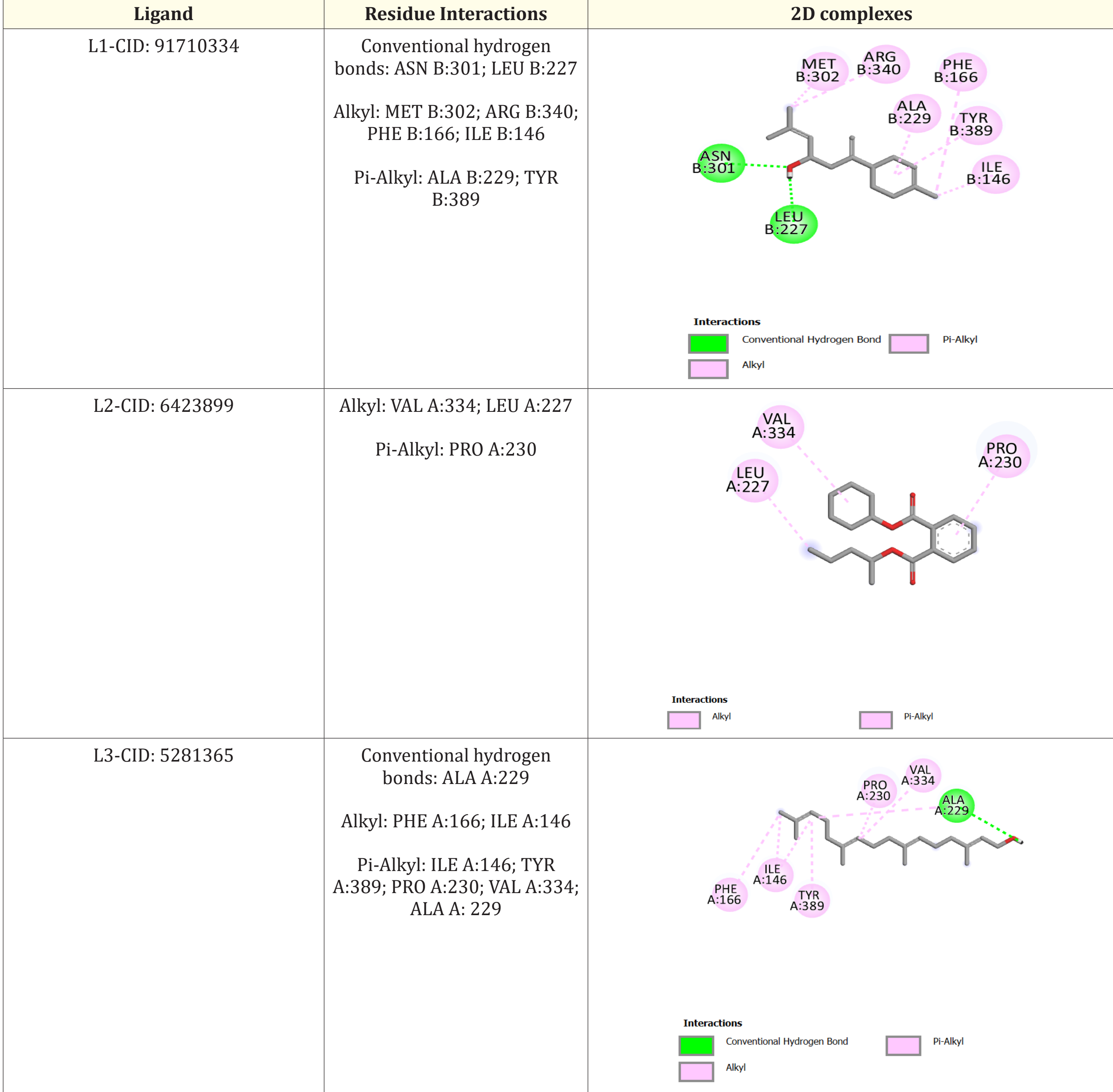
Table 7: Docking analysis of selected ligands from *Oldfieldia dactylophylla* with 1HNY receptor.

All the ligands have moderately good binding energies (Tables 6 and 7). By applying virtual screening, ligands are evaluated according to their binding afnity, which helps in the evaluation of which ligand structure and rotation is most viable concerning the receptor (protein) [42,43]. Although, analysis of the binding efficiencies of natural products and marketed drugs show that therapeutic efficacy is not essentially related with high binding affinity [44]. Inhibition constant values range from 75.02 μM to 268.01. Ligand efficiencies range from -0.23 to -0.47. Cluster RMSD for all the ligands and metformin are 0.00. These docking results support these ligands as prospective drug candidates to be considered for further *in vitro* and *in vivo* investigations.

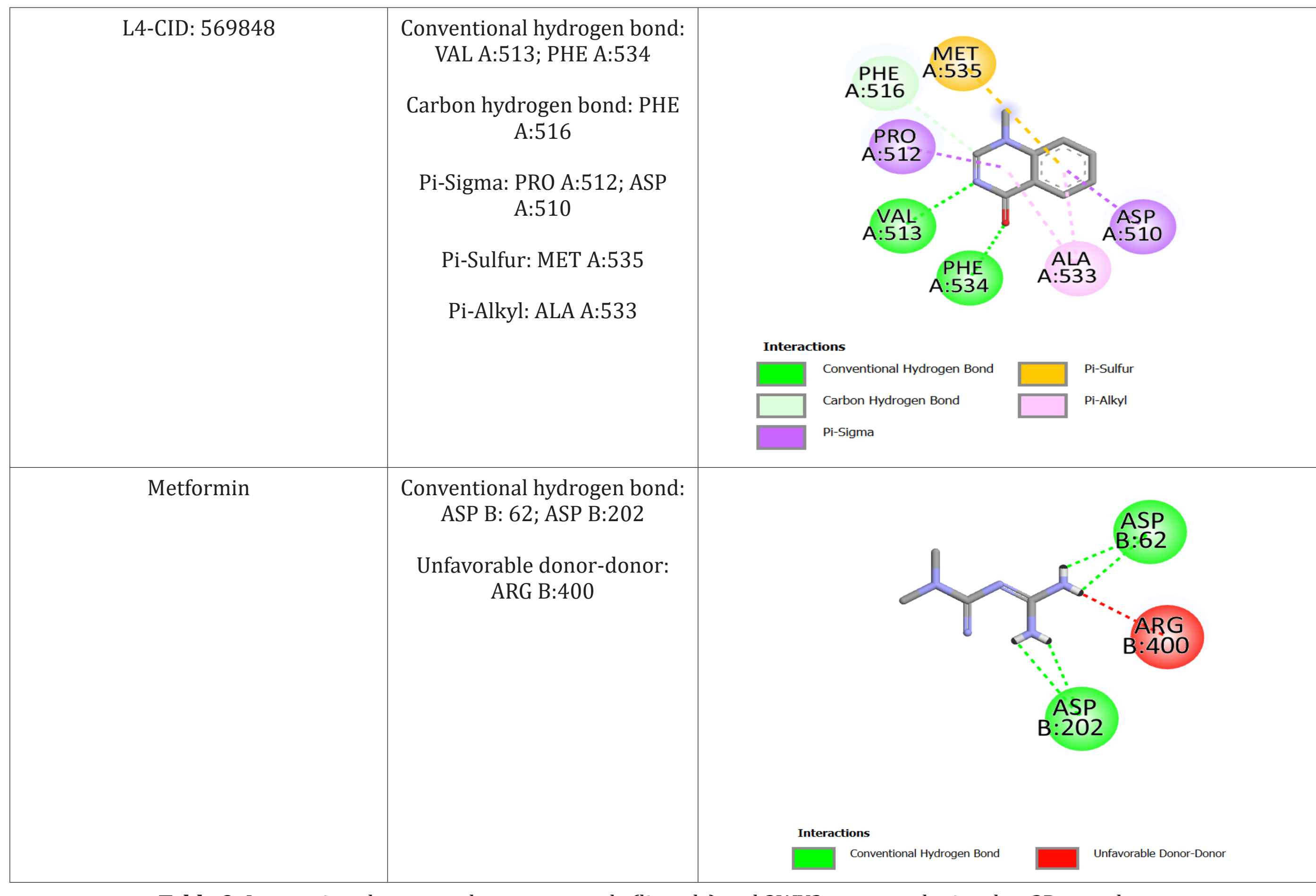
Ligand	Residue Interactions	2D complexes
L2-CID: 6423899	Conventional hydrogen bonds: HIS A:185 Pi-Cation: HIS A:185 Alkyl: VAL A:129; TYR A:67; LYS A:68	
L3-CID: 5281365	Conventional hydrogen bonds: GLU A:233; ARG A:195; ASP A:197 Carbon hydrogen bonds: ASP A:300 Alkyl: LEU A: 165; ALA A:106	
Metformin	Convention hydrogen bonds: ASP A:300; ASP A: 197; GLU A:233	

Table 8: Interactions between phytocompounds (ligands) and 1HNY receptor depicted as 2D complexes.

In the present study, docking of 1HNY with reference drug metformin (C) generated conventional hydrogen bonds (ASP A:300; ASP A: 197; GLU A:233). The docking of L2 with 1 HNY formed conventional hydrogen bond (HIS A:185), Pi-Cation (HIS A:185) and Alkyl (VAL A:129; TYR A:67; LYS A:68). Further, docking of L3 with 1 HNY generated conventional hydrogen bonds (GLU A:233; ARG A:195; ASP A:197), carbon hydrogen bonds (ASP A:300), and alkyl interaction (LEU A: 165; ALA A:106). Apparently, Metformin, with several conventional hydrogen bond interaction with human α-amylase (PDB ID: 1HNY) seems to have more affinity as inhibitor as compared to L2 and L3 with only one conventional hydrogen bonds. However, L2 and L3 have other interaction with α-amylase which are lacking in metformin. In addition, there may be variations in interaction *in vivo* situations.



Ligand	Residue Interactions	2D complexes
L1-CID: 91710334	Conventional hydrogen bonds: ASN B:301; LEU B:227 Alkyl: MET B:302; ARG B:340; PHE B:166; ILE B:146 Pi-Alkyl: ALA B:229; TYR B:389	
L2-CID: 6423899	Alkyl: VAL A:334; LEU A:227 Pi-Alkyl: PRO A:230	
L3-CID: 5281365	Conventional hydrogen bonds: ALA A:229 Alkyl: PHE A:166; ILE A:146 Pi-Alkyl: ILE A:146; TYR A:389; PRO A:230; VAL A:334; ALA A: 229	



L4-CID: 569848	Conventional hydrogen bond: VAL A:513; PHE A:534 Carbon hydrogen bond: PHE A:516 Pi-Sigma: PRO A:512; ASP A:510 Pi-Sulfur: MET A:535 Pi-Alkyl: ALA A:533	
Metformin	Conventional hydrogen bond: ASP B: 62; ASP B:202 Unfavorable donor-donor: ARG B:400	

Table 9: Interactions between phytocompounds (ligands) and 3WY2 receptor depicted as 2D complexes.

In the present study, docking of 3WY2 with reference drug Metformin (C) generated conventional hydrogen bonds (ASP B: 62; ASP B:202), Unfavorable donor-donor (ARG B:400). The docking of L1 with 3WY2 formed conventional hydrogen bond (ASN B:301; LEU B:227), Alkyl (MET B:302; ARG B:340; PHE B:166; ILE B:146), and Pi-Alkyl (ALA B:229; TYR B:389). The docking of L2 with 3WY2 generated Alkyl interactions (VAL A:334; LEU A:227) and Pi-Alkyl interaction (PRO A:230). The docking of L3 with 3WY2 formed conventional hydrogen bond (ALA A:229), Alkyl (PHE A:166; ILE A:146) and Pi-Alkyl (ILE A:146; TYR A:389; PRO A:230; VAL A:334; ALA A: 229). Further, docking of L4 with 3WY2 generated conventional hydrogen bond: (VAL A:513; PHE A:534), carbon hydrogen bond: (PHE A:516), Pi-Sigma (PRO A:512; ASP A:510), Pi-Sulfur (MET A:535), and Pi-Alkyl interaction (ALA A:533).

It is obvious that metformin has conventional hydrogen bond association with two amino acid residues and L4 is also associated with two amino acid residues through conventional hydrogen bonds. In addition, L4 has several additional favourable interactions. Adversely, metformin has one unfavourable donor-donor interaction. Thus, L4 has promising attributes based on ligand-target interaction attributes.

ADMET analysis results

The ADMET analysis of the following ligands (L1, L2, L3, L4) and metformin (C) are stipulated in Tables 10.

In this study, physicochemical, lipophilicity, solubility, drug likeness, pharmacokinetic and medicinal properties of phytocompounds (L1, L2, L3, and L4) from *Oldfieldia dactylophylla* root and control anti-diabetic drug metformin were analysed. On Lipinski scale, phytocompounds L1, L2, L4, and reference drug metformin had no violations, while L3 (MLOGP>4.15) had one violation. Gastrointestinal absorption (GI) is a critical factor determining the ef-

ADMET properties	Phytocompounds				Drug
	L1-CID: 91710334	L2-CID: 6423899	L3-CID: 5281365	L4-CID: 569848	C-CID: 4091
Physicochemical properties					
Molecular weight (g/mol)	220.35	318.41	290.48	398.41	129.16
Topological Surface Area (TPSA) (A°)	20.23	52.60	20.23	110.80	91.49
Num. H-bond acceptors	1	4	1	6	2
Num. H-bond donors	1	0	1	2	3
Molar Refractivity	71.84	90.15	97.52	103.37	36.93
Lipofilicity					
XLOGP3	5.32	5.12	7.27	3.46	-1.27
ILOGP	3.38	3.02	4.75	3.03	0.34
MLOGP	3.46	3.75	4.95	3.41	-0.56
WLOGP	4.01	4.52	6.12	2.71	-1.24
Water Solubility					
Log S (ESOL)	-4.29	-4.70	-5.56	-3.87	0.29
Class	Moderately soluble	Moderately soluble	Moderately soluble	Soluble	Highly soluble
Drug likeness					
Lipinski	Yes, 0 Violation	Yes, 0 Violation	1 violation	Yes, 0 violation	Yes, 0 violation
Bioavailability score	0.55	0.55	0.55	0.55	0.55
Pharmacokinetics					
Gastrointestinal (GI) Absorption	High	High	High	High	High
Blood brain barrier (BBB) permeability	Yes	Yes	No	No	No
P-gp substrate	No	No	No	No	No
CYP1A 2 inhibitor	No	Yes	Yes	No	No
CYP2C 19 inhibitor	Yes	Yes	No	Yes	No
CYP2C 9 inhibitor	Yes	Yes	Yes	No	No
CYP2D 6 inhibitor	No	No	No	No	No
CYP3A 4 inhibitor	No	Yes	No	No	No
Log Kp (skin permeation) cm/s	-3.87	4.61	-2.91	-6.27	-7.99
Medicinal Chemistry					
Pas assay interference compounds (PAINS)	0	0	0	0	0
Brenk	1	1	1	2	2
Synthetic accessibility	3.99	3.21	3.65	2.92	3.02
Lead Likeness	2	2	2	2	1 violation

Table 10: ADMET analysis of phytocompounds and reference anti-diabetic drug metformin.

ficacy of an oral drug. In this study, all the molecules showed high GI values. Five prominent isozymes (CYP1A 2, CYP2C 19, CYP2C 9, CYP2D 6, and CYP3A 4) show negative inhibition in case of metformin while only CYP2C 19 shows inhibition in the case of L3. Isozymes CYP1A 2, CYP2D 6, and CYP3A 4 are not inhibitory in case of L1. Isozyme CYP2D 6 is not an inhibitor in case of ligand L2. Two isozymes CYP1A 2 and CYP2C 9 show inhibition attribute in case of ligand L3. Synthetic accessibility demonstrates the ease of synthesis of the drug. This parameter uses numerical values from 1(easiest to synthesize) to 10 (most difficult to make). The values L1(3.99), L2 (3.21), L3 (3.65), L4 (2.92), and reference drug metformin (3.02) are quite favourable.

The candidate L3 with one Lipinski violation (MLOGP>4.15) two violations for drug likeness. Thus, L3 may not be a good drug candidate as it failed on one critical parameters such as Lipinski criteria. Ligands L1, L2 and L4 with zero violations on Lipinski scale and high GI merit as drug candidate. The ADMET parameter for ligands L1, L2 and L4 are comparable to the control drug metformin with high GI and zero Lipinski violations. It has been reported that compounds containing more hydrogen bond acceptors (HBAs) with less hydrogen bond donors (HBDs) have favorable profile as drug candidate [45,46]. Thus, on hydrogen bond criteria, L4 has the best score. Thus, ADMET analysis demonstrates some phytochemicals as strong drug candidate for future drug development.

Conclusion

The preliminary qualitative screening of *Oldfieldia dactylophylla* root extracts revealed the presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, phenols, steroids, and terpenoids that have been known to have therapeutic properties essential in treatment of various ailments. These findings strongly support the wide application of the root extract in traditional medicine. Based on the IC_{50} (35.71 mg/L) obtained, the crude extract showed strong antioxidant capacity due to presence of polyphenols, thus affirming the reason as to why they might be used as medicinal plants to alleviate aliments. The diverse range of medicinal phytochemicals present in plant support the healing power of the plant. *In silico* analysis strongly support some phytomolecules as viable drug candidate to be considered for further *in vitro* and *in vivo* investigations as diabetic drugs.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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