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# IN SILICO ANALYSIS OF SOME GC-MS SCREENED PHYTOCHEMICALS, ESTIMATION OF TOTAL PHENOLIC, FLAVONOID CONTENT AND IN-VITRO ANTIOXIDANT ACTIVITY OF *OLDFIELDIA DACTYLOPHYLLA* LEAF EXTRACT

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**Key words:**
Phytochemical screening, Oldfieldia dactylophylla, antioxidant activity, total flavonoid content, total phenol content

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## ABSTRACT

*Oldfieldia dactylophylla* is used by many herbalists to treat numerous aliments such as diabetes, hernia, venereal diseases (VD), gastrointestinal complications and many others. This study aimed at screening 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 leaf extract of *Oldfieldia dactylophylla*. ***In silico*** analysis of some selected phytochemicals was also carried out. 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 the presence of tentative compounds such as Catechol, Eugenol, Squalene, Cis 5,8,11,14,17-Eicosapentaenoic acid, 4a,5-dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronapthalene-1-ol and many more. The total flavonoid content was found to be 125.4667 mg/L, while total phenol content was found to be 48.1667 mg/L. The $IC_{50}$ value was found to be 44.71 mg/L. A lower $IC_{50}$ value is characteristic of a stronger antioxidant, while a higher one is indicative of weaker antioxidant potential. The $IC_{50}$ of the standard ascorbic acid antioxidant was 23.19 µg/mL. According to the results, *Oldfieldia dactylophylla* possess strong antioxidative potential, while based on literature the identified compounds could potentially have antidiabetic, antiviral, antifungal, antibacterial, anticancer and anti-inflammatory properties. The ***in silico*** analysis results corroborate some phytocompounds as prospective drug candidates. However, these compounds merit further *in vivo* studies.

## INTRODUCTION:

Throughout history, medicinal plants have occupied a significant role in various societies due to their therapeutic potential, leading to extensive studies. The rapid progress in deriving pharmacologically effective drugs from these plants has profoundly influenced contemporary medicinal practices [1]. Numerous studies have shown that whether isolated or in crude extracts, secondary metabolites can function as anti-inflammatory, anti-mutagenic, anti-carcinogenic, antioxidant, antidiabetic, antibacterial, antiviral, and antifungal agents [2,3]. Plants typically produce secondary metabolites as a defense mechanism against pathogens. These bioactive compounds, acting as natural antioxidants, can supplement human body requirements [4]. Antioxidants can be categorized as either synthetic or natural [5]. Recently, some scientists have expressed

concerns about the use of synthetic phenolic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), citing alleged detrimental effects on human health [5]. The growing concern regarding the adverse effects of synthetic antioxidants has led to a demand for non-toxic, natural preservatives [6,7]. Consequently, there is a widespread global interest in research to identify potent natural antioxidants. It is noteworthy that medicinal plants or herbs have emerged as promising sources of naturally potent antioxidants and antimicrobial agents [5]. Picrodendraceae constitutes a family of flowering plants comprising approximately 24 genera and 80 species [8]. *Oldfieldia dactylophylla*, belonging to the Oldfieldia genus within the Picrodendraceae family, is a modest semi-deciduous tree that typically reaches a height of up to about 15m. It is characterized by a short bole and spreading branches adorned with thick branchlets covered in red-brown hairs. Indigenous to Zambia, Angola, Mozambique, Congo, and Tanzania [9], *Oldfieldia dactylophylla* is locally known by various names such as Kampangwila, Mubonga, Kalikali or Nakali, Kafutu, Kafumbafumba, Mufutu, Kazonga, Mutengulu, Munyasha, and Mutobakusu, among others. In local practices, the plant addresses various health issues like malaria, hernias, STDs, diarrhea, and diabetes and serves as an aphrodisiac. Moreover, the plant provides edible fruits and has decorative applications [9]. The current investigation focuses on exploring the bioactive compounds and antioxidative potential present in the leaves of *Oldfieldia dactylophylla*. As part of this study, an analysis of the total flavonoid and phenolic content in the leaves of *Oldfieldia dactylophylla* has also been conducted.

Diabetes, characterized as a metabolic disorder, occurs when the body fails to produce or absorb insulin adequately, resulting in elevated blood sugar levels [10]. The increasing preference for traditional herbal remedies currently is attributed to their lower incidence of adverse effects than chemical or allopathic treatments. This study focused on the computational screening of selected phytochemicals annotated from *Oldfieldia dactylophylla*, renowned for its anti-diabetic properties. The primary goal was to evaluate the inhibitory effects of these phytochemicals on an essential protein α-glucosidase (PDB ID: 3WY2), the proteins playing pivotal roles in breaking down various carbohydrates into glucose [11]. In pharmaceutical development, computational models like Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) are crucial for swiftly assessing essential properties of potential drug candidates. This initial screening prioritizes compounds for more extensive in vitro and in vivo investigations [12-15]. In the present study, an analysis was carried out to evaluate the ADMET properties of some selected phytocompounds alongside a reference drug. The noteworthy results from the ADMET assessment emphasize the merit of further exploration of these molecules through comprehensive in vitro and in vivo analyses.

Thus, this study focused on GC-MS screening of phytochemicals in *Oldfieldia dactylophylla* extract, total phenolic, flavonoid and antioxidant potential determinations. Further, *in silico* molecular analysis of some selected molecules was also conducted. The study produced interesting results.

## Materials and Methods

**Collection and Authentication of plant samples**: The leaves of Oldfieldia dactylophylla were collected in clean plastic bags from Lufwanyama district (13°25ˈ60"S and 27°45ˈ0" E̊ in Degrees Minutes seconds) located on the Copperbelt province of Zambia. Freshly collected plant sample (leaves) were verified and authenticated by the Zambia Forestry department. Figure 1 shows *Oldfieldia dactylophylla* in its natural environment prior to collection.

### Chemicals, Reagents and Apparatus

The reagents used in this study comprised Folin-Ciocalteu reagent, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 99% methanol, and Sodium hydroxide (97% extra pure pellets Sodium nitrate (97% extra pure), gallic acid (99.5% extra pure), Sodium carbonate

anhydrous (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. The equipment utilized, including a Cary Agilent 60 UV-VIS Spectrophotometer and other laboratory tools, were provided by the Chemistry Department at The Copperbelt University.

**Figure 1**: ***Oldfieldia dactylophylla*** **plant in its natural habitat**

**Extract for preliminary phytochemical screening and GC-MS analysis**:

The collected leaf samples were dried in shade for two weeks and powdered using a pulveriser. The crude leaf extract of Oldfieldia dactylophylla was prepared using Soxhlet extraction following a method reported by Chibuye et al. (2023) [16]. Concisely, 20g of powdered leaves was put in a thimble. 300 mL of 98% ethanol was used as a solvent. The extraction was carried out for 10 hours. The mixture was filtered, and the filtrate concentrated under reduced pressure at a temperature of 80℃ using a rotary evaporator (Büchi Rotovapour 200). The concentrated extracts were used for further analysis.

The crude leaf extract for the evaluation of total flavonoid, total phenols content and in-vitro antioxidant activities was prepared using maceration using the method reported by Chibuye et al., (2023) [16]. In brief, 2.0 g of powdered leaves was macerated with 30 mL of 99% methanol by constantly shaking the mixture for 24 hours at 100 rpm. The mixture was then filtered using White Man No 1 filter paper and stored in sterile amber bottles in readiness for analysis.

**Preliminary phytochemical screening**

Preliminary phytochemical screening tests were carried out using the procedure earlier reported by Devi N. N. et al (2012) to evaluate the presence of alkaloids, flavonoids, saponins, terpenoids, tannins, phenols, steroids, anthraquinones in *Oldfieldia dactylophylla* leaves extract [17].

**Test for Alkaloids**

In a clean test tube, 3 mL of the plant extract was added to 1 mL of dilute HCl and Wagner's reagent. The solution was shaken and allowed to stand briefly. The appearance of a reddish-brown precipitate confirmed the presence of alkaloids.

**Test for Saponins (Foam test)**

Initially, 3 mL of ethanoic crude extract was placed in a test tube, followed by the addition of 5 mL of distilled water. The mixture was vigorously shaken and allowed to stand for 10 minutes. A positive test was indicated by the appearance of a fairly stable emulsion.

**Test for Flavonoids**

Firstly, 3 mL of plant extract was placed in a test tube. Further, 3 drops of 20% NaOH solution was added. Presence of flavonoids was confirmed by the intense yellow color, which changed to colorless upon the addition of a few drops of dilute HCl.

**Test for Phenols and Tannins**

In a test tube, 3 mL of plant extract was placed, followed by an immediate addition of 3 drops of 10% ferric chloride solution. Presence of tannins was indicated by the appearance of black color precipitate.

**Test for Terpenoid**

In a test tube, 3 mL of chloroform and 4 mL of plant extract was placed. Further, 4 drops of concentrated sulfuric acid were then introduced, and the solution kept for a few minutes. A positive test was confirmed by the appearance of a reddish colour.

**Test for anthraquinone:** 3 mL of plant extract was combined with 3 mL of chloroform in a test tube, followed by the immediate addition

of 3 mL of 10% ammonia. The test was found to be negative.

**Test for Steroid:** 3 mL of plant extract was combined with 3 mL of chloroform in a test tube. Then, 4 drops of concentrated sulfuric acid were introduced, and a brief standing time confirmed a positive test after the development of a red color.

**GC-MS Analysis**: The study used Gas Chromatography-Mass Spectrometry (GC–MS) using Thermo GC-Trace Ultra Ver 5.0 and Thermo MS DSQ II for phytochemical screening of ethanolic extract of *Oldfieldia dactylophylla*. The analysis used an Electron Ionization system with a 70eV ionization energy and a High-performance GC capillary column (Scion-5MS) with specific conditions. Helium (99.99%) served as the carrier gas at a constant flow rate of 1 mL/min, and the injector type was S/SL at 250℃ with a 10:1 split ratio. The Auto sampler (8400) and a 10µl micro syringe were used, from a starting temperature of 80℃ (Isothermal), a systematic rate increase to 300℃, and a total GC running time of 34.00 mins. The resulting GC–MS chromatogram (Figure 2) of ethanolic extracts from *Oldfieldia dactylophylla* leaf identified bioactive compounds by comparing peak characteristics with known compounds in the NIST library.

**Estimation of Flavonoid content using Aluminum Chloride:** Total flavonoids content in the leaf extracts of *Oldfieldia dactylophylla* were evaluated by the aluminum chloride colorimetric assay as described by Zhishen et al.,(1999) [18]. Quercetin solution for generating the calibration curve was prepared following the method reported by Zhishen et al.,(1999) [18]. Initially, a quercetin stock solution was prepared by dissolving 100 mg of quercetin in 100 mL methanol, followed by serial dilution to achieve concentrations ranging from 20 to 100µg/mL. After that, 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 introduced and kept for 6 minutes. Then, 200 µL of 1 M NaOH was added. Distilled water was then added to make up to 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 with the standard quercetin were applied to determine the total flavonoid content expressed via the calibration curve.

**Determination of total phenolic content (TPC)** Total phenolic content the leaf extract of *Oldfieldia dactylophylla* was determined by the Folin–Ciocalteu colorimetric method as outlined by Singleton et al. (1999) [19]. Gallic acid solution preparation for the calibration curve followed the method reported by Singleton et al., (1999) [19]. Initially, a gallic acid stock solution was prepared by dissolving 100 mg in 100 mL methanol, followed by serial dilution to concentrations of 20, 40, 60, 80, and 100 µg/mL. For each concentration, 1 mL was mixed with 500 µL of 10% Folin–Ciocalteu reagent (FCR) and 400 µL of 7.5% $Na_2CO_3$, reaching 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. 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, subjected to the same conditions as the standard gallic acid for analysis.

**Antioxidant Activities: DPPH Radical Scavenging Assay:** DPPH radical scavenging activity was determined using a method described by Brand-Williams et al., (1995) [ 20]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a commonly used method to evaluate the antioxidant activity of compounds. In this assay, the ability of a substance to neutralize the stable DPPH free radical is measured, indicating its antioxidant potential [21]. The

protocol involves the reduction of the purple DPPH radical to a yellowish diphenylpicrylhydrazine product by antioxidants, leading to a decrease in absorbance [21].

**Preparation of DPPH, control and Ascorbic acid solution:** A DPPH solution was prepared by dissolving 4 mg in 100 mL of 99% methanol and subsequently stored in a cool, dark place, wrapped in aluminum foil. A control was prepared by combining 1 mL of DPPH solution with 1 mL of methanol. The resultant blend was transferred to a cuvette, and the absorbance at 517 nm was measured in comparison to the methanol blank. The calibration curve for ascorbic acid was prepared following a method similar to Brand-Williams et al., (1995) [20]. Initially, a stock solution of ascorbic acid was prepared by dissolving 100 mg in 100 mL distilled water. This solution was serially diluted to obtain concentrations of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL 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 then incubated 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.

**Preparation of Extract Solutions:** The procedure for preparing extracts to assess antioxidant activity followed the method of Brand-Williams et al. (1995) [20]. Concisely, 1, 2, 3, 4, and 5 mL volumes of the extract were pipetted into five 10 mL volumetric flasks. Methanol was added to each flask to reach a total volume of 10 mL. Subsequently, 1 mL from each flask was drawn and placed into 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) [22].

$$I\% = \left(\frac{Ac-Ao}{Ac}\right) x\ 100 \qquad (1)$$

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* leaf such as (1*R*,4*R*,6*R*,10*S*)-4,12,12-trimethyl-9-methylidene-5-oxatricyclo[8.2.0.04,6]dodecane (L1), 2,5-diphenyl-1,3-oxazole (L2), (*E*)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one (L3), 7-hexyloxepan-2-one (L4), identified in the study plant sample and control ligand metformin, an anti-diabetic drug, 3-(diaminomethylidene)-1,1-dimethylguanidine (L5) 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 [23].

**Receptor preparation:**

The receptor protein α-glucosidase (PDB ID: 3WY2) plays a crucial role in the breakdown of various carbohydrates into glucose [24]. Our study targeted the 3WY2 receptor protein. The crystal structure of the protein complex used in the study was sourced from the Protein Data Bank (www.rcsb.org/pdb.pdb). The processed protein, obtained through ChimeraX software, underwent several modifications, including removing nonstandard atoms and bonds from the selected chain and residue. Following this, using AutoDock 4.2 tools, water molecules were eliminated, hydrogens were added,

nonpolar hydrogens were merged, Collman charges were introduced, and AD4 type atoms were assigned to generate the pdbqt file using AutoDock 4.2 tools [23].

**Docking procedure:**

The grid parameters were adjusted by modifying the X, Y, and Z dimensions to 126. The pdbqt files of ligands and protein were processed to prepare gpf type files using AutoDock 4.2 tools for the autogrid run. Further, dpf files were produced 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 applied. To attain the desired conformations, a total of 70 runs were selected. Subsequently, autogrid and AutoDock runs were performed, and the docking results were extracted from resultant glg files [23].

**ADMET analysis:**

The analysis of Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) is relevant to understanding the pharmacodynamic attributes of a drug molecule. The SWISS ADME web-based server (www.swissadme.ch/; accessed on 18 January 2024) was used to assess these properties for phytomolecules and metformin. The smiles for phytomolecules and metformin were sourced from PubChem and loaded online to SWISS ADME Server to determine ADMET properties [ 24,25].

**Results and Discussion**

**Qualitative preliminary phytochemical screening:**

Table 1 presents the qualitative phytochemical compositions of ethanoic extracts from *Oldfieldia dactylophylla* leaves. The findings reveal the presence of important phytochemical classes, including steroids, phenols, tannins, alkaloids, terpenoids, flavonoids, saponins, and anthraquinones.

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

| SNo | PHYTOCHEMICAL | OUTCOME |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Saponins | + |
| 3 | Flavonoids | + |
| 4 | Phenols | + |
| 5 | Tannins | + |
| 6 | Terpenoids | + |
| 7 | Steroids | + |
| 8 | Anthraquinones | - |

**KEY: + = PRESENT, - = ABSENT**

Alkaloids are known for their therapeutic effects as anesthetics, cardioprotective agents, and anti-inflammatory substances. Notable examples of clinical alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine [26]. In nutrients and herbal medicines, bioactive compounds like flavonoids and various phenolic compounds are known for their diverse advantages. For instance, flavonoids have antioxidant, anticancer, antibacterial, cardioprotective, anti-inflammatory, immune system-enhancing, and UV radiation-protective properties. These attributes position them as valuable candidates for pharmaceutical and medical applications [27]. The tannins observed in the extract also exhibit multiple medicinal attributes [28].

Epidemiological and experimental studies underscore the promising role of monoterpenes in preventing and treating various cancers [29]. Additionally, steroids contribute to their medicinal significance, particularly in terms of anti-inflammatory properties [28]. The varied classes of compounds identified in the leaf extract of *Oldfieldia dactylophylla* affirm its suitability for traditional medicinal use, addressing conditions such as venereal disease, dysentery, diarrhea, malaria, diabetes, and pain relief.

**GC-MS phytochemical profiling**:

The chromatogram generated from GC-MS is depicted in Figure 2. The GC-MS phytochemical profiling revealed the presence of tentative compounds among them catechol, eugenol, Cis-5,8,11,14,17 eicosapentaenoic acid, 4a,5-dimethyl-3-(prop-1-en-2-yl)-

1,2,3,4,4a,5,6,7-octahydronapthalene-1-ol and many others as shown in the Table 2.

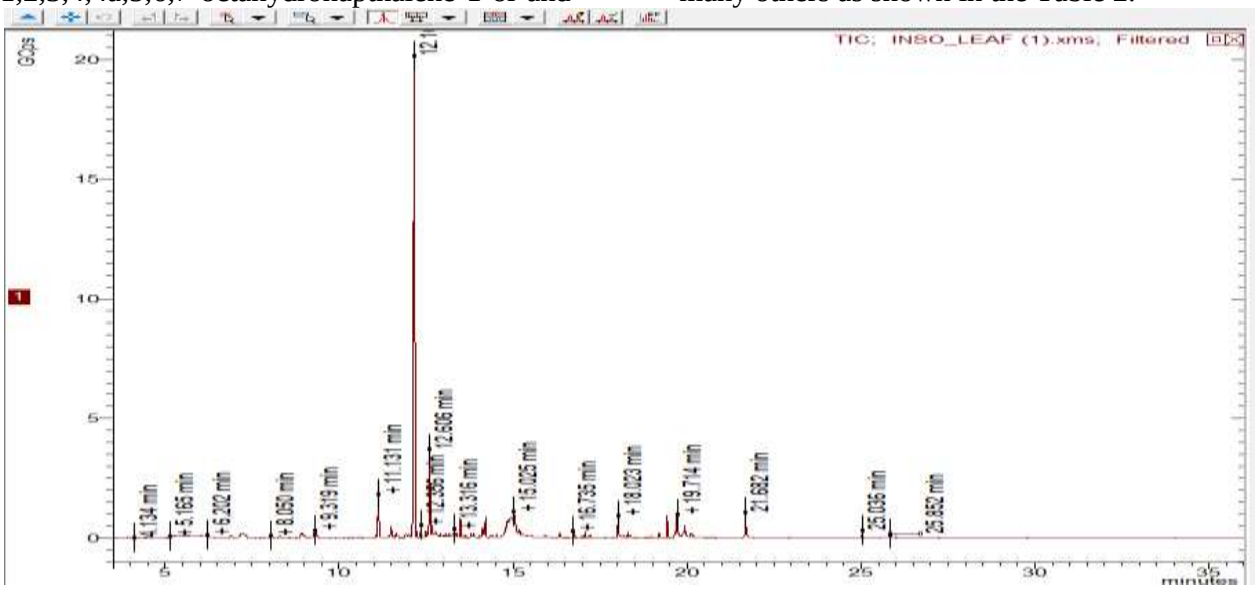



**Figure2: The GC-MS generated chromatogram for *Oldfieldia dactylophylla* leaf extract**

**Table 2**: Tentative bioactive compounds screened in *Oldfieldia dactylophylla* leaf extract

| No | RT | Tentative Compound | CAS | Molecular Weight | Molecular Formula |
|---|---|---|---|---|---|
| 1 | 6.897 | Thymine | 65-71-4 | 126 | $C_5H_6N_2O_2$ |
| 2 | 8.314 | Benzoicacid | 65-85-0 | 122 | $C_7H_6O_2$ |
| 3 | 8.764 | Dodecane,1-flouro- | 334-68-9 | 188 | $C_{12}H_{25}F$ |
| 4 | 8.930 | Catechol | 120-80-9 | 110 | $C_6H_6O_2$ |
| 5 | 9.322 | 5-Hydroxymethylfurfural | 67-47-0 | 120 | $C_6H_6O_3$ |
| 6 | 9.764 | Nonanoicacid | 112-05-0 | 158 | $C_9H_{18}O_2$ |
| 7 | 9.842 | 2-Oxepanone,7-hexyl- | 16429-21-3 | 198 | $C_{12}H_{22}O_2$ |
| 8 | 10.161 | 3-Buten-2-one,4-(2,6,6-trimethyl-2-cyclohexane-1-1yl)- | 6901-97-9 | 192 | $C_{13}H_{20}O$ |
| 9 | 10.287 | 1,2-Benzenediol,4-methyl- | 452-86-8 | 124 | $C_7H_8O_2$ |
| 10 | 11.057 | .alpha.-cubebene | 17699-14-8 | 204 | $C_{15}H_{24}$ |
| 11 | 11.131 | Eugenol | 97-530 | 164 | $C_{10}H_{12}O_2$ |
| 12 | 11.247 | Cis-5,8,11,14,17-Eicosapentaenoic acid | 10417-94-4 | 302 | $C_{20}H_{30}O_2$ |
| 13 | 11.420 | 1H-Cyclopropa[a]naphalene, 1a,2,3,5,7,7a,7b-octahydro- 1,1,7,7a tetramethyl- | 17334-55-3 | 204 | $C_{15}H_{24}$ |
| 14 | 11.498 | Copaene | 3856-25-5 | 204 | $C_{15}H_{24}$ |
| 15 | 12.005 | Bicylo[5.2.0]nonane,4-methyl-2,8,8-trimethyl-2-vinyl | none | 204 | $C_{15}H_{24}$ |
| 16 | 12.160 | Caryophyllene | 87-44-5 | 204 | $C_{15}H_{24}$ |
| 17 | 12.229 | Bicylo[5.2.0]nonane,4-methyl-2,8,8-trimethyl-2-vinyl | none | 204 | $C_{15}H_{24}$ |
| 18 | 12.355 | (E)-farnesene | 18794-84-8 | 204 | $C_{15}H_{24}$ |

| | | | | | |
|---|---|---|---|---|---|
| 19 | 12.493 | 1R,2Z,9S-4,11,11-Trimethyl-8-methyleneBicyclo[7.2.0]undec-3- ene | None | 204 | $C_{15}H_{24}$ |
| 20 | 12.606 | Humulene | 6753-98-6 | 204 | $C_{15}H_{24}$ |
| 21 | 12.666 | Aromandemdrene | 489-39-4 | 204 | $C_{15}H_{24}$ |
| 22 | 12.908 | Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl- (1R,2S,7R,8R)- | 5989-08-2 | 204 | $C_{15}H_{24}$ |
| 23 | 13.017 | e-3-Arachidonicacidmethylether | 132712-70-0 | 318 | $C_{21}H_{34}O_2$ |
| 24 | 13.078 | Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7- dimethyl-(1-methylethenyl)- | 3691-11-0 | 204 | $C_{15}H_{24}$ |
| 25 | 13.164 | (R)-1-methyl-4-(6-6-methylhept-5ene-2-yl)cylohexa-1,4-diene | 28976-2 | 204 | $C_{15}H_{24}$ |
| 26 | 13.317 | 1-isopropyl-4-7-dimethyl-1,2,3,5,6,8a-hexahydronapthalene | 16729-01-4 | 204 | $C_{15}H_{24}$ |
| 27 | 13.383 | Cis-Calamenene | 72937-55-4 | 202 | $C_{15}H_{24}$ |
| 28 | 13.486 | (1R,4S,5S)-1,8-Dimethyl-4-(prop-1-ene-2-yl)spiro[4.5]dec-7-ene | 43219-80-3 | 204 | $C_{15}H_{24}$ |
| 29 | 13.570 | 4a,5-dimethyl-3-(prop-1-ene-2- yl)-1,2,3,4,4a,5,6,7-octahydronapthalen-1-ol | 61847-19-6 | 220 | $C_{15}H_{24}O$ |
| 30 | 14.041 | Pentyltetratriacontylether | none | 564 | $C_{39}H_{80}O$ |
| 31 | 14.128 | Tetracontane,3,5,24-trimethyl | 55162-61-3 | 604 | $C_{43}H_{88}$ |
| 32 | 14.210 | CaryophylleneOxide | 1139-30-6 | 220 | $C_{15}H_{24}O$ |
| 33 | 14.371 | Bicyclo[4.4.4]dec-1-ene,2-isopropyl-5-methyl-9-methylene- | 150320-52-8 | 204 | $C_{15}H_{24}$ |
| 34 | 14.535 | (1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12- oxabicylo[9.1.0]dodeca-3,7-diene | 19888-34-7 | 220 | $C_{15}H_{24}O$ |
| 35 | 14.683 | 7-epi-cisSesquisabinene hydrate | none | 222 | $C_{15}H_{26}O$ |
| 36 | 15.189 | Cis-5,8,11,14,17-Eicosapentaenoic acid | 10417-94-4 | 302 | $C_{20}H_{30}O_2$ |
| 37 | 15.933 | Octadecanoicacid | 57-11-4 | 284 | $C_{18}H_{36}O_2$ |
| 38 | 16.347 | Heptadecane | 629-78-7 | 240 | $C_{17}H_{36}$ |
| 39 | 16.737 | Neophytadiene | 504-96-1 | 278 | $C_{20}H_{38}$ |
| 40 | 16.810 | Hexadecanol,2-methyl | 5519-46-0 | 254 | $C_{17}H_{34}O$ |
| 41 | 16.983 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 102608-53-7 | 296 | $C_{20}H_{40}O$ |
| 42 | 17.066 | 1,2-Benzenedicarboxylicacid, Bis(2-methylpropyl)ester | 84-69-5 | 278 | $C_{16}H_{22}O_4$ |
| 43 | 17.226 | 1-Hexadecanol | 36653-8-4 | 242 | $C_{16}H_{34}O$ |
| 44 | 17.640 | Methyl8-methyl-nonanoate | none | 186 | $C_{11}H_{22}O_2$ |
| 45 | 18.020 | n-hexadecanoicacid | 57-10-3 | 256 | $C_{16}H_{32}O_2$ |
| 46 | 18.177 | octadecanamide | 124-26-5 | 283 | $C_{18}H_{37}NO$ |
| 47 | 18.301 | Eicosanoicacid,ethylester | 18281-05-5 | 340 | $C_{22}H_{44}O_2$ |
| 48 | 18.362 | nonadecane | 629-92-5 | 268 | $C_{19}H_{40}$ |
| 49 | 18.976 | Oxazole,2,5ideiphenyl | 92-71-7 | 221 | $C_{15}H_{11}NO$ |
| 50 | 19.202 | n-nonadecanol-1 | 1454-84-8 | 284 | $C_{19}H_{40}O$ |
| 51 | 19.430 | Phytol | 150-86-7 | 296 | $C_{20}H_{40}O$ |

| | | | | | |
|---|---|---|---|---|---|
| 52 | 19.715 | Cis-7-hexadecanoicacid | 2416-19-5 | 254 | $C_{16}H_{30}O_2$ |
| 53 | 20.111 | Benzeneethanamine,2-fluoro-,Beta,3,4-trihydroxy-N-isopropyl- | 61338-98-5 | 229 | $C_{11}H_{16}FNO_3$ |
| 54 | 20.711 | Cis7,cis-11-hexadecadien-1-yl acetate | 52207-99-5 | 280 | $C_{18}H_{32}O_2$ |
| 55 | 21.681 | 9-octadecanamide,(Z)- | 301-02-0 | 281 | $C_{18}H_{35}NO$ |
| 56 | 22.916 | 1H,-indene,1-ethylidene octahydro-7a-methyl,-cis- | 56362-87-9 | 164 | $C_{12}H_{2O}$ |
| 57 | 23.011 | Bis(2-ethylhexyl)phthalate | 117-81-7 | 390 | $C_{24}H_{38}O_4$ |
| 58 | 25.038 | Squalene | 111-02-4 | 410 | $C_{30}H_{50}$ |
| 59 | 25.851 | 2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-3nyl)oxetane | none | 222 | $C_{15}H_{26}O$ |
| 60 | 29.780 | 2,6,10-dodecatriene-1,12-diol,6-(hydroxylmethyl)-10-methyl-2-(4-methyl-3-penten-1-yl)- | 1083197-50-5 | 364 | $C_{22}H_{36}O_4$ |

As illustrated in Table 2, the potential molecules fall within different classes of phytocompounds outlined in Table 1. Notably, the list of compounds may not be comprehensive, given the limited access to only the NIST library for GC analysis. However, specific molecules from Table 2 were chosen for in silico analysis and molecular docking to evaluate their potential anti-diabetic profiles.

**Total phenol content**

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 3) and found to be 48.1667 mg/L (Table 3). 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. Literature has previously identified these phytochemicals as effective in managing a range of health conditions

Phenolics possess significantly higher antioxidant activity when compared to well-known antioxidant vitamins [30]. The substantial health benefits associated with phenolic compounds are diverse. These compounds find practical applications across various domains, functioning as antioxidants, anticancer agents, antimicrobials, ingredients in skincare products, anti-inflammatories, neuroprotective agents, food preservatives, and more [31]. Additionally, phenolic compounds have demonstrated anti-aging effects by regulating oxidative stress and mitigating inflammation [32]. Consuming foods rich in phenolics has been linked to enhanced gut health, fostering a healthy gut microbiota, and protecting against gastrointestinal disorders [33]. Furthermore, these compounds exhibit promising antibacterial and antifungal activities, indicating their potential for natural food preservation and the development of novel antimicrobial agents [34]. A similar study from Nepal reported a total phenolic content ranging from 72.66 to 292.65 GAE/g [35].

**Evaluation of total flavonoid content (TFC)**

The total flavonoid content (Table 4) was determined using the aluminum chloride colorimetric method. The data were presented as mean values ± standard deviation (n=3). A linear calibration curve (Figure 4) of quercetin (Y = 0.0007x + 0.1233, $R^2$ = 0.9987) was used to estimate the total flavonoid content, with results found to be 125.4667 mg/L.

**Table3:Total phenol content in Oldfieldia dactylophylla ethanolic leaf extract (n=3)**

| S/N | CONCENTRATION | ABSORBANCE | TPC(µg/ml) | STD |
|---|---|---|---|---|
| T1 | 48.20 | 0.8112 | 48.1667 | 0.0577 |
| T2 | 48.10 | 0.8109 | | |
| T3 | 48.20 | 0.8119 | | |

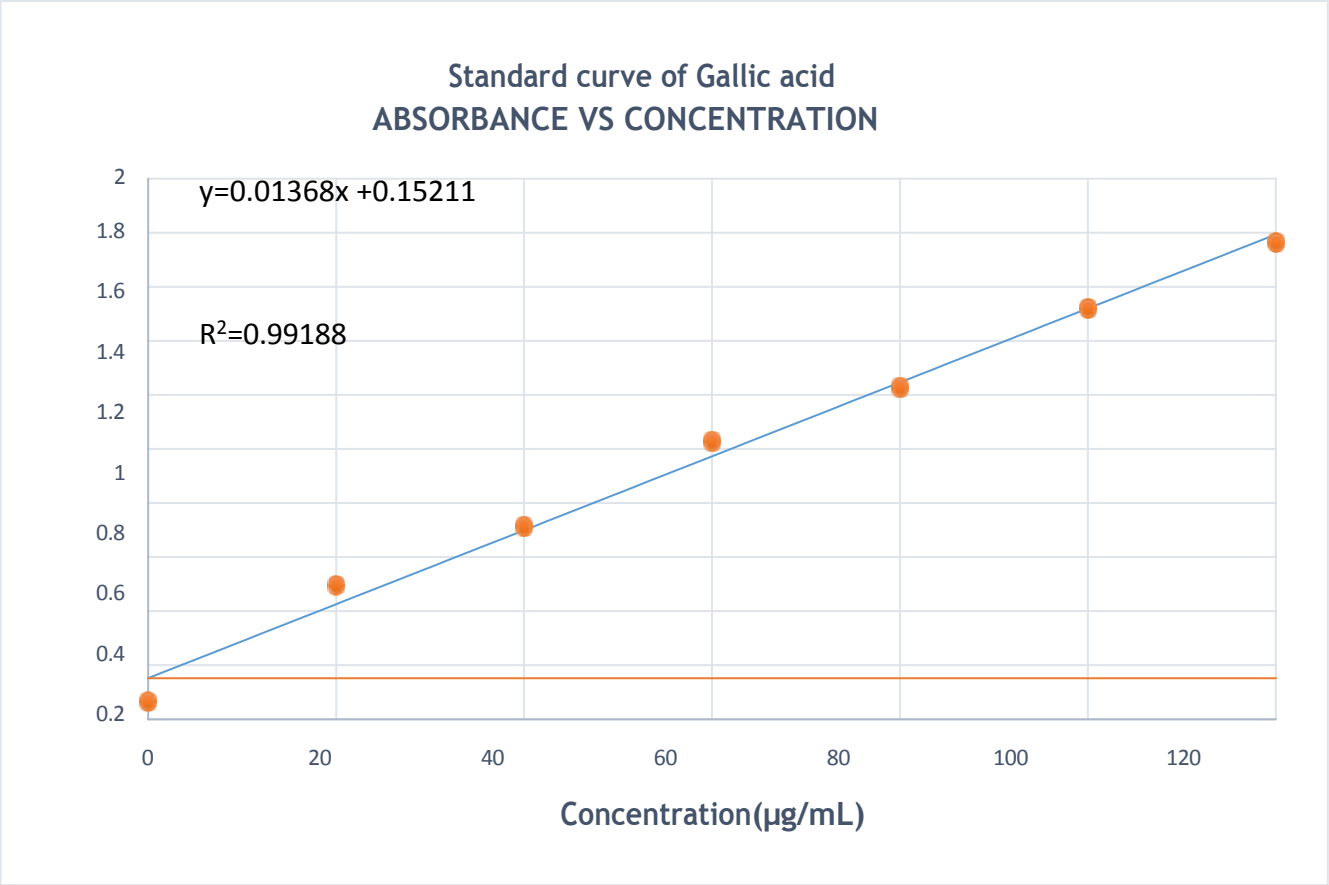



**Figure3: Calibration curve for Gallic acid**

**Table3:Total phenol content in Oldfieldia dactylophylla ethanolic leaf extract (n=3)**

| S/N | CONCENTRATION | ABSORBANCE | TPC(µg/ml) | STD |
|---|---|---|---|---|
| T1 | 48.20 | 0.8112 | 48.1667 | 0.0577 |
| T2 | 48.10 | 0.8109 | | |
| T3 | 48.20 | 0.8119 | | |

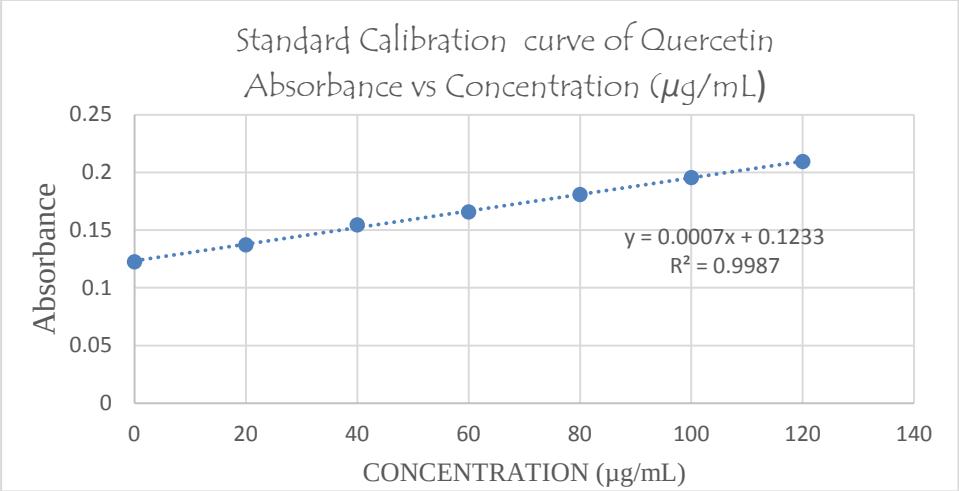



**Figure 4: Calibration curve graph for Quercetin**

**Table4:Total Flavonoid content in Oldfieldia dactylophylla ethanolic leafextract(n=3)**

| S/N | CONCENTRATION | ABSORBANCE | TFC(µg/ml) | STD |
|---|---|---|---|---|
| T1 | 125.30 | 0.2129 | 125.4667 | 0.2887 |
| T2 | 125.30 | 0.2129 | | |
| T3 | 125.80 | 0.2148 | | |

Flavonoids possess therapeutic significance owing to their varied biological functions, encompassing antioxidant, anti-inflammatory, anticancer, antiviral, and neuroprotective properties, as noted by Sharma et al. in 2020[36]. The diverse therapeutic capabilities of flavonoids establish them as valuable compounds for both preventing and treating a range of health conditions. Studies suggest that their antioxidant activity contributes to therapeutic effects against chronic diseases. Furthermore, flavonoids offer cardiovascular benefits, including antiplatelet, anti-inflammatory, and vasodilatory properties, potentially reducing the risk of cardiovascular diseases. Including flavonoid-rich foods into the diet is recommended to support heart health [37-39]. The total flavanol content represented a significant proportion of flavonoids and was present in amounts ranging from 298.8 mg kg−1 in 'Discolor' to 1668.6 mg kg−1 in 'Koncentra' [40].

**Antioxidant activity**

The radical scavenging activity was determined using the free radical DPPH assay. In these experiments, as the electron count rises, the free radical DPPH attracts electrons, resulting in a noticeable transition from a deep purple/violet hue to a yellow color. At concentrations (20-100 mg/L) of the leaf methanoic crude extract, radical scavenging activity (Figure 5) was found to be 27.91, 44.91, 57.22, 62.68 and 76.07%. The $IC_{50}$ value was 44.71 mg/L, while that of the standard antioxidant (ascorbic acid) was $IC_{50}$ = 23.19 mg. Medicinal plants are acknowledged for their substantial antioxidant capacity, with their bioactive compounds playing a vital role in counteracting reactive oxygen species. These plants are instrumental in preserving cellular well-being and are deemed valuable assets in averting diseases associated with oxidative stress (Aruoma, 2003) [41]. The $IC_{50}$ of the crude extract indicates that the leaf is a strong antioxidant in accordance with classification of antioxidative potential by Molyneux (2004) [42]. This quantitative

evidence of the antioxidative capacity of the leaf extract indicates the ability of the leaves to reduce or alleviate oxidative stress as shown in this study. A previous 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 phenolic and flavonoid compounds [43].

**Docking results**

The molecular docking analysis considered L1, L2, L3, and L4 ligands, and metformin (L5) as control (Figure 6). The receptor used was α-glucosidase (PDB ID: 3WY2). The molecular docking results using AutoDock 4.2 are stipulated in the Tables 5. The interactions between ligands (L1, L2, L3, L4, and L5) and 3WY2 are depicted in Table 6.

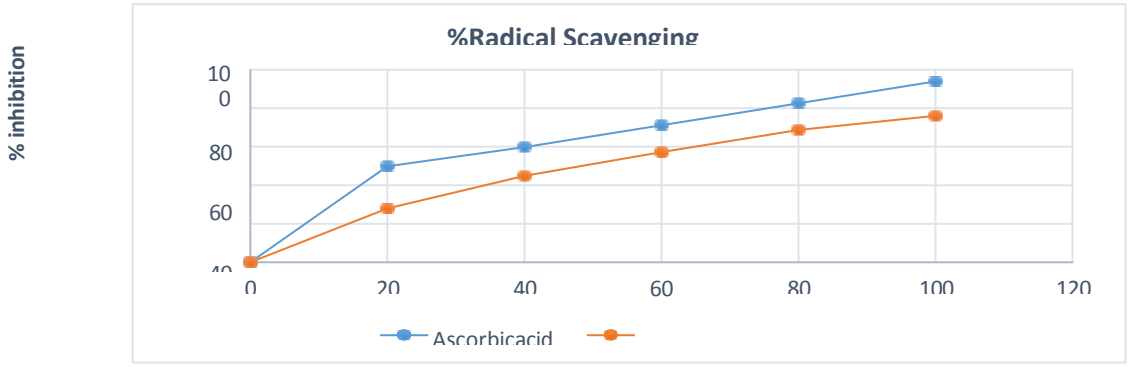
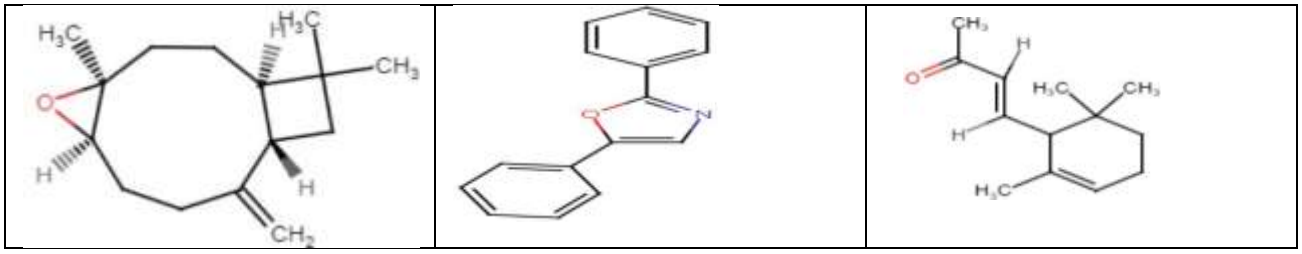



**Figure 5**: Percent Radical scavenging activity of Ascorbic acid and *Oldfieldia dactylophylla*



L1(CID-1742210) L2(CID-7105) L3(CID-5282108)

L4(CID-61835) L5(Metformin-CID:4091)

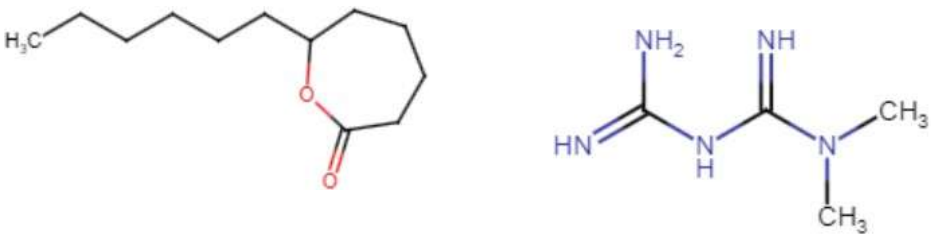


**Figure6: Structure of ligands used for Docking with 3WY2**

**Table5.** Docking analysis of selected ligands from leaf extract of *Oldfieldia dactylophylla* with 3WY2 receptor

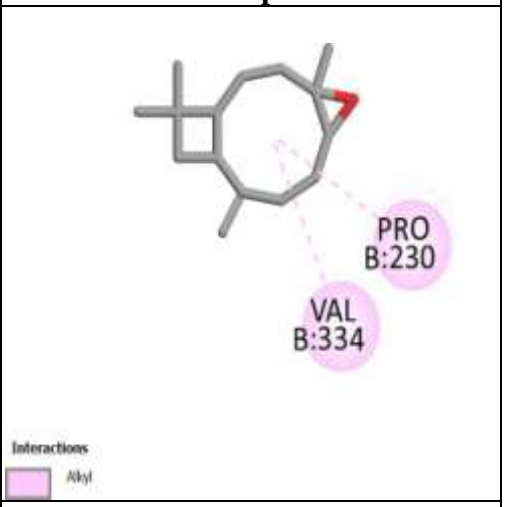
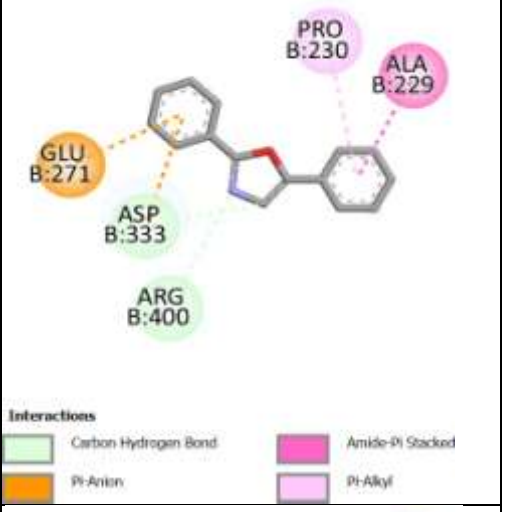
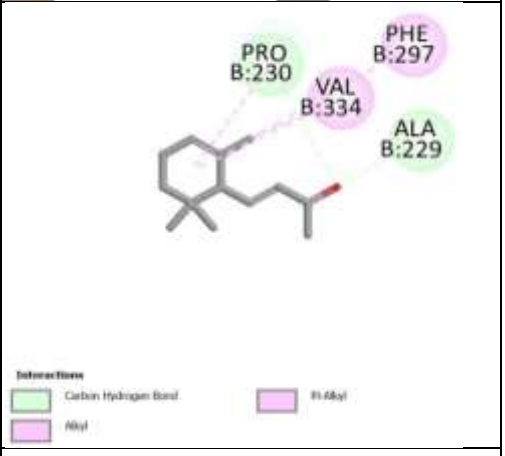
| Ligand | Binding energy Kcal/mole | Inhibition constant (Ki)(μM) | Total internal energy kcal/mol | Torsional free energy kcal/mol | Unbound energy kcal/mol | Cluster RMSD | Ligand efficiency |
|---|---|---|---|---|---|---|---|
| L1 | -6.73 | 43.70 | 0.00 | 0.00 | 0.00 | 0.00 | -0.42 |
| L2 | -6.30 | 24.22 | -0.44 | 0.60 | -0.44 | 0.00 | -0.37 |
| L3 | -6.06 | 115.30 | -0.47 | 0.60 | -0.47 | 0.00 | -0.43 |
| L4 | -5.29 | 695.32 | -0.73 | 1.49 | -0.73 | 0.00 | -0.38 |
| L5 | -8.03 | 1.30 | 0.44 | 0.00 | 0.44 | 0.00 | -0.89 |

All the ligands have moderately good binding energies (Tables 5) except L4 with -5.29 Kcal/mole. Through virtual screening, ligands undergo assessment based on their binding affinity. The binding energies of ligands aid in determining which ligand structure and rotation are most favorable for ligand-receptor interactions [ 44, 45]. Although, analysis of the binding efficiencies of natural products and marketed drugs demonstrate that therapeutic efficacy is not fundamentally linked with high binding affinity [46]. Inhibition constant values for L1-L3 are favorable while that for L4 is highest (695.32 μM). Ligand efficiencies for L1-L4 range from -0.37 to -0.42 while for metformin -0.89. Cluster RMSD for all the ligands and metformin are 0.00. These docking results support these ligands as prospective diabetic drug candidates to be considered for further in vitro and in vivo investigations. In the present study, docking of 3WY2 with reference drug metformin (L5) formed conventional hydrogen bonds (ASP A:300; ASP A: 197; GLU A:233). It showed an unfavourable donor-donor interaction (ARG B:400). The docking of L1 with 3WY2 had alkyl interactions (PRO B:230; VAL B:334). Further, docking of L2 with 3WY2 formed carbon hydrogen bonds (ASP B:333, ARG B:400), Pi-Anion interactions (GLU B:271), Amide-Pi Stacked (ALA B:229), and Pi-Alkyl interactions (ALA B:229). The docking of 3WY2 with L3 involved interactions such as Carbon Hydrogen Bond (PRO B:230, ALA B:229), Alkyl (VAL B:334), and Pi-Alkyl (PHE B:297). Docking of L4 with 3WY2 included interaction as conventional hydrogen bonds (VAL A:335), Alkyl (TYR A:389), and Pi-Alkyl (PRO A:230). From the 2D complexes, it is evident that L1, L2 and L3 with effective binding energies has no unfavourable factors compared to metformin having unfavourable donor-donor interactions. However, there may be some modified interactions in vivo environments. Thus, L1, L2, and L3 are prospective diabetic drug candidates for further in vivo investigations.

**ADMET analysis results**

The ADMET analysis of the following ligands (L1, L2, L3, L4), and (L5) are stipulated in Table 7.

**Table 6. Interactions between phytocompounds from *Oldfieldia dactylophylla* (leaf) and 3WY2 depicted as 2D complexes.**

| Ligand | Residue Interactions | 2D complexes |
|---|---|---|
| **L1** | **Alkyl Interactions: PRO B:230; VAL B:334** |  |
| **L2** | **Carbon Hydrogen Bond: ASP B:333, ARG B:400**<br>**Pi-Anion:GLUB:271**<br>**Amide-Pi Stacked: ALA B:229**<br>**Pi-Alkyl: ALA B:229** |  |
| **L3** | **Carbon Hydrogen Bond:PROB:230, ALA B:229**<br>**Alkyl: VALB:334**<br><br>**Pi-Alkyl:PHEB:297** |  |
| **L4** | **Conventional hydrogen Bond: VAL A:335**<br>**Alkyl: TYR A:389** | |

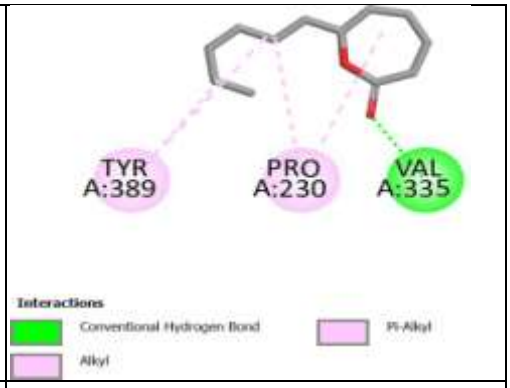
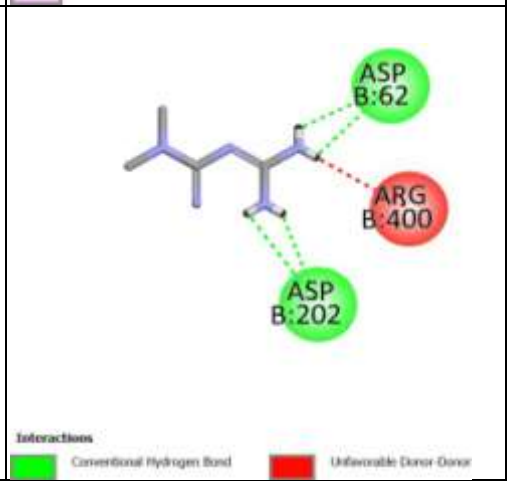
| | | |
|---|---|---|
| | **Pi-Alkyl:PRO A:230** |  |
| **L5** | **Conventional hydrogen bond: ASP B: 62; ASP B:202**<br>**Unfavorable donor- donor: ARG B:400** |  |

**Table7: ADMET analysis of phytocompounds from *Oldfieldia dactylophylla* leaf extract and metformin**

| **ADMET - properties** | **Ligands** | | | | **Drug (ligand)** |
|---|---|---|---|---|---|
| | L1 | L2 | L3 | L4 | L5 |
| Physicochemical properties | | | | | |
| Molecularweight (g/mol) | 220.35 | 221.25 | 192.30 | 199.30 | 129.16 |
| Topological SurfaceArea (TPSA)(A°) | 12.53 | 26.03 | 17.07 | 26.30 | 91.49 |
| Num.H-bond acceptors | 1 | 2 | 1 | 2 | 2 |
| Num.H-bond donors | 0 | 0 | 0 | 0 | 3 |
| Molar Refractivity | 68.27 | 67.38 | 61.48 | 58.97 | 36.93 |
| Lipophilicity | | | | | |
| XLOGP3 | 3.56 | 4.67 | 3.85 | 3.43 | -1.27 |
| ILOGP | 3.15 | 2.84 | 2.81 | 2.97 | 0.34 |
| MLOGP | 3.95 | 2.67 | 2.94 | 2.76 | -0.56 |
| WLOGP | 3.94 | 4.01 | 3.51 | 3.44 | -1.24 |
| Water Solubility | | | | | |
| LogS (ESOL) | -3.45 | -4.76 | 3.33 | -2.90 | 0.29 |
| Class | Soluble | Moderately soluble | Soluble | Soluble | Highly soluble |
| Drug likeness | | | | | |
| Lipinski | Yes:0(zero) violation | Yes:0(zero) violation | Yes:0 (zero) violation | Yes:0(zero) 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 | Yes | Yes | No |
| P-gpsubstrate | No | No | No | No | No |
| CYP1A 2 inhibitor | No | Yes | No | No | No |
| CYP2C 19 inhibitor | Yes | Yes | No | No | No |
| CYP2C 9 inhibitor | Yes | No | Yes | No | No |
| CYP2D 6 inhibitor | No | Yes | No | No | No |
| CYP3A 4 inhibitor | No | No | No | No | No |
| LogKp (skin permeation)cm/s | -5.12 | -4.33 | -4.74 | -5.07 | -7.99 |
| Medicinal Chemistry | | | | | |
| Pas assay interference compounds (PAINS) | 0(zero)alert | 0(zero)alert | 0(zero) alert | 0(zero)alert | 0(zero)alert |
| Brenk | 2 alerts | 0(zero)alert | 2 alerts | 0(zero)alert | 2 |
| Synthetic accessibility | 4.35 | 2.85 | 3.53 | 2.59 | 3.02 |
| LeadLikeness | 2 violations | 2 violations | 2 violations | 1 violation | 1 violation |

In this study, physicochemical, lipophilicity, solubility, drug likeness, pharmacokinetic and medicinal properties of phytocompounds (L1, L2, L3, and L4) from *Oldfieldia dactylophylla* leaaf extract and control anti-diabetic drug metformin were analysed. On Lipinski scale, ligands L1, L2, L3, L4, and reference drug metformin had no violations. 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 and L4 while only CYP2C 9 shows inhibition in the case of L3. Isozymes CYP1A 2, CYP2C 19, and CYP2D 6 are inhibitory in case of L2. Isozymes CYP2C 19 and CYP2C 9 are inhibitory in case of ligand L1. Synthetic accessibility values are L1(4.35), L2 (2.85), L3 (3.53), L4 (2.59), and reference drug metformin (3.02).

Gastrointestinal absorption (GI) is a pivotal factor determining an oral drug's effectiveness; in this context, all the ligands and metformin fulfill this criterion. Synthetic accessibility, rated on a numerical scale from 1 (most accessible to synthesize) to 10 (most difficult to make), indicates favorable values for all the ligands and metformin. Ligands with zero violations on the Lipinski scale hold promise as potential drug candidates. It has been noted that compounds featuring more hydrogen bond acceptors (HBAs) and fewer hydrogen bond donors (HBDs) present a favorable profile as drug candidates [47, 48]. On the criteria of hydrogen bond acceptors and donors, all the ligands meet the specified standards. Therefore, ADMET analysis underscores these phytochemicals as prospective candidates for future drug development, except for L4, which exhibits slightly lower binding energy. However, it is essential to admit the possibility of some modifications in these attributes in in vivo environments.

## CONCLUSION

The preliminary qualitative screening of *Oldfieldia dactylophylla* ethanolic leaf extract 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 the treatment of various ailments. The presence of such diverse classes

of bioactive compounds strongly supports the broad application of leaf extract in traditional medicine. Based on the $IC_{50}$ (44.71 mg/L) obtained, the crude extract showed strong antioxidant potential due to the presence of polyphenols, thus affirming the reason why they might be used as medicinal plants to alleviate diverse ailments. The diverse range of medicinal phytochemicals present in plants supports their healing power. *In silico* analysis strongly supports some phytomolecules as viable drug candidates 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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