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# Identification of potential DPP-4 inhibitors from *Bryophyllum pinnatum* by *in-silico* analysis

**Research Article**

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## Abstract

Background: An estimated 463 million people already live with diabetes and that figure is set to rise to over 700 million by 2045, as per the International Diabetes Federation (IDF). The current form of treatment for Type-2 DM can be done with sulfonylureas, meglitinides, metformin, thiazolidinediones, incretin mimetics: Glucagon-like peptide 1 (GLP-1), Glucose-dependent insulinotropic polypeptide (GIP), and amylin analogues (Pramlintide). *Bryophyllum pinnatum (Lam.) Oken (B. pinnatum)* belongs to the plant family *crassulaceae* used in traditional medicine in Asia. GLP-1 and Glucose-dependant Insulinotropic Polypeptide (GIP), both play a similar role in stimulating insulin secretion and are inactivated by dipeptidyl peptidase-4 (serine protease dipeptidyl peptidase-4) enzymes. Aims: To identify the potential anti-diabetic active compounds of *Bryophyllum pinnatum* against DPP-4 enzyme using *in-silico* methods. Methods and Material: The phytochemicals associated with *Bryophyllum pinnatum* were retrieved from ChEBI and canonical SMILES retrieved, followed by searching for its molecular properties and druglikeness using MolSoft LLC, toxicity test using ADVERPred, Swiss Target Prediction for predicting the DPP-4 inhibitors, molecular docking using Schrodinger software suite. Results: The *in-silico* study identified two phytochemicals from fourteen that have been predicted for DPP-4 inhibition potential against Type-2 DM. In $CH_2CL_2$ and $CH_3OH$ solvent compounds 3,5-dihydroxybenzoic acid (-5.623 kcal/mol) and 3,4-didehydro-N4-deethylbrinzolamide (-4.91 kcal/mol) displayed the highest docking scores against human DPP-4 (5Y7K). Conclusions: The above-mentioned compounds revealed no side effects. The *in-silico* results strongly favour the beneficial use of phytochemicals from *Bryophyllum pinnatum* as a probable herb that can be used for adjuvant therapy. Further *in-vitro* and *in-vivo* tests are needed for confirmation.

**Keywords:** Type-2 diabetes mellitus, *Bryophyllum pinnatum,* Dipeptidyl peptidase-4, In-silico Analysis, ADME/T Prediction, GLP-1, GIP.

## Introduction

Diabetes mellitus (DM) is a metabolic disorder and that affects the kidney, heart, & nervous system in humans. DM is mainly separated into two groups types 1 and 2 (1). DM affected 8.5% of the global population as per records obtained from WHO (World Health Organisation) in 2014, it is also reported that this may increase to 592 million by 2035. As per WHO records in 2016, DM ranked 7 and it exhibited a major threat worldwide (2).

Intestinal endocrine cells produce GLP-1; it is an insulin-promoting polypeptide. The intake of sugar and lipids stimulates the release of GLP-1, and it is also called a G-Protein Coupled Receptor (GPCR) (2). In intestinal endocrine cells, GLP-1 binds to GLP-1R and activates adenylyl cyclise to generate cAMP. The cAMP activates protein kinase A; it enhances glucose-stimulated insulin secretion (3). GLP-1 and Glucose-dependant Insulinotropic Polypeptide (GIP) both play a similar role in stimulating insulin secretion. They are very unstable have short half-lives and are easily degraded and inactivated by DPP-4 enzymes in hypoglycaemic conditions (4). The use of drug delivery systems is a potential solution to overcome this issue, and DPP-4 has a significant hypoglycaemic effect. It is one of the key mechanisms of type 2 DM treatment (5).

*Bryophyllum pinnatum (B. pinnatum)* is mainly present in the plant family *crassulaceae*. The plant holds potassium, malate, ascorbic, malic, and bryophyllin. In preliminary phytochemical analysis tannins, proteins, resin, amino acids, glycosides, phenolic compounds, terpenoids, phytosterol, alkaloids, and flavonoids are present in plant leaf extract. Mainly *B. pinnatum* shows anti-inflammatory, hypoglycaemic, antioxidant, wound healing, anti-diabetic, and anticancer medicinal properties as shown in Figure 1 (6,7).

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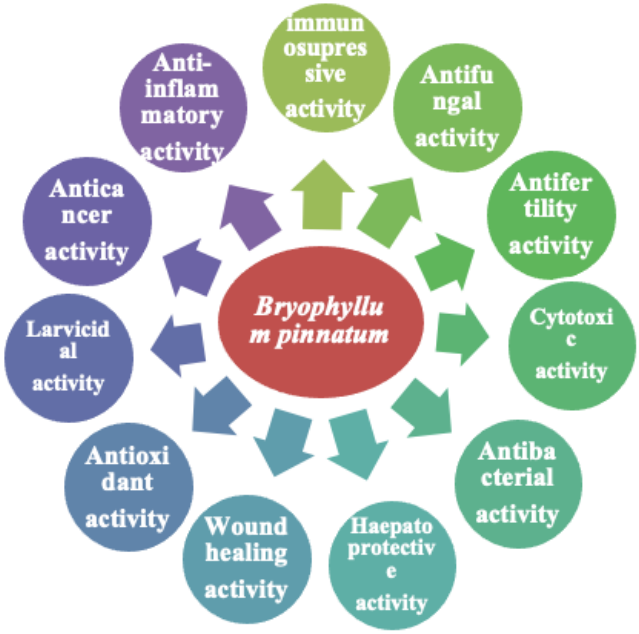
**Figure No. 1: Medicinal properties of *Bryophyllum pinnatum* plant**




*B. pinnatum* is an easily available plant and it does not need any special conditions for its collection and cultivation. Computational methods like molecular dynamics models, molecular docking, drugs-likeness prediction, and ADMET study, are used to screen potential drugs from various databases (8).

Molecular docking has become a well-accepted technique along with X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy in studying drug-target interactions. It is applied in the field of medicinal chemistry to access positive ligand binding poses in the creative originality of structure-based drug design (9). Drug-like properties of compounds are a qualitative approach used in drug design concerning factors such as bioavailability, and it is assessed based on the features of its molecular structure (10).

The ADME/T (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction plays a vital role in the purification of drugs. So, the ADME/T prediction was employed to check for other drug-likeness properties; It is essential to predict the position and association of a drug in the human body during the design of the drug molecule (10).

Therefore, this study was designed to analyze one of the very prominent, economical, and natural sources, *B. pinnatum* for prophylaxis of Diabetes mellitus. *B. pinnatum* possesses 182 phytochemical compounds and it has numerous potential candidates for the treatment of Type-2 diabetes mellitus (11).

## Materials and Methods

### Literature Search

PubMed and Google Scholar were used to identify reports published up until 2021 on the isolation of phytochemicals of *B. pinnatum*. The information obtained from journal article (12) pointed to the use of solvents like $CH_2CL_2$ and $CH_3OH$ for phytochemical extraction. Using these two solvent extraction methods we retrieved 182 compounds from databases.

### Phytochemical identification

The phytochemicals of *B. pinnatum* were retrieved from ChEBI (Chemical entities of biological interest) online tool (https://www.ebi.ac.uk/chebi/). This tool was also used to obtain the PubChem ID, molecular weight, molecular formula, and Canonical SMILE of phytochemicals (11).

### Prediction of DPP-4 inhibitor

The total retrieved phytochemicals were used to predict the DPP-4 inhibition activity using Swiss Target Prediction online tool (http://www.swisstargetprediction.ch/) (13). The query in the form of Canonical SMILE is used for information retrieval.

### Estimation of molecular properties and drug-likeness:

The 2D structures were downloaded from the PubChem database by name or drawn using ChemDraw software (http://www.cambridgesoft.com/Ensemble). The retrieved phytochemicals were used to identify molecular properties and drug-likeness of *B. pinnatum* plant. The MolSoft L.L.C online tool (https://molsoft.com/mprop/) (14) was used, it is based on Lipinski's rule of five.

### Estimation of toxicity

The toxicity study of retrieved phytochemicals was calculated by using the ADVERPred online tool (http://www.way2drug.com/adverpred/) (15). It gave the probable activity, probable inactivity, and side effects of the plant.

### Ligand preparation

All the selected 182 phytochemicals from $CH_2CL_2$ and $CH_3OH$ compounds in *B. pinnatum* extract were downloaded from PubChem. In-step modification and preparation of ligand was conducted by using the LigPrep version 4.8 (Schrodinger LCC) (16).

### Target preparation

For the molecular docking analysis, the crystal structure of human DPP-4 in complex with inhibitor1 (Protein Data Bank ID: 5Y7K) was selected as a receptor, and it was optimized by hydrogenation and CHARMm force field calculations. In the present study, the selected target was downloaded from the RCSB protein data bank database (17) and standard parameters of protein preparation by a wizard (Schrodinger, LLC) were used and the protein structure was prepared until the RMS gradient for heavy atom reached 0.3 A°.

### Grid generation

In this step of receptor grid generation, the crystal-bound ligand was selected to enumerate a binding site grid with a scaling factor of 1.0 and partial charge cut-off of 0.25 for Van Der Waals radius (16). The glide docking produces different poses for each input ligand, and all pose was scored and ranked by using the grid generation method in (Kcal/Mol) (18).

**Molecular docking**

The molecular docking studies were conducted to identify the binding energies of the phytochemicals to the therapeutic protein targets of DPP-4 by using the computational program GLIDE docking module of Schrodinger Suite software (8). The 3D structure of protein was retrieved and modified using the software Discovery Studio 4.5 The refined protein structures were analysed by using the Ramachandran plot. The PDB files of the phytochemicals and proteins were converted into PDBQT format by using the Schrodinger Suite software (8). After screening only 10% of the ligands exhibited affinity and accordingly fourteen ligands retrieved from the 182 phytochemicals of $CH_2CL_2$ and $CH_3OH$ extracts were selected for standard molecular docking. The compounds with docking scores >-8 were selected for result and scoring analysis (19).

# Results

**Literature search data**

In the literature review, NCBI (National Center for Biotechnology Information) was used to search phytochemicals present in the plant, by using the keyword *B. pinnatum*. In this step, we retrieved 182 compounds from published articles (12).

**Phytochemical identification**

PubMed and Google Scholar were used to search for the phytochemicals present in *B. pinnatum* plant and those phytochemicals were retrieved from ChEBI. In the phytochemical identification step, a total of 182 phytochemicals were retrieved, and their Canonical SMILES were documented for generating data required for further work.

**Prediction of DPP-IV inhibitor**

All the retrieved 182 phytochemicals from solvents extraction $CH_2CL_2$ and $CH_3OH$ of selected plant *B. pinnatum* were evaluated for predicted DPP-4 property. Fourteen phytochemicals were identified based on their inhibition potential of DPP-4. In the solvent extraction $CH_2CL_2$ phytochemical compounds, 3, 5-dihydroxybenzoic acid (-5.623 kcal/mol) was predicted to exhibit the highest interaction and docking score represented by Table No. 8. The second solvent compound 3, 4-didehydro-N4-deethylbrinzolamide (-4.91 kcal/mol) exhibited highest interaction and docking score in the listed $CH_3OH$ compound represented by Table No. 9.

**Determination of drug-likeness**

In molecular properties identification, PubChem database was used to download the 2D structure of each compound. The retrieved PubChem data was used to convert each 2D structure to a 3D structure by using Discovery Studio 4.5 software (Accelrys Software Inc., San Diego, CA, USA). The retrieved phytochemicals were used to determine the drug-likeness of *B. pinnatum* plant by using MolSoft L.L.C online tool (https://molso ft.com/mprop/). The drug-like properties such as molecular weight, lipophilicity (MolLogP), Number of hydrogen bond acceptors (NHBA), Number of hydrogen bond donors (NHBD), and drug-likeness score (DLS) were calculated and recorded for the 182 identified compounds. The ranking order of $CH_2CL_2$ extracts phytochemical compound drug-likeness score is as represented in Table No. 1: N-carbamoylaspartic acid >3-hydroxyanthranilic acid >2,2',3-trihydroxydiphenyl ether (2−) >2,3-dihydro-3-oxoanthranilic acid >Gibberellin A34 methyl ester >Glycerol 2-phosphate >Butane-2,3-diol >Meso-butane-2,3-diol >3,5-dihydroxybenzoic acid. The second $CH_3OH$ extracts compound ranking order of DLS was observed and represented in Table No. 2: 2'-deamino-2'-hydroxyneamine (3+) >3, 4-didehydro-N4-deethylbrinzolamide >(R)-3-chloro-1,2-propanediol >(Z)-icos-13-enoic acid >Trimagnesium dicitrate. All $CH_2CL_2$ and $CH_3OH$ extracted phytochemicals follow Lipinski's rule of five.

**Determination of toxicity**

The retrieved and recorded Canonical SMILES of *B. pinnatum* plants were used to determine the toxicity of selected phytochemicals. Based on collected SMILES, the toxicity study of retrieved phytochemicals was calculated by using the ADVERPred online tool (http://www.way2drug.com/adverpred/). It provides the probable activity, probable inactivity, and side effects of the plant. The possible adverse effects (Side effects) of selected $CH_2CL_2$ and $CH_3OH$ extract phytochemicals are listed in Table No. 3 and Table No. 4. In $CH_2CL_2$ compounds: 2, 2', 3-trihydroxydiphenyl ether (2−) and Gibberellin A34 methyl ester revealed no side effects, whereas 3-hydroxyanthranilic acid showed four major side effects such as nephrotoxicity, hepatotoxicity, myocardial infection, and cardiac failure. In $CH_3OH$: Trimagnesium dicitrate and 2'-deamino-2'-hydroxyneamine (3+) compounds revealed no side effects, whereas 3, 4-didehydro-N4-deethylbrinzolamide showed three major side effects such as arrhythmia, myocardial infarction, and cardiac failure. ADVERPred online tool also calculated the probable activity (Pa) and probable inactivity (Pi) of the $CH_2CL_2$ and $CH_3OH$ compounds.

**Molecular docking**

The molecular docking study was performed for fourteen identified phytochemicals that have been predicted for DPP-4 inhibition potential against type-2 DM. Glide module Software from Schrodinger molecular modelling suite (Schrodinger, Inc., USA, 2020-21) was used for molecular docking interaction studies. In the phytochemical identification step, 182 compounds were retrieved from the published article (12) and based on protein-ligand interaction and docking score we obtained fourteen compounds that served as probable candidates with their glide energy.

The first solvent $CH_2CL_2$ extract revealed 3, 5-dihydroxybenzoic acid (-5.623 kcal/mol) as having the highest and Meso-butane-2, 3-diol (-2.941 kcal/mol) as having the lowest docking score. Based on target-ligand interaction and docking score, the ranking order of all compounds was: 3, 5-dihydroxybenzoic acid >3-

hydroxyanthranilic acid >2, 2', 3-trihydroxydiphenyl ether (2−) > N-carbamoylaspartic acid > Glycerol 2-phosphate >2, 3-dihydro-3-oxoanthranilic acid > Gibberellin A34 methyl ester > Butane-2, 3-diol > Meso-butane-2, 3-diol as shown in Table No.7. The compounds exhibit various levels of binding interactions with crystal structure of human DPP-4 in complex with inhibitor1 (Protein Data Bank ID: 5Y7K). These compounds are represented in the form of a number of hydrogen bonds (NHB) and amino acid residue interactions, and this exhibited interaction with conserved catalytic residues like Glu:205 and Glu:206. The maximum interaction and docked poses of 3, 5-dihydroxybenzoic acid revealed that this compound occupies the target of DPP-4 and interacts with Glu: 206, Glu: 205, Arg: 669, Ser: 209 catalytic amino acid residues, refer to Figure 2 The Meso-butane-2, 3-diol shows minimum interactions and docking poses with amino acid residues like Glu:205 and Glu:206, refer to Figure 3.

The second solvent $CH_3OH$ extract revealed a maximum docking score of -4.91 kcal/mol for 3, 4-didehydro-N4-deethylbrinzolamide and a minimum docking score of -3.091 kcal/mol for (Z)-icos-13-enoic acid. Based on this docking score and protein-ligand interaction, the ranking order of compounds was: 3, 4-didehydro-N4-deethylbrinzolamide >2'-deamino-2'-hydroxyneamine (3+) >(R)-3-chloro-1, 2-propanediol > Trimagnesium dicitrate >(Z)-icos-13-enoic acid as shown in Table No. 8. The compounds represent in the form of number of hydrogen bonds (NHB) are arranged accordingly. It revealed the conserved catalytic residues Glu:205 and Glu:206. The highest docking poses of 3, 4-didehydro-N4-deethylbrinzolamide compound exhibited the inhibition activity of DPP-4 and interacted with Glu:205, Glu:206, Ser:209, Hie:126 catalytic amino acid residues, refer to Figure 4 and the (Z)-icos-13-enoic acid exhibited lowest docking score with the presence of amino acid residues like Arg:356 and Arg:358, refer to Figure 5.

The Glide (extra precision (XP) mode) module from Schrodinger molecular docking suite software was used for the calculation of compounds Glide G-score, Glide E-model, and Glide energy studies for the two phytochemical extraction solvents $CH_2CL_2$ and $CH_3OH$, this is represented in Table No. 9 and 10.

**Figure 2: The solvent $CH_2CL_2$ extract compound 3, 5-dihydroxybenzoic acid (-5.623 kcal/mol) had the highest binding interaction and docking score. And the interaction occurs at Glu: 206, Glu: 205, Arg: 669, Ser: 209 catalytic amino acid residues.**

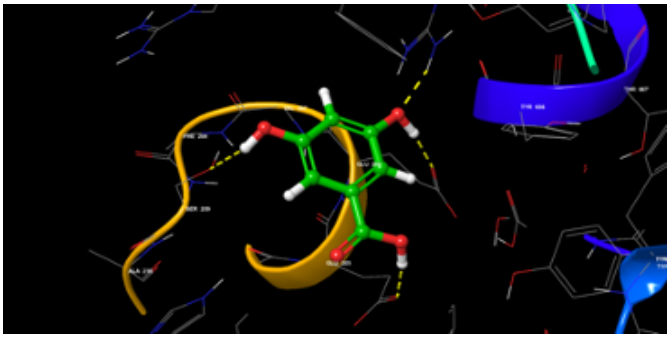


**Figure 3: The solvent $CH_2CL_2$ extract compound Meso-butane-2, 3-diol (-2.941 kcal/mol) had lowest binding interaction and docking score. There are minimum interactions and docking poses with amino acid residues Glu:205 and Glu:206.**

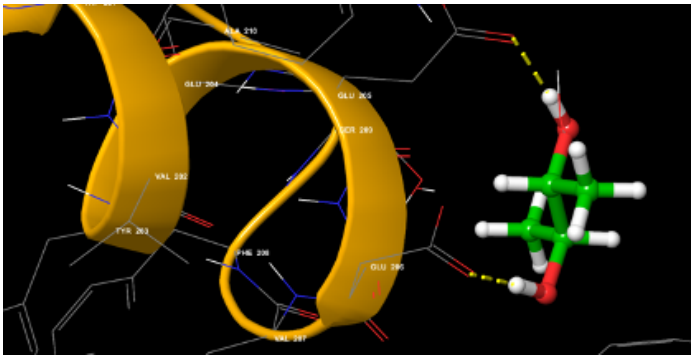


**Figure 4: The solvent $CH_3OH$ extract compound 3, 4-didehydro-N4-deethylbrinzolamide (-4.91 kcal/mol) had maximum binding interaction and docking score. And the interaction occurs with Glu:205, Glu:206, Ser:209, Hie:126 catalytic amino acid residues.**

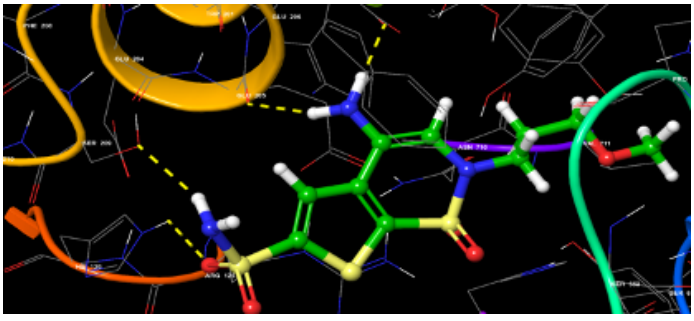


**Figure 5: The solvent $CH_3OH$ extract compound (Z)-icos-13-enoic acid (-3.091 kcal/mol) had minimum binding interaction and docking score. The interaction occurs at amino acid residues Arg:356 and Arg:358.**

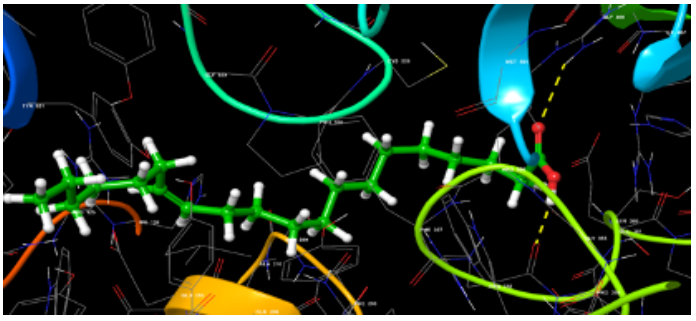


**Table 1: Drug likeness of $CH_2CL_2$ solvent compounds from *B. pinnatum***

| Sr. No. | Compound Name | Sub-Compound Name | PubChem ID | Molecular Formula | Molecular weight (g/ml) | NHBA (<10) | NHBD (≤5) | MolLogP (≤5) | DLS |
|---|---|---|---|---|---|---|---|---|---|
| 1 | (17a)-3-methoxyestra-1,3,5(10)-triene14,17-diyl TMS2 ether | 2,2',3-trihydroxydiphenyl ether (2−) | CHEBI:79174 | $C_{12}H_8O_4$ | 216.1906 | 4 | 1 | 2.97 | -0.76 |
| 2 | 3-Hydroxyanthranilic acid, TMS3 | 3-hydroxyanthranilic acid | CHEBI:15793 | $C_7H_7NO_3$ | 153.13542 | 3 | 4 | 1.03 | 0.01 |
| | | 2,3-dihydro-3-oxoanthranilic acid | CHEBI:15793 | $C_7H_7NO_3$ | 153.1354 | 4 | 3 | -2.33 | -0.92 |
| | | N-carbamoylaspartic acid | CHEBI:64850 | $C_5H_8N_2O_5$ | 176.1274 | 5 | 5 | -2.41 | 0.4 |
| | | 3,5-dihydroxybenzoic acid | CHEBI:39912 | $C_7H_6O_4$ | 154.1201 | 4 | 3 | 1.22 | -1.23 |
| 3 | 9-Octadecenyl TMS ether | Gibberellin A34 methyl ester | CHEBI:73260 | $C_{20}H_{26}O_6$ | 362.4168 | 6 | 2 | 1.21 | -0.92 |
| 4 | Butane-2,3-diol TMS2 ether | Meso-butane-2,3-diol | CHEBI:75460 | $C_4H_{10}O_2$ | 90.121 | 2 | 2 | -0.45 | -1.09 |
| | | Butane-2,3-diol | CHEBI:62064 | $C_4H_{10}O_2$ | 90.121 | 2 | 2 | -0.45 | -1.09 |
| 5 | Glycerol TMS3 | Glycerol 2-phosphate | CHEBI:17270 | $C_3H_9O_6P$ | 172.07372 | 6 | 4 | -1.81 | -0.97 |

The drug likeness score is calculated based on Lipinski's rule of five and the data obtained is tabulated as follows.

**Table No. 2: Drug likeness of $CH_3OH$ solvent compounds from *B. pinnatum*.**

| Sr. No. | Compound Name | Sub-Compound Name | PubChem ID | Molecular Formula | Molecular weight (g/ml) | NHBA (<10) | NHBD (≤5) | MolLogP (≤5) | DLS |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 1, 2 Propanediol TMS2 ether | (R)-3-chloro-1,2-propanediol | CHEBI:18663 | $C_3H_7ClO_2$ | 512.44 (>500) | 5 | 1 | 11.56 (>5) | 0.02 |
| | | 3,4-didehydro-N4-deethylbrinzolamide | CHEBI:43411 | $C_{10}H_{15}N_3O_5S_3$ | 353.02 | 7 | 4 | -0.55 | 0.48 |
| **2** | 2, 3 Butanediol TMS2 ether | Trimagnesium dicitrate | CHEBI:131391 | $C_{12}H_{10}Mg_3O_{14}$ | 456.01 | 14 (>10) | 8 (>5) | -3.41 | -0.6 |
| | | 2'-deamino-2'-hydroxyneamine (3+) | CHEBI:67213 | $C_{12}H_{28}N_3O_7$ | 326.19 | 7 | 14 (>5) | -1.56 | 0.71 |
| 3 | 2-Keto-d-gluconic acid TMS5 | (Z)-icos-13-enoic acid | CHEBI:134479 | $C_{20}H_{38}O_2$ | 310.29 | 2 | 1 | 8.12 (>5) | -0.3 |

The drug likeness score is calculated based on Lipinski's rule of five and the data obtained is tabulated as follows.

**Table 3: Side effects of $CH_2CL_2$ solvent compounds from *B. pinnatum***

| Sr. No. | Compound Name | Sub-Compound Name | Pa | Pi | Side effects |
|---|---|---|---|---|---|
| 1 | (17a)-3-methoxyestra-1,3,5(10)-triene14,17-diyl TMS2 ether | 2,2',3-trihydroxydiphenyl ether (2−) | — | — | — |
| 2 | 3-Hydroxyanthranilic acid, TMS3 | 3-hydroxyanthranilic acid | 0.558 | 0.034 | Nephrotoxicity |
| | | | 0.513 | 0.188 | Hepatotoxicity |
| | | | 0.324 | 0.154 | Myocardial infection |
| | | | 0.275 | 0.198 | Cardiac failure |
| | | 2,3-dihydro-3-oxoanthranilic acid | 0.44 | 0.069 | Nephrotoxicity |
| | | | 0.391 | 0.266 | Hepatotoxicity |
| | | | 0.277 | 0.196 | Cardiac failure |
| | | N-carbamoylaspartic acid | 0.271 | 0.188 | Nephrotoxicity |
| | | 3,5-dihydroxybenzoic acid | 0.413 | 0.249 | Hepatotoxicity |
| | | | 0.307 | 0.15 | Nephrotoxicity |
| 3 | 9-Octadecenyl TMS ether | Gibberellin A34 methyl ester | — | — | — |
| 4 | Butane-2,3-diol TMS2 ether | Meso-butane-2,3-diol | 0.592 | 0.028 | Nephrotoxicity |
| | | | 0.591 | 0.023 | Cardiac Failure |
| | | | 0.288 | 0.23 | Myocardial infraction |
| | | Butane-2,3-diol | 0.592 | 0.028 | Nephrotoxicity |
| | | | 0.591 | 0.023 | Cardiac failure |
| | | | 0.288 | 0.23 | Myocardial infraction |
| 5 | Glycerol TMS3 | Glycerol 2-phosphate | 0.404 | 0.084 | Nephrotoxicity |

The side effects of the compounds are predicted using the online tool http://www.way2drug.com/adverpred.

**Table 4: Side effects of $CH_3OH$ solvent compounds from *B. pinnatum***

| Sr. No. | Compound Name | Sub-Compound Name | Pa | Pi | Side effects |
|---|---|---|---|---|---|
| 1 | 1, 2 Propanediol TMS2 ether | (R)-3-chloro-1,2-propanediol | 0.666 | 0.009 | Cardiac failure |
| | | | 0.501 | 0.05 | Nephrotoxicity |
| | | 3, 4-didehydro-N4-deethylbrinzolamide | 0.876 | 0.004 | Cardiac failure |
| | | | 0.355 | 0.227 | Arrhythmia |
| | | | 0.328 | 0.148 | Myocardial infarction |
| 2 | 2, 3 Butanediol TMS2 ether | Trimagnesium dicitrate | — | — | — |
| | | 2'-deamino-2'-hydroxyneamine (3+) | — | — | — |
| 3 | 2-Keto-d-gluconic acid TMS5 | (Z)-icos-13-enoic acid | 0.693 | 0.013 | Myocardial infraction |
| | | | 0.361 | 0.29 | Hepatotoxicity |

The side effects of the compounds are predicted using the online tool http://www.way2drug.com/adverpred/.

**Table 5: The $CH_2CL_2$ solvent compounds 2D and 3D interactions of ligand with their targets obtained from plant *B. pinnatum***

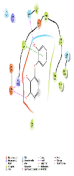
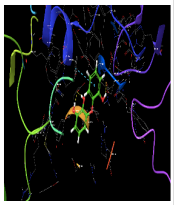
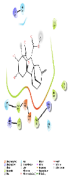
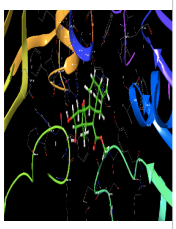
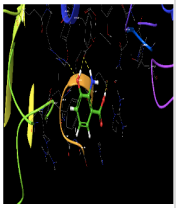
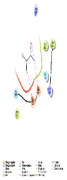
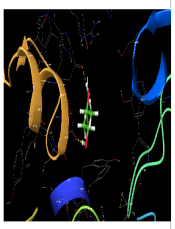
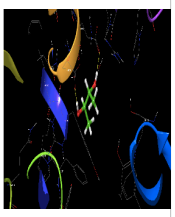
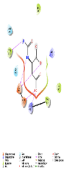
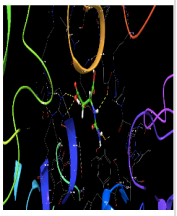
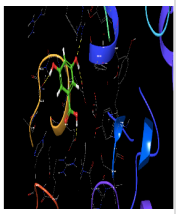
| Compound Number | Sub-Compound name and Structure | 2D Interaction of ligand with target | 3D Interaction of ligand with target | Compound Number | Sub-Compound name and Structure | 2D Interaction of ligand with target | 3D Interaction of ligand with target |
|---|---|---|---|---|---|---|---|
| C1 | 2,2',3-trihydroxydiphenyl ether (2−) |  |  | C3 | Gibberellin A34 methyl ester |  |  |
| C2 | 3-hydroxyanthranilic acid |  |  | C4 | Meso-butane-2,3-diol |  |  |
| | 2,3-dihydro-3-oxoanthranilic acid |  |  | | Butane-2,3-diol |  |  |
| | N-carbamoylaspartic acid |  |  | C5 | Glycerol 2-phosphate |  |  |
| | 3,5-dihydroxybenzoic acid |  |  | The retrieved PubChem data is converted to 2D structure and 3D structure by using Discovery Studio 4.5 software (Accelrys Software Inc., San Diego, CA, USA) and the data was tabulated. | | | |

**Table 6: The $CH_3OH$ solvent compounds 2D and 3D interactions of ligand with their targets obtained from plant *B. pinnatum***

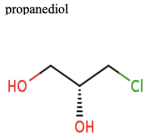
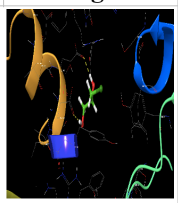
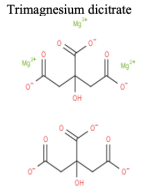
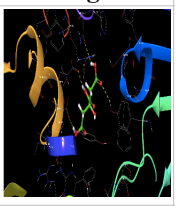
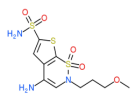
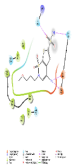
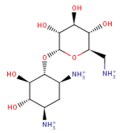
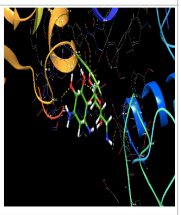
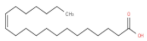
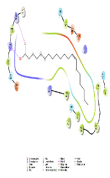
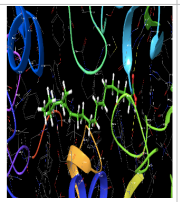
| Compound Number | Sub-Compound name and Structure | 2D Interaction of ligand with target | 3D Interaction of ligand with target | Compound Number | Sub-Compound name and Structure | 2D Interaction of ligand with target | 3D Interaction of ligand with target |
|---|---|---|---|---|---|---|---|
| C1 | (R)-3-chloro-1,2-propanediol  |  |  | C2 | Trimagnesium dicitrate  |  |  |
| | 3, 4-didehydro-N4-deethylbrinzolamide  |  |  | | 2'-deamino-2'-hydroxyneamine (3+)  |  |  |
| C3 | (Z)-icos-13-enoic acid  |  |  | The retrieved PubChem data is converted to 2D structure and 3D structure by using Discovery Studio 4.5 software (Accelrys Software Inc., San Diego, CA, USA) and the data was tabulated. | | | |

**Table 7: Molecular docking score and binding interaction of $CH_2CL_2$ compounds from *B. pinnatum***

| Sr. No. | Compound name | Sub-Compound name | Docking Score (kcal/Mol) | NHB | Amino acid residue |
|---|---|---|---|---|---|
| 1 | (17a)-3-methoxyestra-1,3,5(10)-triene14,17-diyl TMS2 ether | 2,2',3-trihydroxydiphenyl ether (2−) | -5.258 | 4 | Tyr:666<br>Glu:206<br>Asn:710<br>Ser:630 |
| 2 | 3-Hydroxyanthranilic acid, TMS3 | 3-hydroxyanthranilic acid | -5.427 | 4 | Arg:669<br>Glu:206<br>Glu:205<br>Asn:710 |
| | | 2,3-dihydro-3-oxoanthranilic acid | -4.035 | 2 | Glu:206<br>Glu:205 |
| | | N-carbamoylaspartic acid | -5.222 | 4 | Tyr:662<br>Glu:206<br>Glu:205<br>Arg:669 |
| | | 3,5-dihydroxybenzoic acid | -5.623 | 4 | Glu:206<br>Glu:205<br>Arg:669<br>Ser:209 |
| 3 | 9-Octadecenyl TMS ether | Gibberellin A34 methyl ester | -3.404 | 3 | Arg:125<br>Glu:206<br>Gly:549 |
| 4 | Butane-2,3-diol TMS2 ether | Meso-butane-2,3-diol | -2.941 | 2 | Glu:206<br>Glu:205 |
| | | Butane-2,3-diol | -3.132 | 2 | Glu:206<br>Glu:205 |
| 5 | Glycerol TMS3 | Glycerol 2-phosphate | -4.857 | 4 | Arg:669<br>Glu:206<br>Glu:205<br>Ser:209 |

The therapeutic protein targets of DPP-4 were identified using the computational program GLIDE docking module of Schrodinger Suite software.

**Table 8: Molecular docking score and binding interaction of $CH_3OH$ compounds from *B. pinnatum***

| Sr. No | Compound Name | Sub- Compound name | Docking Score (kcal/Mol) | NHB | Amino acid Residue |
|---|---|---|---|---|---|
| 1 | 1, 2 Propanediol TMS2 ether | (R)-3-chloro-1,2-propanediol | -3.538 | 2 | Glu:205<br>Glu:206 |
| | | 3, 4-didehydro-N4-deethylbrinzolamide | -4.91 | 4 | Glu:205<br>Glu:206<br>Ser:209<br>Hie:126 |
| 2 | 2, 3 Butanediol TMS2 ether | Trimagnesium dicitrate | -3.421 | 4 | Glu:205<br>Glu:206<br>Tyr:547<br>Asn:710 |
| | | 2'-deamino-2'-hydroxyneamine (3+) | -4.719 | 3 | Glu:205<br>Glu:206<br>Ser:209 |
| 3 | 2-Keto-d-gluconic acid TMS5 | (Z)-icos-13-enoic acid | -3.091 | 2 | Arg:356<br>Arg:358 |

The therapeutic protein targets of DPP-4 were identified using the computational program GLIDE docking module of Schrodinger Suite software.

**Table No. 9: Glide energy calculation of $CH_2CL_2$ solvent of *B. pinnatum***

| Sr. No. | Compound Name | Sub- Compound Name | Glide G-score | Glide E-model | Glide energy |
|---|---|---|---|---|---|
| 1 | (17a)-3-methoxyestra-1,3,5(10)-triene14,17-diyl TMS2 ether | 2,2',3-trihydroxydiphenyl ether (2−) | -5.623 | -37.638 | -28.013 |
| 2 | 3-Hydroxyanthranilic acid, TMS3 | 3-hydroxyanthranilic acid | -5.427 | -37.641 | -28.639 |
| | | 2,3-dihydro-3-oxoanthranilic acid | -5.258 | -44.224 | -34.019 |
| | | N-carbamoylaspartic acid | -5.222 | -43.59 | -35.164 |
| | | 3,5-dihydroxybenzoic acid | -4.857 | -41.479 | -31.99 |
| 3 | 9-Octadecenyl TMS ether | Gibberellin A34 methyl ester | -4.035 | -32.51 | -25.222 |
| 4 | Butane-2,3-diol TMS2 ether | Meso-butane-2,3-diol | -3.404 | -42.035 | -34.528 |
| | | Butane-2,3-diol | -3.132 | -22.54 | -18.306 |
| 5 | Glycerol TMS3 | Glycerol 2-phosphate | -2.941 | -22.689 | -18.671 |

The therapeutic protein targets of DPP-4 were identified using the computational program GLIDE docking module of Schrodinger Suite software.

**Table 10: Glide energy calculation of $CH_3OH$ solvent of *B. pinnatum***

| Sr. No. | Compound Name | Sub-Compound Name | Glide G-score | Glide E-model | Glide energy |
|---|---|---|---|---|---|
| 1 | 1, 2 Propanediol TMS2 ether | (R)-3-chloro-1,2-propanediol | -4.91 | -49.105 | -39.472 |
| | | 3, 4-didehydro-N4-deethylbrinzolamide | -4.719 | -44.281 | -34.985 |
| 2 | 2, 3 Butanediol TMS2 ether | Trimagnesium dicitrate | -3.538 | -24.886 | -19.145 |
| | | 2'-deamino-2'-hydroxyneamine (3+) | -3.421 | -39.812 | -34.283 |
| 3 | 2-Keto-d-gluconic acid TMS5 | (Z)-icos-13-enoic acid | -3.091 | -43.072 | -39.84 |

The therapeutic protein targets of DPP-4 were identified using the computational program GLIDE docking module of Schrodinger Suite software.

## Discussion

Diabetes mellitus (DM) is a metabolic disorder characterized by long-term high blood glucose levels (1). Over time, DM can severely damage the heart, kidneys, and nervous system. The target chosen for this research is DPP-4. The DPP-4 is also called serine protease dipeptidyl peptidase-4. Its mechanism shows a target of interest for the anti-diabetic action because inhibition of DPP-4 has been shown to be an effective treatment for type-2 DM (5). The currently available DPP-4 drugs in the market namely Vildagliptin, Saxagliptin, and Sitagliptin have the same side effects as headache, dizziness, nausea, feeling weak, weight gain, low blood sugar levels and swelling of the legs and ankles (20). As an alternative, plants have always been a useful source of alternative therapy and a useful source in drug development. The ethnobotanical evidence reports about eight hundred plants that may hold anti-diabetic potential (6). One of the well-documented plants

related to diabetes treatment is *B. pinnatum*. It is used as a medicinal plant in drug development. Current pharmacological studies have established the traditional use of *B. pinnatum* and its extracts in illnesses: fungal, viral, inflammations, ulcers and microbial infections, a reduced immune system, diabetes mellitus, and insecticidal properties (21).

The potential anti-diabetic active components of *B. pinnatum,* were estimated by using the computer-assisted virtual screening of phytochemicals from two solvent extracts ($CH_2CL_2$ and $CH_3OH$) of *B. pinnatum* based on the structure of DPP-4 inhibitors. Significantly, the molecular docking/computer-assisted screening technique was used to estimate the method of target-ligand and docking score of the compounds with the DPP-4 receptor (5Y7K), which gives the essential base for structure optimisation and drug development.

From the study, the first solvent $CH_2CL_2$ extract revealed the highest docking score of -5.623 kcal/mol for 3, 5-dihydroxybenzoic acid and Meso-butane-2, 3-diol had the lowest docking score of -2.941 kcal/mol. The second solvent $CH_3OH$ extract revealed a maximum docking score of -4.91 kcal/mol for 3, 4-didehydro-N4-deethylbrinzolamide and (Z)-icos-13-enoic acid had a minimum docking score of -3.091 kcal/mol.

In $CH_2CL_2$ compounds: 2, 2', 3-trihydroxydiphenyl ether (2−) and Gibberellin A34 methyl ester revealed no side effects, whereas 3-hydroxyanthranilic acid showed high side effects. In $CH_3OH$: Trimagnesium dicitrate and 2'-deamino-2'-hydroxyneamine (3+) compounds have shown no side effects, whereas 3, 4-didehydro-N4-deethylbrinzolamide revealed maximum side effects.

Consequently, *in-silico* molecular docking studies suggested that *B. pinnatum* targeted the DPP-4 and binds effectively and shows a potential for the management of Type-2 DM (11). However, to strengthen this claim it would be necessary to conduct *in-vitro* experiments using the potential phytoconstituents followed by *in-vivo* studies using diabetic mouse models.

## Conclusion

In conclusion, our results strongly favour the beneficial use of phytoconstituents from *B. pinnatum.* The *in-silico* data suggests that it may be used along with the standard drugs; thereby reducing the side effects and improving the life of the DM patients. Hence, may serve as a potential drug candidate for use as an alternative treatment or adjuvant therapy for Type-2 DM.

### Abbreviations

| | |
|---|---|
| DM | Diabetes Mellitus |
| ADMET | Absorption, Distribution, Metabolism, Excretion and Toxicity |
| ChEBI | Chemical Entities of Biological Interest |
| PDB | Protein Data Bank |
| RCSB | Research Collaboratory for Structural Bioinformatics |
| SMILES | Simplified Molecular- Input Line-Entry System |
| NHBA | Number of Hydrogen Bond Acceptor |
| NHBD | Number of Hydrogen Bond Donor |
| DLS | Drug Likeness Score |
| WHO | World Health Organization |
| pH | Potential of Hydrogen |
| % | Percentage |

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