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Study of drug-like characteristics of bioactive compounds identified from *Dicranella pseudosubulata* Müll. Hal. ex Gangulee

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Dicranella pseudosubulata Müll Hal. ex Gangulee is a moss species and a member of the Dicranaceae family, which is known to harbour many secondary metabolites of therapeutic importance. A gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic extract of *D. pseudosubulata* was obtained to determine the metabolites of the moss. The chromatogram revealed 48 compounds, of which four compounds showed the highest concentrations, constituting 73.86% of all the bioactive chemicals detected. The compound d-Glucitol, 1-O-heptyl- showed the highest area percentage of 45.70 per cent. Other compounds with high concentrations are 9,12-Octadecadienoic acid (Z,Z)-, TMS derivative (13.35%); Palmitic acid, TMS derivative (9.46%) and Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester (5.35%). These metabolites were then examined for their drug-like characteristics using SwissADME and predicted for potential target proteins using SwissTargetPrediction tools. Molecular docking analyses with the most probable targets of d-Glucitol, 1-O-heptyl-;9,12-octadecadienoic acid; Palmitic acid, TMS derivative and Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester with Beta-glucocerebrosidase (GBA), Peroxisome proliferator-activated receptor gamma (PPARG), fatty acid binding protein (FABP4) and Thymidylate synthase (TYMS), respectively, showed thermodynamically favourable bindings. The target proteins have been reported to be associated with various ailments and their inhibition by these compounds is necessary to control and maintain cell growth in several cancers as well as in treating malaria and leishmania, obesity, lipidosis, kidney necrosis, liver carcinoma, insulin resistance etc. Some of the compounds have been reported to exhibit antimicrobial, antioxidant, and hepatoprotective activities. Thus, the finding suggests that *D. pseudosubulata* could serve as a potential therapeutic drug against various diseases.

Keywords: Bioactive compounds, *Dicranella pseudosubulata*, GC-MS analysis, Molecular docking, Moss

IPC code; Int. cl. (2021.01)- A61K 36/00, A61K 36/10

Introduction

Dicranella pseudosubulata Müll. Hal. ex Gangulee is a moss species, belonging to the family Dicranaceae. The family Dicranaceae with about 74 genera and 2, 675 species is one of the largest families of the phylum Bryopsida[1]. Moss species have been known to possess numerous metabolites which can be used to treat various diseases[2,3]. Likewise, members of the family Dicranaceae have shown the presence of metabolites as reported in past studies[4,5]. For instance, Kohn *et al.* showed the presence of 9,12,15-Octadecatrien-6-ynoic acid, an inhibitor of platelet aggregation[6] and 9,12-octadecadien-6-ynoic acid in *D. heteromalla* (Hedw.) Brid., *D. jamesonii* (Mitt.) Broth., *D. palustris* (Dick.) Crundw. ex Warb. and *D. schreberiana* (Hedw.) Dix. Similarly, gas chromatography-mass spectrometry (GC-MS) analysis of *D. coarctata* revealed 66 biomolecules from three different extract solutions viz. n-hexane (5), acetic ethyl (38) and methanol extract (23)[5]. In the genus *Dicranella, D. heteromalla* has been widely investigated for tolerance of high tissue levels of cadmium (610 ppm), zinc (55,000 ppm) and copper (2,700 ppm)[7], and cytotoxicity against P388 leukemia cells[8]. Being a member of Dicranaceae, it is expected that *D. pseudosubulata* might contain many secondary metabolites of interest. However, there is no report or literature available on the metabolites or compounds of *D. pseudosubulata* as well as their therapeutic properties. Therefore, this paper presents a first-hand report on the presence of bioactive constituents in the moss species

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D. pseudosubulata, and predicts their drug-likeness which showed possible therapeutic potential against a wide range of diseases.

The plant is green to yellowish green, occasionally reddish, and grows in loose to thick tufts (Fig. 1). Rhizoids at the bases of branches or stems, erect, simple or forked stems. Leaves are short to long-lanceolate, rarely flat, concave to keeled, spreading or upright, falcate-secund or straight. Capsules are usually erect or inclined, typically ovoid, sometimes cylindric or oblong, occasionally straight, sub-globose or arcuate, smooth, without or with struma, frequently constricted below the mouth, plicate or furrowed when dry, oblique occasionally; operculum often arcuate, long-rostrate to conic; peristome single, teeth are 16, split approximately half their length into 2 divisions, vertically pitted Calyptra cucullate, smooth, covering around half of the capsule, and fugitive. Spores 10–25 µm in diameter, round and smooth to barely papillose[1].

Materials and Methods

Plant sample collection and identification

The specimen was collected from Banderdewa circle (at Latitude: 27.0952° N and Longitude: 93.8274° E) of Papum Pare district, Arunachal Pradesh, India in March 2022. The collected specimen was kept inside a ziplock bag and brought to the laboratory. The specimen was then identified from its morphological and anatomical features using standard literature[9]. The identity of the specimen was certified by Dr P L Uniyal, Senior Professor, Department of Botany, University of Delhi and submitted to Delhi University Herbarium with the voucher number DUH15006.

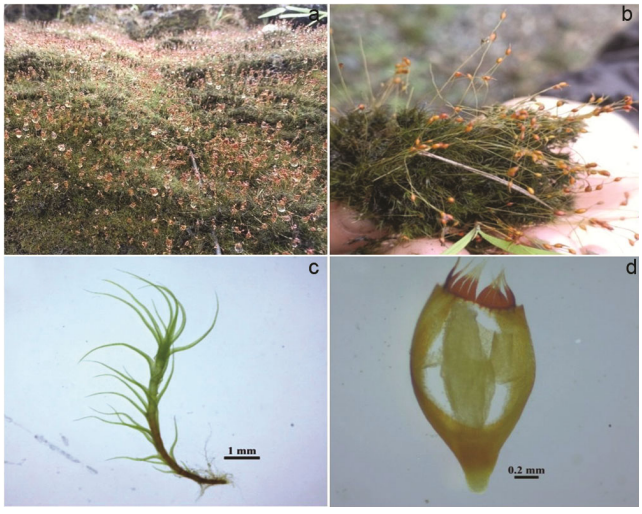

Fig. 1 — *Dicranella pseudosubulata*. a) Habitat photo; b) Plant body; c) Stereo zoom picture of *D. pseudosubulata* and d) V. S. of the sporophyte.

Preparation of extract

In the laboratory, the specimen was washed thoroughly under tap water[10], air dried under shade and pulverized using mortar and pestle. The cold percolation method was performed for the extraction of plant samples using methanol as the extraction solvent. Two grams of pulverized sample was soaked in methanol for 72 h with intermittent shaking and filtered using Whatman No. 1. The filtrate was then concentrated under room temperature for 72 h. It was then resuspended with methanol to get a 10 mg/mL concentration for GCMS analysis.

GC-MS analysis

GC-MS analysis was performed at USIC, AIRF, Jawaharlal Nehru University, New Delhi. The methanol extract of the plant sample (10 mg/mL) was injected into Shimadzu GC MS-QP-2010 plus system. The column used for the analysis was RTx-5Sil MS column (30 m × 0.25 mm id × 0.25 µM film thickness). Helium gas was employed as a carrier gas. The oven temperature program was 100°C with a hold time of 3 min and 280°C with a hold time of 19 min. The column oven temperature was 100°C and the injection temperature was 260°C at a pressure of 90.5 kPa. The column flow was 1.21 mL/min and linear velocity was recorded as 40.9 cm/sec. The total flow was 16.3 mL/min, purge flow 3.0 mL/min, split ratio 10.0, ion source temperature 220°C, interface Temperature 270°C and solvent cut time 3.50 min. The data was obtained as a chromatogram and the compounds were identified with the help of data available from the National Institute of Standards and Technology (NIST14, NIST14s, and Wiley8 libraries)[11].

Study of drug-like characteristics of the compounds

According to GC-MS data, four compounds namely d-Glucitol, 1-O-heptyl-; 9,12-Octadecadienoic acid (Z,Z)-, TMS derivative; Palmitic acid, TMS derivative and Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester were found in highest concentrations in *D. pseudosubulata* extract. These compounds were then selected for further research on their drug-like properties. The first step was to retrieve their chemical structures and canonical smiles formulae from PubChem[12]. The compounds were then examined to determine their physical and biological characteristics using SwissADME[13]. The analysis of the metabolites involved determining their physicochemical properties

viz, molecular weights, hydrogen bond donor atoms, hydrogen bond acceptor atoms, rotatable bonds and topological polar surface area (TPSA). Further, lipophilicity in terms of Log P and water solubility in terms of Log S for the metabolites were calculated. In addition, pharmacokinetics was examined through their gastro-intestinal (GI) absorption, blood-brain barrier (BBB) permeability, skin permeability (Log K_p), being P-glycoprotein (P-gp) substrate, and inhibitors of cytochrome P450 1A2 (CYP1A2), cytochrome P450 2C19 (CYP2C19), cytochrome P450 2C9 (CYP2C9), cytochrome P450 2D6 (CYP2D6) and cytochrome P450 3A4 (CYP3A4). Finally, the drug-likeness of the four metabolites was tested for any violations of the Lipinski filter (rule of five[14]), Ghose filter, Veber filter, Egan filter, Muegge filter and analyzing their bioavailability scores using the Abbott Bioavailability score.

Target predictions

Potential target proteins of the top four compounds were predicted using the Swiss Target Prediction programme[15] to establish their affinity towards the proteins that may predict their therapeutic potential. The target proteins with the highest probability and statistical significance were identified. The predicted targets belonged to enzymes, G-protein coupled receptors, oxidoreductases, phosphatases, nuclear receptors, proteases, kinases, etc.

Pathways and disease association studies of the predicted targets

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database[16] and DisGenNet[17] databases, were used to analyze pathways with GeneCodis4[18] and the disease associated with the 100 predicted potential targets of each of the four compounds, respectively. The pathways and diseases which showed statistically significant associations were plotted for their numbers of genes (proteins) associated against their *–log (p-value adjusted*).

Molecular docking analysis

To validate the findings on predicted potential targets of four compounds, molecular docking analyses of each compound with their highest probable predicted targets were conducted. For the analyses, the three-dimensional (3D) protein structures of the predicted targets were retrieved from a protein data bank i.e., Research Collaboratory for Structural Bioinformatics PDB (RCSB PDB) (https://www.rcsb.org/)[19] and the 3D conformer structures of the compounds were retrieved from PubChem[10]. The PDB IDs of the proteins used in the molecular dockings were 1HZW (Thymidylate synthase, TYMS), 2V3E (Beta-glucocerebrosidase, GBA), 5TWO (Peroxisome proliferator-activated receptor gamma, PPARG) and 1HMR (Fatty acid binding protein-adipocyte, FABP4)[20-23].

To determine the binding affinities between a receptor molecule and a ligand molecule[24], AutoDock Vina was used as the docking tool[25]. Proteins were prepared by removing water molecules and all the non-standard atoms/molecules, then adding polar hydrogen atoms and charges using the Docking preparation tool in Chimera[22]. Ligands were also prepared for docking and blind dockings were performed using Autodock Vina docking software[25] in Chimera[23]. After recording the binding affinities as Gibbs free energy (ΔG), thermodynamically favourable binds were subsequently examined. Using Discovery Studio Visualizer, the ligand binding with the proteins possessing the most thermodynamically advantageous poses was obtained as a 2D ligand-protein interaction diagram[26].

Results

Composition of *D. pseudosubulata*

The moss, *D. pseudosubulata*, contained 48 compounds, according to GC-MS analyses. Table 1 and Fig. 2 enlists the top ten compounds with the highest area percentage obtained from the plant. Out

Table 1 — Names, and area percentage of ten compounds with highest area percentage

S. No.	Compound	Area percentage
1	d-Glucitol, 1-O-heptyl-	45.70
2	9,12-Octadecadienoic acid (Z,Z)-, TMS derivative	13.35
3	Palmitic acid, TMS derivative	9.46
4	Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	5.35
5	9-octadecenoic acid (z)-, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester	2.23
6	Pyrrolo[1,2-a]pyrazine-1,4-dione, 3-[[2-(1,1-dimethyl-2-propenyl)-1H-indol-3-yl]methyl]-2,3,6,7-tetrahydro-, (S)-	1.18
7	DIETHYL PHTHALATE	1.57
8	OCTADECANOIC ACID, METHYL ESTER	1.53
9	TRANS-10-METHYLDECALONE-1	1.28
10	Neotigogenin acetate	1.17

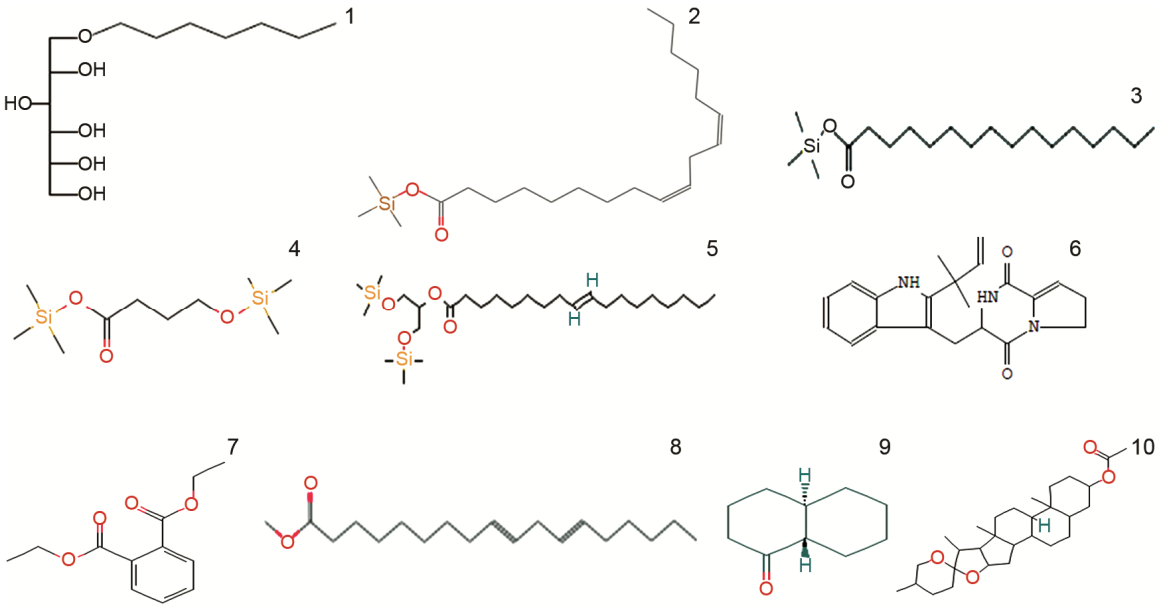

Fig. 2 — Structure of compounds with highest area percentage as given in Table 1.

of those, four compounds displayed the highest concentration, making up 73.86% of all the chemical constituents observed. Among them, d-Glucitol, 1-O-heptyl-exhibited the highest percentage area of 45.70%, which was followed by 9,12-Octadecadienoic acid (Z,Z)-, TMS derivative (13.35); Palmitic acid, TMS derivative (9.46) and Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester (5.35).

Characteristics of metabolites present in *D. pseudosubulata*

The four compounds viz. d-Glucitol, 1-O-heptyl-, 9,12-Octadecadienoic acid, Palmitic acid and Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester were found to obey Lipinsky's rule of five of a drug like physicochemical characteristics[14]. In the case of 9,12-Octadecadienoic acid and Palmitic acid, they showed one violation each since one of the derivatives of lipophilicity measurements, MLOGP>4.15 but other characteristics viz., molecular weights, no. of hydrogen bond acceptors, donors, lipophilicity and solubility were favourable (Table 2). All the four compounds showed high Gastrointestinal (GI) absorptions and synthetic accessibility apart from the other fundamental physicochemical properties (Table 2). Amongst these compounds, Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester, showed no violations of other rules viz. Ghose, Veber, Egan and Muegge rules along with Lipinski rule (Table 2). These characteristics of the metabolites expressed their potential drug-like properties.

Predicted targets and their association with different pathways and diseases

Prediction of potential targets of the four metabolites in SwissTargetPrediction[15], predicted 100 statistically significant target proteins, out of which, the first top 15% are presented in Table 3. For d-Glucitol, 1-O-heptyl, beta-glucocerebrosidase (GBA) was the potential target with the highest probability (Table 3). Peroxisome proliferator-activated receptor gamma (PPARG), Thymidylate synthase (TYMS) and Fatty acid binding protein adipocyte (FABP4) were the highest probable targets of 9,12-Octadecadienoic acid, Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester and Palmitic acid, respectively (Table 3). In addition, examination of the top 15% target genes (proteins) of d-Glucitol, 1-O-heptyl- revealed that 33% of its targets were enzymes, followed by 26.7% lyase which are also enzymes and the rest belonged to the family of G-protein-coupled receptor and electrochemical transporters (Fig. 3a). Top 15% targets of 9,12-Octadecadienoic acid and Palmitic acid were mainly fatty acid binding proteins and nuclear receptors, respectively (Fig. 3b-c). In the case of Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester, the majority of its highest probable 15% targets belong to kinase and family of G-protein-coupled receptors (Fig. 3d).

Pathway and disease association analyses of the highest probable 15% target proteins of the four metabolites also showed they were involved in important pathways and associated with different diseases. Enrichment analysis of the targets of

d-Glucitol, 1-O-heptyl-, showed that they were significantly involved in nitrogen metabolism, antibiotic synthesis, carbohydrate metabolisms and type II diabetes mellitus pathways etc. (Fig. 4a). For, Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester, the targets were shown to be involved in endocrine resistance,

Table 2 — Physicochemical/drug-likeness properties of the compounds (highest per cent content) identified from *D. pseudosubulata*

Molecule	d-Glucitol, 1-O-heptyl-	Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	9,12-octadecadienoic acid	n-Hexadecanoid acid (Palmitic acid)
Canonical SMILES	CCCCCCCOCC(C(C(C(CO)O)O)O)O	O = C(O[Si](C)(C)C)CCCO[Si] (C)(C)C	CCCCCC=CCC=CCCCCCCCC(=O)O	CCCCCCCCCCCCCCCCC(=O)O
Formula	C13H28O6	C10H24O3Si2	C18H32O2	C16H32O2
MW	280.36	248.47	280.45	256.42
Heavy atoms	19	15	20	18
Aromatic heavy atoms	0	0	0	0
Fraction Csp3	1	0.9	0.72	0.94
Rotatable bonds	12	7	14	14
H-bond acceptors	6	3	2	2
H-bond donors	5	0	1	1
MR	71.5	68.29	89.46	80.8
TPSA	110.38	35.53	37.3	37.3
iLOGP	3.59	3.76	4.14	3.85
XLOGP3	-0.2	3.11	6.98	7.17
WLOGP	-0.59	3	5.88	5.55
MLOGP	-0.62	1.7	4.47	4.19
Silicos-IT Log P	1.05	-0.4	5.77	5.25
Consensus Log P	0.65	2.23	5.45	5.2
ESOL Log S	-0.66	-2.88	-5.05	-5.02
ESOL Solubility (mg/ml)	6.13E+01	3.29E-01	2.49E-03	2.43E-03
ESOL Solubility (moL/l)	2.19E-01	1.32E-03	8.87E-06	9.49E-06
ESOL Class	Very soluble	Soluble	Moderately soluble	Moderately soluble
Ali Log S	-1.66	-3.52	-7.58	-7.77
Ali Solubility (mg/mL)	6.11E+00	7.42E-02	7.42E-06	4.31E-06
Ali Solubility (moL/l)	2.18E-02	2.99E-04	2.64E-08	1.68E-08
Ali class	Very soluble	Soluble	Poorly soluble	Poorly soluble
Silicos-IT Log Sw	-0.56	-3.06	-4.67	-5.31
Silicos-IT Solubility (mg/mL)	7.67E+01	2.16E-01	5.93E-03	1.25E-03
Silicos-IT Solubility (moL/l)	2.74E-01	8.69E-04	2.11E-05	4.88E-06
Silicos-IT class	Soluble	Soluble	Moderately soluble	Moderately soluble
GI absorption	High	High	High	High
BBB permeant	No	Yes	Yes	Yes
Pgp substrate	No	No	No	No
CYP1A2 inhibitor	No	No	Yes	Yes
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	No	No	Yes	Yes
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
log Kp (cm/s)	-8.15	-5.61	-3.05	-2.77
Lipinski violations	0	0	1	1
Ghose violations	1	0	1	0
Veber violations	1	0	1	1
Egan violations	0	0	1	0
Muegge violations	0	0	1	1
Bioavailability score	0.55	0.55	0.85	0.85
PAINS alerts	0	0	0	0
Brenk alerts	0	1	1	0
Leadlikeness violations	1	1	2	2
Synthetic accessibility	4.45	3.98	3.1	2.31

Table 3 — The highest probable targets of the four compounds of *D. pseudosubulata*

S. No.	d-Glucitol, 1-O-heptyl-	Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	9,12-octadecadienoic acid	Palmitic acid
1	GBA	TYMS	PPARG	FABP4
2	CA1	VDR	PPARA	PPARA
3	CA12	EGFR	PPARD	FABP3
4	CA9	MAPK1	FFAR1	FABP5
5	SLC5A2	LTA4H	FABP4	PPARD
6	ADK	LRRK2	FABP3	FABP2
7	CA2	MDM2	PTGS1	FFAR1
8	SLC5A1	TRPA1	SCD	SLC22A6
9	ADORA1	KCNH2	FABP5	CDC25A
10	SLC29A1	PDE9A	FAAH	HSD11B1
11	ADORA2A	NPY5R	TERT	AKR1B10
12	ADORA3	MAPK8	FABP1	POLB
13	OGA	P2RX3	CNR1	VDR
14	HK2	TAAR1	ALOX5	NR1H4
15	HK1	KIF11	PTPN1	PHF8

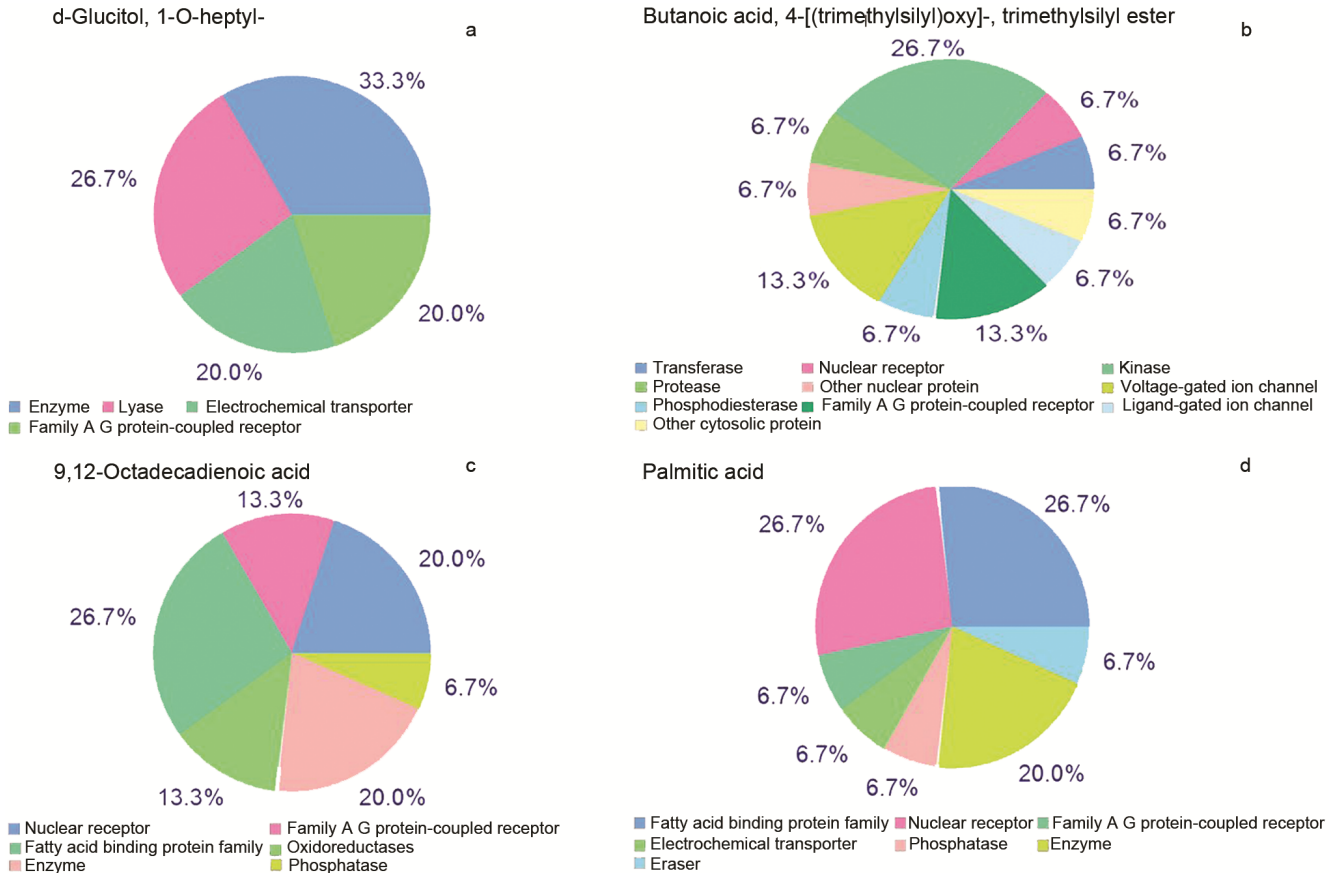

Fig. 3 — Class of the top 15% predicted targets of the four metabolites.

bladder cancer, glioma, pancreatic cancer, ErbB and FoxO signaling pathways etc. (Fig. 4b). Peroxisome proliferator-activated receptors (PPAR) signaling, insulin resistance, lipid metabolism, AMP-activated protein kinase (AMPK) signaling pathways etc. were enriched among the targets of 9,12-Octadecadienoic acid (Fig. 4c). PPAR signaling, chemical carcinogenesis, carbohydrate and lipid metabolisms etc. were highly enriched among the targets of Palmitic acid (Fig. 4d). In addition, disease association analysis of the targets of d-Glucitol, 1-O-heptyl- showed significant association with ventricular dysfunction,

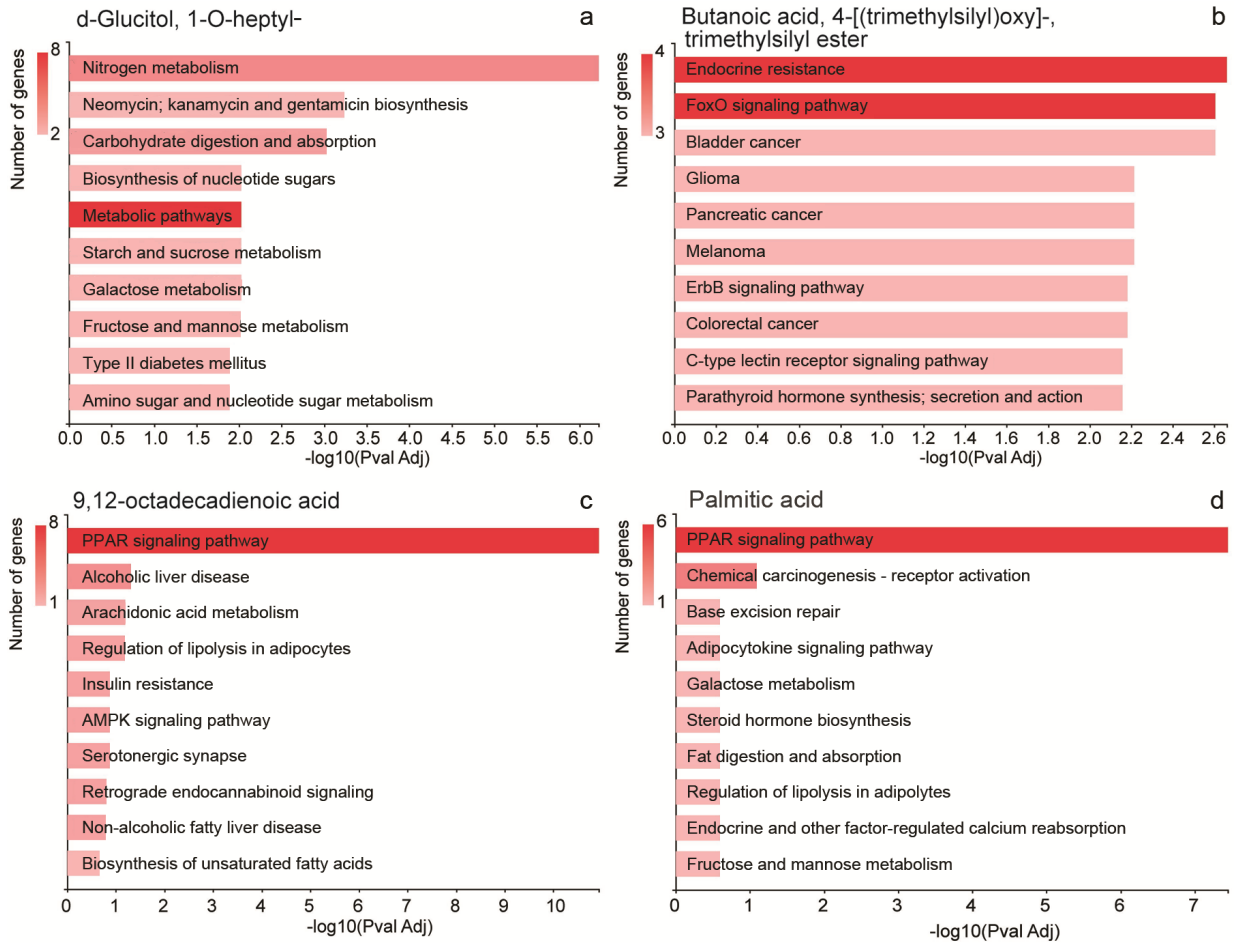

Fig. 4 — Enriched KEGG pathways of the top 15 predicted targets of the four metabolites.

hemolytic anemia, Gaucher disease, neuropathy etc. (Fig. 5a). Targets of Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester were associated with allodynia, hyperalgesia, cancers of head and neck, etc. (Fig. 5b). Reperfusion injury, obesity, lipidosis, kidney necrosis, liver carcinoma, insulin resistance etc., were significantly associated to targets of 9,12-Octadecadienoic acid (Fig. 5c). The targets of Palmitic acid were also significantly associated with neoplasms, fatty liver diseases, steatohepatitis, myopathy, Crohn's disease, cholestasis etc. (Fig. 5d). Thus, these metabolites have potentials to be investigated for their characteristic properties which could be further developed into drugs targeting to the above important pathways and diseases.

Thermodynamically stable interactions between the metabolites and their target proteins

The target prediction was observed to be endorsed by molecular docking interactions between the four compounds and their respective highest probable targets, which showed the interactions in the protein-ligand adducts that were thermodynamically stable (Table 4). The binding affinity between the proteins and the ligands were facilitated by forming hydrogen bonds, alkyl and pi-alkyl bonds and Van der Waal's interactions (Fig. 6). Between d-Glucitol, 1-O-heptyl- and Beta-glucocerebrosidase (GBA), five conventional hydrogen bonds were formed with Glutamine 362, Lysine 346, Tyrosine 313 and Cystine 342 (Fig. 6a). One hydrogen bond was formed between Asparagine 226 of Thymidylate synthase (TYMS) and Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester (Fig. 6b). 9,12-octadecadienoic acid and Peroxisome proliferator-activated receptor gamma (PPARG) formed two hydrogen bonds with Glycine 284 and Serine 342 (Fig. 6c). Three hydrogen bonds formed between Glutamine 95 and Arginine 78 of Fatty acid binding protein-adipocyte (FABP4) protein and Palmitic acid were also observed

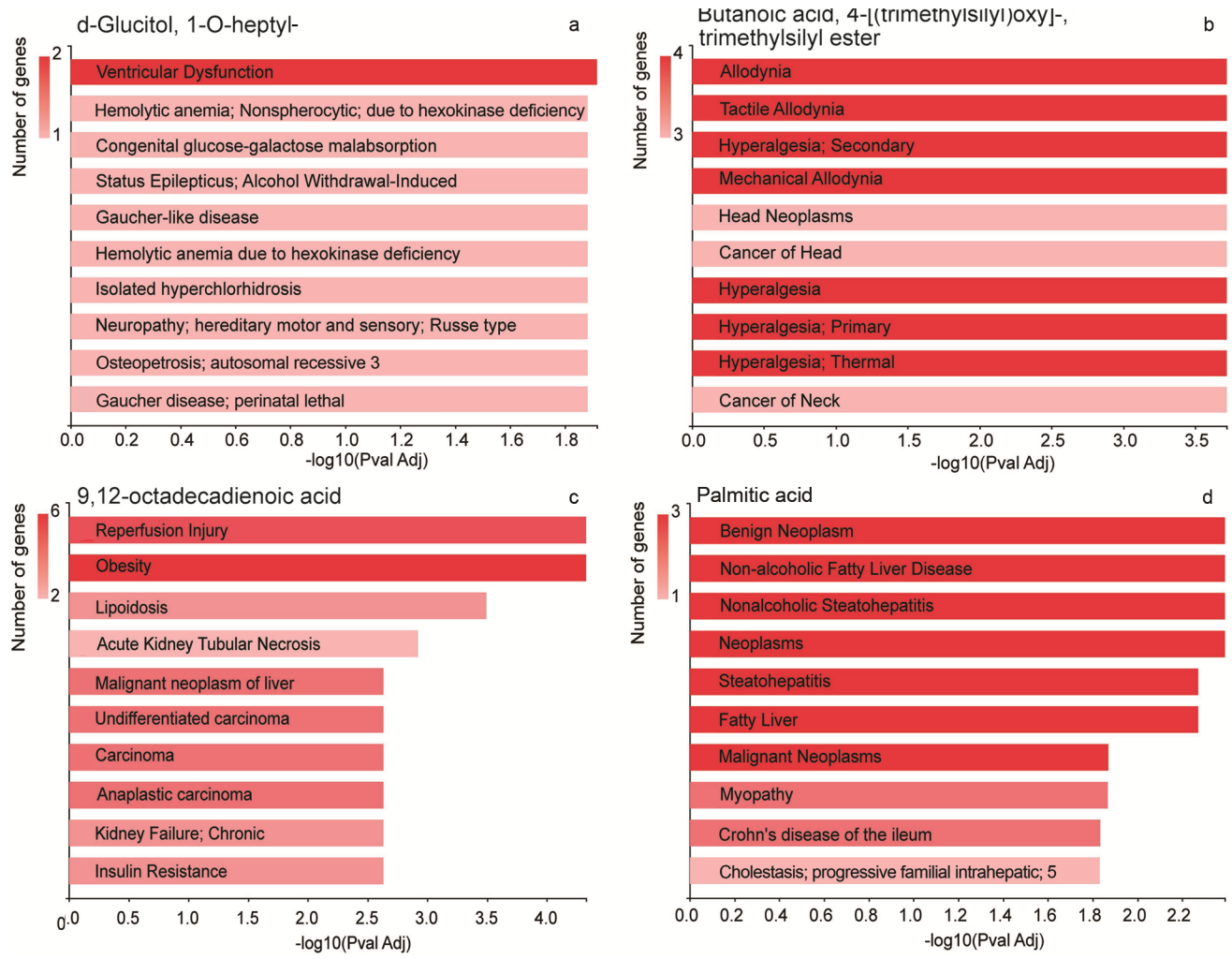

Fig. 5 — Diseases associated with the top 15 predicted targets of the four metabolites.

Table 4 — Molecular docking binding affinities (ΔG) of the four compounds of *D. pseudosubulata* with their respective highest probable targets

Sl. No.	Molecule	Target protein	Binding affinity(ΔG) kcal/mol
1	9,12-octadecadienoic acid	PPARG	-6.3
2	n-Hexadecanoid acid (Palmitic acid)	FABP4	-5.8
3	d-Glucitol, 1-O-heptyl-	GBA	-5.3
4	Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	TYMS	-4.4

(Fig. 6d). Formation of hydrogen bonds and other bonds especially Van der Waal's interactions between these adducts exhibited their stable complexes.

Discussion

The study reports numerous metabolites of therapeutic potential in *D. pseudosubulata* for the first time. Earlier, bioactive compounds in the members of the family Dicranaceae have been reported[4,5,8]. The study revealed four compounds namely d-Glucitol, 1-O-heptyl; 9,12-octadecadienoic acid; Palmitic acid and Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester as the major components of the plant extracts. Physicochemical analysis of these metabolites revealed that they exhibited drug-like characteristics abiding Lipinski's rule as well as other drug-likeness rules. In addition, they displayed affinities for various proteins and predictions of their potential key target proteins revealed their involvement in several pathways and conditions. Among the four major compounds detected, d-Glucitol, 1-O-heptyl- was found to be the highest in the composition. Target prediction analyses

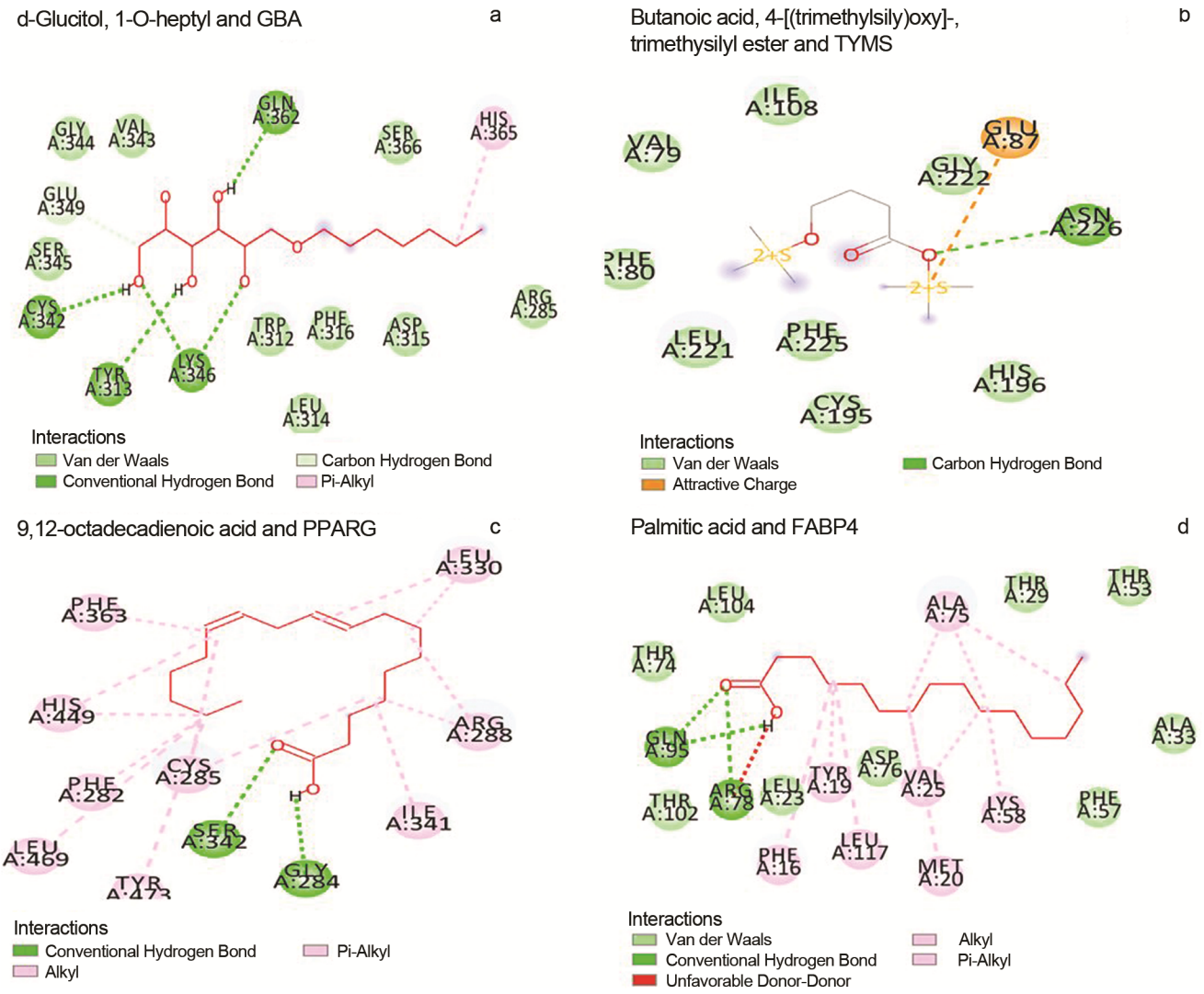

Fig. 6 — Interactions between the four metabolites with their respective targets.

showed that the most probable target of d-Glucitol, 1-O-heptyl- was found to be Beta-glucocerebrosidase (GBA), an enzyme which is involved in glycolipid metabolism and associated with Parkinson's disease and Gaucher's disease[27,28]. Carbonic anhydrase I (CA1), carbonic anhydrase XII (CA12), carbonic anhydrase IX (CA9) and sodium-glucose cotransporter 2 (SLC5A2) were the other highest probable targets of d-Glucitol, 1-O-heptyl- (Table 3). Carbonic anhydrase enzymes catalyze the interconversion of CO_2 and O_2, regulate pH and maintain acid-base and fluid balance in the tissues[29]. Many inhibitor drugs of carbonic anhydrases are used in treating different diseases e.g., gastric ulcers, hypertension, Glaucoma etc[30]. Thus, d-Glucitol, 1-O-heptyl- and its derivatives could serve as potential small molecule drugs against various diseases.

The second most concentrated compound, 9,12-octadecadienoic acid has been reported for numerous pharmacological potencies including antibacterial, anti-inflammatory, anti-fungal, antieczemic, antioxidant and hepatoprotective[31,32]. Target prediction showed that the most likely target protein for 9,12-octadecadienoic acid is Peroxisome proliferator-activated receptor gamma (PPARG), a transcription factor belonging to the nuclear receptor family and involved in glucose metabolism[33]. Thus, its activation by agonists was reported to increase glucose absorption in skeletal muscles under hyperglycemia conditions in diabetes[34]. Peroxisome proliferator-activated receptor alpha (PPARA), peroxisome proliferator-activated receptor delta PPARD, free fatty acid receptor 1 (FFAR1) and fatty acid binding protein adipocyte (FABP4) were other

highest probable targets of 9,12-octadecadienoic acid (Table 3). Peroxisome proliferator-activated receptor family proteins are involved in energy homeostasis by reducing triglyceride levels, inducing insulin sensitivity, enhancing glucose and fatty acid metabolisms etc[35]. Thus, their deregulated expressions have been found to be associated with different diseases viz., diabetes, inflammation, cancers, dyslipidemia, obesity, etc[35]. So, 9,12-octadecadienoic acid and its derivatives could be studied for their therapeutic potential against such diseases.

Palmitic acid was the third-highest compound observed in the plant extract. A high presence of the acid was also reported from *Dioscorea alata* L. (family Dioscoreaceae), a popular food and dietary supplement and is employed in Indian and Chinese ethnopharmacological methods to treat a variety of illnesses[31]. From this instance, it could be established that *D. pseudosubulata* could also serve as a therapeutic plant as it also contains a favourable amount of palmitic acid. In addition, fatty acid binding protein (FABP4) which is also linked to familial partial lipodystrophy and liposarcoma of the bone[36-38] was found to be the highest probable target of Palmitic acid. Thymidylate synthase (TYMS) was found to be the most potential target of Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester. TYMS is an important enzyme involved in DNA biosynthesis and its inhibition is an important strategy in maintaining cell growth in several cancers, malaria, and leishmania. Thus, these illnesses could be controlled by targeting and disrupting the function of TYMS[39,40].

The other highest probable targets of Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester were Vitamin D receptor (VDR), Epidermal growth factor receptor erbB1(EGFR), MAP kinase ERK2 (MAPK1) and Leukotriene A4 hydrolase (LTA4H) (Table 3). VDR mediates the function of vitamin D which maintains body calcium and phosphate balance in the body, thereby involving bone and teeth formation[41]. Several polymorphisms in VDR have been reported to be associated with various diseases including in diabetes, cancer, and cardiovascular diseases[41]. Epidermal growth factor receptor is a tyrosine kinase receptor and is involved in different cell proliferation pro-oncogenic signaling pathways[42].

Dysregulation of these pathways due to the aberration of the receptor led to several cancers viz., gastric, colorectal, breast cancer etc[42]. MAPK1 is a signaling protein in MAPK1/ERK2 pathway which is a very common transduction pathway involved in cell proliferation, inflammation, apoptosis etc[43]. Its dysregulation has been reported in different diseases especially several cancers viz., breast, gastric cancers etc., and inhibition of MAPK1 has been reported to suppress gastric cancer progression[43]. Leukotriene A4 hydrolase (LTA4H) is also involved in inflammation and cancer manifestations, and its inhibition has been recently studied to treat inflammatory response which supports cancer progressions[44]. Therefore, these metabolites could have potential medicinal benefits against different diseases, especially to use as therapeutic drugs against several cancers.

Conclusion

D. pseudosubulata contains numerous bioactive constituents. Four major compounds have been demonstrated for their drug-like properties. The molecular docking analyses of these compounds validated their affinities towards their respective target proteins. Therefore, it is suggestible to carry out further *in vitro* experimentation on these compounds which will provide a clear insight into their activities on the action of their potential targets.

Conflict of interest

The authors declared that there is no conflict of interest.

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References

1 World Flora Online Plant List, https://wfoplantlist.org/plant-list/taxon/wfo-9949999996-2022-12?page=1, 2022.
2 Drobnik J and Stebel A, Four centuries of medicinal mosses and liverworts in European ethnopharmacy and scientific pharmacy: A review, *Plants*, 2021, **10**(7), 1296.
3 Bandyopadhyay A and Dey A, The ethno-medicinal and pharmaceutical attributes of Bryophytes: A review, *Phytomed Plus*, 2022, **11**, 100255.
4 Kohn G, Demmerle S, Vandekerkhove O, Hartmann E and Beutelmann P, Distribution and chemotaxonomic significance of acetylenic fatty acids in mosses of the Dicranales, *Phytochem*, 1987, **26**(8), 2271-2275.
5 Junairiah J, Nurhariyati T and Sulistyorini L, Isolation of bioactive compounds from dicranaceae mosses, *J Kimia Riset*, 2016, **1**(2), 111-121.

6 Guichardant M, Michel M, Borel C, Fay L, Magnolato D, *et al.*, Effects of 9,12,15-octadecatrien-6-ynoic acid on the metabolism of arachidonic acid in platelets and on platelet aggregation, *Thromb Res*, 1992, **65**, 687–698.

7 Nash E H, *Effect of effluents from a zinc smelter on mosses*. PhD thesis, Rutgers-The State University, New Brunswick, 1972.

8 Spjut R W, Suffness M, Cragg G M and Norris D H, Mosses, liverworts, and hornworts screening for antitumor agents, *Econ Bot*, 1986, **40**, 310–338.

9 Gangulee H C, Mosses of Eastern India and adjacent regions: A Monograph, (Fascicle 1-8. Calcutta, India), 1969-1980.

10 Mathew M and Mathew A, GC MS analysis of petroleum ether extract of *Pterobryopsispilifolia* (dixon) magil to identify chemicals that offer antifeedant properties in bryophytes, *Poll Res*, 2022, **41**(2), 668-675. doi:10.53550/PR.2022.v41i02.040.

11 Mastrangelo A, Ferrarini A, Rey-Stolle F, Garcia A and Barbas C, From sample treatment to biomarker discovery: A tutorial for untargeted metabolomics based on GC-(EI)-Q-MS, *Anal Chim Acta*, 2015, **900**, 21-35.

12 Kim S, Chen J, Cheng T, Gindulyte A, He J, *et al.*, PubChem in 2021: New data content and improved web interfaces, *Nucleic Acids Res*, 2021, **49**(D1), D1388–D1395.

13 Daina A, Michielin O and Zoete V, SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, *Sci Rep*, 2017, **7**(1). doi:10.1038/srep42717.

14 Kim S, Chen J, Cheng T, Gindulyte A, He J, *et al.*, PubChem in 2021: New data content and improved web interfaces, *Nucleic Acids Res*, 2021, **49**(D1), D1388–D1395. doi:10.1093/nar/gkaa971.

15 Daina A, Michielin O and Zoete V, SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules, *Nucleic Acids Res*, 2019, **47**(W1), W357-64.

16 Kanehisa M, Furumichi M, Sato Y, Kawashima M and Ishiguro-Watanabe M, KEGG for taxonomy-based analysis of pathways and genomes, *Nucleic Acids Res*, 2023, **51**(D1), D587-D592.

17 Pinero J, Queralt-Rosinach N, Bravo A, Deu-Pons J, Bauer-Mehren A, *et al*., DisGeNET: A discovery platform for the dynamical exploration of human diseases and their genes, *Database*, 2015, **2015**. doi:10.1093/database/bav028.

18 Garcia-Moreno A, López-Domínguez R, Villatoro-García J A, Ramirez-Mena A, Aparicio-Puerta E, *et al.*, Functional enrichment analysis of regulatory elements, *Biomed*, 2022, **10**, 590. doi:10.3390/biomedicines10030590.

19 Burley S K, Bhikadiya C, Bi C, Bittrich S, Chen L, *et al*., RCSB protein data bank: Powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences, *Nucleic Acids Res*, 2020, **49**(D1), D437–D451. doi:10.1093/nar/gkaa1038.

20 Almog R, Waddling C A, Maley F, Maley G F and Van Roey P, Crystal structure of a deletion mutant of human thymidylate synthase Δ (7-29) and its ternary complex with Tomudex and dUMP, *Protein Sci*, 2001, **10**(5), 988–996. doi:10.1110/ps.47601.

21 Brumshtein B, Greenblatt H M, Butters T D, Shaaltiel Y, Aviezer D, *et al*., Crystal structures of complexes ofN-Butyl- and N-Nonyl-Deoxynojirimycin Bound to Acid β-Glucosidase, *J Biol Chem*, 2007, **282**(39), 29052–29058. doi:10.1074/jbc.m705005200.

22 Yi W, Shi J, Zhao G, Zhou X E, Suino-Powell K, *et al.*, Identification of a novel selective PPARγ ligand with a unique binding mode and improved therapeutic profile *in vitro*, *Sci Rep*, 2017, **7**(1). doi:10.1038/srep41487.

23 Young A C, Scapin G, Kromminga A, Patel S B, Veerkamp J H, *et al.*, Structural studies on human muscle fatty acid binding protein at 1.4 å resolution: binding interactions with three C18 fatty acids, *Struct*, 1994, **2**(6), 523–534. doi:10.1016/s0969-2126(00)00052-6.

24 Mangangcha I R, Brojen Singh R K, Lebeche D and Ali S, Xanthone glucoside 2-β-D-glucopyranosyl-1,3,6, 7-tetrahydroxy-9H-xanthen-9-one binds to the ATP-binding pocket of glycogen synthase kinase 3β and inhibits its activity: implications in prostate cancer and associated cardiovascular disease risk, *J BiomolStruct Dyn*, 2022, **40**(17), 7868–7884. doi:10.1080/07391102.2021.1902857.

25 Trott O and Olson A J, AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, *J Comput Chem*, 2010, **31**(2), 455-461.

26 BIOVIA, Dassault Systèmes, Discovery Studio Visualizer, 2020, San Diego: Dassault Systèmes, 2020.

27 Do J, McKinney C, Sharma P and Sidransky E, Glucocerebrosidase and its relevance to Parkinson disease, *Mol Neurodegener*, 2019, **14**(1). doi:10.1186/s13024-019-0336-2.

28 Rosenbloom B E and Weinreb N J, Gaucher Disease: A comprehensive review, *Crit Rev Oncog*, 2013, **18**(3), 163–175. doi:10.1615/critrevoncog.20130060.

29 Occhipinti R and Boron W F, Role of carbonic anhydrases and inhibitors in acid-base physiology: Insights from mathematical modeling, *Int J Mol Sci*, 2019, **20**(15), 3841. doi:10.3390/ijms20153841.

30 Stamper R L, Lieberman M F and Drake M V, Carbonic anhydrase inhibitors*, Becker-Shaffer's Diagnosis and Therapy of the Glaucomas*, E-Book, (Elsevier Health Sciences), 2009, 407–419.

31 Dey P and Chaudhuri T K, Phytochemical characterization of *Dioscoreaalata*leaf and stem by silylation followed by GC-MS analysis, *J Food Biochem*, 2016, **40**(4), 630-635.

32 Fagbemi K O, Aina D A and Olajuyigbe O O, Soxhlet extraction versus hydrodistillation using the clevenger apparatus: A comparative study on the extraction of a volatile compound from *Tamarindus indica* Seeds, *SciWorld J*, 2021, **2021**, 1-8.

33 Mal S, Dwivedi A R, Kumar V, Kumar N, Kumar B, *et al.*, Role of peroxisome proliferator-activated receptor gamma (PPARγ) in different disease states: Recent updates, *CurrMed Chem*, 2021, **28**(16), 3193–3215.

34 Wang Q, Imam M U, Yida Z and Wang F, Peroxisome proliferator-activated receptor gamma (PPARγ) as a target for concurrent management of diabetes and obesity-related cancer, *Curr Pharm Des*, 2017, **23**(25), 3677–3688. doi:10.2174/1381612823666170704125104.

35 Tyagi S, Gupta P, Saini A S, Kaushal C and Sharma S, The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases, *J Adv Pharm Technol Res*, 2011, **2**(4), 236–240. doi:10.4103/2231-4040.90879.

36 Furuhashi M, Saitoh S, Shimamoto K and Miura T, Fatty acid-binding protein 4 (FABP4): Pathophysiological insights and potent clinical biomarker of metabolic and cardiovascular diseases, *Clin Med Insights Cardiol*, 2015, **8**(Suppl 3), 23–33. doi:10.4137/CMC.S17067.

37 Tiemeier G L, Brown J M, Pratap S E, McCarthy C, Kastrenopoulou A, *et al.*, Pleomorphic liposarcoma of bone: A rare primary malignant bone tumour, *Clin Sarcoma Res*, 2018, **8**, 2. doi:10.1186/s13569-018-0089-7.

38 Araújo-Vilar D, Fernández-Pombo A, Victoria B, Mosquera-Orgueira A, Cobelo-Gómez S, *et al.*, Variable expressivity and allelic heterogeneity in type 2 familial partial lipodystrophy: The p.(Thr528Met) LMNA variant, *J Clin Med*, 2021, **10**(7), 1497. doi:10.3390/jcm10071497.

39 Siddiqui M A, Gollavilli P N, Ramesh V, Parma B, Schwab A, *et al.*, Thymidylate synthase drives the phenotypes of epithelial-to-mesenchymal transition in non-small cell lung cancer, *Br J Cancer*, 2021, **124**(1), 281–289. doi:10.1038/s41416-020-01095-x.

40 Shamshad H, Bakri R and Mirza A Z, Dihydrofolate reductase, thymidylate synthase, and serine hydroxy methyltransferase: Successful targets against some infectious diseases, *Mol Biol Rep*, 2022, **49**(7), 6659–6691. doi:10.1007/s11033-022-07266-8.

41 Valdivielso J M and Fernandez E, Vitamin D receptor polymorphisms and diseases, *Clin Chim Acta; Int J Clin Chem*, 2006, **371**(1-2), 1–12. doi:10.1016/j.cca.2006.02.016.

42 Wee P and Wang Z, Epidermal growth factor receptor cell proliferation signaling pathways, *Cancers*, 2017, **9**(5), 52. doi:10.3390/cancers9050052.

43 Jiang T, Xia Y, Lv J, Li B, Li Y, *et al.*, A novel protein encoded by circMAPK1 inhibits progression of gastric cancer by suppressing activation of MAPK signaling, *Mol Cancer*, 2021, **20**(1), 66. doi:10.1186/s12943-021-01358-y.

44 Vo T T L, Jang W J and Jeong C H, Leukotriene A4 hydrolase: An emerging target of natural products for cancer chemoprevention and chemotherapy, *Ann N Y Acad Sci*, **1431**(1), 31-3. doi:10.1111/nyas.13929.