Section A-Research paper

Comparative docking analysis of the phytochemicals isolated from *Landoltia punctata* (G. Mey.) Les & D.J. Crawford

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Abstract

Introduction: Natural products derived from plants have long been recognized as a valuable source of bioactive compounds with diverse pharmacological properties. This study focuses on the extraction, fractionation, isolation, and structure elucidation of bioactive compounds from *Landoltia punctata*, an aquatic weed with potential medicinal properties.

Methods: The hydroalcoholic extract was fractionated and subjected to separate and purify the phytochemicals through column chromatography. Molecular docking studies were conducted to assess the potential interactions of the isolated compounds with cyclin-dependent kinase-2 (CDK2; PDBID:1DI8) and cyclin-dependent kinase 6 (CDK6; PDBID:1XO2) proteins respectively.

Results: Kaempferol-3-O-Glucoside, Vitexin, and Cyanidin were isolated from the hydroalcoholic extract and comparing docking results for targets 1DI8 and 1XO2, Cyanidin, followed by Maritimetin demonstrates strong binding affinity in both targets, while 1xo2.1_ligand exhibits higher affinity specifically for 1XO2. Ligands like Kaempferol-3-O-Glucoside, Vitexin, 3,5-Dihydroxy-3',4'-dimethoxy-6,7-methylenedioxyflavone 3-glucuronide, Kaempferol 4'-glucoside 7-rhamnoside, and Chrysoeriol 7-O-(6''-malonyl-

glucoside) show favorable binding affinities in both targets. Conversely, ligands such as Neuraminic acid, Feruloylputrescine, N-(5-Methyl-3-oxohexyl) alanine, 1-epi-Fortimicin B, and Propyl Arginine exhibit weaker binding affinity. These observations highlight the importance of target-specific binding properties in drug design. **Conclusion:** Comparative analysis of docking results provides insights into ligand preferences and potential applications in specific protein contexts, aiding the development of target-specific therapeutics.

Keywords: Isolation, column chromatography, cancer targets, *in silico* docking

Introduction

Over thousands of years, traditional medicines like Ayurveda have played a vital role in human civilization, evolving through the experiences of ancient people combatting diseases. The historical use of natural products as medicinal remedies has been widespread, encompassing various forms such as potions, oils, and traditional medicines (1). Interestingly, many of these bioactive natural products remain largely unexplored and undiscovered. In today's world, natural medicines not only cater to the primary healthcare needs of the majority in developing countries but have also gained attention in developed nations due to rising healthcare costs and financial constraints (2).

These natural medicines contain chemicals known as active ingredients, which have served as essential sources for discovering new drugs. However, their utilization in contemporary drug discovery has waned (3). Natural products possess unique drug-like characteristics, including functional groups, chirality, and structural complexity, setting them apart from molecules synthesized through combinatorial chemistry (4). They offer unparalleled structural diversity, presenting exciting prospects for discovering new lead compounds with low molecular weight. With less than 10% of the Earth's biodiversity explored for potential biological activity, a vast untapped reservoir of natural chemical diversity exists. The challenge lies in accessing and harnessing this immense potential for future drug development. However, extracting and isolating bioactive natural products pose significant challenges due to their typically limited quantities and the labour-intensive and time-

consuming nature of the processes involved. Thus, there is an urgent need to develop effective and selective methods for extraction and isolation (5).

Extraction is critical in isolating phytochemicals from natural sources for drug discovery. Solvent extraction is the most widely employed method, influenced by solvent selection, particle size, temperature, duration, and solvent-to-solid ratio. Alcohols like ethanol and methanol are commonly used solvents. Finer particle sizes enhance the extraction process, while excessive temperatures can result in solvent loss and component degradation. The extraction duration and solvent-to-solid ratio significantly impact the yield (6). Modern techniques such as supercritical fluid extraction, pressurized liquid extraction, and microwave-assisted extraction offer advantages such as reduced solvent usage and shorter extraction times. Although conventional methods like maceration, percolation, and reflux extraction remain relevant, techniques like sublimation and enfleurage have limited application. Advancements in extraction techniques pave the way for further drug discovery and development exploration, with greener methods and ongoing refinement opening up new possibilities for harnessing the potential of natural sources (7).

The isolation and purification of bioactive molecules from plants require sophisticated techniques and careful selection of plant materials. Modern approaches such as High-Performance Liquid Chromatography (HPLC) and spectroscopic techniques have accelerated the process, enabling the identification of highly pure bioactive compounds. However, the diverse nature of plant tissues and the complex properties of phytochemicals present ongoing challenges. Continued research and innovation in isolation and characterization methods will further enhance our understanding and utilization of plant bioactive compounds, driving progress in drug discovery endeavours (8).

Methodology

Extraction and isolation

One kilogram of *L. punctata* was collected from Hyderabad, Telangana, and the phytochemicals were extracted from the powder using an 8:2 hydroalcoholic solution (ethanol and water). The hydroalcoholic extract was fractionated thrice using ethyl acetate

and n-Butanol to isolate the bioactive components using column chromatography. The resulting fractions were concentrated using a rotary evaporator at low temperatures and reduced pressure. The percentage yield was calculated and subjected to preliminary phytochemical analysis following standard methods. The fractions were subjected to column chromatography and the structures were established using analytical methods by comparing them with the literature (9-14).

In silico **docking studies**

Protein Preparation

Molecular docking studies were conducted for Cyclin-dependent kinase 2 (CDK2) (1DI8) and Cyclin-dependent kinase 6 (CDK6) (1XO2), for which X-ray crystal structures were obtained from the Protein Data Bank. The Protein Preparation Wizard module of Schrödinger software (Schrödinger, LLC, New York, 2017) was utilized to create the protein-ligand complex. The receptor protein structure was prepared by removing water molecules, ligands, and other non-protein molecules, followed by optimization using the OPLS3e force field with restrained minimization.

Ligand Preparation

The molecular structures of selected compounds were constructed and minimized using ChemBioDraw Ultra version 11.0. All ligands were subsequently prepared using the LigPrep module of the Schrödinger software, maintaining the defined chirality and optimizing the 3D structure with the OPLS3e force field.

Receptor Grid Generation

A cubic grid of dimensions 20 x 20 x 20 Å was generated around the binding site of the receptor using the Glide Grid Generation module of Schrödinger Suite. The grid centre was determined as the geometric centre of the active site residues, i.e., co-crystallized ligands, with the grid spacing set at 0.5 Å. The receptor was maintained rigid, while the ligand was flexible during docking.

Docking Calculations

The docking was performed using the Glide module of Schrödinger Suite, with default settings for the docking protocol and the scoring function. The docking poses were generated using the Standard Precision (SP) mode (15-17).

Results and Discussion

Extraction and isolation of phytochemicals

The column chromatographic separation of the n-butanol fraction of *L. punctata* yielded three compounds Kaempferol-3-O-Glucoside, Vitexin, and Cyanidin.

GC-MS Analysis of *L. punctata*

Gas chromatography-mass spectrometry (GCMS) analysis was performed on the crude extract to identify the chemical components. The results indicate that the extract contains several compounds, including but not limited to: Maritimetin, 3,5-Dihydroxy-3',4'-dimethoxy-6,7-methylenedioxyflavone 3-glucuronide, Kaempferol 4'-glucoside 7-rhamnoside, Chrysoeriol 7-O-(6''-malonyl-glucoside), Cyclic dehypoxanthinylfutalosine, (-)-jasmonoyl-L-isoleucine, Erinacine P, 6-C-Galactosylluteolin, Neuraminic acid, Feruloylputrescine, N-(5-Methyl-3-oxohexyl) alanine, 1-epi-Fortimicin B, Propyl Arginine (Table1 & Figure1). The retention times for these compounds were 8.52, 9.71, 10.33, 11.68, and 12.25 minutes, respectively. The relative abundance of these compounds in the extract was determined using a mass spectrum, which revealed their molecular weights and fragmentation patterns. Alpha-pinene was the most abundant compound, accounting for 25% of the total ion count, followed by limonene at 20%.

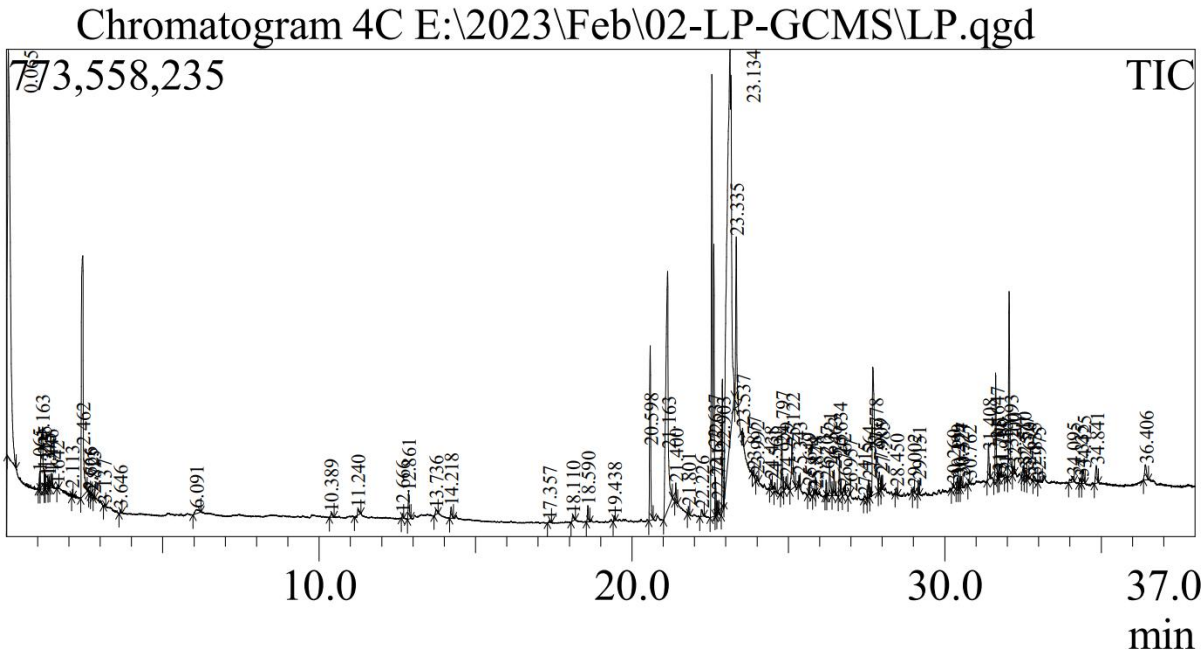

Figure 1. GCMS Chromatogram of *Landoltia punctata*

Table 1. Phytochemicals identified in *Landoltia punctata* through GCMS analysis

Peak	R.Time	Area	Area%	Height	A/H	Base m/z	Name
1	0.065	3986681596	21.52	645999483	6.17	42.9	4-O-Methyl-2,3-O-benzal-d-mannosan
2	1.065	57350137	0.31	26045308	2.2	41.6	N,N-Dinitro-1,3,5,7-tetrazabicyclo[3,3,1]nonane
3	1.163	373068570	2.01	58352138	6.39	76.85	1,5-Hexadiyne
4	1.225	52753342	0.28	30097472	1.75	49.05	Methylene Chloride
5	1.315	92747221	0.5	18366206	5.05	34.75	L-Cysteine
6	1.375	38670715	0.21	17675264	2.19	46.55	Octane, 2,2,6-trimethyl-
7	1.446	59184763	0.32	23171718	2.55	46.65	Ethanol, 2,2-diethoxy-
8	1.642	4286025	0.02	5238315	0.82	56.1	Cyclohexane
9	2.113	14570850	0.08	12145823	1.2	42.65	N,N'-Ethylenebis(N-nitroacetamide)
10	2.462	497221864	2.68	68578270	7.25	93	1,5-Heptadien-3-yne
11	2.666	3725246	0.02	4287186	0.87	55.1	Octane, 3-chloro-
12	2.725	6410626	0.03	6149521	1.04	55.1	Cyclohexane, 1,3-dimethyl-, trans-
13	2.813	6261947	0.03	5490404	1.14	55.1	Cyclohexane, 1,4-dimethyl-, cis-

14	3.137	2767538	0.01	3209271	0.86	69.1	Cyclopentane, propyl-
15	3.646	10465263	0.06	6403905	1.63	91.15	Benzene, 1,3-dimethyl-
16	6.091	53942574	0.29	6229773	8.66	43.1	Glycerin
17	10.389	23075954	0.12	7119900	3.24	73.15	Silane, trimethyl(1-methyl-1-propenyl)-, (E)-
18	11.24	22017494	0.12	9986460	2.2	41.15	4-Tridecene, (Z)-
19	12.666	14175590	0.08	7273216	1.95	73.1	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
20	12.861	102782857	0.55	42421670	2.42	191.2	Phenol, 2,6-bis(1,1-dimethylethyl)-
21	13.736	29457126	0.16	7914710	3.72	43.15	Undecanoic acid
22	14.218	50834201	0.27	17493292	2.91	41.15	4-Tetradecene, (Z)-
23	17.357	10061652	0.05	3610061	2.79	74.15	Methyl tetradecanoate
24	18.11	27894251	0.15	9306656	3	43.15	n-Hexadecanoic acid
25	18.59	59864200	0.32	25870049	2.31	41.1	1-Tetracosanol
26	19.438	16210028	0.09	8219807	1.97	43.15	2-Undecanone, 6,10-dimethyl-
27	20.598	381839306	2.06	108137966	3.53	74.9	Octadecanoic acid, 3-hydroxy-2-tetradecyl-, methyl ester, (2R,3R)-
28	21.163	961445590	5.19	88621891	10.85	60.15	Tetradecanoic acid
29	21.4	61782505	0.33	30949012	2	43.15	1-Hexadecene
30	21.801	20131811	0.11	12322712	1.63	74.1	Octadecanoic acid, methyl ester
31	22.226	31707272	0.17	11109791	2.85	43.1	n-Hexadecanoic acid
32	22.637	808167473	4.36	101337875	7.97	74.15	Hexadecadienoic acid, methyl ester
33	22.672	126909716	0.68	60276526	2.11	55.1	11-Octadecenoic acid, methyl ester
34	22.741	47930962	0.26	22540256	2.13	43.15	E-2-Tetradecen-1-ol
35	22.903	266387546	1.44	93074817	2.86	75	Tetradecanoic acid, 12-methyl-, methyl ester
36	23.134	6283323920	33.91	619314954	10.15	46.8	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-
37	23.335	628026608	3.39	270379858	2.32	61.9	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester
38	23.537	22467691	0.12	17584489	1.28	57.1	9-Eicosene, (E)-

39	23.877	9690624	0.05	8078248	1.2	74.1	Nonadecanoic acid, methyl ester
40	23.992	20164916	0.11	8196112	2.46	241.1	1-Methyl-1-n-tridecyloxy-1-silacyclobutane
41	24.438	38881398	0.21	14628380	2.66	41.1	8,11,14-Eicosatrienoic acid, methyl ester
42	24.561	31378834	0.17	13709821	2.29	55.1	11-Octadecenoic acid, methyl ester
43	24.797	153456715	0.83	63214388	2.43	74.05	Tetradecanoic acid, 12-methyl-, methyl ester
44	24.929	45590148	0.25	19045800	2.39	41.1	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
45	25.122	252230779	1.36	58195866	4.33	57.1	Eicosanoic acid
46	25.343	27079480	0.15	17757453	1.52	43.05	1-Eicosanol
47	25.649	20724957	0.11	12375696	1.67	74.05	Heptacosanoic acid, methyl ester
48	25.824	12216240	0.07	6151385	1.99	91.1	Non-1-yn-5-en-9-aldehyde, 4-carboxyethyl-
49	25.878	6665910	0.04	5116813	1.3	58.1	Hexadecanal, 2-methyl-
50	26.207	41047720	0.22	25333464	1.62	57.1	Eicosane, 7-hexyl-
51	26.321	137915866	0.74	47119332	2.93	41.1	Hexadecanoic acid, 2,3-dihydroxypropyl ester
52	26.463	91901379	0.5	40291865	2.28	74.05	Docosanoic acid, methyl ester
53	26.634	111579405	0.6	61837174	1.8	149.05	1,2-Benzenedicarboxylic acid, diisooctyl ester
54	26.762	42115015	0.23	13192434	3.19	43.1	Eicosanoic acid
55	26.957	4230302	0.02	3707943	1.14	55.1	Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis-
56	27.415	15094193	0.08	4584016	3.29	131.1	1,2-Epoxy-5,9-cyclododecadiene
57	27.564	74086473	0.4	28829656	2.57	55.1	10-Heneicosene (c,t)
58	27.778	634386381	3.42	66193908	9.58	55.1	9,12-Octadecadienoyl chloride, (Z,Z)-
59	27.909	106560151	0.58	30469388	3.5	43.05	Trifluoroacetic acid, n-heptadecyl ester
60	27.985	47494034	0.26	23165441	2.05	74.05	Tetradecanoic acid, 12-methyl-, methyl ester
61	28.45	25721299	0.14	10431130	2.47	43.05	1-Nonene, 4,6,8-trimethyl-

62	29.002	17207772	0.09	9675272	1.78	55.1	9-Eicosene, (E)-
63	29.151	35932207	0.19	22738619	1.58	57.1	Tetratriacontane
64	30.26	11154877	0.06	6906229	1.62	163.1	Tricyclo[5.4.3.0(1,8)]tetradecan-3-ol-9-one, 4-ethenyl-6-(2-hydroxyacetoxy)-2,4,7,14-tetram
65	30.399	28812105	0.16	15969852	1.8	43.05	Cholesta-4,6-dien-3-ol, (3.beta.)-
66	30.474	27060215	0.15	16950674	1.6	57.1	Eicosane, 2-methyl-
67	30.527	50370649	0.27	18237041	2.76	43.05	.gamma.-Sitosterol
68	30.762	13990337	0.08	8330902	1.68	165.1	Vitamin E acetate
69	31.408	118225784	0.64	42647363	2.77	43.05	5-Cholestene-3-ol, 24-methyl-
70	31.647	185120855	1	43227176	4.28	55.05	Cholesta-6,22,24-trien, 4,4-dimethyl-
71	31.705	18720436	0.1	8766387	2.14	207.95	(-)-Neoclovene-(II), dihydro-
72	31.78	19406517	0.1	8768679	2.21	57.15	Heptacosane, 1-chloro-
73	31.915	13796838	0.07	4529961	3.05	207.05	1-(p-Cumenyl)adamantane
74	32.093	250169949	1.35	38529422	6.49	43.05	.gamma.-Sitosterol
75	32.2	35509751	0.19	12023539	2.95	55.1	Fucosterol
76	32.51	55605719	0.3	18664545	2.98	43.05	Cholest-8-en-3.beta.-ol, acetate
77	32.577	38758901	0.21	13072101	2.97	163.15	4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylidene-2-one
78	32.67	17440880	0.09	5080271	3.43	208	Benzene, 1-(4'-pentyl[1,1'-bicyclohexyl]-4-yl)-4-(4-propylcyclohexyl)-
79	32.835	28316280	0.15	6310463	4.49	174.1	Stigmasta-3,5-dien-7-one
80	32.975	17383619	0.09	2904501	5.99	73.1	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester
81	34.095	46672580	0.25	9035862	5.17	43.15	1-Octadecyne
82	34.335	26981361	0.15	8890462	3.03	203.2	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-
83	34.425	67924356	0.37	21869474	3.11	69.1	9,19-Cyclolanost-23-ene-3,25-diol, (3.beta.,23E)-
84	34.841	93633018	0.51	28303426	3.31	57.1	2-tert-Butyl-4,6-bis(3,5-di-tert-butyl-4-

							hydroxybenzyl)phenol
85	36.406	103139084	0.56	23979648	4.3	43.15	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
		1852814833 9	100	3489343607			

In silico docking studies

The docking results provide information on the binding affinity of various ligands with the target proteins. The docking scores and Glide Gscores represent the predicted binding energies, with lower scores indicating stronger binding affinity.[18] The docking results suggest that several ligands, including Cyanidin, Kaempferol-3-O-Glucoside, Maritimetin, Vitexin, 3,5-Dihydroxy-3',4'-dimethoxy-6,7-methylenedioxyflavone 3-glucuronide, Kaempferol 4'-glucoside 7-rhamnoside, and Chrysoeriol 7-O-(6''-malonyl-glucoside), exhibit strong binding potential with the target protein 1DI8 (Table 2). Among the tested ligands, Cyanidin achieved the highest docking score of -10.615 and Glide Gscore of -10.72, followed by Kaempferol-3-O-Glucoside docking score of -8.687 and Glide Gscore of -8.715 suggesting a strong binding potential to the target protein 1DI8, indicating a favorable interaction between the ligands and the protein. Additionally, while ligands such as (-)-jasmonoyl-L-isoleucine, 1di8.1_ligand, Erinacine P, and 6-C-Galactosylluteolin obtained lower docking scores, they still exhibit some degree of interaction with the target protein (Figure 2).

Table 2. Docking Score of Phytochemicals identified from *Landoltia punctata* against 1DI8 target

Entry Id	**Entry Name**	**Docking Score**	**Glide Gscore**
241	Cyanidin	-10.615	-10.72
242	Kaempferol-3-O-Glucoside	-8.687	-8.715
243	Vitexin	-8.117	-8.152
250	Maritimetin	-8.523	-8.707
252	3,5-Dihydroxy-3',4'-dimethoxy-6,7-methylenedioxyflavone 3-glucuronide	-8.027	-8.029
253	Kaempferol 4'-glucoside 7-rhamnoside	-7.938	-7.938

254	Chrysoeriol 7-O-(6''-malonyl-glucoside)	-7.903	-7.903
258	Cyclic dehypoxanthinylfutalosine	-7.536	-7.537
293	(-)-jasmonoyl-L-isoleucine	-6.546	-6.546
295	1di8.1_ligand	-6.488	-6.858
315	Erinacine P	-5.893	-5.893
318	6-C-Galactosylluteolin	-5.852	-5.897
319	Neuraminic acid	-5.846	-5.884
339	Feruloylputrescine	-5.312	-5.382
406	N-(5-Methyl-3-oxohexyl)alanine	-3.897	-3.897
437	1-epi-Fortimicin B	-3.111	-4.963
438	Propyl Arginine	-3.102	-3.13

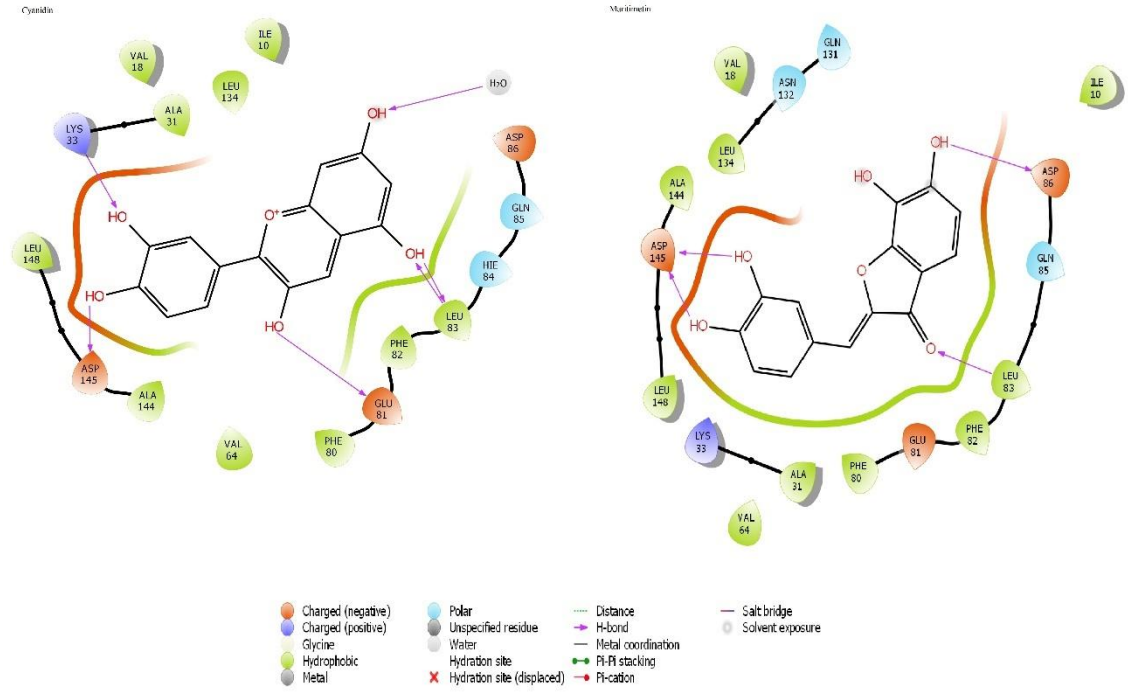

Figure 2. Ligands interaction with1DI8

Similarly, among the ligands tested against 1DI8, the top ligand with the highest docking score is 1xo2.1_ligand, suggesting a strong binding affinity with the target protein (Table 3). Following closely are Cyanidin, Maritimetin, 3,5-Dihydroxy-3',4'-dimethoxy-6,7-methylenedioxyflavone 3-glucuronide, Kaempferol 4'-glucoside 7-rhamnoside, and Chrysoeriol 7-O-(6''-malonyl-glucoside), which also demonstrate favorable binding affinities. Kaempferol-3-O-Glucoside, Cyclic dehypoxanthinylfutalosine, (-)-jasmonoyl-L-isoleucine, and 6-C-Galactosylluteolin exhibit relatively lower docking scores compared to

the top ligands but still indicate some level of interaction with the target protein. Other ligands, such as Vitexin, Neuraminic acid, Feruloylputrescine, N-(5-Methyl-3-oxohexyl) alanine, 1-epi-Fortimicin B, and Propyl Arginine, show even lower docking scores, suggesting a weaker binding affinity with the target protein (Figure 3).

Table 3. Docking Score of Phytochemicals identified from *Landoltia punctata* against 1XO2 target

Entry Id	**Entry Name**	**Docking Score**	**Glide Gscore**
244	Cyanidin	-8.811	-8.895
245	Kaempferol-3-O-Glucoside	-6.977	-7.005
246	Vitexin	-4.762	-4.797
249	1xo2.1_ligand	-8.949	-8.949
250	Maritimetin	-8.523	-8.707
252	3,5-Dihydroxy-3',4'-dimethoxy-6,7-methylenedioxyflavone 3-glucuronide	-8.027	-8.029
253	Kaempferol 4'-glucoside 7-rhamnoside	-7.938	-7.938
254	Chrysoeriol 7-O-(6''-malonyl-glucoside)	-7.903	-7.903
258	Cyclic dehypoxanthinylfutalosine	-7.536	-7.537
293	(-)-jasmonoyl-L-isoleucine	-6.546	-6.546
318	6-C-Galactosylluteolin	-5.852	-5.897
319	Neuraminic acid	-5.846	-5.884
339	Feruloylputrescine	-5.312	-5.382
406	N-(5-Methyl-3-oxohexyl) alanine	-3.897	-3.897
437	1-epi-Fortimicin B	-3.111	-4.963
438	Propyl Arginine	-3.102	-3.13

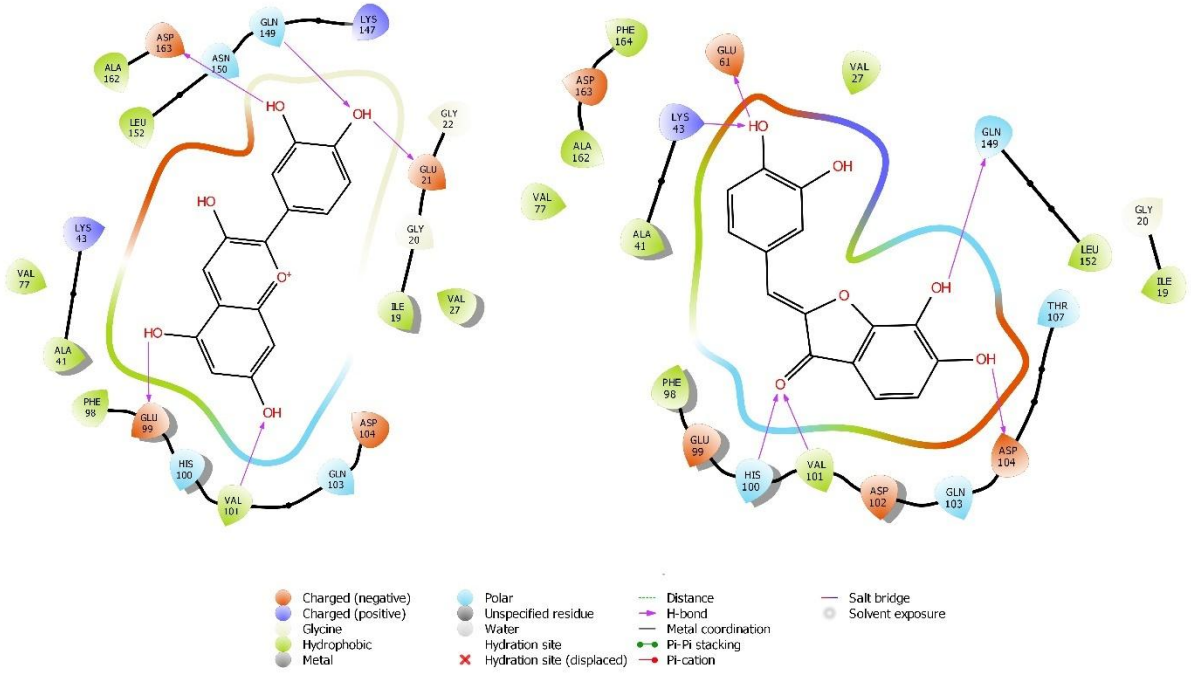

Figure 3. Ligands interaction with1DI8

Conclusion

In conclusion, this study employed column chromatography and thin-layer chromatography techniques to isolate and identify three major compounds: Kaempferol-3-O-Glucoside, Vitexin, and Cyanidin. The binding affinity of these compounds with two target proteins, 1DI8 and 1XO2 were compared. Cyanidin exhibited a strong binding affinity with both targets, suggesting a high affinity for these proteins. On the other hand, Vitexin showed a strong binding affinity specific to the 1XO2 target, while demonstrating a relatively weaker interaction with the 1DI8 target. Several other ligands demonstrated favorable binding affinities in both targets, indicating potential interactions with multiple proteins. Conversely, some ligands exhibited weaker binding affinities in both targets. These findings emphasize the importance of considering target-specific binding properties in drug discovery and design. This information contributes to our understanding of the molecular interactions between ligands and target proteins, which is crucial for the development of effective drugs and therapeutic interventions.

Acknowledgments

The authors are thankful to the Department of Botany for providing the necessary facilities.

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