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Original Research Article

Molecular Docking Evaluation of Phytochemicals in Fruits of *Terminalia pallida* Brandis: Implication on Immunomodulation

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ABSTRACT

Terminalia pallida Brandis, a Combretaceae family plant, has garnered attention due to its traditional medicinal uses and rich phytochemical diversity. In this study, we conducted a comprehensive analysis of the fruit extracts of *Terminalia pallida* Brandis to unravel the intricate composition of its metabolites and explore their potential therapeutic applications. Preliminary phytochemical analysis of the n-Hexane and alcohol extracts revealed distinct compositions containing steroids, lipids, saponins, flavonoids, tannins, proteins, carbohydrates, and amino acids. Advanced techniques such as Gas Chromatography-Mass Spectrometry (GCMS) and Liquid Chromatography-Mass Spectrometry (LCMS) were employed to delve deeper into the composition of these extracts. The GCMS analysis of the n-Hexane extract identified 54 compounds, a complex mixture of hydrocarbons and fatty acids. Notably, (Z)-Docosenoic acid methyl ester, 9(E)-Octadecenoic acid methyl ester, Methyl linoleate, and Stigmast-5-en-3-ol oleate. Furthermore, LCMS analysis of the alcohol extract revealed the presence of compounds like Orientin, Arctiin, Quercetin, Arjunolic acid, and Stigmasterol, etc. Orientin, Quercetin, Purpurin, and Pteropodine exhibited higher concentrations, suggesting their potential significance. *In-silico* docking studies were conducted using the above ligands on protein targets 1M48 and 2AZ5. The different binding affinities and interactions revealed by the docking data revealed the possible modes of action of these drugs. The importance of *Terminalia pallida* Brandis fruit extracts as sources of several bioactive chemicals is highlighted by this study. Integrating advanced analytical techniques with *in-silico* approaches revealed the contribution of phenolic compounds such as Resveratrol and Arctiin in Immunomodulation. This will further guide us to the *in vitro* and *in vivo* models.

Keywords: *Terminalia pallida* Brandis, phytochemical analysis, immunomodulation, *in-silico* studies, gas chromatography-mass spectrometry , liquid chromatography-mass spectrometry

Introduction

Terminalia pallida Brandis, a member of the Combretaceae family, is an evergreen semi-tree species endemic to the Seshachalam hills.[1]*Terminalia pallida* Brandis. holds significance for its phytochemical richness, traditional uses, and potential therapeutic properties across various ailments. *T. pallida* Brandis. is used as a crude drug by local communities and serves as a source for non-wood forest products like tannins, gums, oils, and fodder.[2] Leaves, and bark treat skin ailments and conditions such as inflammation, fever, diabetes, and dysentery. Fruit extracts exhibit antipyretic, thrombolytic, anti-hyperlipidemic, purgative, and antidiabetic properties. *T. pallida* Brandis. fruit also demonstrates antioxidant potential and antidiabetic effects. Studies reveal *T. pallida's* positive effects, such as anti-ulcer activity and cardioprotective actions, attributed to its fruit extracts. Its powdered fruit is used traditionally for diabetes and piles.

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Mineral content analysis indicates that micronutrients and macronutrients are valuable for daily activities.[3-5]

Historically, medicinal plants and phytochemicals have been recognised for their ability to modulate immune system function. These agents primarily function as immune boosters, stimulating innate and adaptive immunity. Additionally, they exhibit mechanisms such as interference with proinflammatory pathways and gut microbiome modulation.[6-7]

The immune system plays a crucial role in defending against infections and maintaining balance within the body. While an appropriate immune response is beneficial, an exaggerated one can led to severe consequences and diseases. Chronic inflammation is linked to various ailments, including asthma, arthritis, and neurological disorders. Immunomodulation, the strategic regulation of the immune response, shows promise in treating these conditions.[8-9]

The immune system operates on multiple levels, from genes to organs, working together to combat diseases. Understanding this complexity allows us to target phytochemicals for more effective treatments. Strategies involve deactivating proinflammatory agents using antibodies, mimicking immune signals, and inhibiting inflammatory factors. Well-established therapies like corticosteroids are being refined for better results.[10]

By integrating the results of GCMS and LCMS analyses with *in-silico* docking studies, this research study can take a comprehensive approach towards unravelling the therapeutic potential of *T. pallida* Brandis fruit extracts. Investigating ligand-protein interactions in the inflammatory and immunological processes framework provides important

information that can guide future efforts in immunomodulation and medication development.

The choice of IL-2, PDB ID: 2AZ5 (Interleukin-2, Protein Data Bank ID: 2AZ5) and TNF-α (Tumour Necrosis Factor-α, PDB ID: 1M48) as protein targets for docking studies is rooted in their pivotal roles within immune and inflammatory processes. IL-2 is a critical cytokine that governs immune response modulation by influencing the proliferation and differentiation of immune cells. Its importance is further highlighted by its potential in cancer immunotherapy. Meanwhile, TNF-α is crucial in initiating inflammation, orchestrating immune cell activation and cytokine production. While essential for immune responses, its overactivity is linked to chronic inflammatory diseases. The present investigation aims to evaluate the phytochemicals identified from the analytical profiling and to perform *in-silico* docking studies on the targets involved in immunity.

Materials and Methods

Plant material

Terminalia pallida Brandis. fruits were collected from Seshachalam hills, Andhra Pradesh. in January 2023 and authenticated from the Department of Botany, Osmania University, Hyderabad India with voucher number 01971. The GPS location of the tree is at the following coordinates Latitude 14.22 and Longitude 79.55.

Extraction and preliminary phytochemical screening

The dried fruits were powdered and subjected to maceration with 80% alcohol and fractionated with n-Hexane. Later, it is subjected to drying using a rotary evaporator (Vedh Instruments, 2020 Model, India). The preliminary phytochemical analysis was performed according to the standard protocols.[11]

Reagents and chemicals

All the chemicals and reagents were procured from Sigma Aldrich (laboratory grade, with the highest purity available).

GC-MS analysis

Gas Chromatography-Mass Spectrometry (GC-MS) is essential for analyzing phytochemicals in plant extracts, allowing precise identification and quantification of compounds, even in trace amounts. This technique supports the exploration of the potential therapeutic benefits of plants. In a study, a Shimadzu (QP 2010 Ultra) GC-MS system, with a 30×0.25 mm column and 0.25 μM particle size, analyzed n-Hexane extracts from *T. pallida* Brandis using a non-split injection. The protocol included a temperature program from 70°C to 310°C, helium as the carrier gas, and ionization at 70 eV. Compounds were identified by comparing mass spectra with the NIST Ver.2.1 database. [12-13]

LC-MS analysis

Liquid Chromatography-Mass Spectrometry (LC-MS) is crucial for analyzing complex compounds, including large or thermally sensitive molecules, with minimal sample preparation due to its ability to handle liquid samples. It stands out by using a liquid mobile phase, enabling the analysis of compounds unsuitable for gas phases. LC-MS separates compounds based on their affinity differences between a liquid solvent and the column's stationary phase, enhanced by ionization techniques like ESI and APCI for broad compound applicability. A Waters Alliance e2695/HPLC-TQD Mass spectrometer analyzed *T. pallida* Brandis extract via LC-ESI/MS, using a Bruker Daltonik C18 column at 30°C, with acetonitrile/methanol and ammonium acetate/acetic acid eluent. Samples in DMSO and acetonitrile were centrifuged and injected, with compound identification based on m/z ratios and retention times. [14-15]

In-silico docking studies

In-silico docking studies of the phytochemicals identified in GC-MS and LC-MS analysis were selected as ligands for the two targets, viz., IL-2 (1M48) and TNF-α (2AZ5). It involves computational simulations to predict their binding interactions and affinities. The 3D structures of the protein targets are retrieved from the Protein Data Bank, while the ligands of interest are selected and prepared in appropriate file formats. [16] The protein structures undergo preparation, including removing nonessential ligands and adding hydrogen atoms with correct protonation states. Docking grids are generated around the active sites, defining the search space for ligand binding. Schrodinger Maestro Software, module version 4.4.12.0 Schrodinger, LLC, New York, NY, 2018 was used for the docking simulations, allowing ligands to explore different orientations within the grid. The results are analyzed to identify ligand poses with favorable interactions, such as hydrogen bonding and hydrophobic interactions. Ligands are ranked based on predicted binding affinities. [17-18] By exploring ligand interactions with IL-2 and TNF-α through docking studies, we can uncover potential therapeutic applications for immune disorders, inflammatory conditions, and cancer treatments. [19-20]

Results and Discussion

Preliminary phytochemical analysis

Preliminary analysis of *T. pallida* Brandis fruit extracts showed varied phytochemicals. The n-Hexane extract contained steroids and lipids, but lacked alkaloids, glycosides, saponins, flavonoids, tannins, proteins, carbohydrates, and amino acids. Conversely, the alcohol extract was abundant in saponins, flavonoids, tannins, proteins, carbohydrates, and amino acids, but devoid of alkaloids, glycosides, steroids, and lipids listed in Table 1.

GC-MS analysis

The n-Hexane fraction of *T. pallida* Brandis. fruits were subjected to GCMS analysis, revealing the identification of 54 compounds. The identified compounds encompass a spectrum of chemical constituents present in the n-Hexane fraction of *T. pallida* Brandis. fruits. Among these, (Z)-Docosenoic acid methyl ester, with a prominent percentage area of 22.34%, emerged as the primary compound, showcasing its abundance within the extract. Notably, the GCMS analysis also highlighted the presence of significant compounds such as 9(E)-Octadecenoic acid methyl ester (9.02%), Methyl linoleate (8.99%), Stigmast-5-en-3-ol oleate (7.71%), 9(Z)-octadecenoic acid oxiranylmethyl ester (6.28%), Tris(2,4-di-tert-butylphenyl) phosphate (4.93%), Cholest-4-en-3-one (3.44%), Hexadecanoic acid methyl ester (3.37%), Drostanolone (2.47%), and 11-Eicosenoic acid methyl ester (2.38%), among others as given in Table 2 and Figure 1.

LC-MS analysis

The LCMS results represent the identification of various phytochemicals in the alcohol extract. The identified compounds include Orientin, Arctiin, Quercetin, 3,4-O-dicaffeoyl-epi-quinic acid methyl ester, Arjunolic acid, Stigmasterol, Terminoside A, β-sitosterol, Arjunetin, Purpurin, Pteropodine, 2-o-acetylorochrine, Quercetin-3-glucuronide, Corilagin, Linoleic acid, Oleic acid, Isoquercetin, Strophanthidol, Lupenone, Resveratrol. But, Orientin, Quercetin, Purpurin, and Pteropodine were in higher concentrations in the extract (Table 3, Figure 2 and 3).

Table 1: Preliminary phytochemical analysis of fruit extract of *T. pallida* using n-Hexane and Alcohol

Phytochemicals	n-Hexane extract	Alcohol extract
Alkaloids	-	-
Glycosides	-	-
Saponins	-	+
Flavonoids	-	+
Steroids	+	-
Tannins	-	+
Proteins	-	+
Carbohydrates	-	+
Amino acids	-	+
Lipids	+	-

Present = +, Absent = -

Table 2: GCMS Analysis of fruit extract of *T. pallida* using n-Hexane

Peak	R. Time	Area%	Name
1	16.326	0.09	Nonanal dimethyl acetal
2	19.951	0.14	Tetradecane
3	22.975	0.1	2,4-Di-tert-butylphenol
4	25.187	0.16	Hexadecane
5	29.861	0.19	Heneicosane
6	32.197	0.25	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6
7	32.555	3.37	Hexadecanoic acid, methyl ester
8	34.093	0.2	Heneicosane
9	35.937	8.99	9,12-Octadecadienoic acid (Z, Z)-, methyl ether
10	36.077	9.02	9-Octadecenoic acid, methyl ester, (E)-
11	36.18	0.43	9-Octadecenoic acid, methyl ester, (E)-
12	36.567	1.55	Methyl stearate
13	37.677	0.31	Hexadecanamide
14	37.952	0.23	Nonacosane
15	39.689	0.56	9-Octadecenoic acid (Z)-, oxiranylmethyl ether
16	39.784	2.38	11-Eicosenoic acid, methyl ester
17	39.899	0.5	11-Eicosenoic acid, methyl ester
18	40.245	0.62	Eicosanoic acid, methyl ester
19	41.355	1.14	Hexadecanamide
20	41.499	0.37	Tetracosane
21	42.67	1.49	9,12-Octadecadien-1-ol, (Z, Z)-
22	42.761	1.91	9-Octadecenoic acid (Z)-, oxiranylmethyl ether
23	43.267	22.34	13-Docosenoic acid, methyl ester, (Z)-
24	43.341	1.46	13-Docosenoic acid, methyl ester, (Z)-
25	43.645	0.94	Docosanoic acid, methyl ester
26	43.703	1.5	Bis(2-ethylhexyl) phthalate
27	44.775	0.29	Tetracosane
28	45.243	0.18	Tricosanoic acid, methyl ester
29	45.493	0.41	9,12,15-Octadecatrienoic acid, ethyl ester
30	45.571	0.64	Heptanoic acid, octyl ester
31	46.034	0.45	9-Octadecenoic acid (Z)-, oxiranylmethyl ether
32	46.32	0.2	Triacontane
33	46.415	1.21	15-Tetracosenoic acid, methyl ester, (Z)
34	46.78	0.68	Tetracosanoic acid, methyl ester
35	47.578	0.29	13-Docosenamide, (Z)-
36	48.009	0.51	Squalene
37	48.33	0.58	9-Octadecenoic acid, 1,2,3-propanetriyl
38	49.089	6.28	9-Octadecenoic acid (Z)-, oxiranylmeth
39	49.406	0.47	9-Octadecenoic acid (Z)-, oxiranylmeth
40	49.768	0.32	Glycerol tricaprylate
41	50.025	0.35	9-Octadecen-1-ol, (*Z*)-
42	51.427	2.01	*γ*-Tocopherol
43	51.914	0.26	9-Octadecenoic acid (Z)-, oxiranylmethyl ether
44	52.327	0.63	Glycerol tricaprylate
45	52.469	0.77	*α*-Tocopherol-*β*-D-mannoside

46	54.349	0.62	Stigmasterol
47	54.905	0.3	Decanoic acid, 1,2,3-propanetriyl ester
48	55.315	7.71	Stigmast-5-en-3-ol, oleate
49	55.964	0.73	1,22-Docosanediol
50	57.548	3.44	Cholest-4-en-3-one
51	59.695	4.93	Tris(2,4-di-tert-butylphenyl) phosphate
52	60.918	2.47	Drostanolone
53	61.565	0.73	Isopropyl linoleate
54	62.138	2.33	9-Octadecenoic acid, 1,2,3-propanetriyl

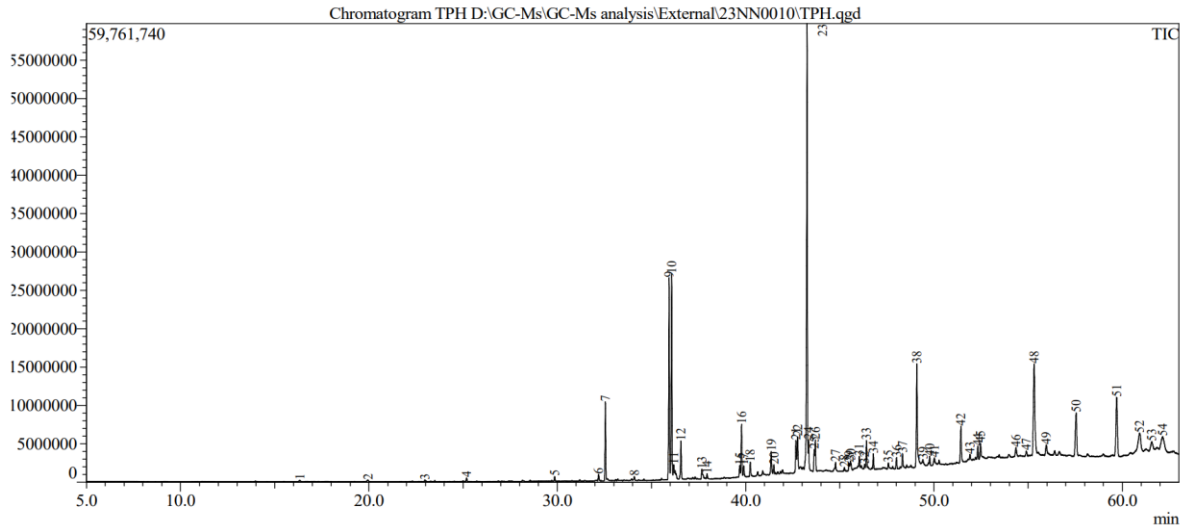

Figure 1: GCMS Chromatogram

LC-MS analysis

The LCMS results represent the identification of various phytochemicals in the alcohol extract. The identified compounds include Orientin, Arctiin, Quercetin, 3,4-O-dicaffeoyl-epi-quinic acid methyl ester, Arjunolic acid, Stigmasterol, Terminoside A, β-sitosterol, Arjunetin, Purpurin, Pteropodine, 2-o-acetylorochrine, Quercetin-3-glucuronide, Corilagin, Linoleic acid, Oleic acid, Isoquercetin, Strophanthidol, Lupenone, Resveratrol. But, Orientin, Quercetin, Purpurin, and Pteropodine were in higher concentrations in the extract (Table 3, Figure 2 and 3).

In-silico Docking studies

In-silico docking reveals key insights into ligand-protein interactions, with binding scores reflecting the predicted affinity. Stronger binding is suggested by more negative scores. Pi-pi interactions involve aromatic rings, while hydrogen bonds are crucial for complex stability. For the IM48 protein, Resveratrol shows strong binding with a -6.565 score, engaging in pi-pi and hydrogen bonds with PHE, THR, and LEU. Quercetin-3-Glucuronide, with a -6.3 score, forms similar interactions. Other compounds like 3,4-Di-O-caffeoylquinic acid methyl ester.1 and Corilagin.1 also show significant interactions, contributing to ligand stability at the protein's binding site. These findings are vital for experimental validation and potential treatment development.

For the protein 2AZ5, notable ligands exhibit strong binding affinities, such as Arctiin with a -6.365 binding score, interacting through pi-pi interactions and hydrogen bonds with TYR and GLN residues. Pteropodine has a score of -6.333, while Terminoside A at -6.32 binds via hydrogen bonds with TYR. Corilagin, with -6.282, engages in pi-pi stacking and hydrogen bonds with TYR, GLU, and GLY, and Quercetin follows with -6.116. These interactions highlight potential action mechanisms and preferences of compound binding with IL-2 (1M48) and TNF-α (2AZ5), underscoring the significance of understanding the distinct binding site characteristics of each target protein, as ligand efficacy varies across different proteins.

Conclusion

To conclude, in this comprehensive exploration of *T. pallida* Brandis. fruit extracts, a multidisciplinary approach was adopted to uncover the potential of its phytochemicals as immunomodulatory agents. The integrated GCMS and LCMS analyses revealed the richness of the extract with phenolic compounds, and *in-silico* docking studies against the selected targets of provided a holistic understanding of the compounds' interactions with the immune system. *In-silico* docking studies predicted interactions between these compounds and immune-related proteins IL-2 and TNF-α with best affinity towards Resveratrol (A Stilbenoid; Phenol) and Arctiin (Lignan; Phenol) respectively. It indicates that the phenolic compounds have good interactions with these targets and validated the effect of the extract on immune responses. This interconnected approach advanced our comprehension of *T. pallida's* potential to modulate immune functions. These studies guide us to prove the immune stimulant properties of *T.pallida* using appropriate *in vitro* and *in vivo* models.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 3: LCMS Analysis of fruit extract of *T. pallida* using alcohol

Sr No.	Phytocompounds	Base	Adduct		ES	Mol.Wt.
1.	Orientin	329.4	[M+H]	1	+	447.5
			[M+K]	37.9		
			[M+2ACN+H]	82.0		
2.	Arctiin	339.3,371	[M + H] 2		+	534.2
3.	Quercetin	205.3	[M+H] 1		+	301
4.	3,4-O-dicaffeoyl-epi-quinic acid methyl ester	485.6	[M+H] 1		+	530.6
5.	Arjunolic acid	385.3	M+2ACN+H -82		+	488
6.	Stigmasterol	158.3	[M+H] 1		+	412.7
7.	Terminoside A	465.4	[M+H] 2		+	650.8
8.	Beta-sitosterol	399.4	[M + H] 2		+	414.3
9.	Arjunetin	411.6	[M + H] 2		+	650.8
10.	Purpurin	255.4	[M-H]2		-	255.03
			[M-H]1		-	255.03
11.	Pteropodine	337.2	[M-H]1		-	368.33
12.	2-o-acetylorochrine	337.2	[M-H]1		-	384.5
13.	Quercetin-3-glucuronide	183.1	[M-H]1		-	478
14.	Corilagin	633.3	[M-H]2		-	636.46
15.	Linoleic acid	227.3	[M-H]1		-	278
16.	Oleic acid	255.4	[M-H]1		-	282
17.	Isoquercetin	281.4	[M-H]1		-	464.4
18.	Strophanthidol	283.4	[M-H]1		-	406
19.	Lupenone	329.6	[M+H] 1		+	424.7
20.	Quercetin	205.4	[M+H] 1		+	301
21.	Resveratrol	227.6	[M-H]1		_	228.25

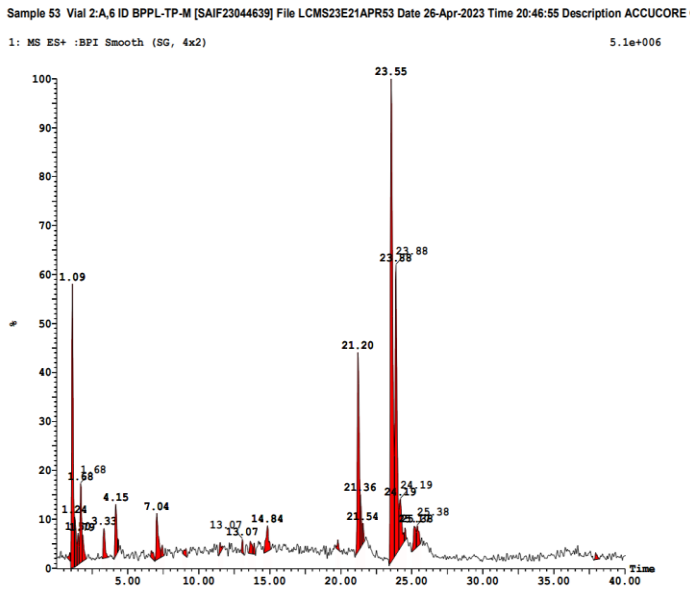

Figure 2: LCMS Chromatogram in Positive Mode

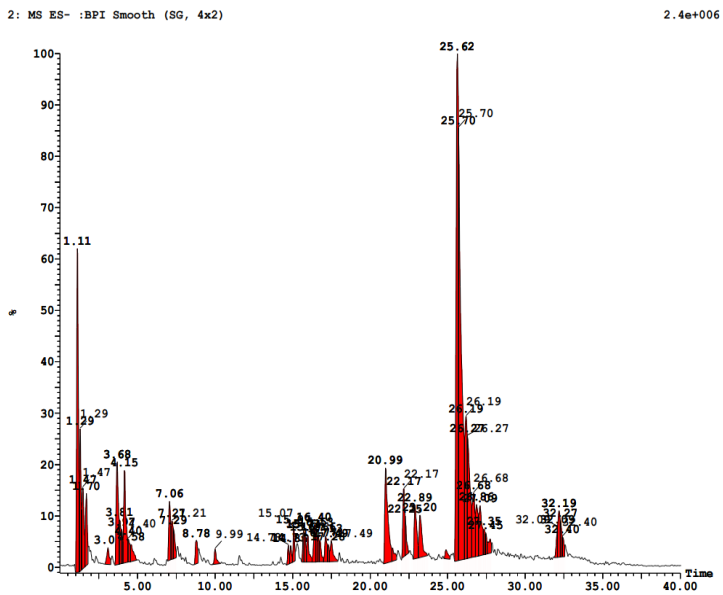

Figure 3: LCMS Chromatogram in Negative Mode

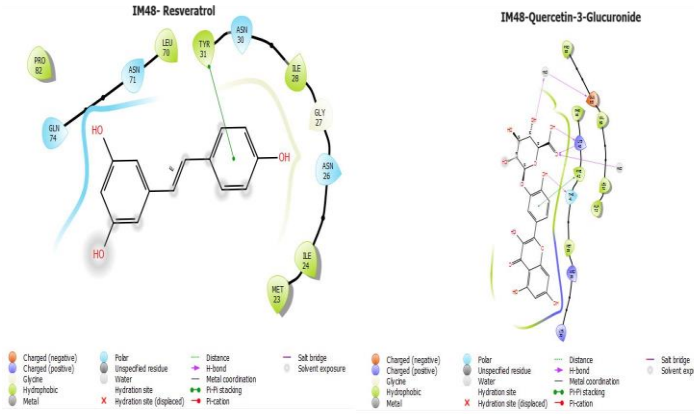

Figure 4: Ligand interaction with IM48

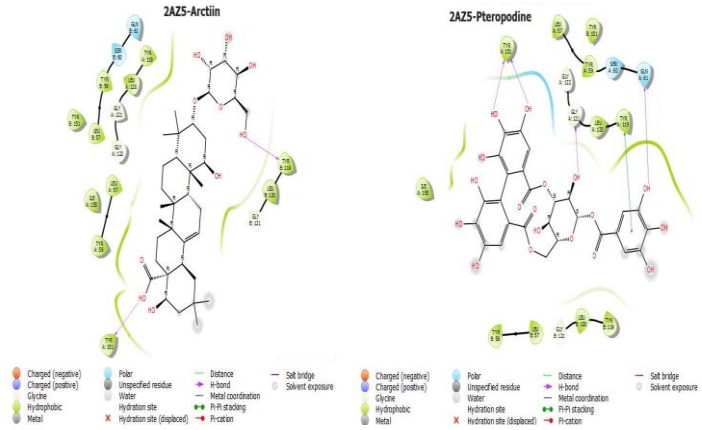

Figure 5: Ligand interaction with 2AZ5

Table 4: Docking results of the selected ligands on Human IL-2 (1M48)

Ligand	Binding score	pi-pi interactions	H-bond
Resveratrol.1	-6.565	PHE	THR, LEU
Quercetin-3-Glucuronide.1	-6.3	PHE	LYS, THR
3,4-Di-O-caffeoylquinic acid methyl ester.1	-5.39	-	LYS, THR, LEU
Corilagin.1	-5.372	-	GLU, LYS
Purpurin.1	-5.025	-	LEU
Pteropodine.1	-5.005	-	LYS
Quercetin.1	-4.776	PHE	LYS
Arctiin.1	-4.293	-	ARG, LEU
Terminoside A.1	-4.171	-	GLU, LYS, THR
Orientin.1	-3.941	-	GLU, LYS
isoquercetin.1	-3.624	-	ARG, THR
2-O-Acetylorochrine.1	-3.412	-	LYS
gamma Tocopherol.1	-3.336	-	-
Squalene.1	-3.074	-	-
Cholest-4-en-3-one.1	-2.94	-	-
Methyl erucate.1	-2.762	-	-
Arjunetin.1	-2.75	-	-
Drostanolone.1	-2.605	-	-
Withaferin-A.1	-2.321	-	-
Arjunolic acid.1	-2.31	-	-
alpha-Tocopherol-beta-D-mannoside.1	-2.27	-	-
Stigmasterol.1	-2.2	-	-
beta-sitosterol.1	-1.6	-	-
lupenone.1	-1.595	-	-
Stigmast-5-en-3-ol oleate.1	-0.752	-	-
Methyl elaidate.1	0.43	-	-
Methyl linoleate.1	0.939	-	-

Table 5: Docking results of the selected ligands on Human TNF-α (2AZ5)

Ligand	Binding score	pi-pi interactions	H-bond
Arctiin.1	-6.365	TYR	TYR-A&B, GLN
Pteropodine.1	-6.333	-	-
Terminoside A.1	-6.32	-	TYR-A&B
Corilagin.1	-6.282	TYR-A&B	GLN, GLY, TYR
Quercetin.1	-6.116	-	-
Drostanolone.1	-5.844	-	-
3,4-Di-O-caffeoylquinic acid methyl ester.1	-5.808	TYR	PRO, TYR-A&B, LYS
Resveratrol.1	-5.777	-	TYR
Purpurin.1	-5.766	TYR-A&B	TYR-A&B
Quercetin-3-Glucuronide.1	-5.752	-	-
alpha-Tocopherol-beta-D-mannoside.1	-5.534	-	-
Squalene.1	-5.501	TYR	TYR-A&B, SER
Orientin.1	-5.434	-	-
2-O-Acetylorochrine.1	-5.393	-	LEU
Stigmasterol.1	-5.261	TYR-A&B, LEU	TYR, SER
gamma Tocopherol.1	-5.26	-	-
beta-sitosterol.1	-5.221	-	TYR
isoquercetin.1	-5.215	-	GLN, SER
Cholest-4-en-3-one.1	-5.164	-	-
Arjunolic acid.1	-4.965	-	TYR-A&B, GLU
Withaferin-A.1	-4.906	TYR	TYR, GLN
Strophanthidol	-4.513	-	SER
(z)-docos-13-enamide	-4.385	-	-
lupenone.1	-4.349	-	-
Methyl erucate.1	-4.347	-	GLY, TYR, LEU, SER
Methyl linoleate.1	-1.994	-	TYR
Methyl elaidate.1	-0.623	-	-

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