Exploring the Antipyretic Activity of Enicostemma axillare (Nahi): A Computational Approach

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Insilico, Enicostemma axillare, Network pharmacology, Pyrexia

Abstract
This study aims to perform the insilico analysis of Enicostemma axillare (Nahi) which are well-known for its antipyretic properties and investigate for its active compounds by using computational results with traditional application. Using network pharmacology, pharmacokinetic properties i.e. ADME (Absorption, Distribution, Metabolism, Excretion), tools for toxicity prediction and analyze, through this it evaluates bioactive compounds against specific molecular targets which linked with pyrexia. The therapeutic efficacy and safety of selected compounds for additional investigation were validated by ADME and toxicity tests. Bioinformatics databases such as IMPPAT, PubChem, SwissADME, and pkCSM are used to identify Nahi bioactive compounds, as well as their structural and pharmacokinetic properties. The bioactive compounds were screened for drug-likeness properties using Limpki's Rule of 5, followed by target prediction and pathway enrichment analysis by using Swiss Target Prediction and KEGG pathway. The important connections were identified using visualization and network design tools i.e. Cytoscape 3.10.2. The binding affinity between the target and the bioactive compounds are identified using Autodock vina. The application of network pharmacology will give an in depth understanding of the plant's medicinal potential, which corresponds with Ayurveda's multi-target technique. This study emphasizes the value of combining traditional medicine into modern healthcare systems, encouraging innovation, and expanding treatment alternatives by showcasing the potential of Ayurvedic plants in current drug development.

Introduction

Pyrexia is the first and most common symptom of a patient. It can be caused through various pathways and cytokines (proinflammatory and anti-inflammatory cytokines), and pyrogen, and is mainly involved in pyrexia. Commonly, the pyrexia is usually treated with NSAIDs. However, they often produce major side effects such as kidney failure, hepatotoxicity, and increased risk of bleeding. (Poir et al., 2024) In Ayurved, Enicostemma axillare a perennial herb is known as "Nahi" and is useful in various diseases such as pyrexia, diabetes, obesity, cough, stomach pain, snake bite, etc. (Nangare, 2023) Previous studies suggested that various bioactive compounds of Nahi are effective against pyrexia. (Nangare, 2023)However, in silico studies have not yet been conducted. Computational studies help to identify the pathways of cytokines for various inflammatory conditions and analyze the binding ability and binding site interaction with bioactive compounds through molecular docking.

This study seeks to investigate the antipyretic potential of E. axillare by identifying bioactives, predicting their targets, mapping their interactions in fever-related pathways, and validating their effectiveness using molecular docking. To achieve this goal, first bioactive compounds were identified using IMPAAT and then their drug-likeness was assessed. The swissTargetPrediction database was utilized to predict biological targets. Following this, protein-protein interaction (PPI) analysis and KEGG pathway analysis were done. Lastly, network pharmacology techniques and molecular docking studies were conducted. This study helps to validate the effectiveness of E. axillare and investigate their expected targets and signaling pathways in inflammation.

Materials and Methods

Identification of Enicostemma axillare bioactives

Bioactives present in Enicostemma axillare were screened through literature and the IMPPAT database (Mohanraj et al., 2018). The IMPPAT database lists phytochemical compounds that can be identified in plants. The structure data file (.sdf) from PubChem's (Kim et al., 2019) database of phytochemical compounds and their phytochemistry was downloaded. This made it possible to screen for phytochemicals with potential pharmacological effects in a methodical manner. These phytochemicals were further investigated to determine their pharmacokinetic features.

Druglikeness of bioactive of Enicostemma axillare

Each phytoconstituent was screened for its Drug likeness property based on 'Rule of 5' that is Lipinski's rules (Chen et al., 2020) using SwissADME (Daina et al., 2017) and pkCSM server (Pires et al., 2015). Using this two software are used to screen the pharmacokinetic properties of phytochemical compounds. This pharmacokinetic analysis is done by using this software that helps to identify the bioactive compound to analyze the further docking process.

Target identification of bioactive from Enicostemma axillare

The targets of all phytochemicals were identified using Swiss Target Prediction (Gfeller et al., 2014), and UNIPROT (Boutet et al., 2016) was used to standardize the projected target gene names. Using Swiss Target Prediction, the targets can predict bioactive compounds in humans and other vertebrates. The genes that are screened through Swiss Target Prediction software can be standardized using UNIPROT database. Disease target genes were identified using Genecards (The Human Gene Database) version 5.21 (Stelzer et al., 2016).

Estimation of overlapping genes

By using VENNY 2.1 (Oliveros, n.d.) overlapping genes of both disease targets and bioactive targets are sorted by generating a Venn diagram of each bioactive target. These sorts the overlapped genes between targets and bioactive compounds. From this, the common genes that are interconnected with disease targets and bioactive targets are sorted. This makes it possible to identify the important molecular participant in the fever response.

Protein-Protein Interaction (PPI)

PPI was screened by inserting the overlapping genes in the multiple protein search tool in STRING (Szklarczyk et al., 2017) for the retrieval of interacting genes and proteins. This STRING software creates protein-protein interaction; this helps to sort out the data according to the pyrexia. Then common genes are inserted into STRING for protein-protein interaction. This step requires finding the regulatory hubs that are essential to the modulation of fever.

KEGG (Kyoto Encyclopedia of Genes and Genome) Pathway Analysis

By analysis through the STRING database, the pathways of interacting genes are screened through KEGG Pathways (Kanehisa et al., 2017) based on the literature and the pathways. By analyzing the protein-protein interaction from STRING we get the pathways through which these genes are involved. This aids in figuring out if E. axillare works by influencing the production of prostaglandins, cytokine signaling, or inflammation, which are key mediators of fever.

Network Construction and Analysis

The network between the bioactive targets and pathways was built using Cytoscape 3.10.2 (Shannon et al., 2003). The network was directed and interpreting the entire network using edge count, the generated network was assessed using the "Network Analyzer" tool. Depending on the information used and the time taken to retrieve the data, the number of nodes for compounds, targets, and pathways may vary.

Docking Analysis

Sorted the pathways according to our requirements by studying and understanding each pathway. By using Autodock vina docking (Trott & Olson, 2010) is carried out by converting the files into pdbqt. To obtain compound-target interaction and visualize for pose scoring minimum binding energy in Autodock Vina. This docking is done by ligand-specific docking with the help of CASTp3.0 (Tian et al., 2018) The grid box for the docking process is done by ligand-specific docking. The grid box is drawn only on the active site of the protein. This helps to study the surface topography of protein and minimum binding energy along with the binding site interaction with bioactive compounds.

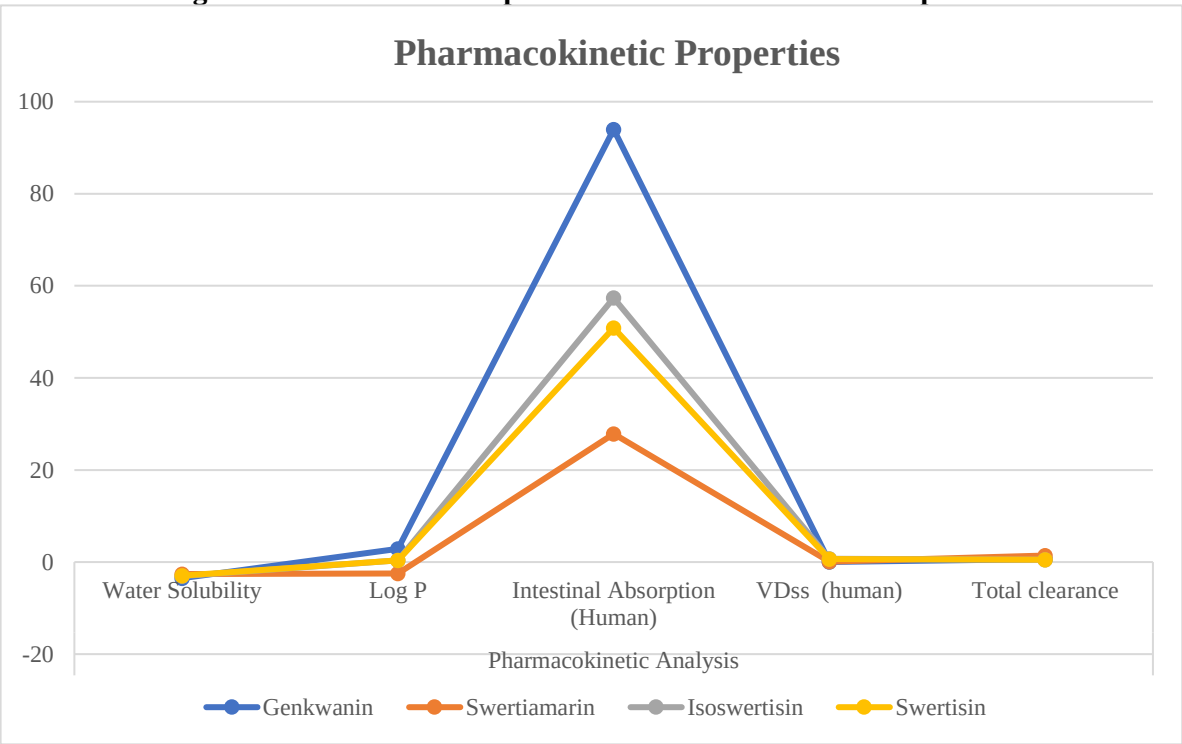
Results

Screening of bioactives and Drug Likeness of Enicostemma axillare

54 active compounds of Enicostemma axillare were found by using the IMPPAT database and literature study and these all compounds were downloaded in sdf format from PubChem. All 54 compounds were analyzed in the pkCSM server and SWISS-ADMET software for the drug-likeness properties, among them 47 compounds showed positive drug-likeness properties along with positive Lipinski's rules based on molecular weight, hydrogen bond donor, hydrogen bond acceptor, partition coefficient (Log P). From 47 phytoconstituents we

selected Genkwanin, Swertiamarin, Isoswertisin, and Swertisin for our antipyretic studies by comparing all data and studies. (Adetunji et al., 2023) In Fig. 1 the Pharmacokinetic properties of selected bioactive compounds are shown.

Fig.1 Pharmacokinetic Properties of Selected Bioactive Compounds.



The chart compares the pharmacokinetic properties of Genkwanin, Swertiamarin, Isoswertisin, and Swertisin. Genkwanin shows the highest Log P (~100), indicating high lipophilicity, with moderate intestinal absorption and VDss. Swertiamarin has the lowest values across all parameters. Isoswertisin and Swertisin show moderate Log P (~60 and ~40, respectively) with significant intestinal absorption and VDss. All compounds exhibit negligible water solubility and total clearance. Overall, Genkwanin stands out for its lipophilicity, while Swertiamarin has the weakest pharmacokinetic profile.

Target identification of bioactives from Enicostemma axillare

Protein targets for 4 phytocompounds were predicted using Swiss Target Prediction. Screened bioactives are summarized below in Table 1

Table 1: -Screened Bioacitve of Enicostemma axillare with their disease targets

Phytoconstituents	Molecular Formula	PubChem IDs	Name of Targets
Genkwanin	C16H12O5	5281617	NOS2, F2, PTGS2, KIT, CFTR, EGFR, PIK3CG, SYK, TERT, ARG1, CSNK2A1, MMP9, ALOX5, CDK6, IKBKB, PLG, PPARG, CALM1, MMP2, PARP1, PIM1, GSK3B
Swertiamarin	C16H22O10	442435	ELANE, IL2, PTGS2, CFTR, EGFR, HSP90AA1, TYR, PTGES, MMP9, PIM1,
Isoswertisin	C22H22O10	44258317	TNF, TP53, IL2, KIT, JAK2, PIK3CA, EGFR, ABCB1, CDK4, CHEK2, HSP90AA1, PTGES ALOX5, CDK6, IKBKB, NQO1, CDK9, CDK2, CALM1, HSP90AB1, PRKACA,

Swertisin	C22H22O10	124034	TNF, NOS2, TP53, IL2, PTGS2, KIT, CFTR, EGFR, SYK, TERT, PTGES, ARG1, CSNK2A1, MMP9, ALOX5, CDK6, PLG, CYP19A1, PPARG, MMP2, PARP1, PIM1, PRKACA, GSK3B, PRKCB,

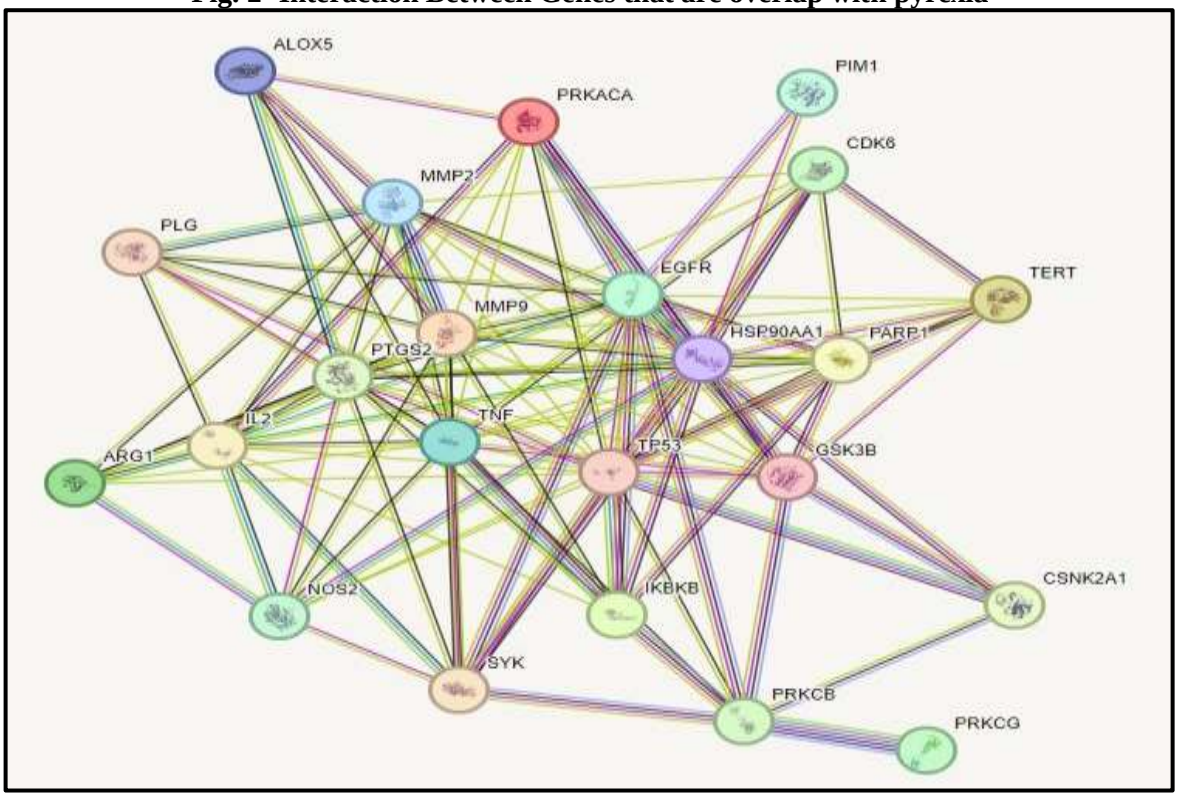
Estimation of Overlapping Genes

Seventy-nine overlapping genes were identified by VENNY 2.1 for Enicostemma axillare

Protein-protein interaction

Out of Seventy-nine overlapping genes, twenty-four targeted genes were contributing to inflammation during any disease. Then, Targeted genes were assessed using the STRING database which gives a protein-protein interaction network with a moderate confidence score of 0.4. By analysis of the protein-protein interaction network, we get confirmed results of the gene set that is responsible for the Inflammation pathway. The 24 genes are: TNF, PRKCB, PRKCG, PTGS2, CSNK2A2, SYK, IKBKB, PARP1, MMP9, GSK3B, HSP90AA1, ARG1, NOS2, PRKACA, IL2, PIM1, NOX4, MMP2, CDK6, EGFR, TP53, ALOX5, TERT, PLG. In Fig 1, the interaction between these genes is shown using the STRING database.

Fig. 2- Interaction Between Genes that are overlap with pyrexia



KEGG Pathway Analysis

By using the KEGG pathway, the study of 24 targeted genes and disease-intersecting targets was done. The proteins that are involved in those pathways are also studied through this database. Around 21 pathways were associated with inflammation which are mentioned in Table 2.

Table 2- Gene set enrichment analysis of Inflammation Pathway which is responsible for Fever

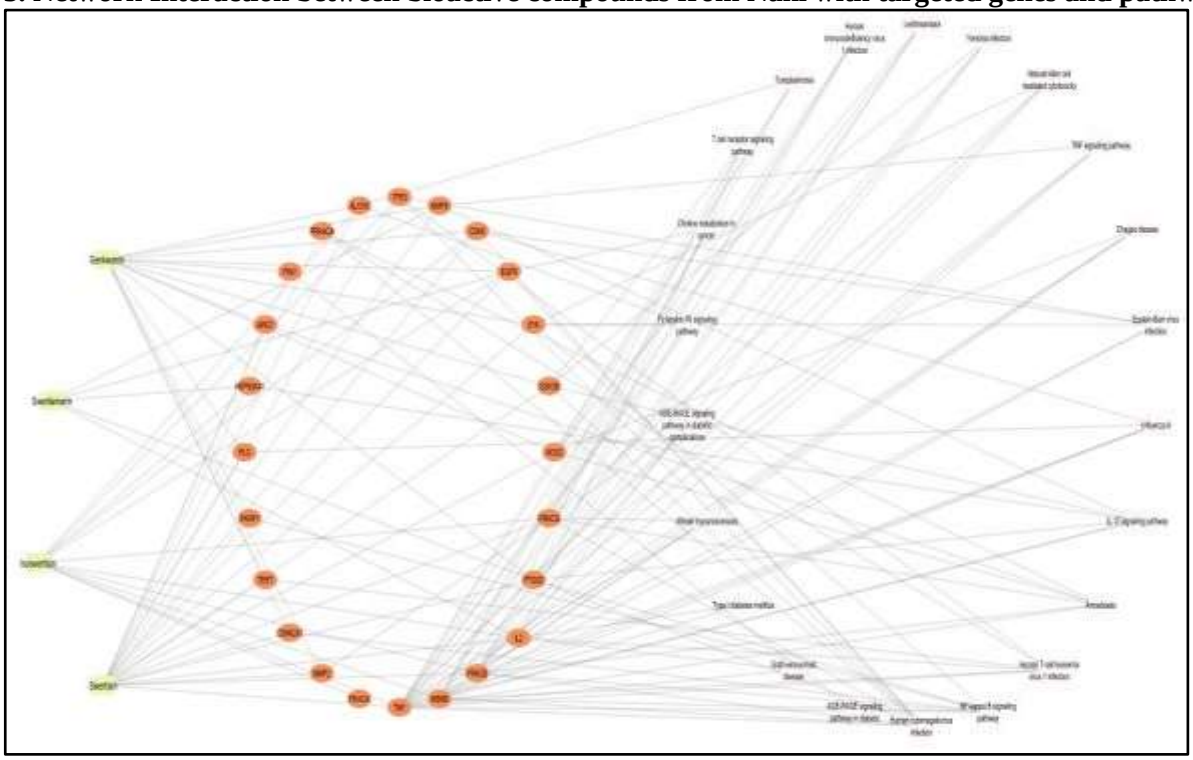
KEGG ID	Pathways	No of targets	Targets
hsa05143	African trypanosomiasis	3	TNF, PRKCB, PRKCG
hsa04064	NF-kappa B signaling pathway	7	PTGS2, PRKCB, CSNK2A1, TNF, SYK, IKBKB, PARP1

hsa04657	IL-17 signaling pathway	6	MMP9, TNF, PTGS2, GSK3B, HSP90AA1, IKBKB
hsa05146	Amoebiasis	6	TNF, PRKCB, PRKCG, ARG1, NOS2, PRKACA
hsa05140	Leishmaniasis	4	PTGS2, PRKCB, TNF, NOS2
hsa05332	Graft-versus-host disease	2	TNF, IL2
hsa04940	Type I diabetes mellitus	2	TNF, IL2
hsa04933	AGE-RAGE signaling pathway in diabetic complications	5	PIM1, NOX4, PRKCB, TNF, MMP2
hsa05163	Human cytomegalovirus infection	10	PTGS2, TNF, PRKCB, PRKCG, PRKACA, GSK3B, CDK6, IKBKB, EGFR, TP53
hsa04664	Fc epsilon RI signaling pathway	3	SYK, TNF, ALOX5
hsa05142	Chagas disease	4	IKBKB, NOS2, TNF, IL2
hsa04660	T cell receptor signaling pathway	4	IKBKB, TNF, IL2, GSK3B
hsa05145	Toxoplasmosis	4	IKBKB, TNF, NOS2, ALOX5
hsa05170	Human immunodeficiency virus 1 infection	4	IKBKB, TNF, PRKCB, PRKCG
hsa05169	Epstein-Barr virus infection	4	TNF, IKBKB, TP53, CDK6, SYK
hsa05166	Human T-cell leukemia virus 1 infection	6	TERT, IKBKB, TNF, PRKACA, TP53, IL2
hsa05164	Influenza A	5	PLG, PRKCB, TNF, IKBKB, CDK6
hsa05231	Choline metabolism in cancer	3	EGFR, PRKCB, PRKCG
hsa05135	Yersinia infection	4	IKBKB, TNF, IL2, GSK3B
hsa04650	Natural killer cell-mediated cytotoxicity	4	TNF, SYK, PRKCB, PRKCG
hsa04668	TNF signaling pathway	4	TNF, IKBKB, MMP9, PTGS2

Construction of Network and analysis

There is a total of 21 pathways and targeted genes that related to inflammation were screened by network analysis. Network analysis was constructed with the use of Cytoscape 3.10.1 version which gave 50 nodes and 146 edges including 21 pathways and 24 targeted genes.

Fig 3. Network Interaction between bioactive compounds from Nahi with targeted genes and pathways



Docking Analysis Results

Molecular docking was performed by using Autodock vina on selected bioactive compounds and the proteins that are involved in inflammation i.e. proinflammatory and anti-inflammatory cytokines (TNF Alpha, IL1 Beta, IL6, IL10, IL13). The interaction site between the bioactive compounds and specific cytokines was predicted by selecting PDB files of cytokines using the Protein Data Bank database.

PDB Files used for molecular docking-

TNF ALPHA- PDB ID 2AZ5 (Proinflammatory cytokines)
IL1 BETA- PDB ID 1ITB (Proinflammatory cytokines)
IL6- PDB ID 1ALU (Proinflammatory as well as Anti-inflammatory cytokines)
IL10- PDB ID 2ILK (Anti-inflammatory cytokines)
IL13- PDB ID 3BPO (Anti-inflammatory cytokines)

This helps to identify the minimum binding energy values from molecular docking of bioactive compounds with 2AZ5, 1ITB, 1ALU, 2ILK, and 3BPO. The grid boxes are made according to the surface topology analysis of PDB files using the CASTp 3.0 (Computed Atlas of Surface Topography of Proteins) server. This identifies the active site of protein according to the grid box prepared for the docking process. This docking process is all done using Autodock Vina and analyzed through Autodock Tools (ADT). The dimensions of the grid box between cytokines and bioactive compounds are shown in Table 3.

Table 3: Grid Box Dimensions are shown between Cytokines and Bioactive compounds

Pro-inflammatory & Anti-inflammatory Cytokines	**Grid Box Dimensions (x, y, z)**			
	Genkwanin	**Swertiamarin**	**Isoswertisin**	**Swertisin**
TNF Alpha	(-10.380, 71.577, 29.992)	(-10.337, 73.547, 27.974)	(-10.604, 71.777, 29.032)	(-17.496, 75.466, 26.191)
IL1 Beta	(20.44, 5.518, 12.881)	(17.336, 4.657, 10.684)	(19.072, 4.069, 14.47)	(21.91, 1.919, 11.588)
IL6	(-1.339, -0.306, 4.119)	(0.1, 1.667, 4.375)	(1.049, 2.442, 2.565)	(-2.865, -2.938, 0.453)

IL10	(6.054, 54.096. 31.739)	(6.555, 52.603, 34.202)	(4.985, 53.287, 33.242)	(6.689, 50.844, 32.739)
IL13	(-12.527, -10.519, -19.642)	(-12.247, -12.166, -20.852)	(-14.382, -10.642, -20.164)	(-15.346, -8.726, -12.587)

Table 3. presents grid box dimensions (in x, y, z coordinates) for molecular docking interactions between various bioactive compounds (Genkwanin, Swertiamarin, Isoswertisin, and Swertisin) and cytokines involved in proinflammatory and anti-inflammatory responses (e.g., TNF Alpha, IL1 Beta, IL6, IL10, IL13). These coordinates likely represent the optimal positions for binding each compound to the respective cytokine, indicating the spatial configuration for potential bioactive interactions. In Table 4 the minimum binding energy among all the bioactive compounds is shown by Swertisin against TNF Alpha and IL1 Beta which is -8.0 Kcal/mol and -7.8 Kcal/mol. Genkwanin also shows the minimum binding energy against TNF Alpha and IL13 which is -7.9 Kcal/mol and -7.8 Kcal/mol according to ligand-specific docking.

Table 4: - Minimum Binding energy and the Binding Site Interaction.

Bioactive Compounds	Proinflammatory & Anti-inflammatory Cytokines	Binding Energy (Kcal/mol)	Interaction
Genkwanin	TNF Alpha	-7.9	ARG73, PHE115,
	IL1 Beta	-7.2	ARG9, PRO28, ILE90, GLU10
	IL6	-6.9	LYS68, PHE175, MET69, SER178, SER171, LEU66
	IL10	-6.8	LEU26, PHE30, LEU94, TYR72, MET77
	IL13	-7.8	ASP75, VAL76, SER69, ILE53, PHE44
Swertiamarin	TNF Alpha	-7.1	TYR110, TYR142, LEU111,
	IL1 Beta	-7.1	LEU26, GLU25, LEU82, TYR24, LEU 80, PHE133, LEU134, THR79, LYS74, GLN81
	IL6	-6.4	SER171, LEU66, GLU174, LEU167, MET69
	IL10	-6.4	ARG107, SO4601
	IL13	-7.1	ASN133, GLN250, ASN289
Isoswertisin	TNF Alpha	-7.9	ILE146, TYR50, GLY112, TYR110, TYR142,
	IL1 Beta	-7.2	VAL132, GLU25, PRO131, MET33, PRO31, LYS74, THR79, GLN81, LEU82, TYR24, LEU80, LEU134, PHE133
	IL6	-6.5	SER171, LYS68, SER178, MET69, PHE175
	IL10	-6.9	PHE30, ILE94, ALA80, PHE37, LEU94, PHE30
	IL13	-7.3	ILE50, LYS48, PHE39, LYS45, PRO82, GLU80, GLN73,
Swertisin	TNF Alpha	-8.0	TYR50, TYR142, TYR110, LEU111, GLY112, HIS8
	IL1 Beta	-7.8	PRO131, LYS77, THR137, GLY136, ASP142, PRO31
	IL6	-6.3	LEU64, LEU66, GLU61, LEU167, SER171, ARG181, PHE76, ARG170

	IL10	-7.1	PHE30, ALA80, VAL91, PHE37, LEU46, ASP41
	IL13	-7.5	PHE39, PRO82, LYS48, MET83, LYS90, SER75, TRP35

The table showcases the binding affinities of various bioactive compounds to different proinflammatory and anti-inflammatory cytokines. A lower binding energy signifies a stronger interaction. The compounds exhibit varied binding patterns, indicating their potential to modulate cytokine activities. This data provides insights into their potential therapeutic applications in inflammatory condition

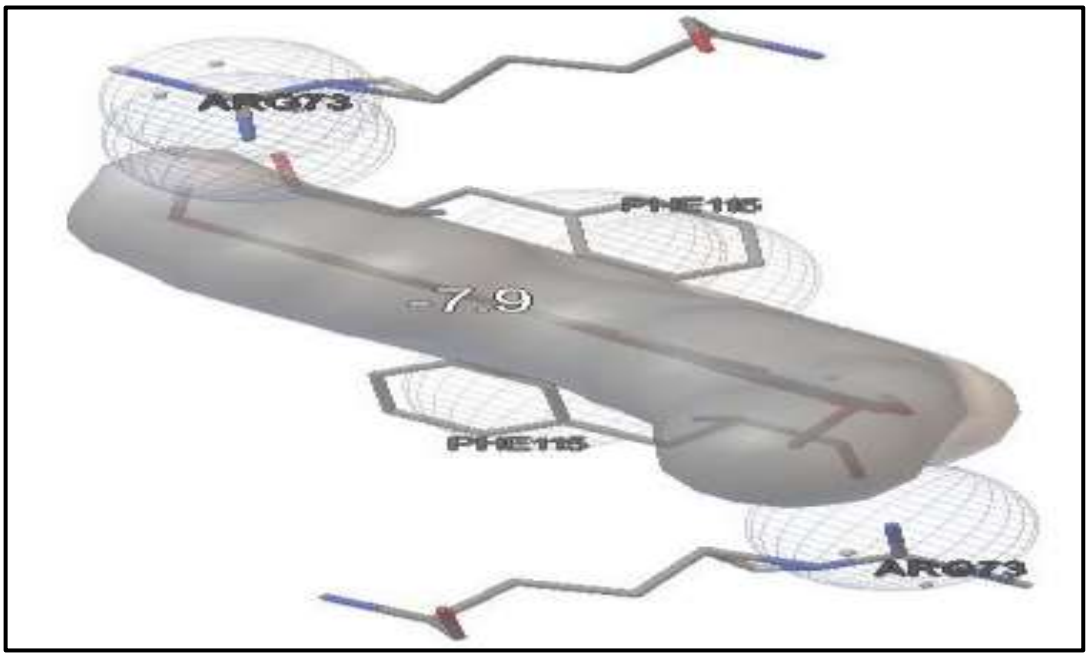


Fig. 4- 3D representation of Interaction of TNF Alpha receptor with Genkwanin

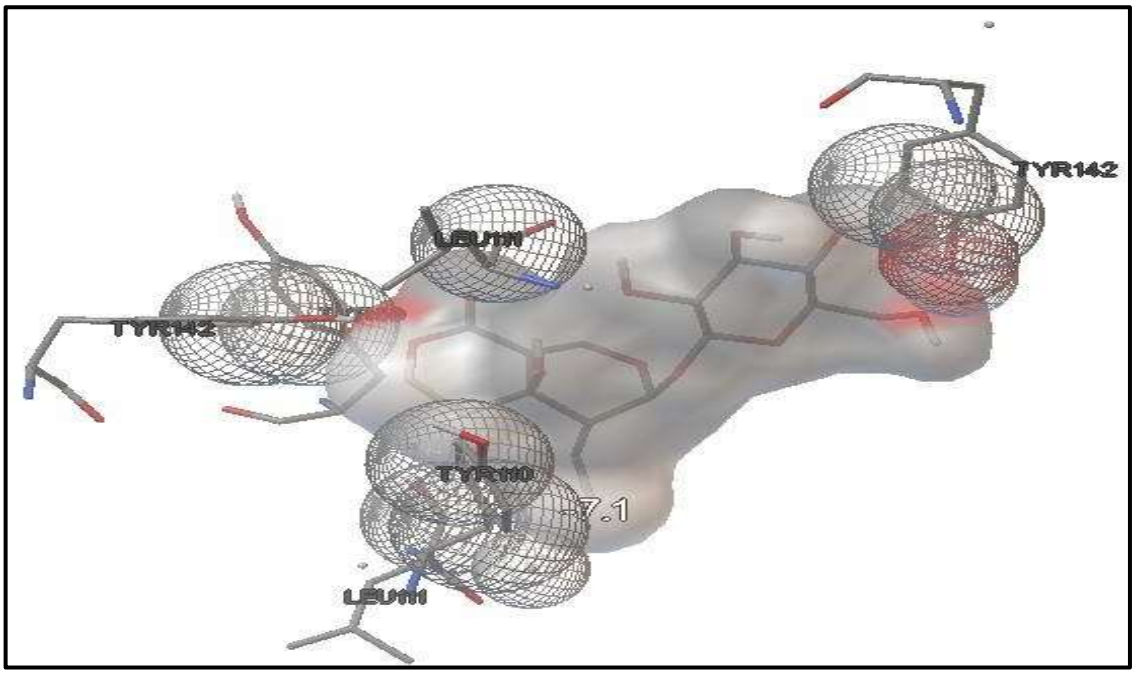


Fig. 5- 3D representation of Interaction of TNF Alpha receptor with Swertiamarin.

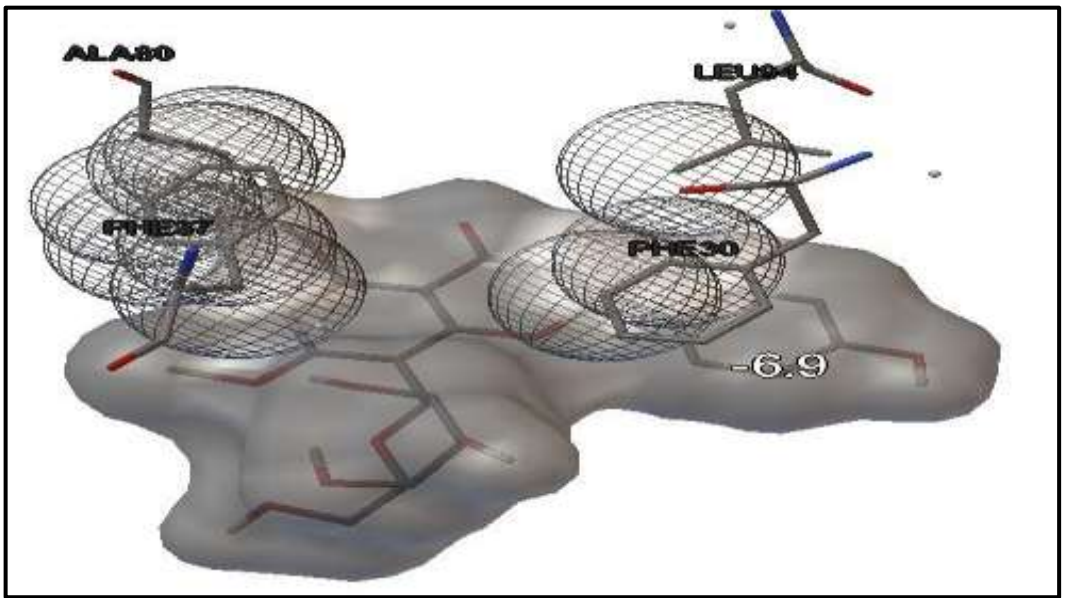


Fig. 6- 3D representation of Interaction of IL10 receptor with Isoswertisin.

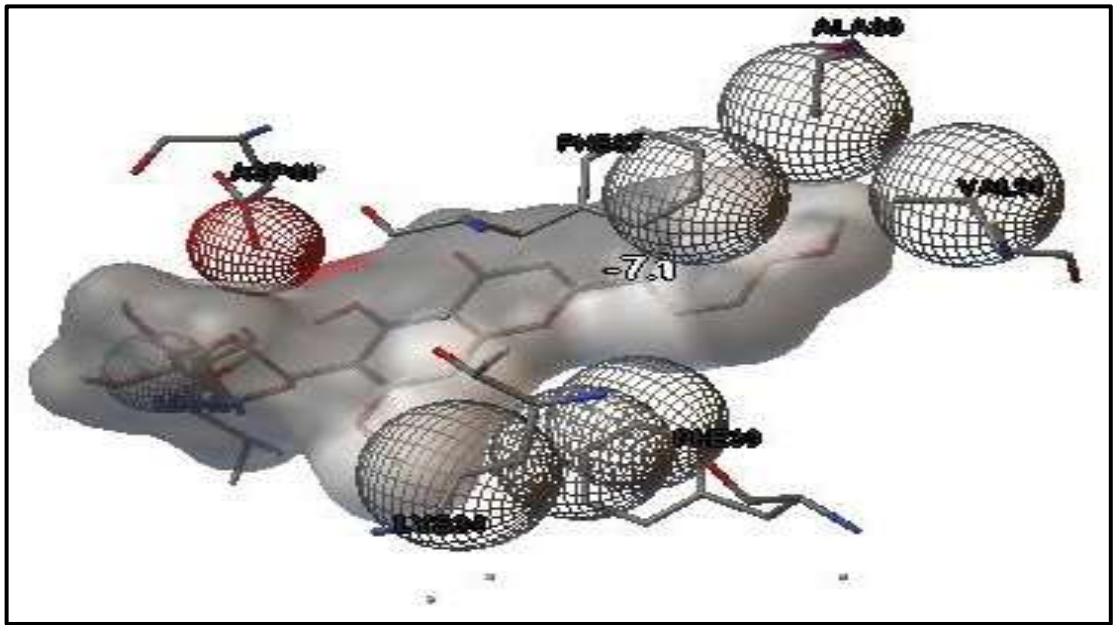


Fig. 7- 3D representation of Interactionof IL10 receptors with Swertisin.

Discussion

The pharmacokinetic parameters (Fig 1) of Genkwanin, Swertiamarin, Isoswertisin, and Swertisin provide important clinical information. Genkwanin has high lipophilicity, intestinal absorption, and tissue distribution, making it potentially useful for oral usage, but it also increases the danger of tissue buildup and toxicity. Swertiamarin, having limited absorption and dispersion, may necessitate alternate administration techniques for therapeutic effectiveness. Because of their moderate distribution and absorption, isoswertisin and swertisin appear to have better-balanced profiles for oral usage with a lesser risk of toxicity. All drugs have low clearance rates, which suggest prolonged activity but call for cautious dosage control to prevent buildup. All things considered, these insights aid in directing formulation, dosage, and safety concerns during the medication development process. Figure 2 depicts a protein-protein interaction (PPI) network in which core proteins such as TP53, EGFR, TNF, and HSP90AA1 serve as regulatory hubs in processes such as inflammation, cell proliferation, and death. MMP9, PTGS2, and ALOX5 proteins are implicated in inflammation and tissue remodeling, which suggests a relationship to disease development. The network focuses on possible treatment targets for inflammatory or cancer-related disorders.

Table 2. provides a gene set enrichment analysis of inflammation-related pathways associated with fever, detailing KEGG pathways like NF-kappa B and TNF signaling. Each pathway includes specific molecular

targets (e.g., TNF, PTGS2, IKBKB) that play roles in triggering inflammation, a common cause of fever. This analysis highlights key pathways and targets that could be explored for therapeutic intervention in fever-related inflammatory conditions.

The docking results might show that the compound has a high binding affinity for pro-inflammatory cytokines (TNF-α, IL-1, IL-6), but it either inhibits or alters their activity. This could be represented by the compound binding to key regions of these cytokines and preventing their normal function or interaction with their receptors. By comparing the minimum binding energy of docking,

Genkwanin showed binding energies ranging from -6.8 to -7.9 kcal/mol across different cytokines, with the highest affinity for TNF Alpha (-7.9 kcal/mol).

Isoswertisin displayed binding energies from -6.5 to -7.9 kcal/mol, also with the highest affinity for TNF Alpha (-7.9 kcal/mol).

Swertiamarin had slightly lower binding energies ranging from -6.4 to -7.1kcal/mol, showing consistent interaction with multiple cytokines but with slightly less affinity compared to Genkwanin and Isoswertisin.

Swertisin exhibited binding energies from -6.3 to -8 kcal/mol, with the highest affinity for TNF Alpha (-8 kcal/mol).

TNF Alpha consistently shows the highest binding affinity for all the ligands, suggesting these compounds could be strong inhibitors of TNF Alpha, a cytokine heavily involved in inflammation and autoimmune diseases.

IL-6 and **IL-1 Beta** also showed good affinity across all ligands, indicating potential anti-inflammatory activity by inhibiting these pro-inflammatory cytokines.

IL-10 and **IL-13** displayed variable affinity, with some ligands like Isoswertisin and Swertisin showing significant binding, suggesting a potential for modulating immune responses.

Conclusion

The strong binding affinity of these ligands, notably with TNF Alpha, IL-6, and IL-1 Beta, implies that they might be used to treat inflammatory illnesses such as rheumatoid arthritis, Crohn's disease, and other autoimmune disorders where these cytokines play an important role. These ligands might be turned into new anti-inflammatory or immunomodulatory medications, particularly for situations where present treatments are ineffective or have considerable adverse effects. Because of their high binding affinity and interaction with various cytokines implicated in inflammatory pathways, genkwanin, and isoswertisin appear to be excellent lead molecules for therapeutic development. Swertiamarin and Swertisin may also be useful, particularly in diseases where regulating IL-10 and IL-13 is advantageous, such as allergies or certain chronic inflammatory illnesses. To ensure proper binding and neutralization of therapeutic antibodies or fusion proteins targeting TNF-α, it's important to understand the critical residues involved in its interaction with the receptor. Understanding the involvement of Arg73 can help engineers develop more effective biologics that disrupt these relationships without interfering with other immune system processes.

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