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Research

In Silico Screening of Phytoconstituents from *Hypericum* sp. as JAK3 Inhibitors for Autoimmune Disorders

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Abstract:
Janus kinase (JAK)s has become a viable therapeutic target, for autoimmune and inflammatory diseases. However, its use is complicated by over 50 different cytokines involved in immune responses, potentially leading to adverse consequences. Among JAKs JAK3 has substantial immunomodulatory effects. Indeed, JAK3 emerges as an exceptionally appealing target for therapeutic interventions in autoimmune and inflammatory disorders. Therefore, it is critical to develop specific JAK3 inhibitors. The present investigation utilized Pyrx and to conduct virtual screening and molecular docking analysis on the active components from one of the species of *Hypericum* i.e. *Hypericum perforatum*, with the JAK3 receptor. Among the phytoconstituents hypericin, demonstrated an exceptional affinity (-12.4 kcal/mol) for the JAK3 protein. Additionally, hypericin exhibited favorable ADMET properties as predicted by pkCSM and Swiss ADME, supporting its potential as a drug candidate. The findings suggest that hypericin might serve as a viable therapeutic alternative for autoimmune disorders. However, in vivo and in vitro investigations specific to JAK3 are required to validate and extend these results.

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1-Introduction

Recent studies have shown that more than 50 million people have autoimmune diseases serving as third most common diseases after cancer and heart disease [1]. Autoimmune diseases arise due to dysfunctions within the immune system, contributing substantial rates of both mortality and incidence [2]. The pathological response in autoimmune and inflammatory diseases are triggered by the release of cytokines such as interleukins, tumor necrosis factor (TNFα), interferon gamma (INFγ) which signal via Janus kinase or JAK-STAT (Janus kinase–Signal transducer and activator of transcription) signaling system [3]. Among JAK (Janus Kinase) family members, JAK3 exhibits greatest immunomodulation, yielding a more profound role in autoimmune disorders. The IL-2 receptor cytokines are involved in immune regulation, binding with cytokine receptor causes the activation of JAK3 (which is associated with γc subunit) resulting in the development of NK (natural killer) cell, Thymus cell and B lymphocytes. However, JAK1 JAK2 and TYK2 affects a wider range of immune as well as non-immune cells. Several JAK inhibitors, including tofacitinib a (pan JAK inhibitor), are approved for autoimmune diseases but may exhibit drug resistance as well as risk of cancer, induction of mutation, and fertility disorders [4]. The European Medicine Agency

has recently issued new recommendations to minimize the risk of adverse effects suggesting that it should only be used in chronic conditions such as chronic autoimmune disease [5]. This is due to the fact that they lack specificity to the JAKs. So, there is a need to explore inhibitors which selectively inhibit JAK3 for autoimmune disorders. Plants resources obtained from natural origin have been documented as incredible role in drug development because they have better biocompatibility and diverse range in phytochemical structure. In this context *Hypericum genus* such as *H. perforatum* exhibits an anti-inflammatory, antidepressant, antimicrobial, anticancer, antiviral, and immunomodulator activity [6]. Despite being used to treat various ailments; the molecular mechanism of this genus remains unclear. Due to the diverse chemical constituents present the exact compound responsible for the effect needs to be scrutinized to find out the hit compound. Thus, this study, aims to screen phytochemical constituents from *H. perforatum* against targeted JAK3 proteins through molecular docking analysis for the management of autoimmune disorders.

2. Materials and Methods

2.1 Molecular docking studies of *H. perforatum* components

2.1.1 Ligand Preparation

The chemical structure of phytoconstituents of *H. perforatum* were collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). FDA-approved Janus kinase (JAK) inhibitors were also utilized for molecular docking in order to establish comparison benchmarks (Table 2).

2.1.2 Protein Preparation

The JAK3 structure in complex with CP-690550 (PDB ID: 3LXK) was downloaded from the Protein Data Bank database (https://www.rcsb.org/) and further processed using Discovery Studio Visualizer (Jain et al., 2019) and Auto Dock tools (https://ccsb.scripps.edu/mgltools).

2.1.3 The Active Site determination for JAK3 by CASTp

Using the CASTp (Computed Atlas of Surface Topography of protein) servers, the active site for JAK3 was predicted (Dundas et al., 2006).

2.1.4 Center Grid Box and Running for Pyrx for *H. perforatum* constituents.

Virtual screening was conducted using phytoconstituents of *H. perforatum* and FDA-approved drugs JAK inhibitors. PyRx, downloaded (http://pyrx.sourceforge.net.) facilitated the process. The grid box covered the entire protein, and blind docking was performed.

2.1.5 Center Grid Box for Auto Dock Vina with Discovery Studio Visualizer

For this particular docking procedure, the values for center grid box were x=0.835783, y=15.106652, and z=4.943739 with dimensions along the x, y, and z axes were 22×22×22.

2.1.6 Docking Simulation of *H. perforatum* constituents with JAK3

Initially, virtual screening was conducted using the PyRx tool and the top scoring compounds were subsequently subjected to further docking using the Auto Dock Vina (http://autodock.scripps.edu/) and supporting software MGLTools 1.5.4 for the docking process. The ligands and JAK3 were docked individually, with each ligand manually docked one at a time to the protein using Auto Dock Vina [7].

2.1.7 Visualizing Interactions

Biovia Discovery Studio 3.5 was employed in order to visualize and analyze the two-dimensional, three-dimensional and surface interactions between the protein and the ligand**.**

2.2 ADMET studies of hypericin

Swiss ADME [8] and pkCSM was used for the ADMET properties of *Hypericum perforatum* ligands. [9]

3. Results and Discussion

The historical use of *H. perforatum* preparations in the treatment of autoimmune disorders and various ailments spans centuries. Empirical evidence supports its efficacy, but the need for a deeper understanding of the underlying molecular pathways is paramount. The intricate signaling cascades orchestrated by JAK3 in immunomodulation makes it a compelling therapeutic target. Despite the longstanding traditional use of *H. perforatum*, the precise mechanisms through which its components exert their effects, particularly in inhibiting JAK3, in autoimmune conditions remain to be elucidated.

JAK3 is related to hematopoietic cells, providing cytokines to interact with the gamma C subunit, which plays a role in immune cells. Various JAK inhibitors are available in the pharmaceutical

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market, but the use is limited due to drug resistance and adverse effects. Therefore, a novel JAK inhibitor which selectively inhibit JAK3 is required. The screening of phytoconstituents of *H. perforatum* with JAK3 was carried out and compared with the standard inhibitor tofacitinib, utilizing a molecular modeling strategy. Thus, this study with the explicit purpose of inhibiting JAK3, we aim to unravel the molecular intricacies that underlie its therapeutic potential.

3.1 In silico screening of *H. perforatum* constituents

3.1.1 Molecular docking studies

The 3D crystal structure of the JAK3 protein and processed protein is given in (Figure 1A) (Figure 1C). The permitted and prohibited areas of torsion angle values of JAK3 (PDBID: 3LXK), were analyzed by Ramachandran plot and 94.62% in the most favored region, and 7.5% in additional allowed regions leading to favorable protein for docking (Figure 1B).

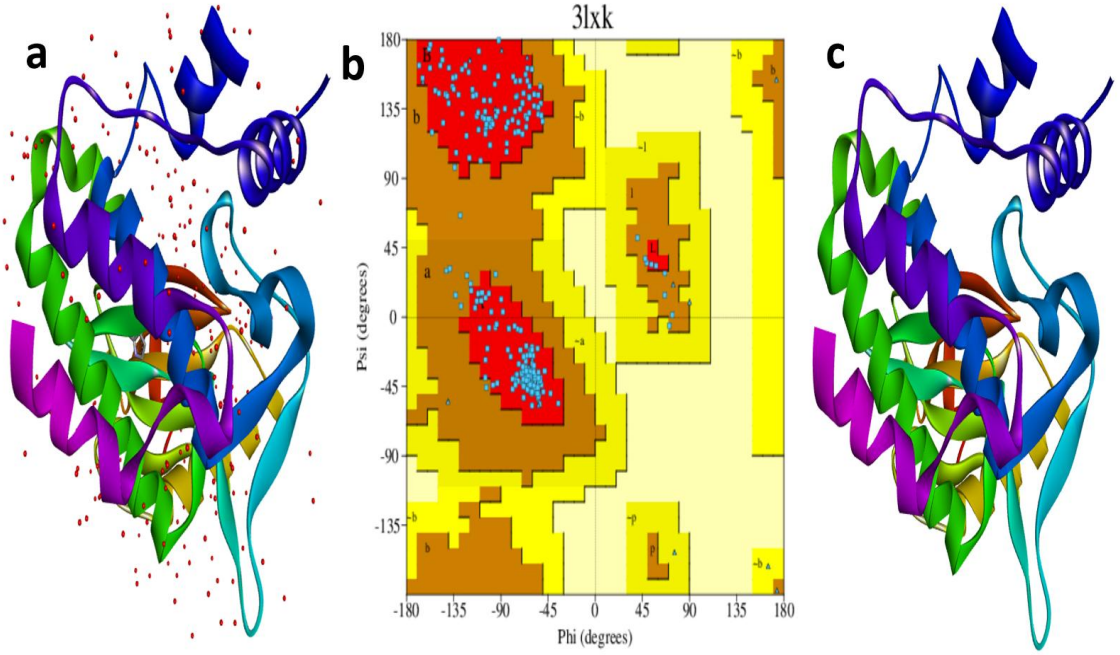

Figure 1: Structure of JAK3 (3LXK). (A) Represents the raw structure of JAK3 with a small-molecule inhibitor retrieved from protein data bank. (B) Ramachandran plot for the predicted structure (C) Represents the processed protein JAK3 obtained following the protein preparation wizard

JAK3 has a molecular wt of 120–130 kDa and containing seven JAK homology domains (JH1–JH7). The catalytically active domain having kinase activity is the C-terminal portion (JH1), which also contains the ATP-binding site [10]. The JH1 domain of JAK3 was utilized for the study. CASTp server was used to predict the active site [11]. The active site consists of amino acid residues. Therefore, the active sites were chosen based on the amino acid residues present in the inhibitor (Table 1). The ATP binding site was located at pocket 2 having an area of 325.795(SA) Å^2 and a volume of 185.585 Å^3.

Table 1: Details of pocket areas of JAK3 (3LXK) using CASTp server.

Pocket	Area (SA) Å ^{2}	Volume Å ^{3}
Pocket1	442.391	308.217
Pocket 2	325.75	185.585
Pocket3	54.267	47.032
Pocket4	60.924	24.639

The components of *H. perforatum* were selected by a literature survey and docked with JAK3. The binding affinity of FDA-approved drugs (Table 2) and *H. perforatum* phytoconstituents are summarized in

(Table 3). Drugs namely CMP-6 (-11 kcal/mol), peficitinib (-9 kcal/mol), and tofacitinib (-6.6kcal/mol) and these FDA drugs were used for comparison. From the docking simulation, the phytoconstituents have shown the potential to inhibit JAK3 (Table 3) with binding energy ranging from -12.4 to -4.8, with the best result achieved using hypericin (−12.4 kcal/mol). Various researches have shown that the pharmacological activity of *H. perforatum* constituent is also due to the presence of hypericin [12].

Table 2: List of binding affinities of the FDA approved drugs against JAK3

S. No.	FDA approved drugs	Binding affinities (Kcal/mol)	Hydrogen bonds
1.	CMP-6	-11	Leu 828, Leu 905, Glu 903
2.	Peficitinib	-9	Cys 909, Leu 828, Glu 903
3.	Tofacitinib	-6.6	Asp 967, Ala 966, Leu 905, Tyr 904, Arg 911, Arg 953, Asn 954

Table 3: List of binding affinities and hydrogen bond interaction of *H. perforatum* phytoconstituents against JAK3

S. No.	H. Perforatum components	Binding affinity (Kcal/mol)	Hydrogen bonds
1	Hypericin	-12.4	Asp 967, Ala 966, Asn 954, Arg 953, Arg 911, Leu 905, Tyr 904
2	Isohypericin	-11.5	Glu 903
3	Pseudohypericin	-10.7	Leu 905, Leu 828, Arg 911
4	Quercetin	-9.3	Ala 966, Asn 954, Asp 967, Lys 855, Lys 830
5	Norathyriol	-9.2	Leu905, Lys 830, Asp 967
6	Protopseudohypericin	-9	Asn 954, Arg 953, Arg 911
7	Hyperoside	-8.9	Lys 830, Ala 966, Asn 832, Asp 967
8	Guajaverin	-8.9	Ala 966, Asp 967, Lys 855, Gly 831
9	Protohypericin	-8.9	Asn 954, Arg 953
10	Catechin	-8.8	Asn 954, Glu 903, Leu 905
11	Epicatechin	-8.7	Leu 905, Glu 903, Leu 905
12	Neochlorogenic acid	-8.5	Gly 831, Lys 855, Cys909
13	Biapgenin	-7.2	Lys 830, Pro 906
14	Protocatechuic acid	-6	Ala:966
15	Hyperforine	-5.8	Tyr 904
16	Beta-ocimene	-5.7	-
17	Adhyperforin	-5.4	-
18	2-Methyldecane	-5.2	-
19	2-Methyloctane	-4.9	-

Hypericin, a naphthodianthrone constituent confirmed highest binding affinity of -12.4 kcal/mol with JAK3 precede by Isohypericin 11.5, pseudohypericin -10.7, quercetin -9.3, Norathyriol with -9.2 kcal/mol respectively (Table 3) which were further confirmed by comparing with the FDA approved JAK3 inhibitors namely tofacitinib, peficitiib and CMP-6 (Table 2).

Hypericin, which exhibited potent cytotoxic and proa poptotic effects on cancer cells(Mirmalek et

al.,2016), formed seven hydrogen bonds with Asp 96 7, Ala 966, Asn 954, Arg 953, Arg 911, Leu 905, Tyr 904 in its active site and bound to 20 amino acids with different interactions such as carbon hydrogen bond, Vander Waals, pi-sulphur and pi-sigma (Figure 2-3.).

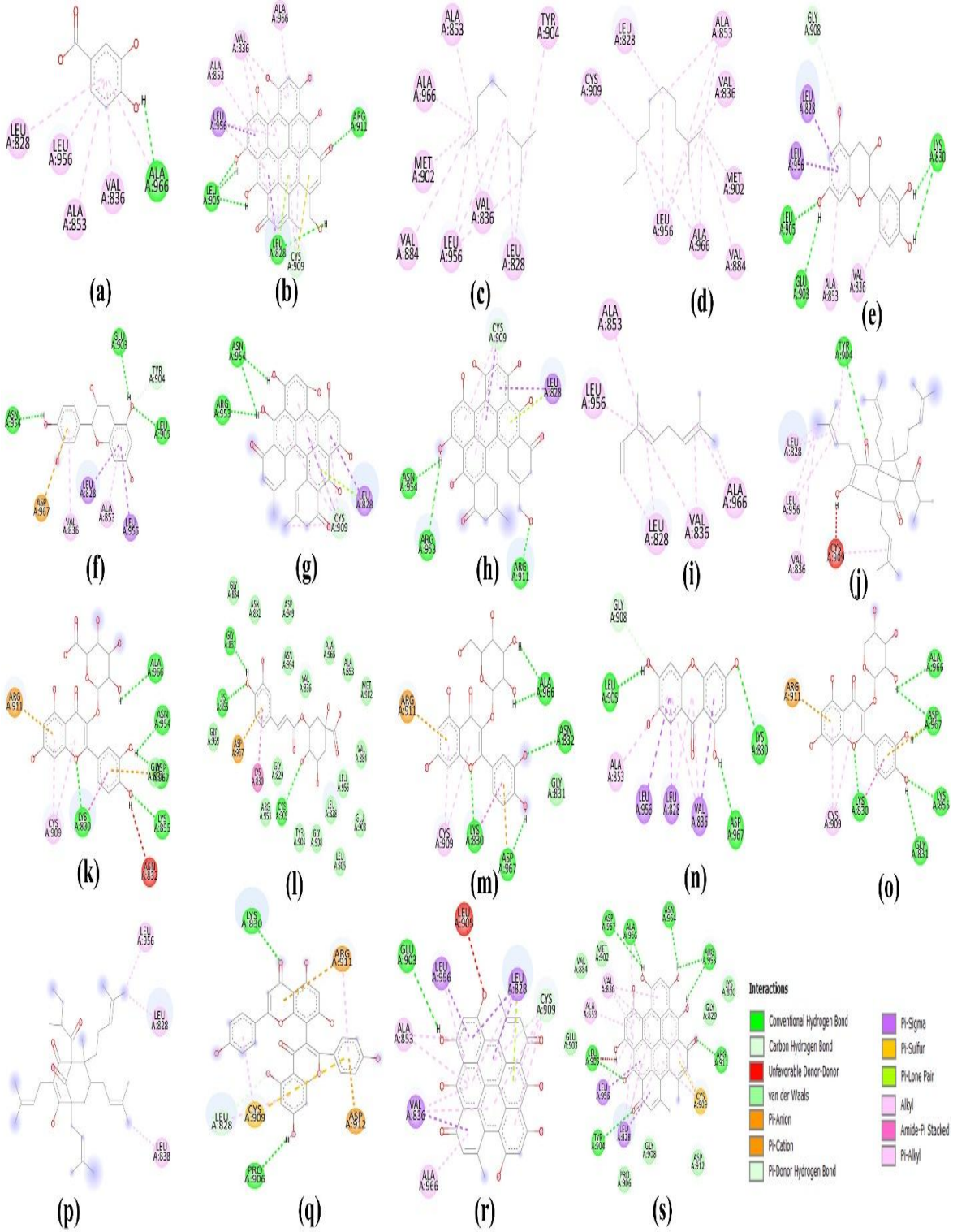

Figure 2: 2D interactions of the compounds docked with JAK3. Protein-ligand interactions were mapped using Discovery Studio. (a. Protocartechuic acid, b. Pseudohypericin, c.2-Methyloctane, d. Betaocimene, e. 2-

Methyloctane. f. Catechin, g. Protohypericin, h. Protopseudohypericin, i. Betaocimene, j. Hyperforin, k.Quercetin, l. Neochlorogenic acid, m. Hyperoside, n. Norathyriol, o. Guaijaverin, p. Adhyperforin, q. Biapgenin, r. Isohypericin, s. Hypericin)

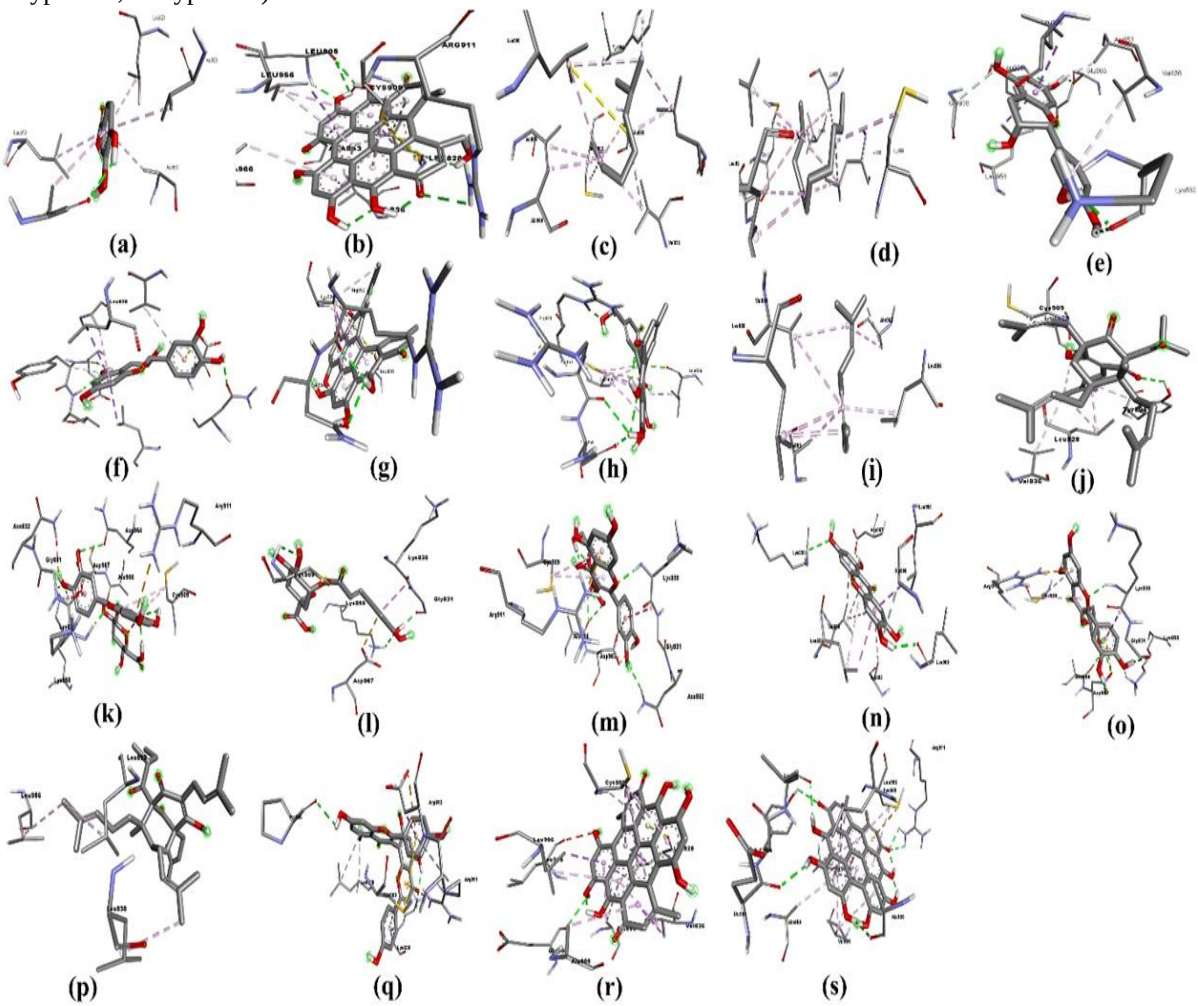

Figure 3: 3D interactions of the compounds docked with JAK3. Protein-ligand interactions were mapped using Discovery Studio. (a. Protocartechuic acid, b. Pseudohypericin, c.2-Methyloctane, d. Betaocimene, e. 2-Methyloctane. f. Catechin, g. Protohypericin, h. Protopseudohypericin, i. Betaocimene, j. Hyperforin, k.Quercetin, l. Neochlorogenic acid, m. Hyperoside, n.Norathyriol, o. Guaijaverin, p. Adhyperforin, q. Biapgenin, r. Isohypericin, s. Hypericin)

3.1.2 ADMET analysis of *H. perforatum* constituents

The efficacy of a drug candidate depends not just on its promising potential but also on its favorable ADMET profile[14]. The drug likeliness and ADMET were assessed using online tools such as pkCSM (Table 4) and SwissADME (Table 5) [9]. The use of *H. perforatum* preparation as herbal supplement is quite extensive, with its application ranging from the treatment of mental illness and insomnia to gastrointestinal tract diseases, skin wounds, eczema, and burns. This wide range of uses indicates that there is evidence supporting its safety and efficacy [15]. Our study showcased the effectiveness and safety of using ADMET analysis to treat autoimmune diseases According to the pharmacokinetic analysis, most of the *H. perforatum* phytoconstituents do not cross blood–brain barrier (BBB) except 2 methyloctane (Table 5). Moreover, most compounds showed no inhibition of cytochrome P450 isomers (CYP2C9) (Table 5). CYP2C9 is a major cytochrome P450 enzyme involved in the metabolic clearance of various drugs [8]. Inhibition of CYP2C9 activity results in decreased metabolism of therapeutic agents,

thus enhancing their plasma concentration, which may cause serious adverse effects. Some of the compound in *H. perforatum* had CYP2C9 inhibitory activity such as pseudohypericin, betaocimence, protohypericin, isohypericin and hypericin. Therefore, caution must be taken while using these drugs as it may cause drug interaction and dosing should be done carefully as low dose may be sufficient for these compounds. Drug likeness prediction was further conducted using Lipinski's Rule, and Veber rule and Ghose rule as mentioned in the study by Yalçın et al. (2021). The drug-likeness analysis, guaijaverin, epicatechin, catechin, norathyriol, protocatechuic acid, beta-ocimene and 2-methyloctane were identified as meeting Lipinski's, Veber's, or Ghose's rule (Table 5). Nevertheless, the application of Lipinski's rule of five may not be suitable for natural compounds. Just 50% of the small-molecule drugs approved by the FDA are both utilized and in line with the "rule of five." [16]. Bioavailability is defined as the probability of a compound having at least 10% oral bioavailability in rats or measurable Caco-2 cell (human colon adenocarcinoma) permeability [17]. The bioavailability score of most of the selected compounds was 0.55, indicating the presence of drug-like properties (Table 5) but some phytoconstituents such as pseudohypericin, protohypericin, protopseudohypericin, quercitin, neochlorogenic acid, hyperoside, cryptochlorogenic acid, Biapgenin, Isohypericin and hypericin had bioavailability less than 0.55 indicating less availability in systemic circulation. So, during drug designing the bioavailability should be enhanced by using different formulations such as nano formulation. However, in some cases if the application is local then bioavailability is not a problem such as if the drug has to be used for intestinal disorders there is no requirement of systemic availability of drug. Synthetic accessibility (SA) is the ability of drug to be easily synthesized. The SA scores range from 1 (extremely easy) to 10 (extremely difficult) [18]. Our study revealed that all the phytoconstituents were in the range of 1.07 and 7.58 demonstrating easily synthesizable. (Table 5). The intestine is the primary site for the absorption of oral drugs [19]. The ADMET analysis revealed that all the phytoconstituents had an intestinal absorption of >30% (Table 5). Furthermore, the skin sensitization of the phytoconstituents were assessed to test the suitability of the product when it is applied dermally, it may also cause skin sensitization [20]. So, it is necessary to test for skin sensitization. The phytoconstituents had no skin sensitization except. The hERG (human ether-a-go-go-related gene) codes the potassium channel and inhibition of this activity leads to prolongation of QT interval causing ventricular arrythmia [21]. The phytoconstituents had no hERG inhibitory properties (Table 4). The Ames test determines mutagenic potential of compounds utilizing bacteria (Salmonella typhimurium) [22]. All the phytoconstituents cleared Ame's test except norathyriol (Table 4). The renal uptake transporter also known as Organic Cation Transporter 2 (OCT-2) is responsible for the disposition as well clearance of pharmaceuticals and endogenous chemicals from kidney [23]. There is a chance of interaction with the OCT-2 substrate if the phytoconstituent has renal OCT2-2 inhibitory activity. In our study the phytoconstituents did not have the potential to act as OCT-2 substrates, representing rarer probabilities of contradictions (Table 4). The hepatoxicity indicated that the phytoconstituents had no hepatotoxicity indicating the use of these compound in patient with hepatic insufficiency. The skin permeability (log Kp) measures the substance's penetration ability through the skin. The logarithmic form of the skin permeability coefficient (Kp), is expressed in cm/s and is used to predict the rate of penetration of a compound through the skin (Scheler et al., 2015). According to Fick's law of diffusion, log Kp depends on factors like molecular size, solubility, and skin partitioning behavior. The value of log Kp > -2.5 is the indication of high skin permeability [24]. In our study, all phytoconstituents had log Kp values around -2.7, with one exception, 2 methyloctane (-0.877) show higher skin permeability, indicating that they are more likely to be absorbed through the skin than the other compounds (Table 4). However, most phytoconstituents indicated low permeability, suggesting that they will likely not penetrate the skin effectively, resulting less suitable for topical applications. The ADMET tools, SwissADME and pKCSM proposed that hypericin had good drug-like characteristics and hence was used for further study.

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Vol. 2, Issue 4, April, 2025
Page No.: 155-163

Table 5: ADMET properties of selected compounds using Swiss ADME

S. No	Name of compound	Intestinal absorption (human) %	Skin Permeability (log Kp)	BBB permeability log BB	CYP2C9 inhibitor	Renal OCT2 substrate	AMES toxicity	hERG I inhibitor	Skin Sensitisation	Hepatotoxicity
1	Protocatechuic acid	71.174	-2.727	-0.683	No	No	No	No	No	No
2	Pseudohypericin	100	-2.735	-1.775	No	No	No	No	No	No
3	2- Methyloctane	93.886	-0.877	0.803	No	No	No	No	No	No
4	Betaocimene	93.34	-2.7	-1.054	Yes	No	Yes	No	No	No
5	2- Methyldecane	93.198	-1.091	0.841	No	No	No	No	Yes	No
6	Epicatechin	68.829	-2.735	-1.054	No	No	No	No	No	No
7	Catechin	68.829	-2.735	-1.054	No	No	No	No	No	No
8	Protohypericin	98.376	-2.735	-1.252	No	No	No	No	No	No
9	Protopseudohypericin	90.145	-2.735	-1.288	No	No	No	No	No	No
10	Hyperforin	98.386	-2.715	-0.237	No	No	No	No	No	No
11	Quercetin	25.112	-2.735	-1.614	No	No	No	No	No	No
12	Neochlorogenic acid	36.377	-2.735	-1.407	No	No	No	No	No	No
13	Hyperoside	47.999	-2.735	-1.688	No	No	No	No	No	No
14	Norathyriol	78.127	-2.735	-1.091	No	No	Yes	No	No	No
15	Guaijaverin	51.884	-2.735	-1.473	No	No	No	No	No	No
16	Adhyperforin	97.4	-2.715	-0.24	No	No	No	No	No	No
17	Biapgenin	90.723	-2.735	-1.659	No	No	No	No	No	No
18	Isohypericin	100	-2.735	-1.594	No	No	No	No	No	No
19	Hypericin	100	-2.735	-1.561	No	No	No	No	No	No

Table 6: ADMET properties of selected compounds using pkCSM

S. No	Molecule	MR	TPSA	GI absorption	BBB permeant	Pgp substrate	CYP2C9 inhibitor	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Bioavailability Score	Synthetic Accessibility
1	Protocatechuic acid	37.45	77.76	High	No	No	No	0	3	0	0	0.56	1.07
2	Pseudohypericin	145.99	175.75	Low	No	No	Yes	2	2	1	1	0.17	3.95
3	2- Methyloctane	45.38	0	Low	Yes	No	No	1	1	0	0	0.55	1.52
4	Betaocimene	77.18	127.17	High	No	No	Yes	0	0	0	0	0.55	2.3
5	2- Methyldecane	54.99	0	Low	No	No	No	1	1	0	0	0.55	1.72
6	Epicatechin	74.33	110.38	High	No	Yes	No	0	0	0	0	0.55	3.5
7	Catechin	74.33	110.38	High	No	Yes	No	0	0	0	0	0.55	3.5
8	Protohypericin	144.53	155.52	Low	No	No	Yes	2	2	1	1	0.17	4.31
9	Protopseudohypericin	145.69	175.75	Low	No	No	Yes	2	2	1	1	0.17	4.37
10	Hyperforin	165.15	71.44	Low	No	Yes	No	2	4	1	1	0.85	7.33
11	Quercetin	110.77	227.58	Low	No	Yes	No	2	1	1	1	0.11	5.26
12	Neochlorogenic acid	83.5	164.75	Low	No	No	No	1	1	1	1	0.11	4.16
13	Hyperoside	110.16	210.51	Low	No	No	No	2	1	1	1	0.17	5.32
14	Norathyriol	68.08	111.13	High	No	No	No	0	0	0	0	0.55	2.87
15	Guaijaverin	104.19	190.28	Low	No	No	No	2	0	1	1	0.17	5.05
16	Adhyperforin	169.96	71.44	Low	No	Yes	No	2	4	1	1	0.85	7.58
17	Biapgenin	146.97	181.8	Low	No	No	No	2	2	1	1	0.17	4.24
18	Isohypericin	144.83	155.52	Low	No	No	Yes	2	3	1	1	0.17	3.86
19	Hypericin	144.83	155.52	Low	No	No	Yes	2	3	1	1	0.17	3.89

The overall study suggests hypericin potential as JAK3 inhibitor and can be used in the treatment of autoimmune disease.

4-Conclusion

Hypericum sp. has long been used as a medicinal plant in a variety of treatments, but it has lately acquired prominence in research due to its various properties. The *in-silico* study provided a valuable insight concerning the use of one of its active phytoconstituent hypericin, as a potential JAK3 inhibitor. Our findings may pave the way for novel approaches to treating JAK3-targeted diseases. These findings add to the changing landscape of natural product research and open the path for future studies on the medicinal potential of hypericin and other phytoconstituents from *H. perforatum.*

Declaration of competing interest

The authors declared no potential conflicts of interest.

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Data availability

Data will be made available on request.

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