VNU Journal of Science: Medical and Pharmaceutical Sciences
Journal homepage: https://js.vnu.edu.vn/MPS

Original Article

In silico Screening of Compounds of *Piper betle* L. leaves for Potential TNF-α Cytokine Inhibition

Bui Thi Hao[1,2], Tran Minh Quan[3], Tran Hoang Mai[3], Nguyen Thi Anh Hong[3], Trinh Thi Duong[3], Nguyen Thuc Thu Huong[3], Nguyen Minh Khoi[2], Bui Thanh Tung[4,*]

[1]*Vietnam University of Traditional Medicine, 02 Tran Phu, Dai Mo, Hanoi, Vietnam*
[2]*National Institute of Medicinal Materials (NIMM), 3B Quang Trung, Cua Nam, Hanoi, Vietnam*
[3]*VNU University of Medicine and Pharmacy, 144 Xuan Thuy, Cau Giay, Hanoi, Vietnam*
[4]*People's Police University of Technology and Logistics, Thuan Thanh, Bac Ninh, Vietnam*

Received 01st July 2025
Revised 26th July 2025; Accepted 15th September 2025

Abstract: Tumor necrosis factor alpha (TNF-α) is a key pro-inflammatory cytokine that plays a central role in immune regulation, inflammatory responses, and host defense against infectious agents. It is closely associated with the pathogenesis of various chronic inflammatory conditions, including rheumatoid arthritis, Crohn's disease, psoriasis, ankylosing spondylitis, and other autoimmune disorders. *Piper betle* L. is a medicinal plant with potential inhibitory activity against the TNF-α cytokine. In this study, molecular docking was employed to assess the potential of 37 compounds identified in *Piper betle* L. leaves to bind directly to the TNF-α cytokine protein (PDB ID: 2AZ5) and potentially inhibit its pro-inflammatory activity. The results showed that two compounds, 3-ethyl-3-hydroxy-5α-androstan-17-one and β-sitosterol, exhibited stronger binding affinity to the TNF-α cytokine protein than the positive control, SPD-304. In addition, both compounds also satisfied Lipinski's rule of five for drug-likeness and demonstrated favorable pharmacokinetic properties and toxicity predictions. Therefore, further research is warranted to explore the potential of these two compounds as TNF-α cytokine inhibitors for the treatment of inflammatory and autoimmune diseases.

Keywords: Autoimmune diseases, inflammation, TNF-α, molecular docking, *Piper betle* L., *In silico*.

* Corresponding author.
E-mail address: tungbt.ump@vnu.edu.vn

https://doi.org/10.25073/2588-1132/vnumps.4807

1. Introduction

Tumor necrosis factor alpha (TNF-α) is a key proinflammatory cytokine produced by macrophages and other immune cells, playing a central role in immune regulation, inflammatory responses, and host defense against infections. Functionally, TNF-α acts as a ligand that binds to two specific cell surface receptors (TNFR1 and TNFR2) to initiate downstream signaling cascades involved in inflammation, apoptosis, and cell survival. However, dysregulated or sustained overexpression of TNF-α contributes to the development of chronic inflammatory diseases such as rheumatoid arthritis, Crohn's disease, psoriasis, and other autoimmune disorders. Therefore, modulation of TNF-α activity has emerged as an important therapeutic strategy, guiding the development of novel anti-inflammatory and immunomodulatory agents [1].

TNF-α exists in two biologically active forms: soluble and transmembrane, and it exerts its effects by binding to two principal cell surface receptors: TNFR1 (Tumor Necrosis Factor Receptor 1) and TNFR2 (Tumor Necrosis Factor Receptor 2). TNFR1 is widely expressed in many tissues and is involved in inflammation, apoptosis, and necrosis through signaling complexes I, IIa, IIb, and IIc. Meanwhile, TNFR2 is primarily expressed on immune cells, lacks a death domain, and is mainly associated with homeostatic biological processes, including tissue remodeling, cell proliferation, and cell survival. Overall, TNFR1 plays a critical role in mediating cytotoxic and proinflammatory TNF-α responses, whereas TNFR2 predominantly contributes to cell activation, migration, and proliferation [1-3]. TNF-α inhibitors currently used to treat chronic inflammatory diseases include monoclonal antibodies such as infliximab, adalimumab, golimumab, and certolizumab pegol, as well as soluble TNF-α cytokine fusion proteins such as etanercept. These agents function by binding to free TNF-α in the circulation or at sites of inflammation, thereby preventing its interaction with cell surface receptors and reducing the activation of inflammatory pathways. As a result, they contribute to the alleviation of clinical symptoms. The remarkable efficacy of these therapies in various autoimmune diseases has stimulated the development and clinical evaluation of novel TNF-α inhibitors [4].

Piper betle L. has long been used in traditional medicine to treat colds, bronchial asthma, coughs, stomachaches, rheumatism, bad breath, constipation, conjunctivitis, swollen gums, abscesses, and trauma. Modern studies have demonstrated that this medicinal plant possesses antibacterial, antifungal, anti-inflammatory, antioxidant, and analgesic properties largely attributed to its natural bioactive compounds. In particular, preliminary phytochemical analyses of *Piper betle* L. have revealed the presence of various classes of active constituents, including alkaloids, flavonoids, tannins, sterols, phenols, glycosides, saponins, and terpenoids [5, 6].

Molecular docking is a modeling technique that predicts the optimal binding position and conformation of a substrate molecule (ligand) within the active site of a target protein. The main advantage of this approach is that it is time-efficient and more cost-effective than conventional experimental methods for screening potential bioactive compounds [7]. In this study, we employed molecular docking to identify compounds from *Piper betle* L. leaves with potential inhibitory activity against the TNF-α target.

2. Materials and Methods

2.1. Model Docking

Preparation of protein structure: The X-ray crystal structure of the TNF-α cytokine (PDB ID: 2AZ5) was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/). This structure represents the soluble trimeric form of TNF-α protein, which plays a central role in initiating pro-inflammatory signaling by binding to its receptors. The TNF-α structure contains a co-crystallized ligand, SPD-304, which is a known TNF-α inhibitor. The co-crystallized ligand and water molecules were removed using

Discovery Studio 2025 software. Then, hydrogen atoms were added to the protein, and Kollman charges were assigned. The enzyme active site was determined using MGL Autodock tools 1.5.6 software. The active site of TNF-α is enclosed within a grid box of 40 Å× 40 Å× 40 Å, with a spacing of 0.375 Å (centered at x = -13.678; y = 71.607; z = 27.002). The prepared protein structure was then saved in pdbqt format.

Preparation of ligands: A total of 37 compounds were identified in *Piper betle* L. leaves [5, 6]. The 3D structures of the ligands were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and initially saved in SDF format. These files were subsequently converted to PDB format using Chimera 1.19 software. Next, the ligands were energy-minimized using Avogadro software with the Conjugate Gradients method and finally converted to .pdbqt format using AutoDock Tools software.

Performance of molecular docking: The ligands were docked into the active site of the protein using Autodock Vina software. The software was used to identify the optimal binding conformations based on the evaluation of binding free energy (ΔG) and the number of physical interactions. The binding affinities of the ligands were assessed by analyzing their interactions with amino acid residues at the active site. The interaction energies were calculated using the scoring function of AutoDock Vina.

2.2. Evaluation of Docking Results

To validate the docking protocol, the co-crystallized ligand was separated from the protein and re-docked into the active site of the target. The docking results were considered reliable if the root mean square deviation (RMSD) value was less than 1.5 Å. For the test compounds, binding affinity was evaluated based on their interactions with amino acid residues within the binding cavity, and the interaction energies were calculated using the scoring function of AutoDock Vina.

2.3. Evaluation of Lipinski's Rule of Five

Lipinski's rule of five was used to evaluate the drug-likeness of the compounds. An online tool was employed to assess compliance with Lipinski's criteria (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) [8].

2.4. Prediction of ADMET by Computational Analysis

The pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the potential compounds were predicted using the pkCSM tool (http://biosig.unimelb.edu.au/pkcsm/prediction) [9]. In addition to pkCSM, the ADMET properties of the top compounds were further evaluated using SwissADME (http://www.swissadme.ch/), a web-based tool that predicts physicochemical properties, pharmacokinetics, drug-likeness, and medicinal chemistry friendliness [10].

3. Results

3.1. Evaluation of the Docking Model

Before screening the compounds, the co-crystallized ligand was re-docked into the active site of the target protein to determine the root mean square deviation (RMSD) and assess the suitability of the docking parameters. The similarity of the conformations was evaluated by calculating the RMSD using Chimera 1.19. The superposition of the co-crystallized ligand before and after docking yielded an RMSD value of 1.057 Å < 1.5 Å, indicating that the docking protocol was reliable [11] (Figure 1).

The docking result of SPD-304 gave a binding energy of ΔG = -8.6 kcal/mol. The SPD-304 ligand formed alkyl and π-alkyl bonds interacting with Leu57, Tyr59, and Tyr119, as well as a halogen bond with Gly121 (Figure 2). The docking score of -8.6 kCal/mol was used as a reference for screening potential compounds.

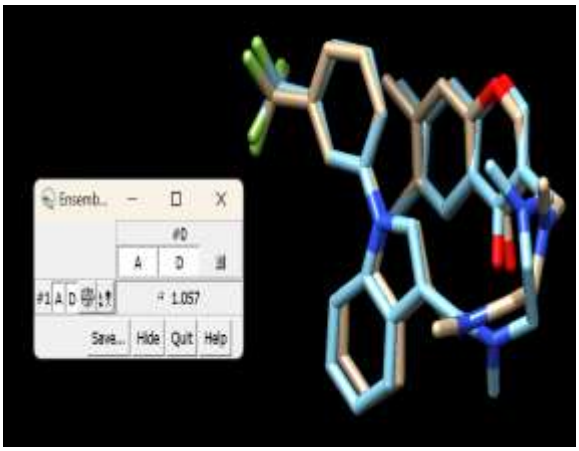


Figure 1. Re-docking validation results of the TNF-α co-crystallized ligand (SPD-304).

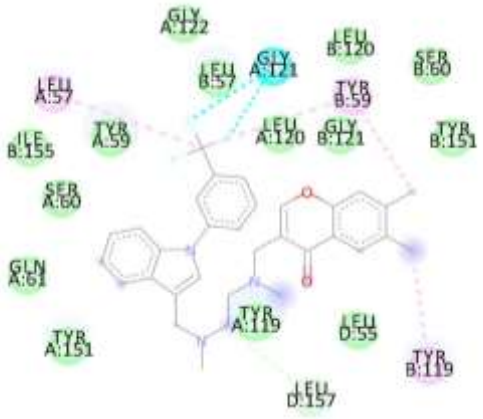


Figure 2. Representation of SPD-304 binding to the active site of TNF-α.

3.2. Molecular Docking of Compounds to the Target Protein

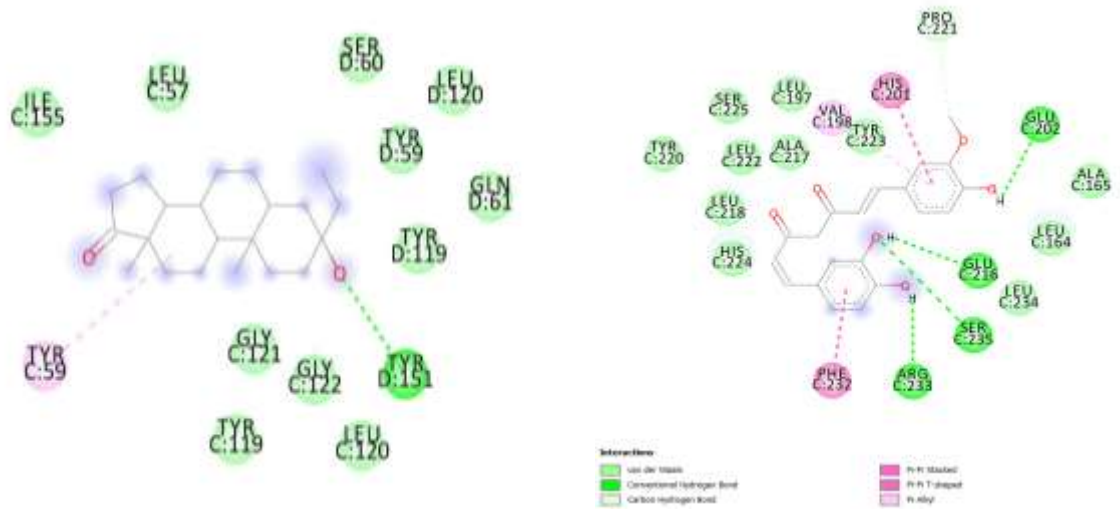
After preparing the ligands, molecular docking was performed for 37 compounds from *Piper betle* L. leaves against the TNF-α cytokine protein to identify potential inhibitors. By comparing the binding energy of the co-crystallized ligand (SPD-304) with those of the 37 compounds, two compounds were selected with binding energies $\Delta G \leq -8.6$ kcal/mol, equivalent to or better than that of SPD-304. These compounds were β-sitosterol (-8.7 kcal/mol) and 3-ethyl-3-hydroxy-5α-androstan-17-one (-8.8 kcal/mol). Given that these differences are small and approach the reliability limits of docking scoring functions, they should be regarded as indicative rather than definitive, thus requiring further experimental validation. The docking results are presented in Table 1.

Table 1. The docking results of 37 compounds and positive controls with the TNF-α cytokine protein

No.	Name	PubChem ID	Binding energy (kcal/mol)
1	3-ethyl-3-hydroxy-5**α**-androstan-17-one	14681481	-8.8
2	4-allyl 1,2-diacetoxybenzene	166872	-6.3
3	4-Terpineol	11230	-5.7
4	Acetylisoeugenol	876160	-6.3
5	Allylpyrocatechol	292101	-5.6
6	Allylpyrocatechol diacetate	46700759	-6.3
7	α-pinene	6654	-5.8
8	α-terpinene	7462	-5.7
9	α-terpineol	17100	-5.9
10	α-thujene	12444324	-5.6
11	β-cadinene	10657	-7.3
12	β-caryophyllene	20831623	-7.0
13	β-caryophyllene oxide	1742210	-7.2
14	β-ocimene	18756	-5.2
15	β-selinene	442393	-7.2
16	β-sitosterol	222284	-8.7

17	Chavibetol	596375	-5.8
18	Chavicol	68148	-5.7
19	Chavicol acetate	523825	-6.0
20	Estragole	8815	-5.4
21	Eugenol	3314	-5.8
22	Eugenol acetate	7136	-6.0
23	γ-terpinene	7461	-5.6
24	Germacrene B	5281519	-6.9
25	Germacrene D	5317570	-7.1
26	Globulol	12304985	-7.2
27	Humulene	5281520	-7.1
28	Hydroxychavicol	70775	-6.1
29	Isoeugenol	853433	-6.2
30	Limonene	22311	-5.6
31	Methyleugenol	7127	-5.7
32	Neophytadiene	10446	-5.7
33	Phytol	5280435	-5.2
34	Phytol acetate	6428538	-6.0
35	ρ-cymene	7463	-5.8
36	Safrole	5144	-5.8
37	Squalene	638072	-6.8
+	SPD-304		-8.6

The interactions between 3-ethyl-3-hydroxy-5α-androstan-17-one and β-sitosterol with TNF-α were visualized using Discovery Studio 2025, as shown in Figure 3.



R1 R2

Figure 3. Interactions between 3-ethyl-3-hydroxy-5α-androstan-17-one (R1) and β-sitosterol (R2) with TNF-α.

3.3. Lipinski's Rule of Five

Compounds are considered to be "drug-like" if they have at least 2 of the 5 criteria defined of Lipinski's rule of five: molecular weight (MW) below 500 Daltons; high lipophilicity (expressed as LogP less than 5); less than 5 hydrogen bond donors (HBD); less than 10 hydrogen bond

acceptors (HBA1) and molar refractivity (MR) should be between 40-130.

Based on the results presented in Table 2, both compounds met the drug-likeness criteria. Subsequently, these two compounds were further evaluated for their pharmacokinetic and toxic profiles.

Table 2. Results of Lipinski's rule parameters of 2 compounds

No.	Name	MW	HBD	HBA1	LogP	MR	Drug-likeness
1	3-ethyl-3-hydroxy-5α-androstan-17-one	318	1	2	4.739300	91.974762	Yes
2	β-sitosterol	414	1	1	8.024803	128.216736	Yes

Table 3. ADMET prediction results using pkCSM

Properties	3-ethyl-3-hydroxy-5α-androstan-17-one	β-sitosterol
Absorption		
Water solubility (log mol/L)	-5.322	-6.773
CaCO2 permeability (log Papp in 10^{-6} cm/s)	1.614	1.201
Intestinal absorption (human) (% Absorbed)	95.249	94.464
Skin Permeability (log Kp)	-3.103	-2.783
P-glycoprotein substrate	No	No
P-glycoprotein I inhibitor	Yes	Yes
P-glycoprotein II inhibitor	No	Yes
Distribution		
VDss (human) (log L/kg)	0.49	0.193
Fraction unbound (human) (Fu)	0.051	0
BBB permeability (log BB)	0.047	0.781
CNS permeability (log PS)	-2.328	-1.705
Metabolism		
CYP2D6 substrate	No	No
CYP3A4 substrate	Yes	Yes
CYP1A2 inhibitor	No	No
CYP2C19 inhibitor	Yes	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	No
Excretion		
Total Clearance (log ml/min/kg)	0.666	0.628
Renal OCT2 substrate	No	No
Toxicity		
AMES toxicity	No	No
Max. tolerated dose (human) (log mg/kg/day)	-0.641	-0.621
hERG I inhibitor	No	No
hERG II inhibitor	No	Yes
Oral Rat Acute Toxicity (LD50) (mol/kg)	1.863	2.552
Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	1.977	0.855
Hepatotoxicity	Yes	No
Skin Sensitisation	No	No
T. pyriformis toxicity (log ug/L)	1.164	0.43
Minnow toxicity (log mM)	0.143	-1.802

Table 4. SwissADME prediction results

Properties	3-ethyl-3-hydroxy-5α-androstan-17-one	β-sitosterol
Lipinski rule	Yes	Yes
GI absorption	High	Low
BBB permeant	Yes	No
P-gp substrate	No	No
CYP1A2 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	No
PAINS	0	0
Synthetic accessibility (0–10)	4.11	6.30

3.4. Prediction of ADMET Profile

To further assess the potential efficacy of the two compounds, their pharmacokinetic and toxicity (ADMET) parameters were predicted using the pkCSM tool and SwissADME. SwissADME offers a complementary set of predictive models, including detailed profiling of gastrointestinal absorption, cytochrome P450 inhibition, blood–brain barrier permeability, and medicinal chemistry filters such as PAINS alerts and synthetic accessibility. By integrating predictions from both platforms, the study mitigates model-specific biases and enhances the robustness of compound evaluation. This dual-tool approach reinforces confidence in the identified compounds and aligns with best practices in computational drug discovery, where multi-platform validation is increasingly recommended.

In terms of absorption, both compounds demonstrated favorable pharmacokinetic behavior, with predicted high intestinal absorption and efficient passive diffusion across Caco-2 membranes. However, SwissADME indicated lower gastrointestinal absorption for β-sitosterol, likely due to its larger molecular size and poor water solubility. Neither compound was identified as a P-glycoprotein (P-gp) substrate, suggesting a low risk of active efflux and favorable bioavailability.

Regarding distribution, 3-ethyl-3-hydroxy-5α-androstan-17-one demonstrated better predicted tissue distribution and a higher unbound fraction, indicating better systemic exposure compared to β-sitosterol. In addition, two parameters of permeability through the blood-brain barrier (BBB) and the central nervous system (CNS) are important for assessing the neurological safety of drugs. A logBB value greater than 0.3 is considered to readily cross the BBB, while molecules with a logBB lower than -1 are predicted to be poorly distributed to the brain. Similarly, compounds with a logPS value higher than -2 are considered likely to penetrate the CNS, whereas those with a logPS lower than -3 are predicted to be unable to cross the CNS [9]. The results showed β-sitosterol was predicted to cross both the BBB and the CNS barrier, whereas 3-ethyl-3-hydroxy-5α-androstan-17-one showed minimal permeability across these barriers.

In terms of metabolism, both compounds were predicted to be substrates of CYP3A4, implying hepatic metabolism via this major isoenzyme. However, only 3-ethyl-3-hydroxy-5α-androstan-17-one was identified as a CYP2C19 inhibitor, which may affect the metabolism of co-administered drugs. Neither compound showed inhibitory activity against other major CYP isoforms (CYP1A2, CYP2C9, CYP2D6, CYP3A4), reducing the likelihood of broader drug-drug interactions.

Regarding elimination, both compounds demonstrated renal clearance and were not predicted to be substrates of OCT2 transporters, indicating low risk of transporter-mediated nephrotoxicity.

For toxicity, both compounds were predicted to be non-mutagenic (negative AMES test) and non-sensitizing to skin. Nevertheless, 3-ethyl-3-hydroxy-5α-androstan-17-one may pose a risk of hepatotoxicity, whereas β-sitosterol may have cardiotoxic potential due to predicted hERG II inhibition, and was classified as highly toxic in minnow toxicity testing.

In conclusion, both compounds satisfied Lipinski's rule of five, showed no PAINS alerts, and had acceptable synthetic accessibility scores, supporting their potential as orally bioavailable drug candidates. Overall, 3-ethyl-3-hydroxy-5α-androstan-17-one appeared more favorable in terms of absorption, whereas β-sitosterol may offer benefits in CYP safety, albeit with lower GI absorption and cardiotoxicity concerns. Further research and experimental validation are required to address and optimize the safety profiles and limitations of these compounds.

4. Discussion

In this study, 37 compounds of *Piper betle* L. leaves were screened using structures obtained from the PubChem chemical library. The results showed that two compounds, 3-ethyl-3-hydroxy-5α-androstan-17-one and β-sitosterol, showed stronger binding affinities to TNF-α than the control compound SPD-304 (-8.6 kcal/mol), indicating their potential as TNF-α inhibitors. Given the limited data on the TNF-α inhibitory activity of *Piper betle* L. constituents, this study adopts an application-driven approach using existing computational frameworks to identify novel lead compounds.

3-ethyl-3-hydroxy-5α-androstan-17-one is a steroid compound identified in *Piper betle* L. leaf extract. A study by Fatimawali et al. demonstrated its antimalarial activity through strong inhibition of plasmepsins, which are aspartic proteases of *Plasmodium falciparum*. Specifically, this compound exhibited strong inhibitory effects on plasmepsin-1 and plasmepsin-2 via hydrogen bonding, hydrophobic, and electrostatic interactions at the enzymes' active sites. Molecular dynamics simulations further confirmed the stability of the ligand-receptor complexes, suggesting that this compound holds promise as an antimalarial agent [12]. In addition, a study by Kalalo et al. reported that 3-ethyl-3-hydroxy-5α-androstan-17-one may exert immunoregulatory effects and suppress cytokine storms by binding effectively to several inflammatory targets, including TNF-α, IL-1β, IL-6, and NF-κB p65, with binding energies of -8.7, -7.1, -7.0, and -6.8 kcal/mol, respectively [13]. These findings are consistent with our current results, which also indicate strong binding affinity of this compound to TNF-α, highlighting its potential as a multi-target anti-inflammatory agent. In our study, 3-ethyl-3-hydroxy-5α-androstan-17-one exhibited good binding affinity to TNF-α with a docking score of -8.8 kcal/mol. This compound demonstrated favorable absorption and bioavailability profiles, with no indication of P-glycoprotein-mediated efflux and balanced renal and hepatic elimination. However, it was predicted to have limited ability to cross the BBB and the CNS, which may restrict potential neurological effects. Metabolic modeling suggests that 3-ethyl-3-hydroxy-5α-androstan-17-one is processed via CYP3A4 and could inhibit CYP2C19, raising a possible risk of drug-drug interactions. Although predicted to be non-mutagenic and non-sensitizing, its potential hepatotoxicity remains a concern and requires further assessment. Therefore, despite its promising binding interactions and favorable drug-like properties, further studies are required to investigate its biological activity *in vitro* and *in vivo*, and to comprehensively assess its safety and therapeutic efficacy in humans.

β-sitosterol is a phytosterol compound found in various plant species, including *Hymenocrather calycinus*, *Salvia hypoleuca*, *Lomatopodium staurophyllum*, *Tephrosia uniflora*, *Alpinia galangal*,... [14]. It possesses a wide range of biological activities, such as antibacterial, antifungal, antioxidant, anti-inflammatory, immunomodulatory, and anticancer effects [14-16]. Among these, its anti-

inflammatory activity has been extensively studied, particularly its ability to inhibit TNF-α. Pei-Chun Liao et al. demonstrated that β-sitosterol at concentrations ranging from 7.5 to 30 μM dispersed well in the medium as nanoparticles with a mean diameter of 50 ± 5 nm and significantly inhibited the secretion of inflammatory mediators, including TNF-α, IL-1β, IL-6, IL-8, and reactive oxygen species (ROS) in keratinocytes and macrophages [16]. Similarly, In-Ah Lee et al. reported that this compound at a dose of 20 mg/kg significantly downregulated the expression of TNF-α (45%), IL-1β (42.5%), IL-6 (60.4%), COX-2, and suppressed the NF-κB signaling pathway in the colonic tissue of mice with 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis [17]. In another study, Rafael et al. showed that β-sitosterol inhibited the activity of inflammatory enzymes such as myeloperoxidase and adenosine deaminase, and reduced the expression of IL-1β and TNF-α. Specifically, at doses of 0.25 and 0.5 mg/kg, TNF-α levels were reduced by 15.1 ± 3.1% and 64.3 ± 9.5%, respectively ($P < 0.05$) [18]. In our study, β-sitosterol exhibited good binding affinity to TNF-α, with a docking score of -8.7 kcal/mol. This compound demonstrated favorable absorption properties and the ability to cross both the BBB and CNS barriers, which may be relevant for its potential pharmacological effects. Although predicted to be non-mutagenic and non-sensitizing, *in silico* toxicity assessments suggested a possible cardiotoxicity risk due to hERG II inhibition, as well as high toxicity in the minnow aquatic model, raising concerns about its environmental impact. β-sitosterol is expected to be metabolized in the liver via CYP3A4, with no major interactions anticipated with other CYP isoforms. These findings highlight both the therapeutic promise and safety considerations that warrant further experimental validation.

While this study is limited to *in silico* analysis, future validation is crucial to confirm biological relevance. Proposed experiments include *in vitro* TNF-α inhibition assays using macrophage-derived cell lines (e.g., RAW 264.7) and *in vivo* models of inflammation, such as LPS-induced edema in mice. These assays would assess the ability of the lead compounds to modulate TNF-α production and related signaling pathways.

In addition to biological validation, future computational efforts may benefit from the integration of more advanced simulation techniques. While the present study was limited to static molecular docking, applying molecular dynamics (MD) simulations would allow for time-dependent evaluation of ligand-protein stability, and MM-PBSA (Molecular Mechanics Poisson-Boltzmann Surface Area) analysis could offer more refined estimates of binding free energy. Moreover, consensus docking strategies using multiple scoring functions or docking engines may help mitigate potential algorithm-specific biases. These enhancements would further strengthen the predictive power and reliability of *in silico* screening results.

5. Conclusion

Among the screening of 37 compounds from *Piper betle* L. leaves, β-sitosterol and 3-ethyl-3-hydroxy-5α-androstan-17-one emerged as promising TNF-α inhibitor candidates based on the *in silico* docking results and ADMET parameters analysis. Therefore, further *in vitro* and *in vivo* studies are required to validate their activity and support the development of these compounds into clinical drug candidates.

Acknowledgments

This research has been done under the research project QG 23.70, “Study on formulation and evaluation of the therapeutic support effect for acute gout treatment in experimental model of hard capsules containing quantified extract of *Piper betle* L., Piperaceae leaves” of Vietnam National University, Hanoi.

References

[1] A. K. Palucka, J. P. Blanck, L. Bennett, V. Pascual, J. Banchereau, Cross-regulation of TNF and IFN-alpha in Autoimmune Diseases, Proc Natl Acad Sci U S A, Vol. 102, No. 9, 2005, pp. 3372-3377, https://doi.org/10.1073/pnas.0408506102.

[2] K. Zia, S. Ashraf, A. Jabeen, M. Saeed, E. A. M. Nur, S. Ahmed et al., Identification of Potential TNF-alpha Inhibitors: from in Silico to *in Vitro* Studies, Sci Rep, Vol. 10, No. 1, 2020, pp. 20974, https://doi.org/10.1038/s41598-020-77750-3.

[3] J. Bystrom, F. I. Clanchy, T. E. Taher, P. Mangat, A. S. Jawad, R. O. Williams et al., TNF-alpha in the Regulation of Treg and Th17 Cells in Rheumatoid Arthritis and Other Autoimmune Inflammatory Diseases, Cytokine, Vol. 101, 2018, pp. 4-13, https://doi.org/10.1016/j.cyto.2016.09.001.

[4] D. I. Jang, A. H. Lee, H. Y. Shin, H. R. Song, J. H. Park, T. B. Kang et al., The Role of Tumor Necrosis Factor Alpha (TNF-alpha) in Autoimmune Disease and Current TNF-alpha Inhibitors in Therapeutics, Int J Mol Sci, Vol. 22, No. 5, 2021, pp. 2719, https://doi.org/ 10.3390/ijms22052719.

[5] M. Madhumita, P. Guha, A. Nag, Bio-actives of Betel Leaf (Piper betle L.): A Comprehensive Review on extraction, isolation, characterization, and Biological Activity, Phytother Res, Vol. 34, No. 10, 2020, pp. 2609-2627, https://doi.org/10.1002/ptr.6715.

[6] N. Nayaka, M. M. V. Sasadara, D. A. Sanjaya, P. Yuda, N. Dewi, E. Cahyaningsih et al., Piper betle (L): Recent Review of Antibacterial and Antifungal Properties, Safety Profiles, and Commercial Applications, Molecules, Vol. 26, No. 8, 2021, pp. 2321, https://doi.org/10.3390/molecules26082321.

[7] J. Fan, A. Fu, L. J. Q. B. Zhang, Progress in Molecular Docking, Vol. 7, 2019, pp. 83-89, https://doi.org/10.1007/s40484-019-0172-y.

[8] B. Jayaram, T. Singh, G. Mukherjee, A. Mathur, S. Shekhar, V. Shekhar, Sanjeevini: a Freely Accessible Web-Server for Target Directed Lead Molecule Discovery, BMC Bioinformatics, Vol. 13, No. Suppl 17, 2012, pp. S7, https://doi.org/10.1186/1471-2105-13-S17-S7.

[9] D. E. Pires, T. L. Blundell, D. B. Ascher, pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures, Journal of Medicinal Chemistry, Vol. 58, No. 9, 2015, pp. 4066-4072, https://doi.org/10.1021/acs.jmedchem.5b00104.

[10] A. Daina, O. Michielin, V. Zoete, SwissADME: a Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules, Sci Rep, Vol. 7, 2017, pp. 42717, https://doi.org/10.1038/srep42717.

[11] H. Gohlke, M. Hendlich, G. Klebe, Knowledge-Based Scoring Function to Predict Protein-Ligand Interactions, J Mol Biol, Vol. 295, No. 2, 2000, pp. 337-356, https://doi.org/10.1006/jmbi.1999.3371.

[12] Fatimawali, T. E. Tallei, B. J. Kepel, M. Alorabi, A. M. E. Shehawi, W. Bodhi et al., Appraisal of Bioactive Compounds of Betel Fruit as Antimalarial Agents by Targeting Plasmepsin 1 and 2: A Computational Approach, Pharmaceuticals (Basel), Vol. 14, No. 12, 2021, pp.1285. http:// doi: 10.3390/ph14121285.

[13] M. J. Kalalo, S. B. Simanjuntak, T. A. Hebber, B. J. Kepel, T. E. J. J. O. A. P. S. Tallei, Immunomodulatory Potential of Bioactive Compounds of Betel Leaf Extract Targeting COVID-19 Immunological Human Host Proteins: An in Silico Study, Vol. 12, No. 2, 2022, pp. 075-088, https://doi.org/10.7324/JAPS.2021.120208.

[14] S. Saeidnia, A. Manayi, A. R. Gohari, M. Abdollahi, The Story of Beta-Sitosterol-a Review, European Journal of Medicinal Plants, Vol 4, Iss. 5, pp. 590-609, https:// doi.org/10.9734/EJMP/2014/7764.

[15] H. Wang, Z. Wang, Z. Zhang, J. Liu, L. Hong, Beta-Sitosterol as a Promising Anticancer Agent for Chemoprevention and Chemotherapy: Mechanisms of Action and Future Prospects, Adv Nutr, Vol. 14, No. 5, 2023, pp. 1085-1110, https://doi.org/10.1016/j.advnut.2023.05.013.

[16] P. C. Liao, M. H. Lai, K. P. Hsu, Y. H. Kuo, J. Chen, M. C. Tsai et al., Identification of Beta-Sitosterol as *in Vitro* Anti-Inflammatory Constituent in Moringa Oleifera, J Agric Food Chem, Vol. 66, No. 41, 2018, pp. 10748 - 10748, https://doi.org/10.1021/acs.jafc.8b04555.

[17] I. A. Lee, E. J. Kim, D. H. Kim, Inhibitory Effect of Beta-Sitosterol on TNBS-Induced Colitis in Mice, Planta Med, Vol. 78, No. 9, 2012, pp. 896, https://doi.org/10.1055/s-0031-1298486.

[18] R. Liz, L. Zanatta, G. O. Dos Reis, H. Horst, M. G. Pizzolatti, F. R. Silva et al., Acute Effect of Beta-Sitosterol on Calcium Uptake Mediates Anti-Inflammatory Effect in Murine Activated Neutrophils, J Pharm Pharmacol, Vol. 65, No. 1, 2013, pp. 115, https://doi.org/10.1111/j.2042-7158.2012.01568.x.