LUPINE PUBLISHERS

Lupine Online Journal of Pharmacology & Clinical Research

DOI: 10.32474/LOJPCR.2023.03.000162

ISSN: 2644-1373

Research Article

An alternative approach to used Boswellic acid α and Capsaicin as relive pain pathophysiology

Abdulaziz Alzahrani*

Pharmaceuticals Chemistry Department, Faculty of Pharmacy, Al-Baha University,

***Corresponding author:** Abdulaziz Alzahrani, Pharmaceuticals Chemistry Department, Faculty of Pharmacy, Al-Baha University, Saudi Arabia

Received: August 20, 2023 **Published:** September 10, 2025

Abstract

Inflammation, a key component in the pathophysiology of pain, involves a complex interplay of immune, vascular, and cellular biochemical reactions, leading to the release of various pro-inflammatory molecules. IL-6 and TNF-α are pro-inflammatory cytokines implicated in various inflammatory and autoimmune diseases, as well as in the development of pain. In the present study, we tested the potentiality of natural compounds such as Boswellic acid α and Capsaicin targeting several key interleukins that are secreted as a result of pain. We used molecular docking analyses, molecular dynamics simulations, and pharmacokinetic properties to test the stability of the complexes. The compound's binding energy with some cytokines was over -6 to -7.2 Kcal/mol. Furthermore, a post-molecular dynamics (MD) study revealed that Capsaicin was extremely stable with these cytokines. The compound's pharmacokinetic measurements demonstrated excellent properties in terms of adsorption, distribution, metabolism, and excretion. Our research revealed that this compound may be effective in lowering pain and treating inflammatory.

Keywords: Boswellic acid α and Capsaicin; Rheumatoid Arthritis; pain

Introduction

Natural products are increasingly recognized for their potential to alleviate pain pathophysiology through the modulation of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [1]. Chronic pain, affecting approximately 20% of the global adult population, poses significant healthcare and economic challenges, necessitating the exploration of novel therapeutic avenues [2]. Inflammation, a key component in the pathophysiology of pain, involves a complex interplay of immune, vascular, and cellular biochemical reactions, leading to the release of various pro-inflammatory molecules [1] [3]. Cytokines, including IL-6 and TNF-α, play a crucial role in this inflammatory cascade, contributing to the sensitization of the nervous system and the development of chronic pain conditions [4] [5]. IL-6 and TNF-α are pro-inflammatory cytokines implicated in various inflammatory and autoimmune diseases, as well as in the development of pain [6] [7] [8]. Specifically, TNF-α binding to its receptor TNFR1 can stimulate the production of reactive oxygen species (ROS), activating downstream signaling pathways involving MEK and ERK1/2, ultimately leading to the activation of NF-κB and the transcription of genes involved in inflammation and pain [9]. IL-6 also promotes inflammation and can lead to neuronal degeneration [5]. Given their central role in inflammation and pain, IL-6 and TNF-α have become therapeutic targets for pain management. Natural products, derived from various sources such as plants, marine organisms, and fungi, have demonstrated the ability to modulate the production and activity of IL-6 and TNF-α, offering a promising approach for pain alleviation [10] [11] [12].

These natural compounds can target multiple steps in the inflammatory cascade, including the triggering of signaling cascades, the activation of transcription IL-6 is a cytokine that, in

different contexts, can exhibit both pro-inflammatory and anti-inflammatory properties. For instance, IL-6 plays a protective role in some infections by suppressing TNF-alpha production, which suggests an interaction where IL-6 downregulates TNF-alpha, mitigating inflammation and its associated pain [13]. On the other hand, increased IL-6 levels have been observed in conditions like inflammatory bowel disease (IBD), indicating its role in ongoing immune responses in the inflammatory mucosal environment [14]. TNF-alpha, another crucial cytokine, can induce significant inflammatory responses. For example, TNF-alpha's upregulation has been implicated in lung injury and inflammation induced by interleukin-2 (IL-2), which can exacerbate tissue damage and pain [15]. However, its expression can be regulated by various physiological conditions, such as hyperthermia, which attenuates TNF-alpha production while maintaining IL-6 expression [16]. The interaction between IL-6 and TNF-alpha is complex and can vary depending on the biological context. For instance, the synergistic action of TNF-alpha and IL-6 has been shown to enhance T cell proliferation, indicating a cooperative role in immune activation [17]. However, these cytokines are also subject to modulation by external agents and stresses, such as exercise and psychological stress, which differentially affect their production [18].

Overall, the modulation of IL-6 and TNF-alpha by natural products holds potential in alleviating pain by controlling their levels and mitigating inflammatory processes. The ability to fine-tune these cytokines' expression may lead to novel therapeutic strategies for managing inflammatory and pain-related conditions. While natural products are promising in this domain, further research is necessary to fully understand their potential and efficacy in clinical settings. Boswellic acid α and capsaicin are bioactive compounds with distinct pharmacological properties. Boswellic acid α, a pentacyclic triterpene derived from Boswellia serrata resin, exhibits potent anti-inflammatory and anti-arthritic effects by inhibiting 5-lipoxygenase (5-LOX) and modulating NF-κB signaling [19]. It has been studied for its potential in treating chronic inflammatory diseases, including osteoarthritis and inflammatory bowel disease [20]. In contrast, capsaicin, the pungent alkaloid found in chili peppers (Capsicum spp.), acts primarily on the transient receptor potential vanilloid 1 (TRPV1) channel, inducing analgesia and anti-nociceptive effects [21]. Capsaicin is utilized in pain management, particularly for neuropathic pain, and has demonstrated metabolic benefits in obesity-related studies [22]. Both compounds highlight the therapeutic potential of plant-derived molecules in modern medicine.

Materials and methods

Preparation of compounds and receptor

Two small natural compounds named Capsaicin and Boswellic acid α were selected for this study. The crystal structures of the IL1β,TNF-α and IL-6 were retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB ID: 3BIK) [23]. Discovery Studio 4.0 client (http://accelrys.com/products/discovery-studio/) was used to remove all the bonded ligand molecules, hetero atoms and the water molecules of these [24]. Later, the 3D structure of 4 compounds was retrieved from PubChem database and used for molecular docking.

Molecular Docking Simulation of 2 compounds with IL1β, TNF-α and IL-6

PyRX, an open-source virtual screening program, was used to perform molecular docking to determine receptor-ligand interactions [25]. The CASTp server (https://sts.bioe.uic.edu/castp/) provided the essential ligand binding site residues, which were then enclosed with the appropriate grid box parameters. All docking simulations were carried out using PyRX's Auto Dock Vina (ADV) [26] implementation, with the predefined parameters. Additionally, Pymol 1.1 and Discovery studio 4.0 (https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/) client were used to visualize the protein-ligand interaction [27]. To validate the docking protocol, we collected the 3D structures of 2 known inhibitors of IL6 [28] and TNFα [29], performed molecular docking in the predefined site of these 2 cytokines and the results were analyzed.

Molecular Dynamics (MD) Simulation Analysis

Molecular dynamics (MD) simulation of the four cytokine-apigenin complexes was performed using GROMACS 2021.1 (University of Groningen, City of Groningen, Northern Netherlands) MD engine [30] on Linux 5.4 package. The GROMOS96 54a7 [31] forcefield was used for the cytokines while the ligand topologies were obtained from the PRODRG [32] server. Solvation of the complexes were done using simple point charge (SPC) water molecules in a rectangular box. The simulation systems were neutralized by adding required number of Na+ and Cl− ions while salt concentrations were set as 0.15 mol/L in all the systems. All the solvated systems were subjected to energy minimization for 5000 steps utilizing the steepest descent algorithm. Afterwards, NVT (constant number of particles, volume, and temperature) series, NPT (constant number of particles, pressure, and temperature) series, and the production run were performed in the MD simulation [33]. Both the NVT and the NPT series were performed at a 300 K temperature and 1 atm pressure for 300 ps (picoseconds) duration. V-rescale thermostat and Parrinello-Rahman barostat were used of the performed simulations. Finally, the production run was conducted at 300 K for a duration of 100 ns (nanoseconds). Thereafter, by measuring root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA) and hydrogen bonds, a comparative analysis was performed to analyze their stability. The Qtgrace [34] program was utilized to represent the analyses in the form of plots.

ADME analysis

SwissADME [35] online tool (http://www.swissadme.ch) was utilized to assess the pharmacokinetics properties of 4 compounds.

Results

Molecular Docking Analysis of 2 compounds with the IL1β,TNF-α and IL-6

Nine possible binding positions for each compound was obtained from AutoDock Vina as output, from where the best position was selected for each compound depending on the lowest binding energy. The docking energy score of the three cytokines with IL1β, TNF-α and IL-6 ranged from -6to -7.2 Kcal/mol (Table 1). 4 compounds established at least interactions with the PD-1 shown in Figure 2 (A-B). all 2 compounds showed higher docking energy of-6.2 -7.2 Kcal/mol with IL-6 Molecular docking results.

Table 1: Docking energy score of the four compounds with cytokines.

Compounds	Binding energy (Kcal/mol) with IL1β	Binding energy (Kcal/mol) with TNF-α	Binding energy (Kcal/mol) with IL6
Lavender lactone	+5.8	-4.4	-4.0
Capsaicin	-3.8	-6.0	-6.2
Eucalyptus oil	-4.2	-3.9	-4.6
Boswellic acid α	-3.6	-3.9	-7.2

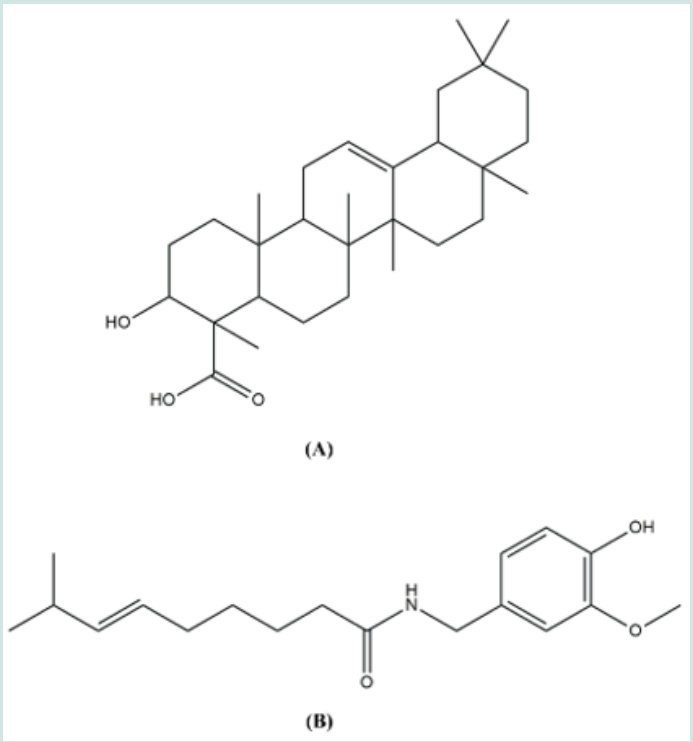

Figure 1: Chemical structure of A) Boswellic acid α and B) Capsaicin.

Molecular dynamics (MD) simulation results

The dynamic movements of atoms and conformational variations of Cα atoms of the IL-6 and TNF-α complexes were calculated by RMSD to detect their stability. It is observed that the Boswellic acid α and Capsaicin exhibit a slightly higher but stable RMSD wherein the remaining complexes exhibit lower RMSD (<2.0 nm) with no major fluctuations indicating their greater stability shown in Figure 3A. The flexibility of each residue was calculated in terms of RMSF to get better insight on the region of cytokines that are being fluctuated during the simulation. It can be understood that the binding of Boswellic acid α and Capsaicin makes the IL-6 slightly flexible in 100-150 residue areas (Figure 3B). No major fluctuations were observed in any residue areas of the remaining complexes. The compactness of the complex was represented by the radius of gyration (Rg). The lower degree of fluctuation throughout the simulation period denotes the greater compactness of a system. The Rg of the complexes were found to be similar while the Boswellic acid α and Capsaicin complexed to IL-6 and TNF-α complex was found to be slightly opposite (Figure 3C). Interaction

between protein-ligand complexes and solvents was measured by solvent accessible surface area (SASA) over the simulation period. So, SASA of the complex was calculated to analyze the extent of the conformational changes occurred during the interaction. Interestingly, all the cytokines featured a slight reduction of the surface area showing relatively lower SASA value than the starting period (Figure 3D). Hydrogen bonding between a protein-ligand complex is essential to stabilize the structure. It was observed that the highest number of conformations of IL6 and TNF- α formed up to three hydrogen bonds with apigenin (Figure 3E).

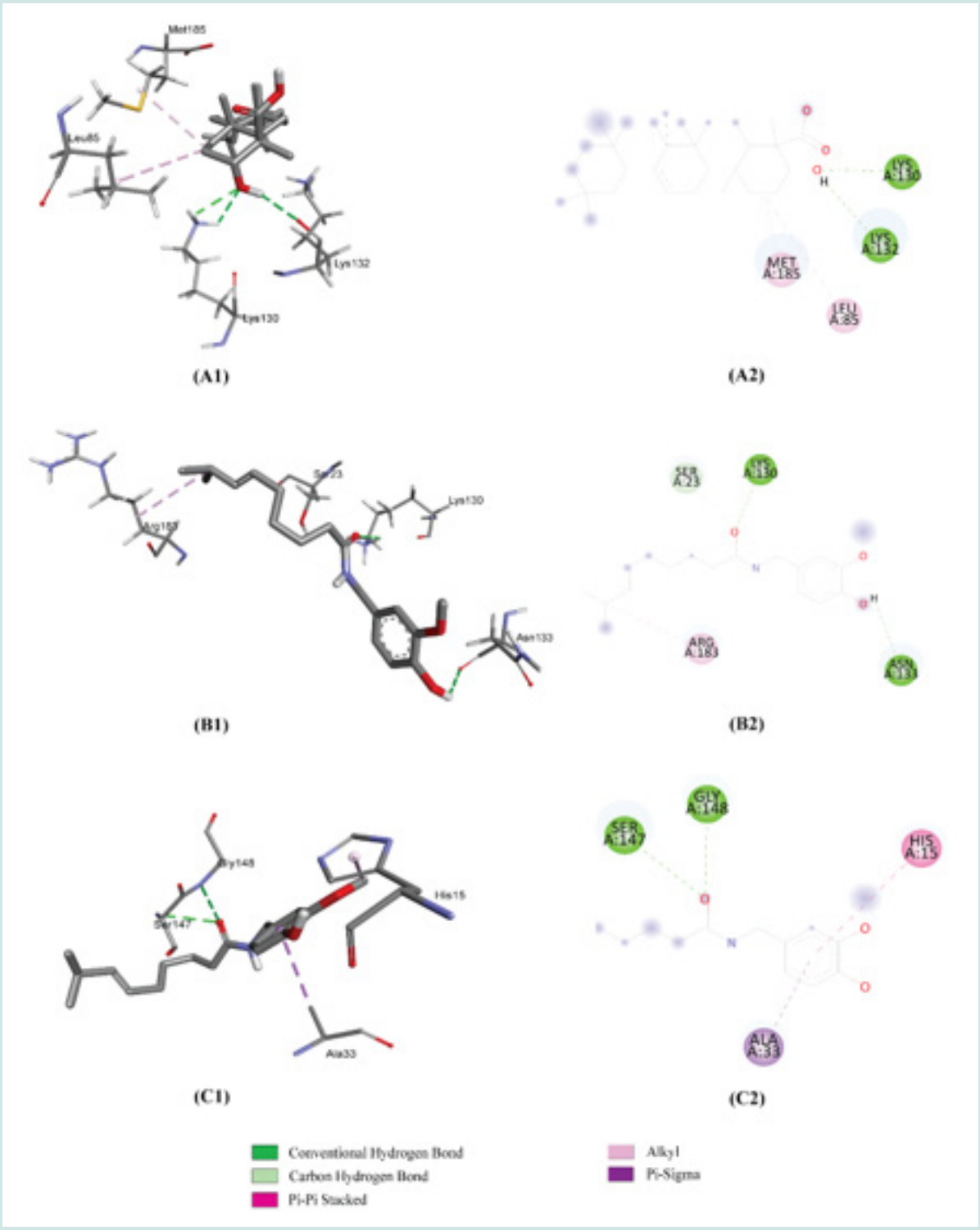

Figure 2: Interactions of IL6-Boswellic acid α (A), IL6-Capsaicin (B), and TNF-α-Capsaicin (C).

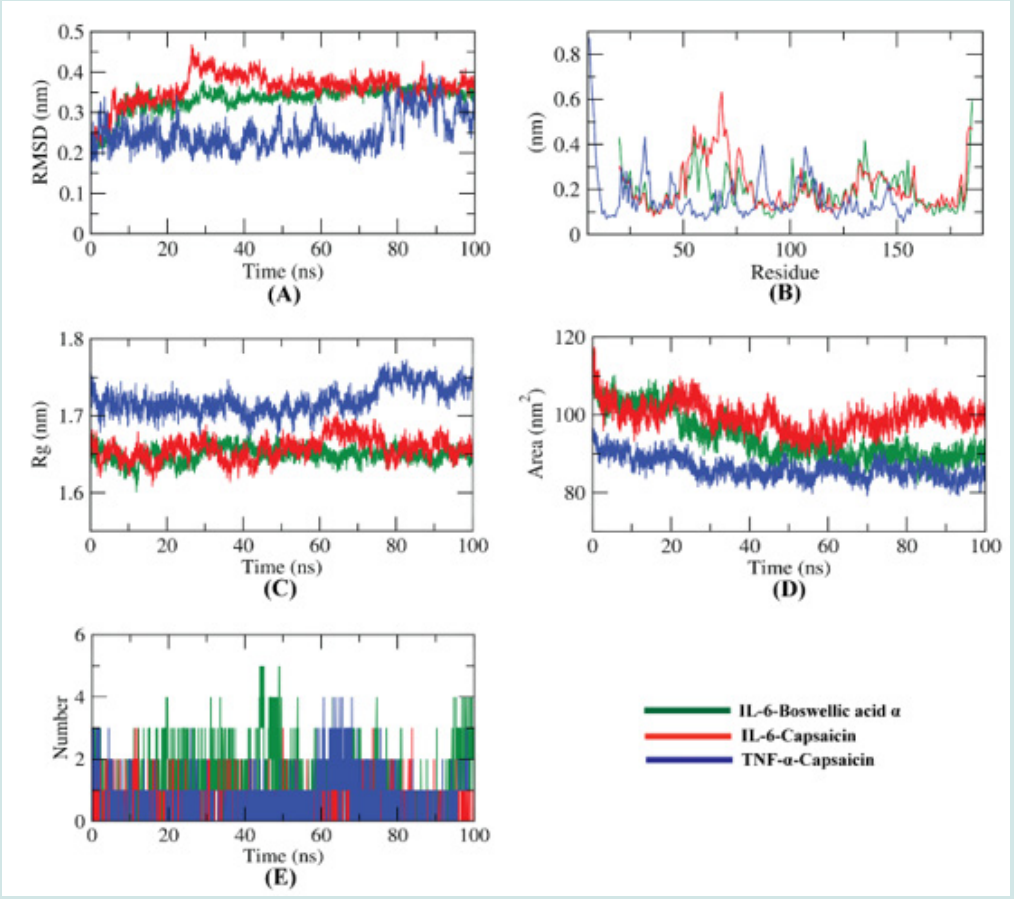

Figure 3: RMSD (A), RMSF (B), Rg (C), Hydrogen bond (D) and SASA (E) results of the four compounds complexes after 100ns simulation.

ADME analysis

ADME properties of Boswellic acid α and Capsaicin were predicted using Swiss ADME server (Table 2). In many attributes apigenin exhibited significant values. Notably, it doesn't violate Lipinski's rule of five and has high GI absorption. The compound does not seem to inhibit any isoform of the cytochrome P450 enzyme. The log kp (skin permeation) was in good range and was unable to cross blood brain barrier. PAINS and brenk alerts confirmed absence of any catechol moieties in the compound and synthetic accessibility values were also found to be low.

Table 2: Important ADME properties of 2 compounds.

Parameters	Boswellic acid α	Capsaicin
Num. H-bond acceptors	3	3
Num. H-bond donors	2	2
Molecular weight (g/mol)	456.7	305.41
Lipinski violation	1 violation	0 violation
GI absorption	Low	High
BBB permeant	No	No
CYP1A2 inhibitor	No	Yes
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	Yes	No
CYP2D6 inhibitor	No	Yes

CYP3A4 inhibitor	No	Yes
Log K_p (skin permeation) (cm/s)	-3.11	-5.62
PAINS	0 alert	0 alert
Brenk	1 alert: isolated_alkene	1 alert: isolated_alkene
Synthetic accessibility	6.04	2.32

Discussion

Apart from traditional methods, computer-assisted drug design (CADD) approaches are now used efficiently in the drug discovery and development process. Molecular docking and molecular dynamics simulations are commonly used to perform the virtual screening of compounds to examine how ligands inhibit a target and confirm their stability towards specific target proteins, among numerous approaches to CADD that are recognized as promising techniques. Additionally, the rising development of robust computational techniques allows for the assessment of the pharmacological properties of drugs prior to engaging in an experimental trial. As a result, in this study, rigorous CADD methodologies were used to examine the drug capabilities of Boswellic acid α and Capsaicin against a range of IL6 and TNF- α cytokines.

Considering the significance of cytokines pain management, particularly for neuropathic pain, we tested Boswellic acid α and Capsaicin efficacy against different cytokines by looking at both its binding affinity and ADMET characteristics. Boswellic acid α and Capsaicin were tested against IL6 and TNF- α cytokines for this purpose. When binding with IL6, the compound had the maximum binding affinity of −7.2 kcal/mol and interacted with four amino acid residues in the cytokines' active site. We further investigated their stability with Boswellic acid α and Capsaicin, conducting MD simulation for a 100 ns time period, even though they had strong binding affinity with other cytokines as well.

The compound was further used for molecular docking analysis to determine their binding affinities towards different cytokines. The result of the analysis indicated good binding energies of most cytokines ranging between −6.2 and −7.2 kcal/mol respectively. (Table 1). The compound formed stable interactions with several of the active site residues of the target cytokines. Almost all the cytokines were stabilized by at least one hydrogen bonding interaction and several other interactions such as carbon–hydrogen bonding and Pi-Alkyl interactions. The stability of Boswellic acid α and Capsaicin with the respected cytokines was confirmed by the MD simulation findings of the top 4 complexes. The RMSD plot demonstrated that all of the complexes are stable, and the RMSD values did not fluctuate significantly over time. The complexes did not show significant variability and were within an acceptable range, according to the RMSF analysis. The radius of gyration (Rg) data revealed that each complex had a substantially comparable compactness characteristic. The SASA data indicated that the volume of the complexes had dropped somewhat. Throughout the simulation, a significant number of hydrogen bonds were identified in all the complexes, which once again explained their conformational stability. As a result, Andrographolide can bind to these cytokines, interfering with their action. Thus, by inhibiting these cytokines, several functions such as the production of granulocyte and monocyte, and the proliferation of B and T cells can be altered, thereby reducing the cytokine inflammatory as result pain reliefed.

Boswellic acid α and Capsaicin has a high gastrointestinal absorption potency, with a skin permeability coefficient (Log Kp) of −6.90, according to its ADME characteristics. It does not break Lipinski's rule of five; therefore, it might readily be mimicked by other natural cytokine ligands. Furthermore, the permeability of the blood–brain barrier membrane revealed that the compound is unable to cross the blood–brain barrier. Pan-assay interference chemicals (PAINS) are chemical compounds that react nonspecifically with a variety of biological targets, resulting in false positive results in high-throughput screens. It does not have any catechol groups, according to the PAINS filter. It has a low synthetic accessibility score, indicating that the compound can considerably more easily be produced in experimental laboratories. There may be some limitations in this study because it was conducted via in silico analysis. Despite the positive results with the cytokines, the compound has yet to be tested in an animal model. To assure its therapeutic efficacy, sufficient experimental validations are required.

Conclusions

Our study proposed and tested a new compound, Boswellic acid α and Capsaicin, against IL6 and TNF- α crucial cytokines involved in pain relief. Binding of the compound to the target cytokines may eventually create an alteration in entering in their action and producing a minor amount. The compound showed better results in both binding with the proteins in efficient oral bioavailability and less toxicity. Considering binding affinities, interactions with target proteins, and ADME profile, we have analyzed and validated the stability of protein–ligand complexes using an MD simulation approach and found intriguing results for all of them. The results of this study might be valuable references for further experimental research in designing new potent cytokine regulators. Hence, it can be concluded, after further in vitro and in vivo experiments, that Boswellic acid α and Capsaicin, against IL6 and TNF- α can be used as a therapeutic against inflammatory diseases.

References

1. Andrade PB, Valentão P (2018) Natural products as anti-inflammatory agents: Role in pain management *Pharmacol Res* 132: 239-250.
2. Belda Antolí M, Martínez Sanz JM, Gil Martínez A (2025) Global burden of chronic pain: Epidemiology and healthcare challenges. Pain Rep 10(2): e850.
3. Vichare R, Teli P, Ojha S (2025) Inflammatory pathways in chronic pain: Mechanisms and interventions. Pharmacol Rep 77(1): 45-58.
4. Körtési T, Tuka B, Tajti J, Vécsei L (2024) Cytokine modulation in migraine and cluster headache: Implications for treatment strategies. J Headache Pain 25(1): 12.
5. Nashtahosseini S, Mahmoudi M, Rezaei N (2025) Cytokine-targeted therapies for chronic inflammatory pain. Expert Rev Clin Immunol 21(3): 345-60.
6. Lee JH, Kim HJ, Park JW (2023) Role of IL-6 and TNF-α in neuropathic pain: Mechanisms and therapeutic implications. Exp Neurol 359: 114240.
7. Jia Y, Jing J, Bai Y, Li Z, Liu L, et al. (2011) Amelioration of experimental autoimmune encephalomyelitis by plumbagin through down-regulation of JAK-STAT and NF-κB signaling pathways. PLoS One 6(10): 27006.
8. Afsar T, Afsar B (2023) Role of cytokines in pathophysiology of pain. Cytokine Growth Factor Rev 70: 22-34.
9. Kumar A, Vinayak M (2020) TNF-α signaling in pain pathophysiology: Mechanisms and therapeutic targeting. Cytokine 125: 154861.
10. Pal A, Das S, Kundu R (2023) Natural anti-inflammatory compounds as cytokine modulators in pain management. Bioorg Chem 130: 106234.
11. Kim SH, Lee JH, Chung HS (2021) Natural products targeting cytokine networks in chronic inflammatory diseases. Phytomedicine 90: 153645.
12. Agung A, Sari N, Prasetyo R (2024) Natural products as modulators of IL-6 and TNF-α in pain management. J Ethnopharmacol 320: 117432.
13. Diao H, Kohanawa M (2005) Endogenous interleukin-6 plays a protective role in streptococcal toxic shock syndrome via suppression of tumor necrosis factor-alpha production. Infect Immun 73(7): 4085-92.
14. Stevens C, Walz G, Singaram C, Lipman ML, Zanker B, et al (1992) Tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 expression in inflammatory bowel disease. Dig Dis Sci 37(6): 818-26.
15. Rabinovici R, Neville LF, Rudolph AS, Feuerstein G (1996) Interleukin-2-induced lung injury: Role of tumor necrosis factor-alpha. Am J Respir Cell Mol Biol 14(1): 47-54.
16. Ensor JE, Wiener SM, McCrea KA, Viscardi RM, Crawford EK, et al (1994) Differential effects of hyperthermia on macrophage interleukin-6 and tumor necrosis factor-alpha expression. Am J Physiol Cell Physiol 266(4): C967-C974.
17. Kuhweide R, Van Damme J, Ceuppens JL (1990) Tumor necrosis factor-alpha and interleukin-6 synergistically induce T cell growth. Eur J Immunol 20(5): 1019-25.
18. Goebel MU, Mills PJ, Irwin MR, Ziegler MG (2000) Interleukin-6 and tumor necrosis factor-alpha production after acute psychological stress, exercise, and infused isoproterenol: Differential effects and pathways. Psychosom Med 62(4): 591-8.
19. Ammon HP (2016) Boswellic acids and their role in chronic inflammatory diseases. Adv Exp Med Biol 928: 291-327.
20. Siddiqui MZ (2011) Boswellia serrata, a potential anti-inflammatory agent: An overview. Indian J Pharm Sci 73(3): 255-61.
21. Bley K, Boorman G, Mohammad B, McKenzie D, Babbar S et al (2012) A comprehensive review of the carcinogenic and anticarcinogenic potential of capsaicin. Toxicol Pathol 40(6): 847-73.
22. Whiting S, Derbyshire E, Tiwari BK (2014) Capsaicinoids and capsinoids: A potential role for weight management? A systematic review of the evidence. Appetite 73: 183-8.
23. Zardecki C, Dutta S, Goodsell DS, Voigt M, Burley SK et al (2016) RCSB Protein Data Bank: A Resource for Chemical, Biochemical, and Structural Explorations of Large and Small Biomolecules. J Chem Educ 93: 569-75.
24. Gao YD, Huang JF (2011) An extension strategy of Discovery Studio 2.0 for non-bonded interaction energy automatic calculation at the residue level. Zool Res 32: 262-6.
25. Dallakyan S, Olson AJ (2015) Small-molecule library screening by docking with PyRx. Methods Mol Biol 1263: 243-50.
26. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 31: 455-61.
27. Rigsby RE, Parker AB (2016) Using the PyMOL application to reinforce visual understanding of protein structure. Biochem Mol Biol Educ 44: 433-7.
28. Yoo J, Kim D, Park J, Kim YK, et al (2022) Novel Small Molecule Inhibitors Targeting the IL-6/STAT3 Pathway or IL-1β. Molecules 27: 2696.
29. He MM, Smith AS, Oslob JD, Flanagan WM, Braisted AC, et al (2005) Small-molecule inhibition of TNF-α. Science 310: 1022-5.
30. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, et al (2015) GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX.1-2: 19-25.
31. Schmid N, Eichenberger AP, Choutko A, Riniker S, Winger M, et al (2011) Definition and testing of the GROMOS force-field versions 54A7 and 54B7. Eur Biophys J 40: 843-56.
32. Schüttelkopf AW, van Aalten DMF (2004) PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. Acta Crystallogr D Biol Crystallogr 60: 1355-63.
33. Berendsen HJC, van der Spoel D, van Drunen R (1995) GROMACS: A message-passing parallel molecular dynamics implementation. Comput Phys Commun 91: 43-56.
34. Soman SS, Sivakumar KC, Sreekumar E (2010) Molecular dynamics simulation studies and in vitro site directed mutagenesis of avian beta-defensin Apl_AvBD2. BMC Bioinformatics 11(1): S7.
35. Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7: 42717.

DOI: 10.32474/LOJPCR.2023.03.000162