IN SILICO STUDY OF BIOACTIVE COMPOUNDS IN BLUE SHARK (*Prionace glauca*) CARTILAGE AND ITS EXTRACT AS ANTI-INFLAMMATORY AGENTS

Titiek Indhira Agustin[1,2*], Happy Nursyam[3], Muhamad Firdaus[3], Muhaimin Rifa'i[4], Maria Dolorosa Sare[2,6], Ersanda Nurma Praditapuspa[5]

[1]Graduate Program of Fisheries and Marine Science, Faculty of Fisheries and Marine Science, Brawijaya University
Veteran st., Malang, Indonesia 65149

[2]Fisheries Study Program, Faculty of Engineering and Marine Science, Hang Tuah University
Arief Rahman Hakim st. 150, Surabaya, Indonesia 60111

[3]Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science, Brawijaya University
Veteran st., Malang, Indonesia 65149

[4]Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University
Veteran st., Malang, Indonesia 65149

[5] Departement of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Hang Tuah
Arief Rahman Hakim st. 150, Surabaya, Indonesia 60111

[6]Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, IPB University Raya Dramaga st., Bogor, Indonesia 16680

Submitted: 21 September 2025/Accepted: 13 March 2026
*Correspondence: titiek.indhira@hangtuah.ac.id

How to cite (APA Style 7th): Agustin, T. I. Nursyam, H., Firdaus, M., Rifa'i, M., Sare, M. D., & Praditapuspa, E. N. (2026). In silico study of bioactive compounds in blue shark (*Prionace glauca*) cartilage and its extract as anti-inflammatory agents. *Jurnal Pengolahan Hasil Perikanan Indonesia,* 29(5), 398-419. http://dx.doi.org/10.17844/rf3rmd10

Abstract

Blue shark *(Prionace glauca)* cartilage contains various bioactive compounds that may be beneficial to human health. This study aimed to identify bioactive compounds in shark cartilage and evaluate their potential as anti-inflammatory agents by targeting NF-kB p65 and TNF-α convertase. Shark cartilage powder and its extract were analyzed using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS), while ligand-protein interactions were evaluated in silico via molecular docking using Autodock on the NF-kB p65 (PDB ID: 2RAM) and TNF-α convertase (PDB ID: 3EWJ) targets. LC-HRMS analysis identified 64 bioactive compounds in the cartilage powder and 141 in the extract. A total of 20 powdered compounds (powder) and 37 compounds (extract) showed mzCloud Best Match values >80%. Thirteen compounds were successfully identified, including betaine, creatine, hypoxanthine, L-norleucine, choline, caprolactam, nicotinamide, L-phenylalanine, creatinine, guanine, trigonelline, trans-3-indoleacrylic acid, and D-(+)-proline, with isocytosine and lactamide specific to the powder, and 3,5-di-tert-butyl-4-hydroxybenzaldehyde, TEMPO, and piperine specific to the extract. Docking results indicated that l-phenylalanine and trans-3-indoleacrylic acid possess high binding affinities and stably interact with the active sites of NF-kB p65 and TNF-α convertase, potentially inhibiting the pro-inflammatory pathways mediated by these two proteins. These findings indicate that shark cartilage powder and its extract show promise as candidates for anti-inflammatory therapeutic agents with low toxicity potential, which requires further validation in vitro and in vivo studies.

Keywords: bioactive compounds, inflammation, LC-HRMS, molecular docking

Studi *In Silico* Senyawa Bioaktif Tulang Rawan Hiu Biru (*Prionace glauca*) dan Ekstraknya sebagai Agen Antiinflamasi

Abstrak

Tulang rawan hiu biru (*Prionace glauca*) diketahui mengandung berbagai senyawa bioaktif yang berpotensi bermanfaat bagi kesehatan manusia. Penelitian ini bertujuan mengidentifikasi senyawa bioaktif tulang rawan hiu serta mengevaluasi potensinya sebagai agen antiinflamasi melalui penargetan NF-kB p65 dan *TNF-α convertase*. Tepung tulang rawan hiu dan ekstraknya dianalisis menggunakan *Liquid Chromatography-High Resolution Mass Spectrometry* (LC-HRMS), sedangkan interaksi ligan-protein dievaluasi secara *in silico* dengan *molecular docking* menggunakan *Autodock* pada target NF-kB p65 (PDB ID: 2RAM) dan *TNF-α convertase* (PDB ID: 3EWJ). Analisis LC-HRMS menemukan 64 senyawa bioaktif pada tepung tulang rawan dan 141 senyawa pada ekstraknya. Sebanyak 20 senyawa (tepung) dan 37 senyawa (ekstrak) menunjukkan nilai *mzCloud Best Match* >80%. Tiga belas senyawa berhasil diidentifikasi, termasuk *betaine*, *creatine*, *hypoxanthine*, *L-norleucine*, *choline*, *caprolactam*, *nicotinamide*, *L-phenylalanine*, *creatinine*, *guanine*, *trigonelline*, *trans-3-indoleacrylic acid*, dan *D-(+)-proline*, dengan *isocytosine* dan *lactamide* spesifik pada tepung, serta *3,5-di-tert-butyl-4-hydroxybenzaldehyde*, TEMPO, dan *piperine* spesifik pada ekstrak. Hasil *docking* menunjukkan bahwa *L-phenylalanine* dan *trans-3-indoleacrylic acid* memiliki afinitas ikatan tinggi dan mampu berinteraksi stabil dengan situs aktif NF-kB p65 dan TNF-α convertase, sehingga berpotensi menghambat jalur proinflamasi yang dimediasi kedua protein tersebut. Temuan ini mengindikasikan bahwa tepung tulang rawan hiu dan ekstraknya menjanjikan sebagai kandidat agen terapeutik antiinflamasi dengan potensi toksisitas rendah yang perlu divalidasi lebih lanjut secara in vitro dan in vivo.

Kata kunci: inflamasi, LC-HRMS, *molecular docking*, NF-kB, TNF-α

INTRODUCTION

Indonesia is the largest archipelagic country with a vast marine area and various underwater living organisms, such as blue sharks (*Prionace glauca*). Several studies have shown that blue sharks are not classified as protected animals in Indonesia because the species are not listed under national protection regulations and are legally permitted as fishery bycatch. These species are often found in open oceans, including the Indian Ocean and South China Sea, at a depth of 800 m and can migrate long distances (Rochman *et al.,* 2021). Despite their current legal status, concerns regarding population decline and overexploitation have been increasingly reported globally, highlighting the need for sustainable utilization and careful resource management of shark-derived materials for future use.

According to Nursanto *et al.* (2019), sharks have high economic value because almost all their body parts can be used as products. Although the meat contains high protein, it is not consumed by the majority of people in Indonesia. Agustin *et al.* (2025) reported that blue sharks are marine fish that inhabit the deep ocean and possess fully cartilaginous skeletons. These species have existed for hundreds of millions of years, with cartilage forming the structure of all their organs. This unique biological characteristic makes shark cartilage a promising source of bioactive compounds, particularly for pharmaceutical applications in humans. However, the increasing demand for shark-derived products raises sustainability concerns, emphasizing the importance of maximizing the value of bycatch over promoting targeted shark harvesting. Shark cartilage possesses many useful substances for health, leading to high economic value. In addition, it is mostly used to manufacture medicines and cosmetics. Cartilage is often dried and exported to several countries. Fahmi and Dharmadi (2013) stated that shark cartilage processing involves

http://dx.doi.org/10.17844/rf3rmd10

various steps, namely separating cartilage from meat, cleansing the remaining meat, and drying cartilage under sunlight. Agustin *et al.* (2016) successfully extracted bioactive compounds from shark cartilage. FTIR spectroscopic analysis revealed the presence of bioactive compounds similar to standard chondroitin sulfate, with strong absorptions at 1627.87 cm-1 and 1413.72 cm-1, indicating the presence of carboxyl groups with amine and sulfate. These compounds exhibited significant anti-inflammatory activity in vitro using the Peripheral Blood Mononuclear Cell (PBMC) method. The procedures were carried out using IL-6 antibody and in vivo tests on Wistar rats infected with carrageenan, demonstrating that shark cartilage extract can inhibit edema in the feet of the mice.

High-resolution mass spectrometry (HRMS) can provide extensive information concerning the accurate mass. Information on the mass and accuracy values can be used to identify the molecular formula of the compound. Several molecular formulas with an accurate mass within the accuracy range were searched, and the most accurate molecular formula was selected for further analysis. LC-HRMS has played an important role in the identification and characterization of novel polyphenolic compounds (Gao *et al.,* 2024). Recently, advanced LC-HRMS platforms, particularly those utilizing Quadrupole Time-of-Flight (Q-TOF) and Orbitrap mass analyzers, have become widely used for the comprehensive profiling of polyphenolic compounds owing to their superior mass accuracy and resolution (Demarque *et al.,* 2016; Gao *et al.,* 2020). In the present study, this method was used to detect compounds in shark cartilage meal and its extract, which then served as activity predictors using molecular docking.

According to Furman *et al.* (2019), when inflammation persists for more than 6 weeks, it shifts from the subacute to chronic stage. The chronic stage occurs when blood cells, such as T lymphocytes and plasma cells, move to the inflammatory area. Failure to treat chronic inflammation can cause tissue damage and fibrosis. Inflammation is a vital response of the body to protect it from hazards. However, excessive or prolonged inflammation leads to hyperinflammation, resulting in various health problems, such as autoimmune diseases, chronic inflammation, and tissue damage. Hyperinflammation is often related to high proinflammatory cytokine production, particularly Tumor Necrosis Factor (TNF)-α.

IL-6 is a cytokine that functions in inflammation and is involved in immune response, bone metabolism, and embryonic development. It plays a role in chronic inflammation (Tan *et al*., 2021) and is mainly produced by macrophages. The TNF precursor is broken down by the TNF enzyme, which causes inflammation and cell death in various diseases. Several drugs that inhibit TNF-α, such as *etanercept* (E), *infliximab* (I), *adalimumab* (A), *certolizumab pegol* (C), and *golimumab* (G), belong to the group of biological agents established by the Food and Drug Administration (FDA). These agents work by triggering apoptosis in inflammatory cells in the mucosa, regulating the signaling pathway in the cell, and reducing the production of proinflammatory cytokines and chemokines (Gerriets *et al*., 2023). Furthermore, the transcription factor NF-kB p65 acts as a master regulator driving the expression of various pro-inflammatory cytokines, whereas TNF-α convertase (TACE) is essential for processing the membrane-bound TNF precursor into its active soluble form (Sharma *et al.,* 2014). Synergistically targeting both NF-kB p65 and TACE provides a comprehensive mechanism for suppressing the inflammatory cascade at both the transcriptional and post-translational stages. Therefore, anti-inflammatory drug development needs to be done at specific and selective targets

New drug invention processes are costly and time-consuming; however, the availability of Computer-Aided Drug Design (CADD) technology can shorten the process (Giordano *et al*., 2022; Vemula *et al*., 2023). This technology can be used to design new compounds at certain receptor targets *in silico* or through safety potential evaluation of the compound (Praditapuspa *et al*., 2021). *In silico* methods can be used to understand how a compound interacts with target molecules,

such as receptors. The interactions between compounds and receptors can be visualized using computer simulations to identify the pharmacophore. A pharmacophore is a molecular structure that exhibits pharmacological activity and interacts with biological receptors (Tran *et al*., 2022). This technique can be applied as a preliminary trial for novel drug discovery. This approach is regarded as more effective because it requires less time and is cost-efficient (Coumar, 2021).

Molecular docking is a computational method used to virtually investigate potential drug candidates. It aims to identify the optimal binding interaction between a ligand and its target receptor protein while estimating the lowest free energy involved in the binding process. In addition, this technique helps pinpoint the precise active site on the protein where a ligand or drug compound may interact and assess the binding energies of different ligands to develop more effective and potent compounds (Mukesh & Rakesh, 2011). Recently, *in silico* methodologies, such as molecular docking, have become strategic and resource-efficient approaches for discovering and evaluating anti-inflammatory therapeutics. Current virtual screening studies frequently utilize docking to evaluate the interactions of various compounds with key pro-inflammatory mediators, particularly cytokines, such as TNF-α, IL-6, and IL-1β. Structural insights into these targets, including the trimeric binding groove of TNF-α, allow study teams to computationally screen small molecules capable of blocking the receptor interface to prevent downstream inflammatory signaling (Enni & Maraj, 2022). Despite established *in vitro* and *in vivo* evidence of the anti-inflammatory properties of shark cartilage, a comprehensive profiling of its bioactive compounds and their precise molecular interactions with key inflammatory mediators remains uncharacterized. Therefore, this study aimed to identify the bioactive compounds in shark cartilage meal and its extract using LC-HRMS. The inhibitory potential of the compound against NF-kB p65 and TNF-α convertase was evaluated using an *in silico* molecular docking approach.

MATERIAL AND METHODS

Sample Preparation

The sample preparation procedure was adopted and modified from the method described by Solihah *et al.* (2019). In this study, preparations began by removing any remaining meat from shark cartilage. The clean cartilage was then cut into small pieces to accelerate drying, washed, drained for approximately 30 min, and weighed. The pieces were dried in a drying machine at 50±2°C for 2 days. Drying was conducted in two phases to prevent color change. A total of 286 g of dry cartilage was obtained and milled into a flour. This resulted in 265 g of shark cartilage meal, which was stored in a plastic bag in a freezer at -20°C until extraction.

Sample Extraction

The extraction procedure was adapted from the method described by Agustin *et al.* (2016). Shark cartilage meal was first washed with n-hexane at a 1:10 (w/v) ratio. A total of 15 g of cartilage meal was mixed with 150 mL of hexane and stirred on a magnetic stirrer for one hour. The meal was separated from hexane by decantation and dried overnight to evaporate any residual hexane. Subsequently, the samples were placed in an oven for 15 min, cooled, and reweighed. Hexane defatting was performed as an initial step to remove non-polar lipids and impurities that could interfere with extraction. Subsequent water extraction was specifically selected to effectively isolate and maximize the yield of the targeted polar bioactive compounds, such as glycosaminoglycans and water-soluble proteins. The washed shark cartilage meal was then extracted with 150 mL of distilled water using a hot plate magnetic stirrer for 8 h at 45±2°C. The mixture was centrifuged at 4,000 rpm for 10 min. The resulting filtrate was collected in an Erlenmeyer flask and stored at -20°C until chemical screening.

LC-HRMS Analysis

Bioactive compound screening was conducted using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS). The analysis was performed using LC-HRMS (Thermo Fisher Scientific, USA).

http://dx.doi.org/10.17844/rf3rmd10

Samples were injected with a volume of 100 µL, and mass detection was carried out using a Q Exactive™ Orbitrap Mass Spectrometer in full-scan mode at 70,000 resolution. The solution was vortexed at 2,000 rpm for approximately 2 min and centrifuged at 6,000 rpm for 2 min. The sample preparation procedures before LC-HRMS injection differed depending on the sample type. For the solid shark cartilage meal, 50 mg of the sample was diluted with distilled water to a final volume of 1,500 µL. For the liquid meal extract, 1,500 µL of the previously stored filtrate was used directly. Both prepared solutions were vortexed at 2,000 rpm for approximately 2 min and centrifuged at 6,000 rpm for 2 min. The supernatant was collected, passed through a 0.22 µm syringe filter, and transferred to a vial. The sample was placed in an autosampler and injected into the LC-HRMS system with an operation time of 30 min.

In Silico Analysis

The pharmacokinetics of the bioactive compounds were evaluated using the SwissADME platform (https://www.swissadme.ch) based on key drug-likeness parameters, including Lipinski's rule of 5, Veber, and Egan criteria. Following the methodology of Rai *et al.* (2023), compounds with higher drug-likeness scores were considered to have greater potential as drug candidates. Subsequently, compounds that met the drug-likeness criteria were assessed for toxicity using the ProTox online tool (https://tox.charite.de/protox3/) to identify those that were safe and non-toxic for human use. As stated by Banerjee *et al.* (2018), compounds in toxicity classes 4 and 5 are considered non-toxic when ingested.

The PerMM web server (https://permm.phar.umich.edu/) was used to evaluate the ability of each compound to penetrate the lipid bilayer of the cell membranes. Membrane permeability is a crucial characteristic that regulates the transport of solutes and solvents across cellular boundaries (Frallicciardi *et al.*, 2022).

The pharmacokinetic properties of the compounds, including absorption, distribution, metabolism, and excretion (ADME), were predicted using the pkCSM application (https://biosig.unimelb.edu.au/pkcsm/prediction). Predictions were performed using the SMILES representations of compounds obtained from the PubChem database.

This study focused on NF-kB p65 (PDB ID: 2RAM) and TNF-α convertase (PDB ID: 3EWJ) as potential anti-inflammatory targets. Molecular docking procedures were performed using AutoDock Tools in PyRx Software to investigate the interactions between the identified ligands and target proteins.

Molecular docking was performed within the specific active sites of each protein, such as Lys221, Gln241, Asp243, Val244, Arg246, and Gln247 for NF-kB p65 protein with grid setting Center X:19.1730 Y: 19.9956 Z:61.2699 and Dimensions (A) X: 16.8862 Y: 17.6627 Z: 19.0387, while the active site of TNF-α were Glu406, Leu348, Asn389, Ala439, Gly346, Tyr390, Ala439, Val402, Ile438, His405 with grid setting Center X: 4.3066 Y:6.8428 Z: 27.990 and Dimension (A) X: 18.5942, Y: 24.5849 Z: 18.4775. The selection of these specific amino acid residues for the active sites was based on previously validated structural studies and active pocket characterizations of NF-kB p65 (Giridharan & Srinivasan, 2018) and TNF-α convertase (Zheng & Wu, 2011). The binding affinity was recorded in kcal/mol, and the binding poses of the ligands were saved and visualized using Discovery Studio software.

RESULTS AND DISCUSSION

Cartilage Meal Rendement of Blue Shark

A total of 2,030 g of fresh cartilage was processed into 256 g meal. The rendement of the cartilage meal of blue shark was estimated to be 12.7 %. This indicates that the water content of fresh shark cartilage is very high. Sulistyowati *et al.* (2015) observed that the water content of fresh shark bones was 74%, and that of shark cartilage meal was 5.40%. According to Putranto *et al.* (2016), the higher the rendement, the higher the economic value and effectiveness of the product.

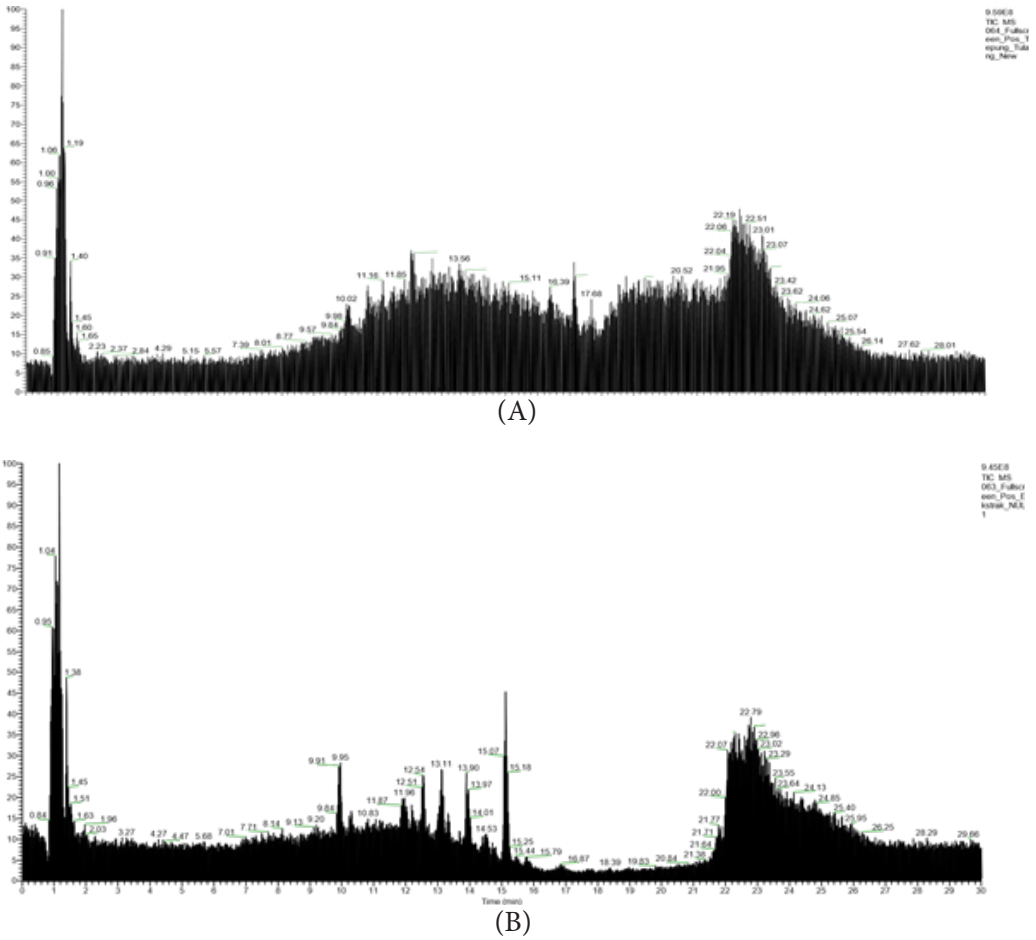
Blue Shark Cartilage Extract

Blue shark cartilage was extracted with 150 mL of distilled water, repeated six times, obtaining an average extract of 75.17±6.88 mL, then weighed to obtain an average weight of 74.21±7.00 g. Wijaya and Satriawan (2023) stated that the amount of rendement obtained is affected by the type of solvent, while according to Damaiyanti (2015), the rendement is inversely proportional to the extract quality.

Bioactive Compounds in Shark Cartilage Meal and Shark Cartilage Extract

LC-HRMS enables thorough protein analysis and identification of peptide markers in samples, offering a strong, reliable, and standardized approach for authentication (Windarsih *et al*., 2024). The results of LC-HRMS analysis of the blue shark cartilage meal and its extract are presented as a whole chromatogram (graphic chromatogram) in Figure 1.

The chromatograms of shark cartilage meal (A) and shark cartilage extract (B) show that the compounds began to elute at approximately 0.84 min, with well-defined chromatographic peaks. LC-HRMS analysis detected 64 compounds in the shark cartilage meal and 141 compounds in the extract. Of these, only compounds with an mzCloud Best Match value >80% were subjected to in silico analysis. In the shark cartilage meal sample, there were 20 compounds with a match value >80% (Table 1), whereas 37 compounds were identified in the extract (Table 2). According to Ortiz *et al.* (2024), compounds with a spectral similarity of more than 80% to the database and that have been visually validated are considered positively identified and documented, along with their m/z values and retention times.



(A)

(B)

Figure 1 Total ion chromatogram (TIC) of blue shark cartilage meal (A) and extract (B)

http://dx.doi.org/10.17844/rf3rmd10

Table 1 Shark cartilage meal compounds

Amino acid type	Formula	Calc. MW	RT [min]	Area (Max.)	mzCloud Best Match
Betaine	$C_5H_{11}NO_2$	117.0788	0.948	8.15×10^8	98.6
Hypoxanthine	$C_5H_4N_4O$	136.0380	1.397	4.53×10^8	99.8
Creatine	$C_4H_9N_3O_2$	131.0690	0.968	3.31×10^8	99.0
Choline	$C_5H_{13}NO$	103.0997	1.126	1.66×10^8	97.5
L-Norleucine	$C_6H_{13}NO_2$	131.0944	1.616	1.45×10^8	99.7
Caprolactam	$C_6H_{11}NO$	113.0840	10.069	1.26×10^8	99.6
Creatine	$C_4H_9N_3O_2$	131.0690	1.256	6.95×10^7	98.7
Nicotinamide	$C_6H_6N_2O$	122.0477	1.402	6.14×10^7	98.5
Betaine	$C_5H_{11}NO_2$	117.0788	1.256	5.82×10^7	95.8
L-Phenylalanine	$C_9H_{11}NO_2$	165.0786	2.246	5.72×10^7	99.2
Creatinine	$C_4H_7N_3O$	113.0588	1.230	5.24×10^7	99.5
Guanine	$C_5H_5N_5O$	151.0491	1.604	4.44×10^7	90.7
Isocytosine	$C_4H_5N_3O$	111.0433	1.388	3.56×10^7	81.5
Guanine	$C_5H_5N_5O$	151.0491	1.365	3.11×10^7	88.5
Trigonelline	$C_7H_7NO_2$	137.0474	0.954	2.58×10^7	97.1
trans-3-Indoleacrylic acid	$C_{11}H_9NO_2$	187.0626	4.058	2.12×10^7	93.7
Uracil	$C_4H_4N_2O_2$	112.0273	1.386	1.87×10^7	82.2
Trigonelline	$C_7H_7NO_2$	137.0474	1.249	1.64×10^7	96.6
Lactamide	$C_3H_7NO_2$	89.04782	0.954	1.12×10^7	87.5
D-(+)-Proline	$C_5H_9NO_2$	115.0632	0.955	1.07×10^7	98.6

LC-HRMS analysis revealed a diverse profile of low-molecular-weight metabolites in both the meal and extract. While several identified bioactive compounds are amino acids (such as L-norleucine, L-phenylalanine, and D-(+)-proline), others belong to distinct chemical classes. Hypoxanthine and guanine are naturally occurring purine derivatives, whereas uracil and isocytosine are pyrimidines, which are typical products of nucleic acid and energy metabolism in animal tissues. Furthermore, other nitrogenous metabolites and quaternary ammonium compounds, such as betaine, choline, and creatine were detected.

However, it is important to note the detection of synthetic substances such as MDPV, DEET, and α-Pyrrolidinopropiophenone (α-PVP) in the extract. These compounds are highly unlikely to be endogenous to shark cartilage and are presumed to be environmental contaminants, marine bioaccumulation products, or artifacts introduced during sample handling and extraction.

Drug-likeness and Membrane Permeability

Drug-likeness evaluation was performed using the Lipinski, Ghose, Veber, and Egan rules to determine which compounds m*et al*l the criteria. High drug-likeness scores indicate that molecules have a greater potential to serve as effective drug candidates (Rai *et al*., 2023). Tables 3 and 4 demonstrate the drug-likeness predictions for shark cartilage meal and shark cartilage extract, respectively.

Table 3 shows that two compounds (L-Phenylalanine and trans-3-Indoleacrylic acid) in shark cartilage meal passed four drug-likeness parameters. Table 4 shows that the shark cartilage extract has 8

Table 2 Shark cartilage extract compounds

Amino acid type	Formula	Calc. MW	RT [min]	Area (Max.)	mzCloud Best Match
Betaine	$C_5H_{11}NO_2$	117.078	0.948	1.41×10^9	98.4
Creatine	$C_4H_9N_3O_2$	131.069	0.954	4.79×10^8	97.9
Hypoxanthine	$C_5H_4N_4O$	136.038	1.394	2.77×10^8	99.3
L-Norleucine	$C_6H_{13}NO_2$	131.094	1.504	2.48×10^8	99.8
Choline	$C_5H_{13}NO$	103.099	1.154	2.21×10^8	98.1
Caprolactam	$C_6H_{11}NO$	113.083	21.811	1.66×10^8	99.6
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	234.160	14.482	1.20×10^8	99.3
2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	$C_9H_{19}NO$	157.146	11.945	7.70×10^8	92.4
L-Phenylalanine	$C_9H_{11}NO_2$	165.078	1.947	7.64×10^7	99.3
13(S)-HOTrE	$C_{18}H_{30}O_3$	294.218	11.931	5.97×10^7	91.9
Creatinine	$C_4H_7N_3O$	113.058	1.249	5.86×10^7	97.4
Creatine	$C_4H_9N_3O_2$	131.069	1.274	5.29×10^7	99
13(S)-HOTrE	$C_{18}H_{30}O_3$	294.218	12.548	4.96×10^7	91.6
Nootkatone	$C_{15}H_{22}O$	218.166	13.345	4.18×10^7	81.6
7-Hydroxycoumarine	$C_9H_6O_3$	162.031	13.328	4.04×10^7	91.7
D-(+)-Proline	$C_5H_9NO_2$	115.063	0.943	3.61×10^7	99.5
DL-Arginine	$C_6H_{14}N_4O_2$	174.111	1.555	3.14×10^7	89.2
Trigonelline	$C_7H_7NO_2$	137.047	0.945	2.89×10^7	97.4
Olomoucine	$C_{15}H_{18}N6O$	298.152	14.534	2.88×10^7	95.3
DL-Carnitine	$C_7H_{15}NO_3$	161.104	0.969	2.65×10^7	98.9
1,2,3,4-Tetramethyl-1,3-cyclopentadiene	C^9H^{14}	122.109	12.498	2.37×10^7	84.8
Sipecolic acid	$C_6H_{11}NO_2$	129.078	1.387	2.27×10^7	85.4
Piperine	$C_{17}H_{19}NO_3$	285.135	12.952	2.25×10^7	98.1
D-(+)-Proline	$C_5H_9NO_2$	115.063	1.388	2.23×10^7	85
MDPV	$C_{16}H_{21}NO_3$	275.150	14.021	2.03×10^7	88.1
Glycyl-L-leucine	$C_8H_{16}N_2O_3$	188.115	2.572	1.78×10^7	98.7
Valine	$C_5H_{11}NO_2$	117.078	1.385	1.71×10^7	97.3
Uracil	$C_4H_4N_2O_2$	112.027	1.38	1.66×10^7	92.9
DEET	$C_{12}H_{17}NO$	191.130	11.874	1.59×10^7	96.4
α-Pyrrolidinopropiophenone	$C_{13}H_{17}NO$	203.130	14.333	1.38×10^7	94
Nootkatone	$C_{15}H_{22}O$	218.166	15.097	1.14×10^7	83.2
Hexadecanamide	$C_{16}H_{33}NO$	255.254	17.396	1.06×10^7	97.8
7-Hydroxycoumarine	$C_9H_6O_3$	162.031	11.049	1.03×10^7	88.1
Piperanine	$C_{17}H_{21}NO_3$	287.150	12.831	8.61×10^7	99.2
4-Aminophenol	C_6H_7NO	109.0527	1.384	8.34×10^7	81.8
L-Norleucine	$C_6H_{13}NO_2$	131.094	0.152	8.04×10^7	97.5
13(S)-HOTrE	$C_{18}H_{30}O_3$	294.218	12.299	7.93×10^7	88.4

http://dx.doi.org/10.17844/rf3rmd10

Table 3 Druglikeness of shark cartilage meal compounds

Bioactive compound	Formula	Lipinski	Ghose	Veber	Egan
Betaine	$C_5H_{11}NO_2$	Yes	No	Yes	Yes
Hypoxanthine	$C_5H_4N_4O$	Yes	No	Yes	Yes
Creatine	$C_4H_9N_3O_2$	Yes	No	Yes	Yes
Choline	$C_5H_{13}NO$	Yes	No	Yes	Yes
L-Norleucine	$C_6H_{13}NO_2$	Yes	No	Yes	Yes
Caprolactam	$C_6H_{11}NO$	Yes	No	Yes	Yes
Nicotinamide	$C_6H_6N_2O$	Yes	No	Yes	Yes
L-Phenylalanine	$C_9H_{11}NO_2$	Yes	Yes	Yes	Yes
Creatinine	$C_4H_7N_3O$	Yes	No	Yes	Yes
Guanine	$C_5H_5N_5O$	Yes	No	Yes	Yes
Trigonelline	$C_7H_7NO_2$	Yes	No	Yes	yes
trans-3-Indoleacrylic acid	$C_{11}H_9NO_2$	Yes	Yes	Yes	Yes
D-(+)-Proline	$C_5H_9NO_2$	Yes	No	Yes	Yes

compounds, namely 3,5-di-tert-butyl-4-hydroxybenzaldehyde, L-Phenylalanine, 13(S)-HOTrE, Olomoucine, Piperine, DEET, α-Pyrrolidinopropiophenone, and Piperanine, which passed 4 drug-likeness parameters (Lipinski, Veber, Ghose, and Egan). According to da Silva (2023), a common approach to determine whether a molecule possesses suitable pharmacological traits (drug-likeness) is to evaluate its adherence to the criteria outlined in Veber's and Lipinski's Rule of 5. The drug-likeness prediction in this study showed that all compounds in shark cartilage meal and its extract had no violations based on Lipinski, Veber, and Egan parameters (Fig 1A and Fig 2A). According to Ursu. (2011), the concept of drug-likeness was developed to provide a useful direction during the initial stages of drug discovery, aiming to enhance the chances of a compound progressing into and through clinical trials.

Drug-likeness parameters, such as Lipinski's Rule of Five, serve as preliminary physicochemical filters for oral bioavailability rather than definitive predictors of pharmacological efficacy. Furthermore, a clear distinction must be made between the identified compounds based on their primary biological roles. Many of the compounds that passed these filters, such as betaine, creatine, choline, and various amino acids (e.g., L-phenylalanine, L-norleucine, and D-(+)-proline), are fundamental nutritional metabolites and dietary components. Although biological functions were identified, no traditional drug candidates were observed. However, the more complex secondary metabolites and unique structural derivatives identified in this study, such as trans-3-indoleacrylic acid, piperine, piperanine, and olomoucine, represent actual lead compounds and potential drug candidates for further pharmacological development.

The membrane permeabilities of these compounds were predicted to evaluate their ability to cross the cell membrane and assess their therapeutic effectiveness. Figure IB shows that the 13 compounds in shark cartilage meal possess the potential for membrane penetration, and the energy transfer value of the 13 compounds penetrating the phospholipid membrane is the minimum transfer energy. According to Frallicciardi *et al*. (2022), membrane permeability is a key characteristic of cell membranes that regulates the transport of solutes and solvents across cells and within their internal compartments.

As shown in Figure 2, the 23 compounds were evaluated to predict their membrane permeability, determine their ability to cross

Table 4 The druglikeness prediction of the compound in the shark cartilage extract

Bioactive compound	Formula	Lipinski	Ghose	Veber	Egan
Betaine	$C_5H_{11}NO_2$	Yes	No	Yes	Yes
Hypoxanthine	$C_5H_4N_4O$	Yes	No	Yes	Yes
L-Norleucine	$C_6H_{13}NO_2$	Yes	No	Yes	Yes
Choline	$C_5H_{13}NO$	Yes	No	yes	Yes
L-Norleucine	$C_6H_{13}NO_2$	Yes	No	Yes	Yes
Caprolactam	$C_6H_{11}NO$	Yes	Yes	Yes	Yes
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	Yes	No	Yes	Yes
2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	$C_9H_{19}NO$	Yes	Yes	Yes	Yes
L-Phenylalanine	$C_9H_{11}NO_2$	Yes	Yes	Yes	Yes
13(S)-HOTrE	$C_{18}H_{30}O_3$	Yes	No	Yes	Yes
Creatinine	$C_4H_7N_3O$	Yes	No	Yes	Yes
Creatine	$C_4H_9N_3O_2$	Yes	No	Yes	Yes
7-Hydroxycoumarine	$C_9H_6O_3$	Yes	No	Yes	Yes
D-(+)-Proline	$C_5H_9NO_2$	Yes	No	Yes	Yes
Trigonelline	$C_7H_7NO_2$	Yes	Yes	Yes	Yes
Olomoucine	$C_{15}H_{18}N_6O$	Yes	No	Yes	Yes
DL-Carnitine	$C_7H_{15}NO_3$	Yes	Yes	Yes	Yes
Piperine	$C_{17}H_{19}NO_3$	Yes	No	Yes	Yes
Glycyl-L-leucine	$C_8H_{16}N_2O_3$	Yes	No	Yes	Yes
Valine	$C_5H_{11}NO_2$	Yes	No	Yes	Yes
Uracil	$C_4H_4N_2O_2$	Yes	Yes	Yes	Yes
DEET	$C_{12}H_{17}NO$	Yes	Yes	Yes	Yes
α-Pyrrolidinopropiophenone	$C_{13}H_{17}NO$	Yes	Yes	No	Yes
Hexadecanamide	$C_{16}H_{33}NO$	Yes	Yes	Yes	Yes
Piperanine	$C_{17}H_{21}NO_3$	Yes	No	Yes	Yes

the cell membrane, and assess their therapeutic effectiveness. The identified compounds have the potential to penetrate the membrane barrier. Among the bioactive compounds, betaine requires relatively high energy to pass through the phospholipid bilayer, suggesting low lipophilicity. This reduced lipophilicity is attributed to the higher molecular weight of the compound compared to the other compounds. Membrane permeability is crucial in drug development (Bennion *et al*., 2017). A drug intended to act on an intracellular target is largely ineffective when it cannot cross the cell membrane.

Pharmacokinetic and Toxicity Properties

The Pharmacokinetic and Toxicity Community System (pkCSM) is a web-based tool designed to predict the pharmacokinetic properties and toxicity profiles of chemical compounds (Pires *et al*., 2015). The pharmacokinetic characteristics of a compound describe its absorption,

http://dx.doi.org/10.17844/rf3rmd10

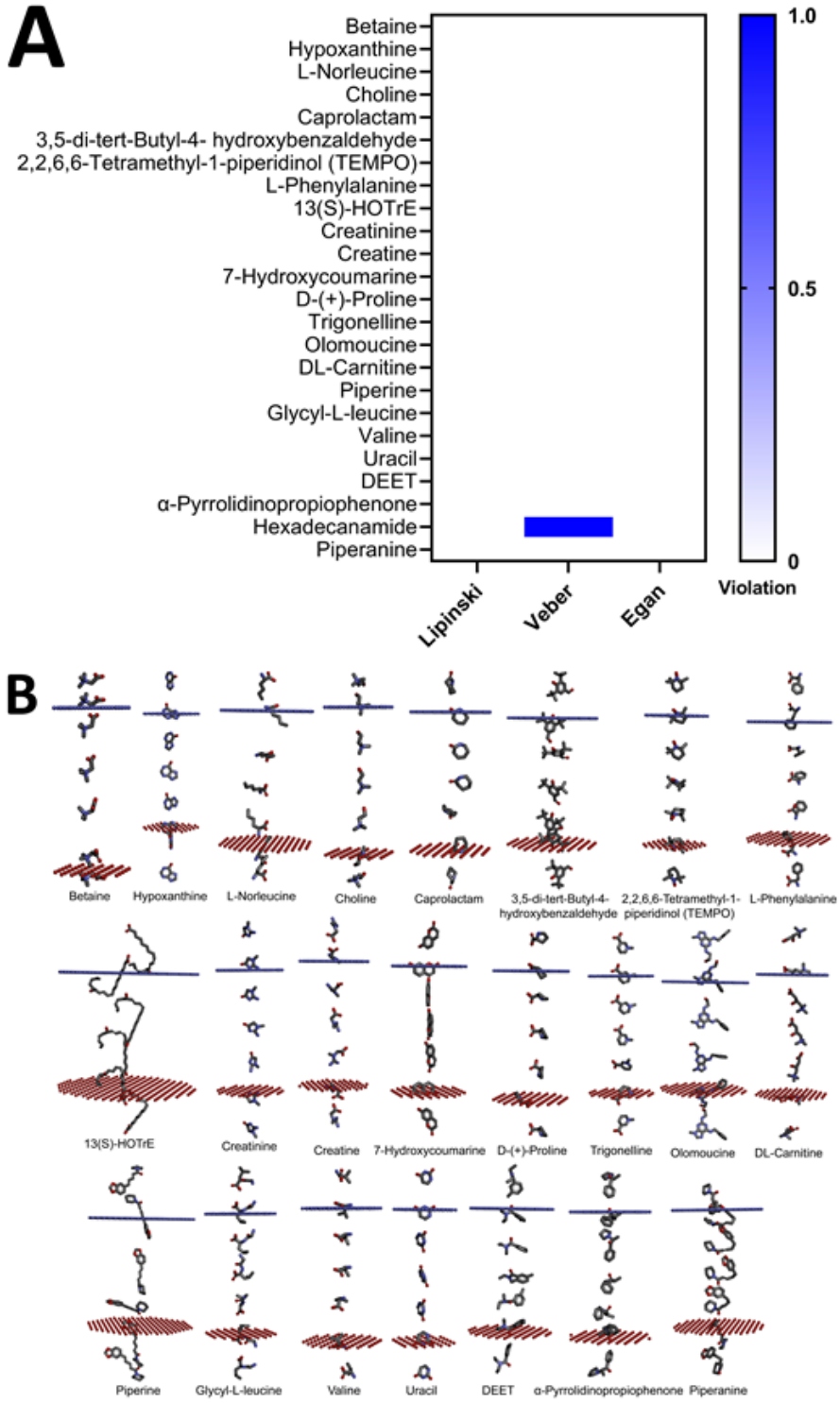


Figure 2 Drug-likeness and membrane permeability analysis of shark cartilage extract: (A) Drug-likeness screening using the Lipinski, Veber, and Egan parameters (B) Simulation of the seven compounds penetrating the lipid bilayer (C) The energy transfer value of the seven compounds penetrating the phospholipid membrane showed that with the minimum transfer energy required.

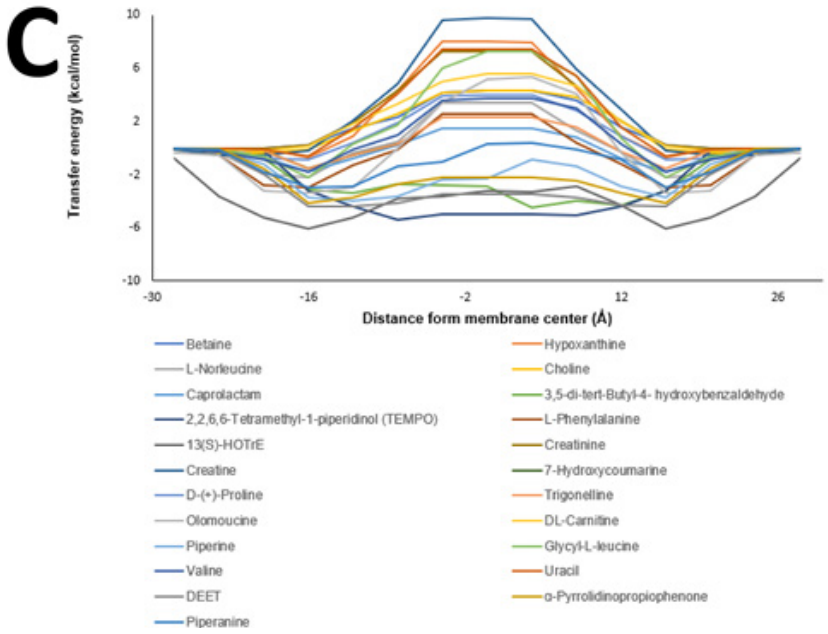


Figure 2 Drug-likeness and membrane permeability analysis of shark cartilage extract: (A) Drug-likeness screening using the Lipinski, Veber, and Egan parameters (B) Simulation of the seven compounds penetrating the lipid bilayer (C) The energy transfer value of the seven compounds penetrating the phospholipid membrane showed that with the minimum transfer energy required.

distribution throughout the body, metabolism, and excretion (ADME). The pharmacokinetic and toxicity properties of shark cartilage meal and shark cartilage extract are shown in Tables 5 and 6, respectively.

According to Chander *et al.* (2017), a compound quality is categorized as very good at an absorption rate of >80%, good at >30%-80%, and poor at <30%. As presented in Table 5, there are nine compounds in shark cartilage meal with very good absorption (absorption >80%): betaine, hypoxanthine, choline, caprolactam, nicotinamide, creatinine, D-(+)-proline, trigonelline, and trans-3-Indoleacrylic acid. Table 6 demonstrates the presence of 17 compounds in the shark cartilage extract with very good absorption (absorption >80%), such as betaine, hypoxanthine, choline, caprolactam, 3,5-di-tert-Butyl-4-hydroxybenzaldehyde, 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO), 13(S)-HOTrE, Creatinine, 7-Hydroxycoumarine, D-(+)-Proline, Trigonelline, Olomoucine, DL-Carnitine, Piperine, Uracil, DEET and Piperanine.

Skin permeability is frequently regarded as a key factor influencing the potential of a chemical to cause sensitization (Alves *et al.*, 2015). This property reflects a molecule's capacity to penetrate the skin, which is largely determined by both its physicochemical characteristics and the physicochemical and biological properties of the skin. A compound with relatively low skin permeability has a log Kp value of >-2.5. Table 5 reveals that there are 11 compounds with a log Kp value <-2.5 and two compounds with a log Kp value >-2.5, L-norleucine and L-phenylalanine, in shark cartilage meal. Table 6 shows that shark cartilage extract contains 19 compounds with log Kp value <-2.5 and five compounds with log Kp value >-2.5, namely L-norleucine, L-Phenylalanine, 7-Hydroxycoumarine, and valine. Therefore, both shark cartilage meal and its extract have relatively high skin permeabilities. Compounds with favorable skin permeability are potential candidates for the development of new drugs for transdermal delivery (Krihariyani *et al.*, 2020).

According to Smith *et al.* (2015), the volume of distribution at steady state (VDss) refers to the theoretical volume that a drug would need to occupy to achieve the observed plasma or blood concentration relative to

Table 5 Pharmacokinetic properties of 13 compounds in shark cartilage meal

Bioactive compound	A		D		M		E	
	HIA	Skin permeability	VDss	BBB permeability	CYP2D6 substrate	CYP2D6 inhibitor	Total clearance	Renal OCT2 substrate
Betaine	100	-3.19	-0.54	-0.21	No	No	0.297	No
Hypoxanthine	88.93	-2.74	0.49	-1.02	No	No	0.649	No
Creatine	57.59	-2.74	0.01	-1.24	Yes	No	0.133	No
Choline	100	-3.10	0.22	0.09	No	No	0.932	No
L-Norleucine	77.12	-2.49	-0.66	-0.32	Yes	No	0.489	No
Caprolactam	99.22	-3.11	0.02	-0.05	No	No	0.333	No
Nicotinamide	84.22	-3.05	-0.38	-0.36	No	No	0.635	No
L-Phenylalanine	73.93	-2.50	-1.24	-0.25	No	No	0.452	No
Creatinine	81.18	-3.10	0.38	-0.37	No	No	0.866	No
Guanine	59.24	-2.74	-0.20	-1.21	No	No	0.742	No
Trigonelline	96.53	-2.77	-0.52	-0.22	No	No	0.349	No
trans-3-Indoleacrylic acid	92.72	-2.65	-0.40	0.12	No	No	0.906	No
D-(+)-Proline	87.29	-2.96	-0.69	-0.32	No	No	0.600	No

the administered dose, while an excessively high or low volume of distribution leads to a reevaluation of the compound's suitability as a drug candidate (Yates & Arundal, 2007). A compound with a low volume of distribution has a log VD value of <-0.15 and >0.45 (Pires *et al*., 2015). Table 5 shows that eight compounds have a log VD value <-0.15, four compounds have a value of -0.15< log VD <0,45, and one >0.45, namely hypoxanthine in shark cartilage meal. Table 6 shows that the shark cartilage extract has 11 compounds with a log VD value of <-0.15, 10 compounds with 0.15< log VD <0.45, and 2 compounds with log VD >0.45, which are hypoxanthine and olomoucine. Mansoor and Mahabadi (2023) added that drugs with a high volume of distribution tend to exit the bloodstream and accumulate in tissues outside the vascular system, implying that a larger dose is necessary to reach the desired plasma concentration. However, drugs with a low Vd tend to remain in the plasma. A smaller dose is sufficient to achieve the same plasma concentration.

The blood-brain barrier (BBB) functions as a selective and partially permeable restraint that helps regulate internal stability in the central nervous system (CNS). As drugs must reach the CNS to exert their intended effects, evaluating their ability to cross the BBB is essential for the early development of CNS-targeted therapies (Kumar *et al*., 2022). Based on PreADMET, a compound with high absorption to the CNS has a log BB value >0.3, moderate absorption to the CNS >-0.1 but <0.3, and low absorption to the CNS <-1.0 (Kang, 2015). Table 5 shows that there are 10 compounds in shark cartilage meal with a log BB value >-0.1 but <0.3, while Table 6 shows the presence of 19 compounds in shark cartilage meal with a log BB value >-0.1 but <0.3. This indicates that a middle absorption to the CNS was exhibited. However, one compound (DEET) had a high absorption to the CNS with a log BB value >0.3, indicating a high absorption to the CNS. According to Huang *et al*. (2024), assessing BBB permeability is important to avoid unwanted CNS side effects, including drowsiness, respiratory suppression, nausea, vomiting, dizziness, confusion, and anxiety in patients. In silico prediction of a compound's ability to cross the BBB plays a major role in the discovery of new drugs for brain and neurological disorders.

Table 6 Pharmakokinetic properties of 24 compounds in shark cartilage extract

Bioactive compound	A		D		M		E	
	HIA	Skin permeability	VDss	BBB permeability	CYP2D6 substrate	CYP2D6 inhibitor	Total clearance	Renal OCT2 substrate
Betaine	100	-3.19	-0.54	-0.21	No	No	0.30	No
Creatine	57.59	-2.74	0.01	-1.25	Yes	No	0.13	No
Hypoxanthine	88.93	-2.74	0.49	-1.02	No	No	0.65	No
L-Norleucine	77.12	-2.49	-0.66	-0.32	Yes	No	0.49	No
Choline	100	-3.10	0.22	0.09	No	No	0.93	No
Caprolactam	99.22	-3.11	0.02	-0.05	No	No	0.33	No
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	93.98	-2.74	0.18	-0.23	No	No	0.93	No
2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	93.27	-2.66	0.13	0.22	No	No	1.10	No
L-Phenylalanine	73.95	-2.50	-1.24	-0.25	No	No	0.45	No
13(S)-HOTrE	92.07	-2.64	-0.96	-0.34	Yes	No	2.01	No
Creatinine	81.18	-3.10	0.38	-0.37	No	No	0.87	No
Creatine	57.59	-2.74	0.01	-1.25	Yes	No	0.13	No
7-Hydroxycoumarine	95.44	-2.33	-0.13	-0.22	No	No	0.74	No
D-(+)-Proline	87.29	-2.96	-0.69	-0.32	No	No	0.6	No
Trigonelline	96.53	-2.77	-0.52	-0.22	No	No	0.35	No
Olomoucine	94.96	-2.74	0.79	-0.90	No	No	0.96	No
DL-Carnitine	93.62	-2.82	-0.41	-0.25	No	No	0.23	No
Piperine	94.38	-2.82	0.27	0.27	No	No	0.24	No
Glycyl-L-leucine	47.70	-2.74	-0.86	-0.93	Yes	No	0.20	No
Valine	77.07	-2.47	-0.82	-0.36	Yes	No	0.20	No
Uracil	83.37	-3.93	-0.61	-0.47	No	No	0.76	No
DEET	94.26	-1.99	0.16	0.34	No	No	0.54	No

The toxicity profile of a compound plays a significant role in determining its therapeutic potential. Favorable toxicity characteristics can facilitate the efficient and rapid advancement of lead compounds into viable drug candidates (Pires *et al*., 2015). The toxicity evaluations of the 13 bioactive compounds in shark cartilage meal and the 23 bioactive compounds in shark cartilage extract are presented in Figures 3 and 4, respectively. Figure 3 shows that the toxicity evaluation of the 13 bioactive compounds in shark cartilage meal indicated that they were non-toxic in terms of immunotoxicity, mutagenicity, and cytotoxicity. Moreover, the screening results suggested that nicotinamide, guanine, and trans-3-indoleacrylic acid may cause liver damage. In addition, toxicity screening showed that trans-3-indoleacrylic acid and D-(+)-proline potentially caused nerve damage. Despite this, these compounds are included in toxicity classes 4 and 5, except for hypoxanthine, L-norleucine, and guanine, which are in class 3. According to Banerjee *et al*. (2018), compounds that fall under toxicity classes 4 and 5 are considered non-toxic when ingested.

Toxicity evaluation revealed 13 bioactive compounds in shark cartilage meal and 23 in shark cartilage extract (Figures 3 and 4).

http://dx.doi.org/10.17844/rf3rmd10

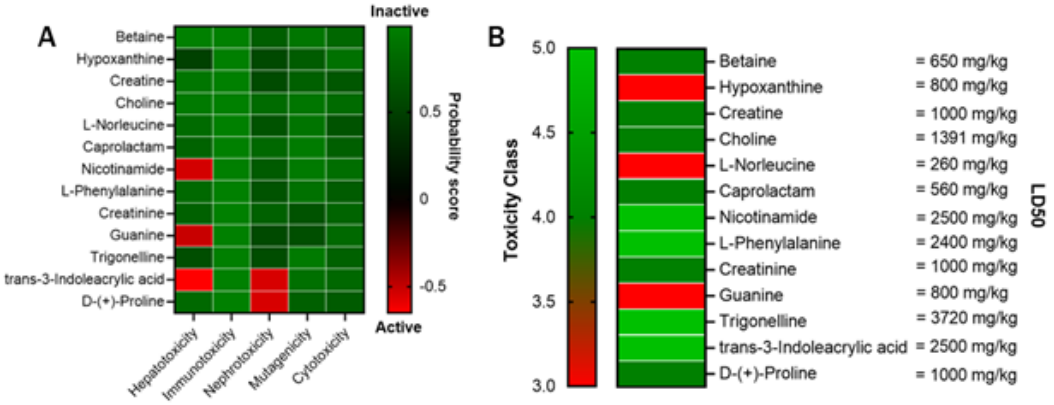


Figure 3 Toxicity analysis of shark cartilage meal; (A) Toxicity analysis for the probability of of inducing toxicity, and (B) The LD50 analysis

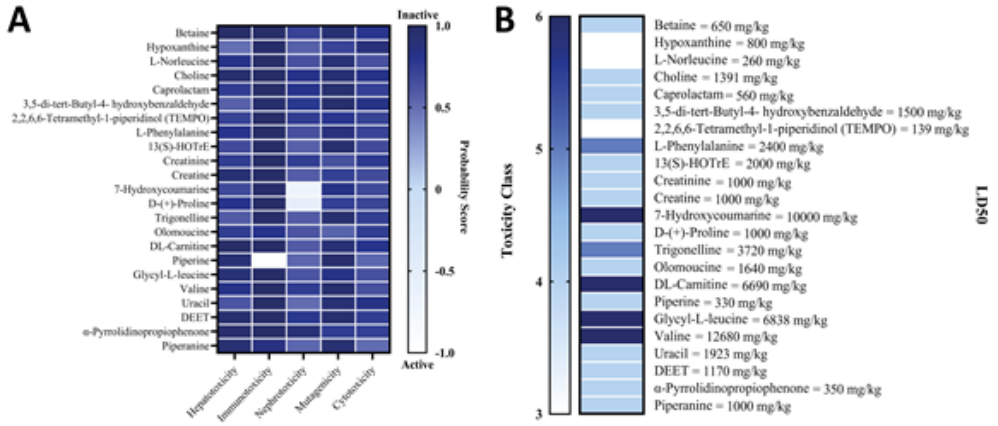


Figure 4 Toxicity analysis of shark cartilage extract; (A) Toxicity analysis for the probability of of inducing toxicity, and (B) The LD50 analysis

Figure 4 shows that the toxicity evaluation of the 23 bioactive compounds indicated that shark cartilage extract was non-toxic and safe in terms of hepatotoxicity, mutagenicity, and cytotoxicity. The screening results suggested that piperine causes immune damage. Toxicity screening showed that 7-Hydroxycoumarine and D(+)-proline potentially caused nerve damage. However, the toxicity class also indicates that the compounds are still in classes 4 and 5, except for hypoxanthine, L-norleucine, and 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO), which are included in class 3. Based on Banerjee *et al.* (2018), the compounds were considered non-toxic when ingested by fish.

Molecular Docking Analysis

A total of 13 bioactive compounds from shark cartilage meal and its extract that met the criteria for drug-likeness, membrane permeability, and pharmacokinetics, with non-toxic properties, were subsequently analyzed for molecular docking. Molecular docking analysis showed that the most favorable binding interaction against the NF-kB p65 protein was exhibited by emetine, which was used as a control drug (-4.9 kcal/mol). Among the identified constituents, Trans-3-indoleacrylic acid (-4.6 kcal/mol) and L-phenylalanine (-4.4 kcal/mol) demonstrated the lowest binding energies to NF-kB p65. These compounds interacted with the protein through hydrogen and hydrophobic bonds at residues Arg246, Gln247 and Lys221 (Table 7). However, it is crucial to clarify that L-phenylalanine is a fundamental proteinogenic amino acid. Its moderate docking score merely reflects the structural binding plausibility within the active site and should not be overinterpreted as evidence of pharmacological inhibition.

Table 7 NF-kB p65 protein and ligand interaction

Compounds	Binding affinity (kcal/mol)	Residue	Category interaction
Drugs (emetine)	**-4.9**	**HIS245, ALA242**	**Hydrogen bond**
		ARG246	**Electrostatic**
Betaine	-2.7	GLN247	Hydrogen bond
Creatine	-3.4	ARG246, GLN241, VAL244	Hydrogen bond
Hypoxanthine	-3.8	ILE224, GLN241	Hydrogen bond
L-norleucine	-3.4	ARG246	Hydrogen bond
		LYS221	Hydrophobic bond
Choline	-2.6	ARG246, GLN247	Hydrogen bond
Caprolactam	-3.4	GLN247, VAL244	Hydrogen bond
Nicotinamide	-3.6	GLN247, GLU222, GLN220	Hydrogen bond
		LYS221	Hydrophobic bond
L-phenylalanine	**-4.4**	**ARG246, GLN247**	**Hydrogen bond**
		LYS221	**Hydrophobic bond**
Creatinine	-3.1	ARG246, GLN241, VAL244, VAL244	Hydrogen bond
Trigonelline	-3.5	GLN247, A:VAL244	Hydrogen bond
		LYS221	Hydrophobic bond
Trans-3-indoleacrylic acid	**-4.6**	**ARG246, GLN247**	**Hydrogen bond**
		LYS221	**Hydrophobic bond**
D-(+)-Proline	-3.4	ARG246, VAL244	Hydrogen bond

A similar binding trend was observed for the TNF-α convertase protein. Trans-3-indoleacrylic acid and L-phenylalanine displayed the most favorable binding affinities among the extracted compounds (-7.0 and -6.5 kcal/mol, respectively), although their affinities remained lower than that of the control ligand (-7.3 kcal/mol) (Table 8). The control ligand interacted with TNF-α through Glu406 (electrostatic interaction), Leu348, Asn389, Ala439, Gly346, and Tyr390 (hydrogen bonds), and Ala439, Val402, and His405 (hydrophobic bonds). The same residues were also included in the interaction between phenylalanine and TNF-α, such as Ala439, His405, and Val402. While Ala439, His405, and Val402 are also involved in Trans-3-indoleacrylic acid (Table 8).

The binding affinities of L-phenylalanine (-4.4 kcal/mol) and Trans-3-indoleacrylic acid (-4.6 kcal/mol) against NF-kB p65 showed moderate binding potential comparable to that of the control drug (-4.9 kcal/mol). Furthermore, both compounds exhibited moderate binding plausibility with TNF-α protein, with affinities of -6.5 kcal/mol and -7.0 kcal/mol, compared to the control ligand (-7.3 kcal/mol). This strong interaction is facilitated by hydrogen bonds formed between the ligand and the protein (Maarif *et al*., 2025). Docking scores in the range of -4 to -7 kcal/mol denote moderate binding affinity. Furthermore, molecular docking merely suggests binding plausibility and does not provide definitive evidence of biological inhibition. Subsequent *in vitro* and *in vivo*

http://dx.doi.org/10.17844/rf3rmd10

Table 8 TNF-α convertase protein and ligand interaction

Compounds	Binding affinity (kcal/mol)	Residue	Category interaction
TNF-α inhibitor	**-7.3**	**GLU406**	**Electrostatic**
		LEU348, ASN389, ALA439, GLY346, TYR390	**Hydrogen bond**
		ALA439, VAL402, HIS405	**Hydrophobic bond**
L-norleucine	-5	ILE438, TYR433, VAL434, TYR436	Hydrogen bond
Choline	-3.8	VAL440, TYR433, ASN447, TYR436, ILE438	Hydrogen bond
Caprolactam	-5.3	HIS405, TYR436	Hydrogen bond
		LEU401, VAL434	Hydrophobic bond
Nicotinamide	-5.7	TYR436, ILE438	Hydrogen bond
		LEU401, VAL434, ALA439	Hydrophobic bond
L-phenylalanine	**-6.5**	**TYR433, VAL434**	**Hydrogen bond**
		ALA439, HIS405, VAL402	**Hydrophobic bond**
Creatinine	-5.2	VAL440, TYR436	Hydrogen bond
Trigonelline	-5.5	TYR436, ILE438, PRO437	Hydrogen bond
		HIS405, VAL402, ALA439	Hydrophobic bond
Trans-3-indoleacrylic acid	**-7**	**ILE438**	**Hydrogen bond**
		ALA439, HIS405, VAL402	**Hydrophobic bond**
D-(+)-Proline	-5.1	ILE438, TYR433, VAL434	Hydrogen bond

biological assays are required to validate their true inhibitory activities.

CONCLUSION

In shark cartilage, 13 bioactive compounds were discovered, with the extract demonstrating a wider variety of compounds and superior drug-like characteristics compared to the whole cartilage. Molecular docking revealed that trans-3-indoleacrylic acid and L-phenylalanine exhibited the highest potential for interaction with NF-kB p65 and TNF-α convertase. Nonetheless, these findings were derived from in silico analysis and require further experimental confirmation.

ACKNOWLEDGMENT

Sincere gratitude is addressed to the Rector of Hang Tuah University for the financial support provided throughout the PhD program of Titiek Indhira Agustin

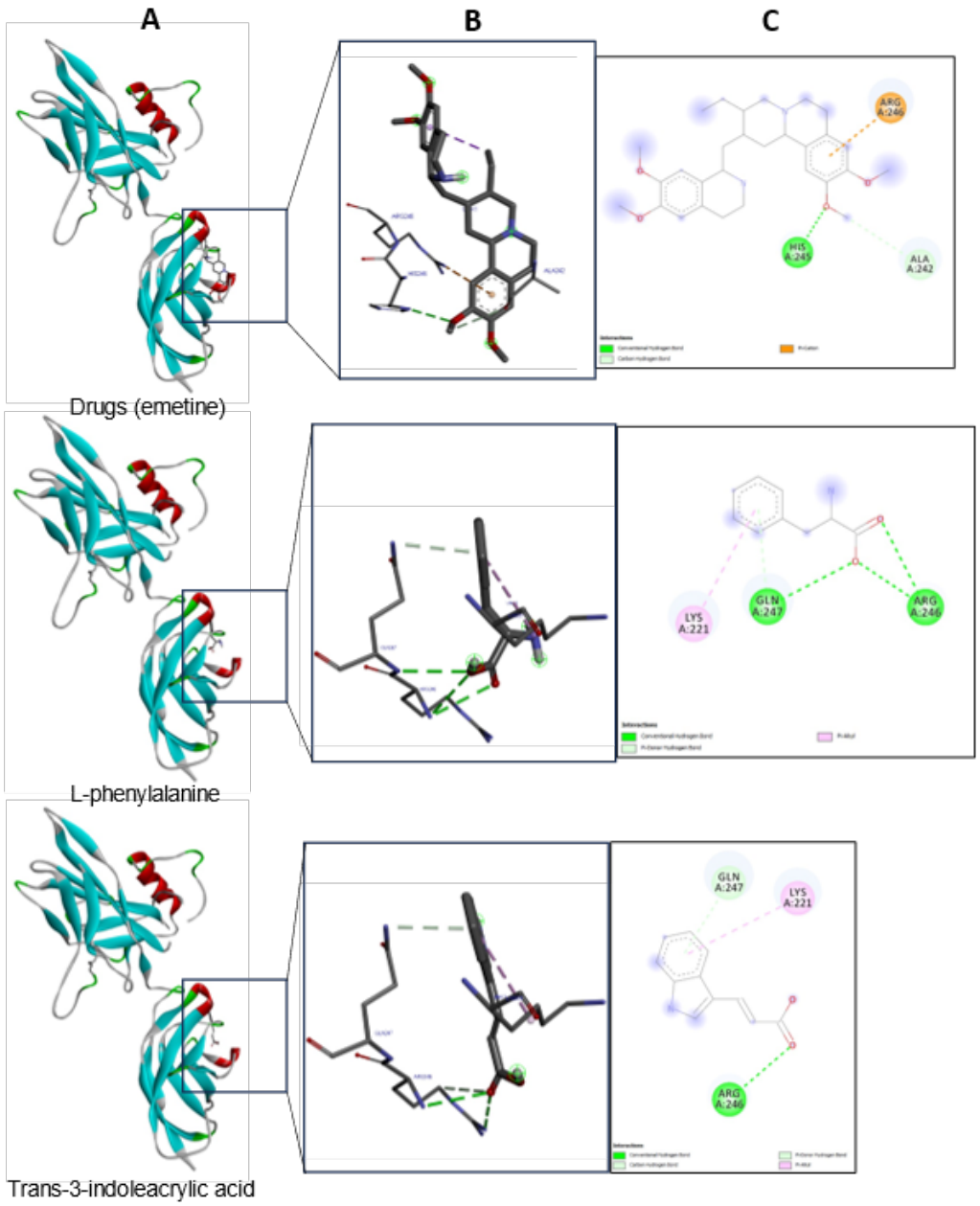


Figure 5 NfKB p65 interaction against emetine, L-phenylalanine, and Trans-3-indoleacrylic acid; (A) 3D visualization of protein and ligand, (B) 3D visualization of protein and ligand interaction, and (C) 2D visualization of protein and ligand interaction

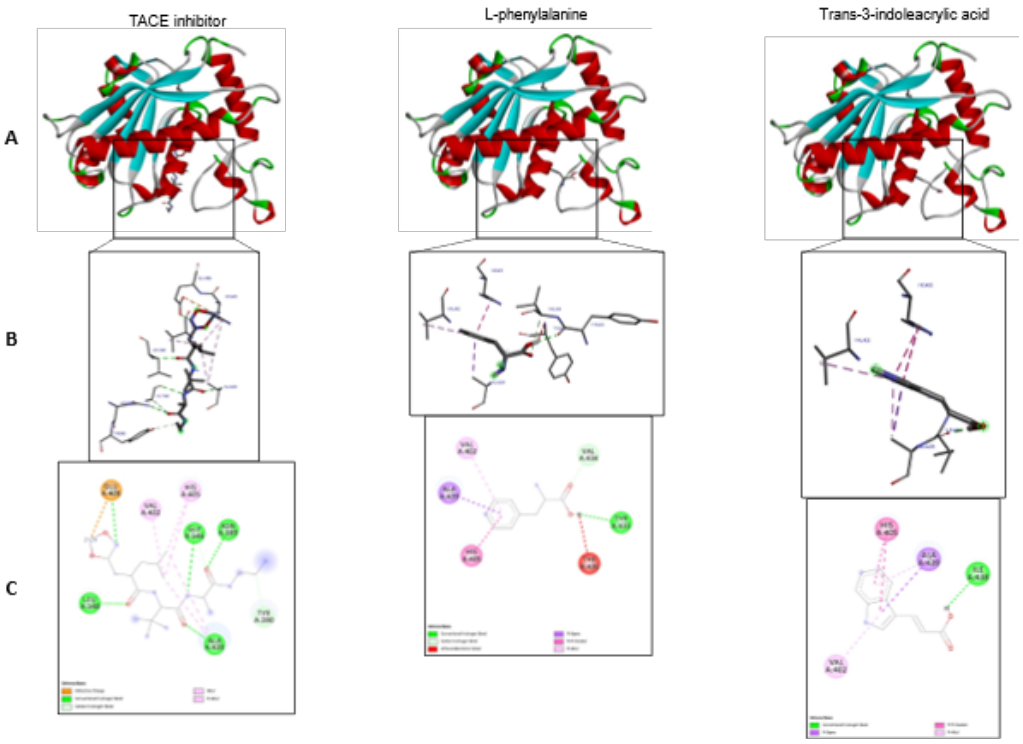


Figure 6 TNF-α interaction against inhibitor, L-phenylalanine, and Trans-3-indoleacrylic acid; (A) 3D visualization of protein and ligand, (B) 3D visualization of protein and ligand interaction, and (C) 2D visualization of protein and ligand interaction

(No.: PIB/009A/UHT. A0/IV/2020). We also thank the Integrated Research Laboratory, Universitas Brawijaya, Malang, for their valuable assistance with the sample analyses.

REFERENCES

Agustin, T. I., Wahyu, S., & Yatmasari, E. (2016). Study on the bioactive compounds of shark (*Prionace glauca*) cartilage and its inflammatory activity. *International Journal of PharmTech Research,* 9(1), 171–178.

Agustin, T., Nursyam, H., Firdaus, M., Rifa'I, M., Ekaputri, I. D., & Mahmiah. (2025). The characterization of blue shark (*Prionace glauca*) cartilage potential as nature-derived drug material. *In IOP Conference Series: Earth and Environmental Science* (Vol. 1473). https://doi.org/10.1088/1755-1315/1473/1/012049

Alves, V. M., Muratov, E., Fourches, D., Strickland, J., Kleinstreuer, N., Andrade, C. H., & Tropsha, A. (2015). Predicting chemically-induced skin reactions. Part II: QSAR models of skin permeability and the relationships between skin permeability and skin sensitization. *Toxicology and Applied Pharmacology,* 284(2), 273–280. https://doi.org/10.1016/j.taap.2014.12.013

Banerjee, P., Dehnbostel, F. O., & Preissner, R. (2018). Prediction is a balancing act: Importance of sampling methods to balance sensitivity and specificity of predictive models based on imbalanced chemical data sets. *Frontiers in Chemistry,* 6(AUG). https://doi.org/10.3389/fchem.2018.00362

Bennion, B. J., Be, N. A., McNerney, M. W., Lao, V., Carlson, E. M., Valdez, C. A., Malfatti, M., Enright, H., Nguyen, T., Lightstone, F., & Carpenter, T. S. (2017). Predicting a drug's membrane permeability: a computational model validated with in vitro permeability assay data. *Journal of Physical Chemistry B,* 121(20), 5228–5237. https://doi.org/10.1021/acs.jpcb.7b02914

Castaño-Ortiz, J. M., Gago-Ferrero, P.,

Barceló, D., Rodríguez-Mozaz, S., & Gil-Solsona, R. (2023). HRMS-based suspect screening of pharmaceuticals and their transformation products in multiple environmental compartments: An alternative to target analysis?. *Journal of Hazardous Materials,* 465, 132974. https://doi.org/10.1016/j.jhazmat.2023.132974

Chander, S., Tang, C. R., Al-Maqtari, H. M., Jamalis, J., Penta, A., Hadda, T. B., Sirat, H., Zheng, Y., & Sankaranarayanan, M. (2017). Synthesis and study of anti-HIV-1 RT activity of 5-benzoyl-4-methyl-1,3,4,5-tetrahydro-2H-1,5-benzodiazepin-2-one derivatives. *Bioorganic Chemistry,* 72, 74–79. https://doi.org/10.1016/j.bioorg.2017.03.013

Coumar, M. S. (2021). Molecular docking for computer-aided drug design: fundamentals, techniques, resources and applications. *Elsevier,* pp. 1–503. Academic Press. https://doi.org/10.1016/B978-0-12-822312-3.01001-8

da Silva, C. P. M., das Neves, G. M., Poser, G. L. von, Eifler-Lima, V. L., & Rates, S. M. K. (2023). In silico prediction of ADMET/Drug-likeness properties of bioactive phloroglucinols from *Hypericum* genus. *Medicinal Chemistry,* 19(10), 1002–1017. https://doi.org/10.2174/1573406419666230601092358

Damaiyanti, D.W. (2015). Karakterisasi esktrak air teripang emas (*Stichopus hermanii*). *Jurnal Kedokteran Gigi,* 9(1), 74–81.

Demarque, D. P., Crotti, A. E. M., Vessecchi, R., Lopes, J. L. C., & Lopes, N. P. (2016). Fragmentation reactions using electrospray ionization mass spectrometry: an important tool for the structural elucidation and characterization of synthetic and natural products. *Natural Product Reports,* 33(3), 432–455. https://doi.org/10.1039/c5np00073d

Dwi Krihariyani Dwi, Retno Sasongkowati, & Edy Haryanto. (2020). Studi *in silico* sifat farmakokinetik, toksisitas, dan aktivitas imunomodulator brazilein kayu secang terhadap enzim 3-Chymotrypsin-Like cysteine protease coronavirus. *Journal of Indonesian Medical Laboratory and Science (JoIMedLabS),* 1(1), 76–85. https://doi.org/10.53699/joimedlabs.v1i1.14

Enni, M. A., & Maraj, M. A. A. (2022). In silico drug repurposing for inflammatory diseases: a systematic review of molecular docking and virtual screening studies. *American Journal of Advanced Technology and Engineering Solutions,* 02(04), 35–64. https://doi.org/10.63125/j1hbts51

Fahmi & Darmadi. (2013). Tinjauan status perikanan hiu dan upaya konservasinya di indonesia. Edisi pertama. Direktorat konservasi kawasan dan jenis ikan. Direktorat jendral kelautan, pesisir dan pulau pulau kecil. *Kementrian kelautan dan perikanan*. ISBN : 978-602-913- 09-7.

Fitrial, Y., & Khotimah, I. K. (2017). Antibacterial activity of melanin from cuttlefish and squid ink. *Jurnal Pengolahan Hasil Perikanan Indonesia,* 20(2), 266. https://doi.org/10.17844/jphpi.v20i2.17907

Frallicciardi, J., Melcr, J., Siginou, P., Marrink, S. J., & Poolman, B. (2022). Membrane thickness, lipid phase and sterol type are determining factors in the permeability of membranes to small solutes. *Nature Communications,* 13(1), 1605. https://doi.org/10.1038/s41467-022-29272-x

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature Medicine,* 25(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Gao, S., Jennings, E. K., Han, L., Koch, B. P., Herzsprung, P., & Lechtenfeld, O. J. (2024). Detection and exclusion of false-positive molecular formula assignments via mass error distributions in UHR mass spectra of natural organic matter.

Analytical Chemistry, 96(25), 10210–10218. https://doi.org/10.1021/acs.analchem.4c00489

Gao, X., Wang, N., Jia, J., Wang, P., Zhang, A., & Qin, X. (2020). Chemical profliling of Dingkun Dan by ultra High performance liquid chromatography Q exactive orbitrap high resolution mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis,* 177, 112732. https://doi.org/10.1016/j.jpba.2019.06.029

Gerriets, V., Goyal, A., & Karam Khaddour. (2023, July 3). *Tumor Necrosis Factor Inhibitors*. Nih.gov; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK482425

Giridharan, S., & Srinivasan, M. (2018). Mechanisms of NF-κB p65 and strategies for therapeutic manipulation. *Journal of Inflammation Research,* 11, 407–419. https://doi.org/10.2147/jir.s140188

Hannoodee, S., & Nasuruddin, D. N. (2021). *Acute Inflammatory Response*. PubMed; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK556083

Huang, E. T. C., Yang, J.-S., Liao, K. Y. K., Tseng, W. C. W., Lee, C. K., Gill, M., Compas, C., See, S., & Tsai, F.-J. (2024). Predicting blood–brain barrier permeability of molecules with a large language model and machine learning. *Scientific Reports,* 14(1), 15844. https://doi.org/10.1038/s41598-024-66897-y

Kumar, R., Sharma, A., Alexiou, A., Bilgrami, A. L., Kamal, M. A., & Ashraf, G. M. (2022). DeePred-BBB: A blood brain barrier permeability prediction model with improved accuracy. *Frontiers in Neuroscience,* 16, 858126. https://doi.org/10.3389/fnins.2022.858126

Lei, M., Xue, C.-H. ., Wang, Y.-M. ., Li, Z.-J. ., Xue, Y., & Wang, J.-F. (2008). Effect of squid ink melanin-fe on iron deficiency anemia remission. *Journal of Food Science,* 73(8), H207–H211. https://doi.org/10.1111/j.1750-3841.2008.00930.x

Ma'arif, N., Sari, A. N., Nafisah, W., Krama, A., Isfaeni, H., Rusdi, R., & Gholam, G. M. (2025). In silico studies of stylissa carteri-compounds against EGFR and RAF proteins in triple-negative breast cancer. *Tropical Journal of Natural Product Research,* 9(6), 2922–2932. https://doi.org/10.26538/tjnpr/v9i6.77

Mukesh, B., & Rakesh, K. (2011). Molecular docking : a review. *International Journal of Research in Ayurveda & Pharmacy,* 2(6), 1746–1751.

Nursanto, R. M., Mustofa, A., & Widanti, Y. A. (2019). Nugget ikan hiu (*Carcharhinus amblyrhynchos*) dengan variasi penambahan jamur tiram (*Pleurotus* Sp.). *Jurnal Teknologi dan Industri Pangan,* 4(1), 15-21. https://doi.org/10.33061/jitipari.v4i1.3014

Pagadala, N. S., Syed, K., & Tuszynski, J. (2017). Software for molecular docking: a review. *Biophysical Reviews,* 9(2), 91–102. https://doi.org/10.1007/s12551-016-0247-1

Pires, D. E. V., Blundell, T. L., & Ascher, D. B. (2015). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry,* 58(9), 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104

Praditapuspa, E. N., Siswandono S, S., & Widiandani, T. (2021). In Silico analysis of pinostrobin derivatives from *Boesenbergia pandurata* on ErbB4 kinase target and QSPR linear models to predict drug clearance for searching anti-breast cancer drug candidates. *Pharmacognosy Journal,* 13(5), 1143–1149. https://doi.org/10.5530/pj.2021.13.147

Putranto, H. F., Asikin, A. N., & Kusumaningrum, D. I. (2015). Karakterisasi tepung tulang ikan belida (*Chitala* sp.) sebagai sumber kalsium dengan metode hidrolisis protein. *Journal ZIRAA'AH,* 40(1), 11–20.

Rai, M., Singh, A., Namuna Paudel, Anurag Kanase, Faletta, E., Pranali Kerkar, Heyda, J., Barghash, R. F., Singh, S., & Miroslav Šoóš. (2023). Herbal concoction Unveiled: A computational analysis of phytochemicals' pharmacokinetic and toxicological profiles using novel approach methodologies (NAMs).

Current Research in Toxicology, 5, 100118–100118. https://doi.org/10.1016/j.crtox.2023.100118

Rochman, F., Wujdi, A., Levi Arnenda, G., & Kurniawan, R. (2021). Blue sharks (*Prionace glauca*) by-catch in the Indonesian industrial tuna longline fishery in the eastern Indian Ocean. *IOP Conference Series: Earth and Environmental Science,* 860(1), 012115. https://doi.org/10.1088/1755-1315/860/1/012115

Sharma, M., Mohapatra, J., Wagh, A., Patel, H. M., Pandey, D., Kadam, S., Argade, A., Deshpande, S. S., Shah, G. B., Chatterjee, A., & Jain, M. R. (2014). Involvement of TACE in colon inflammation: A novel mechanism of regulation via SIRT-1 activation. *Cytokine,* 66(1), 30–39. https://doi.org/10.1016/j.cyto.2013.12.010

Solihah, H., Agustin, T. I., & Sulistyowati, W. (2019). Pengaruh prosentase absorben yang berbeda terhadap karakteristik hasil ekstrak tulang hiu (*Prionace glauca*). *Fisheries: Jurnal Perikanan dan Ilmu Kelautan,* 1(1), 12. https://doi.org/10.30649/fisheries.v1i1.12

Sulityowati, W., Titiek Indhira, A., Arsiniati, A., & Erina, Y. (2015). Glucosamine and chondroitin sulphate content of shark cartilage (Prionace glauca) and its potential as anti-aging supplements. *International Journal of ChemTech Research,* 8(10), 163–168.

Tambunan, U. S. F., & Alamudi, S. (2010). Designing cyclic peptide inhibitor of dengue virus NS3-NS2B protease by using molecular docking approach. *Bioinformation,* 5(6), 250–254. https://doi.org/10.6026/97320630005250

Tan, L. Y., Komarasamy, T. V., & RMT Balasubramaniam, V. (2021). Hyperinflammatory immune response and COVID-19: A double edged sword. *Frontiers in Immunology,* 12, 742941. https://doi.org/10.3389/fimmu.2021.742941

Ursu, O., Rayan, A., Goldblum, A., & Oprea, T. I. (2011). Understanding drug-likeness. *Wiley Interdisciplinary Reviews: Computational Molecular Science,* 1(5), 760–781. https://doi.org/10.1002/wcms.52

Wijaya, A., & Satriawan, B. (2023). Pengaruh perbedaan jenis pelarut terhadap nilai rendemen ekstrak daun pepaya (*Carica papaya*. L). *Jurnal Ilmiah JOPHUS: Journal Of Pharmacy UMUS*, 5(1), 10-17.

Windarsih, A., Nor, Rohman, A., Yuliana, N. D., & Dachriyanus. (2024). Untargeted metabolomics using liquid chromatography-high resolution mass spectrometry and chemometrics for analysis of non-halal meats adulteration in beef meat. *Animal Bioscience*. https://doi.org/10.5713/ab.23.0238

Zheng, C., Yin, Q., & Wu, H. (2010). Structural studies of NF-κB signaling. *Cell Research,* 21(1), 183–195. https://doi.org/10.1038/cr.2010.171