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Small peptide WNWKL and Scabraside D Compound of Sea Cucumber Might Inhibit NFκB: Implication on Their Anti-inflammatory and Anticancer Potentials

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ABSTRACT

Myriad studies have shown that sea cucumber is a promising anti-inflammatory and anticancer agent. Therefore, a study to identify the active compounds contained in sea cucumber, which have the potential as nuclear factor kappa-light-chain-enhancer of activated B cell (NFκB) inhibitors was conducted. NFκB plays a significant role in regulating inflammation and carcinogenesis, so the inhibition of NFκB activity by active compounds from sea cucumber hold the potential to be further investigated as anti-inflammatory and anticancer agents. This research was carried out *in silico* with various tools including molecular docking and molecular dynamics to determine the inhibitor potential of sea cucumber compounds. Small peptides and other active compounds from sea cucumbers were collected from various references, and then, using bioinformatic tools, their tertiary structures were modeled. The structure of NFκB was obtained from the Protein Data Bank. The results of this study show that small peptide WNWKL and Scabraside D may bind to the active site of the NFκB protein, similar to the binding site of caffeic acid phenethyl ester, utilized as a positive control for NFκB inhibitors. The dynamic molecular result analysis also shows that the two active compounds can form stable complexes with NFκB for up to 70 ns. These findings predict that small peptide WNWKL and Scabraside D can be NFκB inhibitors, rendering them promising candidates for further analysis as anti-inflammatory and anticancer agents.

Keywords: nuclear factor-kappaB, Scabraside D, sea cucumber, small peptide

Introduction

Cancer has become one of the deadliest diseases threatening human lives worldwide. In many cancers, the nuclear factor kappa-light-chain-enhancer of activated B cell (NFκB) protein functions as a tumor promoter. Particularly in chronic inflammation-related cancers, aberrant NFκB activation has been involved in the pathogenesis of several human diseases.[1,2] NFκB is a transcription factor for genes encoding proinflammatory cytokines in immune cells.[3] Additionally, activated NFκB binds to specific DNA sequences in target genes and activates the transcription of hundreds of genes. Most genes are involved in carcinogenesis, tumor surveillance, angiogenesis, and metastasis.[2,4,5] NFκB activity can also induce MDM2 activity, resulting in p21 degradation, which triggers cancer cell migration.[6]
Several studies have reported that the expressions of NFκB linked to tumor promotion and progression in cancer cells are remarkably reduced by sea cucumber active compounds.[7–11] Sea cucumbers, class Holothuroidea, are marine invertebrates shaped like gelatinous-bodied cucumbers that habitually live in benthic areas.[12] Some sea cucumbers are consumed for food and have been utilized in Asiatic traditional medicine.[13,14]

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Reportedly, some species of sea cucumbers have anti-inflammatory and antiproliferative effects on cancer cells. *Holothuria polii* inhibited the proliferation of MDA-MB-23I cells and reduced the expression of proinflammatory cytokines in animal models.[15] The *H. scabra* extract decreases the overexpression of human epidermal growth factor receptor 2 (HER2) and matrix metalloproteinase-9 (MMP9) in a breast cancer mice model[16] and has strong activity against nitric oxide (NO) radical.[17] *Isostichopus badionotus* extract has an anti-inflammatory effect by modulating the expression of NFκB, inducible NO synthase (iNOS), and cyclooxygenase.[18] However, no research focusing on the activity of the ingredients in sea cucumber as anticancer and inflammatory targets for NFκB exists.
This study aims to identify the sea cucumber compounds that have the potency to inhibit NFκB using an *in silico* approach. The inhibition of the NFκB proteins should be beneficial for cancer treatment.[19]

Material and Methods

Preparation of Protein and Ligands
The 3D structure of the NFκB protein was obtained from RCSB (https://www.rcsb.org/) with the PDB ID for NFκB (1SVC).[20] The protein was prepared using the BIOVIA Discovery Studio 2019 software (Dassault Systèmes BIOVIA, San Diego, California, USA).
The peptides of sea cucumbers (*Cucumaria frondosa*) were VMLGMLWTLLLR, VELWR, WNWKL, YDWRF, WPPNYQW, KMLWK, EMEWR, EEELAALVLDNGSGMCK, RMCCCSPLK, MMSLHL, TEFHLL, and WNWKV.[21] Peptide structures were established using PEP-FOLD3 (webserver modeled://263bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/). The other sea cucumber compounds studied were Holotoxin A (CID: 3085093), Stichoposide C (CID: 76871760), and Scabraside D (CID: 159134).[22] Those compound structures and the 3D structure of the caffeic acid phenethyl ester (CAPE) (CID: 5281787) as an inhibitor

of NFκB[23] were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov).

Protein–Ligand Docking

The bondings between proteins and ligands were examined using Autodock Vina in Pyrx 0.8 to obtain binding affinities between them.[24] Dockings were carried out in active sites of the NFκB protein, which are Tyr: 60 and His: 144.[20] Visualizations of dockings and interaction bonds were analyzed using BIOVIA Discovery Studio 2019 software (Dassault Systèmes BIOVIA, San Diego, California, USA).

Analysis of Potential Ligands

To determine the anticancer activity based on the Pa (probability to be active) value, the potential of peptides and compounds from sea cucumber was analyzed using Way2Drug Pass Server prediction (www.way2drug.com/passonline).

Molecular Dynamic Simulation

To examine the stability of the interaction between the protein and ligands in the specified system environment, molecular dynamic simulations were carried out. Using Yet Another Scientific Artificial Reality Application software, molecular dynamic analysis was performed.[25] System conditions were set based on the physiological conditions of human cells, namely, pH 7.4, temperature of 37°C, pressure of 1 atm, and salt content of 0.9%. The programs used were the macro md_run for running simulations, md_analyze for displaying the root-mean-square deviation (RMSD) values, md_analyzeres for displaying the root-mean-square fluctuation (RMSF) values, and md_bindenergy for analyzing complex molecular dynamic binding energies during simulations.

Results and Discussion

There are two types of inflammation: acute and chronic. Acute inflammation is beneficial for eliminating injurious agents, disposing of necrotic cells and damaged tissues, and initiating tissue repair. Unfortunately, chronic inflammation lasts for prolonged periods and is associated with chronic diseases like cancer.[26] Chronic inflammation plays a vital role in various mechanisms of tumor growth, including proliferation, cellular transformation, survival, angiogenesis, invasion, and metastasis. NFκB is a proinflammatory transcription factor that regulates more than 500 cancer-related genes.[26] Reportedly, NFκB activity is associated with inflammation and cancer development. NFκB modulates the expression of cytokines and chemokines, which causes inflammation.[3] Therefore, NFκB inhibition can inhibit inflammation and cancer development.

The results of the dockings (Table 1) showed that the binding affinity values between NFκB and peptides (−6.8 to −10.5 kcal/mol) and other compounds of sea cucumbers (−6.6 to −7.2 kcal/mol) are lower than CAPE as control (−5.8 kcal/mol). Lower binding values (kcal/mol) show reduced desolvation energy, representing the stability of ligand–protein complexes.[23] The results revealed that the compounds from sea cucumber have biological activity potential against cancer by inhibiting NFκB.

Furthermore, the dockings between NFκB and peptides showed that the bond between NFκB and WNWKL had the highest binding affinity (−10.5 kcal/mol). Meanwhile, the dockings between NFκB and the other compounds presented that the bond between NFκB and Holotoxin A had the highest binding affinity (−7.2 kcal/mol).

To assess the potential of ligands from sea cucumber for inhibiting NFκB, the analysis of the binding site between NFκB and ligands was conducted. The binding sites of ligands were compared to the binding site of CAPE, an inhibitor of NFκB.[23] It was shown that small peptide WNWKL and small molecule of Scabraside D could bind in the CAPE binding site of the NFκB (Figure 1). The result suggested that the two active compounds might inhibit NFκB activity.

The molecular dynamic simulation results revealed that all complexes tend to be stable during simulation. Complex dynamics represent complex stability during simulation. The NFκB–WNWKL complex showed high RMSD at 0–40 ns and then remained stable until the end of the simulation. The NFκB-Scabraside D complex was stable and had low fluctuation. RMSD values with minimum fluctuations show that the NFκB–ligand complex was stable during the simulation. RMSD ligand movement value showed that NFκB–Scabraside D and NFκB–WNWKL had stable values like NFκB–CAPE from 40 ns until the end of the simulation. RMSD ligand configuration also emphasized that Scabraside D and WNWKL structure tend to be stable during the simulation because these complexes had minimum fluctuation. Based on the binding energy of molecular dynamic simulation, the bond of Scabraside D with NFκB is more stable than with WNWKL. After interacting with the ligand, the protein structure tends to be stable, as shown by the RMSF value (Figure 2).

Analysis of WNWKL and Scabraside D as potential ligands indicated that sea cucumber can exhibit anticancer activities, that is, anticarcinogenic, immunostimulant, free radical scavenger, anti-inflammatory, antioxidant, antineoplastic, and cytokine release inhibitor activities (Table 2). Pa > 0.7 indicated that the peptide/compound is predicted to have a high potential as an anticancer. Moreover, a compound with 0.3 < Pa < 0.7 has a lower potential as an anticancer. The results show that peptide WNWKL is predicted to have the highest potential as an immunostimulant (Pa = 0.831) and the lowest potential as an antineoplastic (Pa = 0.204). Scabraside D is predicted to have the highest potential as an anticarcinogenic (Pa = 0.855), the second-highest potential as a free radical scavenger (Pa = 0.735), and the lowest potential as a cytokine release inhibitor (Pa = 0.116).

Many studies revealed that blocking the NFκB pathways may have consequences for anticancer modulation.[19,27] Targeting NFκB can trigger apoptosis in cancer cells. The NFκB pathway activates the transcription of antiapoptotic genes such as cIAPs, c-FLUP, BCL-XL, and A20. Inhibited NFκB will increase the JNK protein's activity, which causes apoptosis.[28] Besides inhibiting apoptosis, NFκB plays a role in controlling the cell cycle (c-Myc, cyclin D1, cyclin D2, cyclin D3, and cyclin E), cell migration (MMP9, VCAM-1, CXCR4, and CXCL8), and inflammation (iNOS and IL8).[29] Therefore, targeting NFκB is remarkably effective in inhibiting cancer development.

Table 1: Binding affinity values between protein NFκB and ligands from sea cucumber

Protein	Ligand	Binding Affinity (kcal/mol)
	Peptide compound:	
	VMLGMLWTLLLR	-8.9
	VELWR	-9.2
	WNWKL	-10.5
	WPPNYQW	-10.1
	YDWRF	-9.7
	EMEWR	-8.6
	EEELAALVLDNGSGMCK	-8.3
	KMLWK	-8.0
NFκB	MMSLHL	-9.0
	RMCCCSPLK	-9.0
	TEFHLL	-8.0
	WNWKV	-6.8
	Compound:	
	Holotoxin A	-7.2
	Stichoposide C	-7.0
	Scabraside D	-6.6
	Control:	
	CAPE	-5.8

Anticancer agents derived from natural sources (nutraceuticals) have good potential for affordable, safe, and long-term use.[30]

Previous studies have shown that cancer conditions enhance proinflammatory cytokines via NFκB activation.[26,31] NFκB inhibition as the transcription factor can suppress the hyper-expression of those cytokines.[32] Tyr 60 and His 144 are important residues in NFκB's enzymatic activity in the DNA-binding site.[20] One strategy to prevent chronic inflammation is by targeting this NFκB DNA-binding site.[26,33] Therefore, compounds of sea cucumber in the NFκB DNA-binding domain may have reduced inflammation by altering the transcription process of proinflammatory cytokines. This study shows that small peptide WNWKL and small molecule of Scabraside D may bind to the NFκB's active site, that is, Tyr 60 and His 144, as well as CAPE as an NFκB inhibitor. Molecular dynamic results showed the stability of the interaction between NFκB with Scabraside D and WNWKL peptide. RMSD values with minimal fluctuations indicate that the interaction between the protein–ligand is stable during the simulation.[34] These results are supported by the molecular dynamic binding energy value. The more positive the molecular dynamic binding energy value, the more stable the interaction between the protein and the ligand.[35]

This in silico study shows that sea cucumber compounds exhibit anticancer because molecular dockings between compounds and NFκB have shown their binding affinities. The study is in line with several studies that have reported NFκB expressions linked to tumor promotion and progression in cancer cells remarkably reduced by sea cucumber active compounds.[7–10]

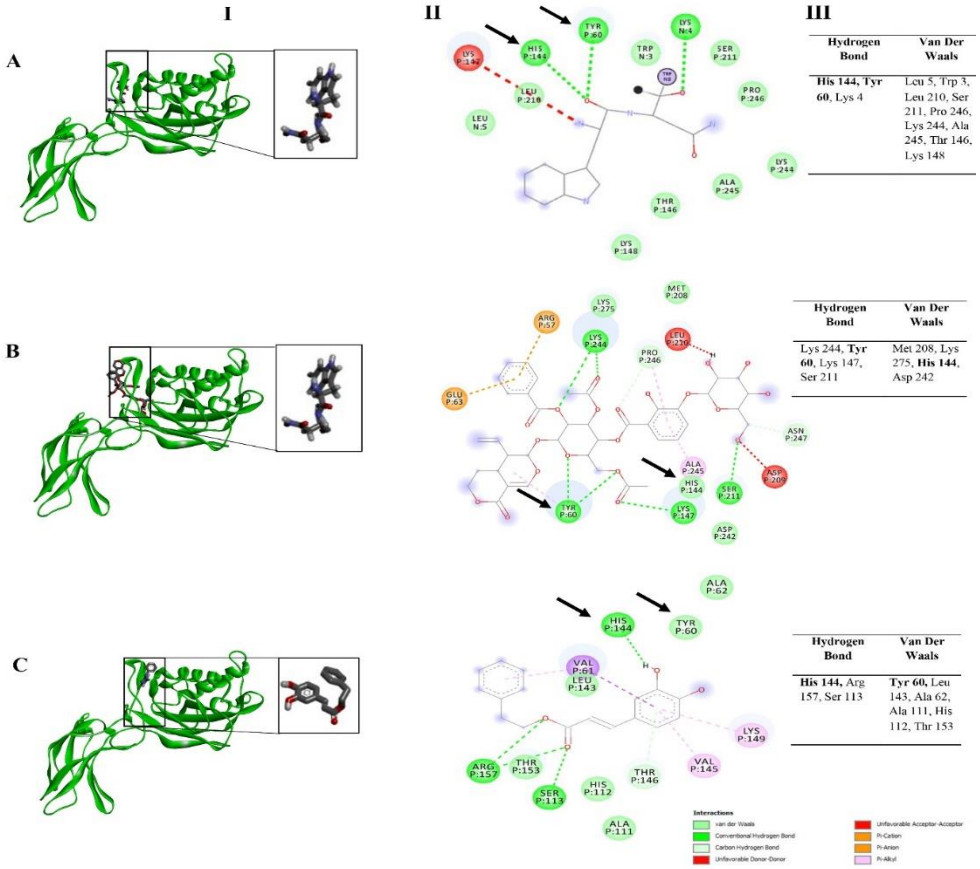

Figure 1: Interaction between NFκB and compounds from sea cucumbers: (A) WNWKL, (B) Scabraside D, (C) CAPE, a known NFκB inhibitor. (I) 3D structure of NFκB is presented in a green ribbon with ligands, (II) 2D structure interaction, (III) a list of amino acids which are binding to the active site of NFκB.

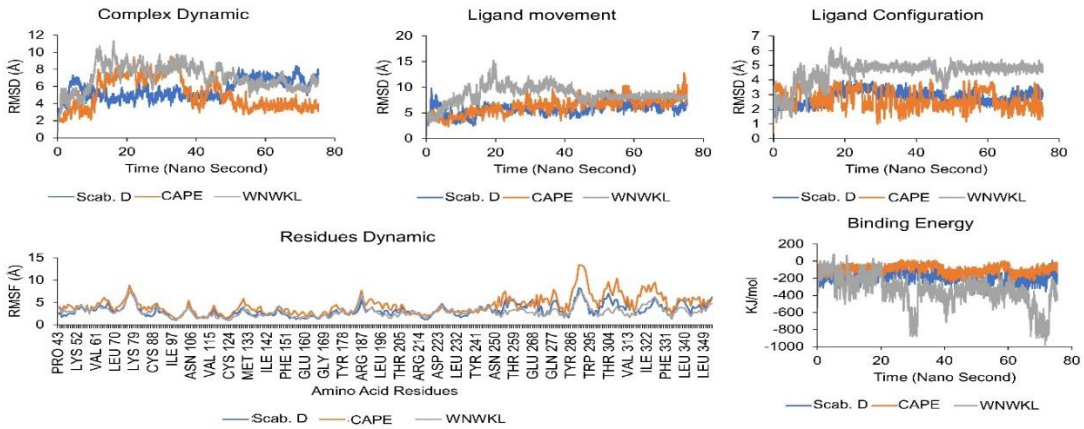

Figure 2: The stability of the interaction between NFκB with Scabraside D, CAPE, and WNWKL

Table 2: Potential of WNWKL and Scabraside D as anticancer

Compound	Activity	Pa value
WNWKL	Immunostimulant	0.831
	Cancer associated disorders treatment	0.439
	Antineoplastic	0.204
Scabraside D	Anticarcinogenic	0.855
	Free radical scavenger	0.735
	Antiinflammatory	0.697
	Antioxidant	0.524
	Cytokine release inhibitor	0.116

Conclusion

In conclusion, the active compounds from sea cucumber, which can potentially function as NFκB inhibitors, are small peptide WNWKL and Scabraside D. Thus, to validate the potential use of the compounds for developing therapy agents for inflammation-related cancers, further studies are required.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Xia Y, Shen S, Verma IM. NF-κB, an active player in human cancers. Cancer Immunol Res. 2014; 2(9):823–830.
2. Li F, Zhang J, Arfuso F, Chinnathambi A, Zayed ME, Alharbi SA, Kumar AP, Ahn KS, Sethi G. NF-κB in cancer therapy. Arch Toxicol. 2015; 89(5):711–731.
3. Nennig SE, Schank JR. The role of NFkB in drug addiction: Beyond inflammation. Alcohol Alcohol. 2017; 52(2):172–179.
4. Erstad DJ, Cusack JC. Targeting the NF-κB pathway in cancer therapy. Surg Oncol Clin N Am. 2013; 22(4):705–746.
5. Lin Y, Bai L, Chen W, Xu S. The NF-κB activation pathways, emerging molecular targets for cancer prevention and therapy. Expert Opin Ther Targets. 2010; 14(1):45–55.
6. Jana A, Krett NL, Guzman G, Khalid A, Ozden O, Staudacher JJ, Bauer J, Baik SH, Carroll T, Yazici C. NFkB is essential for activin-induced colorectal cancer migration via upregulation of PI3K-MDM2 pathway. Oncotarget. 2017; 8(23):37377–37393.
7. Fedorov SN, Dyshlovoy SA, Kuzmich AS, Shubina LK, Avilov SA, Silchenko AS, Bode AM, Dong Z, Stonik VA. In vitro anticancer activities of some triterpene glycosides from holothurians of cucumariidae, stichopodidae, psolidae, holothuriidae and synaptidae families. Nat Prod Commun. 2016; 11(9):1239–1242.
8. Zhao Q, Xue Y, Wang JF, Li H, Long TT, Li Z, Wang YM, Dong P, Xue CH. In vitro and in vivo anti-tumour activities of echinoside A and ds-echinoside A from *Pearsonothuria graeffei*. J Sci Food Agric. 2012;92(4):965–74.
9. Zhao Q, Liu ZD, Xue Y, Wang JF, Li H, Tang QJ, Wang YM, Dong P, Xue CH. Ds-echinoside A, a new triterpene glycoside derived from sea cucumber, exhibits antimetastatic activity via the inhibition of NF-κB-dependent MMP-9 and VEGF expressions. J Zhejiang Univ Sci B. 2011; 12(7):534–544.
10. Park SY, Kim YH, Kim Y, Lee SJ. Frondoside A has an anti-invasive effect by inhibiting TPA-induced MMP-9 activation via NF-κB and AP-1 signaling in human breast cancer cells. Int J Oncol. 2012;41(3):933–40.
11. Zhao Q, Xue Y, Liu ZD, Li H, Wang JF, Li ZJ, Wang YM, Dong P, Xue CH. Differential effects of sulfated triterpene glycosides, holothurin A1, and 24-dehydroechinoside A, on antimetastasic activity via regulation of the MMP-9 signal pathway. J Food Sci. 2010;75(9).
12. Bordbar S, Anwar F, Saari N. High-value components and bioactives from sea cucumbers for functional foods—A review. Mar Drugs. 2011 ;9(10):1761–1805.
13. Cuong NX, Vien LT, Hoang L, Hanh TTH, Thao DT, Thanh NV, Nam NH, Thung DC, Kiem PV, Minh CV. Cytotoxic triterpene diglycosides from the sea cucumber *Stichopus horrens*. Bioorganic Med Chem Lett. 2017; 27(13):2939–42.
14. Shahinozzaman M, Ishii T, Takano R, Halim MA, Hossain MA, Tawata S. Cytotoxic desulfated saponin from *Holothuria atra* predicted to have high binding affinity to the oncogenic kinase PAK1: A combined in vitro and in silico study. Sci Pharm. 2018; 86(3):1–13.
15. Kareh M, El-Nahas R, Al-Aaraj L, Al-Ghadban S, Al Deen NN, Saliba N, El-Sabban M, Talhouk R. Anti-proliferative and anti-inflammatory activities of the sea cucumber *Holothuria polii* aqueous extract. SAGE Open Med. 2018; 6:2050312118809541.
16. Pranoto D, Ratnawati H, Wargasetia TL, Octavallen A, Rachmat MAA. Sea cucumber reduces breast cancer tumor size through inhibition of MMP9 dependent HER2 expression in breast cancer mice model. Trop J Nat Prod Res. 2023; 7(9):4010–4015.
17. Wargasetia TL, Ratnawati H, Widodo N, Widyananda MH. Antioxidant and anti-inflammatory activity of sea cucumber (*Holothuria scabra*) active compounds against KEAP1 and iNOS protein. Bioinform Biol Insights. 2023; 17:1–10.
18. Olivera-Castillo L, Grant G, Kantún-Moreno N, Barrera-Pérez HA, Montero J, Olvera-Novoa MA, Carrillo-Cocom LM, Acevedo JJ, Puerto-Castillo C, May Solís V, Pérez-Vega JA, Gil-Zamorano J, Hernández-Garibay E, Fernández-Herrera MA, Pérez-Tapia M, Medina-Contreras O, Villanueva-Toledo JR, Rodriguez-Canul R, Dávalos A. A glycosaminoglycan-rich fraction from sea cucumber *Isostichopus badionotus* has potent. Nutrients. 2020;12(1698):1–15.
19. Shishodia S, Aggarwal BB. Nuclear factor-κB: A friend or a foe in cancer? Biochem Pharmacol. 2004;68(6):1071–80.
20. Müller CW, Rey FA, Sodeoka M, Verdine GL, Harrison SC. Structure of the NF-kappa B p50 homodimer bound to DNA. Nature. 1995; 373(6512):311–317.
21. Zhang Y, He S, Bonneil É, Simpson BK. Generation of antioxidative peptides from Atlantic sea cucumber using alcalase versus trypsin: *In vitro* activity, *de novo* sequencing, and *in silico* docking for *in vivo* function prediction. Food Chem. 2020; 306:125581.
22. Wargasetia T, Permana S, Widodo N. The role of sea cucumber active compound and its derivative as an anti-cancer agent. Curr Pharmacol Reports. 2018; 4.
23. Ali A, Shah FA, Zeb A, Malik I, Alvi AM, Alkury LT, Rashid S, Hussain I, Ullah N, Khan A, Koh PO, Li S. NF-κB inhibitors attenuate MCAO induced neurodegeneration

and oxidative stress—a reprofiling approach. Front Mol Neurosci. 2020; 13:33.
24. Shityakov S, Förster C. In silico predictive model to determine vector-mediated transport properties for the blood–brain barrier choline transporter. Adv Appl Bioinform Chem. 2014; 7:23–36.
25. Krieger E, Vriend G. YASARA View - molecular graphics for all devices - from smartphones to workstations. Bioinformatics. 2014;30(20):2981–2.
26. Gupta SC, Sundaram C, Reuter S, Aggarwal BB. Inhibiting NF-κB activation by small molecules as a therapeutic strategy. Biochim Biophys Acta. 2010;1799(10–12): 775–787.
27. Philip S, Kundu G. Osteopontin induces nuclear fctor κB-mediated promatrix metalloproteinase-2 activation through IκBα/IKK signaling pathways, and curcumin (diferulolylmethane) down-regulates these pathways. J Biol Chem. 2003; 278(16):14487–14497.
28. Sakamoto K, Maeda S. Targeting NF-κB for colorectal cancer. Expert Opin Ther Targets. 2010;14(6): 593–601.
29. Wargasetia TL, Ratnawati H, Widodo N. Sea cucumber compounds targeting NF-κB in cancer treatment. Bioinform Biol Insights. 2022;16: 11779322221091740.
30. Gupta SC, Kunnumakkara AB, Aggarwal S, Aggarwal BB. Inflammation, a double-edge sword for cancer and other age-related diseases. Front Immunol. 2018; 9:5–10.
31. Del Prete A, Allavena P, Santoro G, Fumarulo R, Corsi MM, Mantovani A. Molecular pathways in cancer-related inflammation. Biochem Medica. 2011; 21(3):264–275.
32. Martins GR, Gelaleti GB, Moschetta MG, Maschio-Signorini LB, Zuccari DAP de C. Proinflammatory and anti-inflammatory cytokines mediated by NF-κB factor as prognostic markers in mammary tumors. Mediators Inflamm. 2016:e9512743.
33. Gilmore TD, Herscovitch M. Inhibitors of NF-kappaB signaling: 785 and counting. Oncogene. 2006; 25(51):6887–6899.
34. Widyananda MH, Wicaksono ST, Rahmawati K, Puspitarini S, Ulfa SM, Jatmiko YD, Masruri M, Widodo N. A potential anticancer mechanism of finger root (*Boesenbergia rotunda*) extracts against a breast cancer cell line. Scientifica (Cairo). 2022; 9130252.
35. Wargasetia TL, Ratnawati H, Widodo N, Widyananda MH. Bioinformatics study of sea cucumber peptides as antibreast cancer through inhibiting the activity of overexpressed protein (EGFR, PI3K, AKT1, and CDK4). Cancer Inform. 2021; 20: 11769351211031864.