Prediction of the activity of carbohydrate moiety of bromelain as immunomodulator using an in silico approach

by Afriza Yelnetty 21

Submission date: 15-Jun-2023 07:30AM (UTC+0700)
Submission ID: 2116259835
File name: ction_of_the_activity_of_carbohydrate_moiety_of_bromelain_as.pdf (1.04M)
Word count: 4902
Character count: 26452

Prediction of the activity of carbohydrate moiety of bromelain as immunomodulator using an in silico approach

Conference Paper · January 2023

DOI: 10.1063/5.0103885

CITATIONS
0

READS
29

12 authors, including:

Trina Tallei
Sam Ratulangi University
209 PUBLICATIONS **1,726** CITATIONS
SEE PROFILE

Fatimawali Umar
Sam Ratulangi University
69 PUBLICATIONS **580** CITATIONS
SEE PROFILE

Afriza Yelnetty
Sam Ratulangi University
17 PUBLICATIONS **80** CITATIONS
SEE PROFILE

Nurdjannah Niode
Sam Ratulangi University
43 PUBLICATIONS **360** CITATIONS
SEE PROFILE

RESEARCH ARTICLE | MAY 09 2023

Prediction of the activity of carbohydrate moiety of bromelain as immunomodulator using an in silico approach

Trina Ekawati Tallei ✉; Fatimawali; Afriza Yelnetty; ... et. al

65 *Conference Proceedings* 2480, 050001 (2023)
https://doi.org/10.1063/5.0103885

Prediction of The Activity of Carbohydrate Moiety of Bromelain as Immunomodulator Using an in Silico Approach

Trina Ekawati Tallei[1,a)], Fatimawali[2,b)], Afriza Yelnetty[3,c)], Nurdjannah Jane Niode[4,d)], Diah Kusumawaty[5,e)], Billy Johnson Kepel[6,f)], Souvia Rahimah[7,g)], Yunus Effendi[8,h)], Rinaldi Idroes[9,i)], Sefren Geiner Tumilaar[2,j)] Shafi Mahmud[10,k)] and Talha Bin Emran[11,l)]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia*
[2]*Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia*
[3]*Animal Products Technology Study Program, Faculty of Animal Husbandry, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia*
[4]*Department of Dermatology and Venereology, Faculty of Medicine, University of Sam Ratulangi, RD Kandou Hospital, Manado 95163, North Sulawesi, Indonesia.*
[5]*Department of Biology Education, Faculty of Mathematics and Science Education, Universitas Pendidikan Indonesia, Bandung 40154, Jawa Barat, Indonesia*
[6]*Department of Chemistry, Faculty of Medicine, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia*
[7]*Food Technology Study Program, Department of Food Industrial Technology, Faculty of Agroindustrial Technology, Universitas Padjadjaran, Jatinangor 45363, West Java, Indonesia*
[8]*Biology Study Program, Faculty of Science and Technology, Universitas Al Azhar, Jakarta 12110, Indonesia*
[9]*Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Kopelma Darussalam, Banda Aceh 23111, Indonesia*
[10]*Microbiology Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi 6205, Bangladesh*
[11]*Department of Pharmacy, BGC Trust University Bangladesh, Chittagong 4381, Bangladesh*

[a)]*Corresponding author: trina_tallei@unsrat.ac.id*
[b)]*fatimawali@unsrat.ac.id*
[c)]*ayelnetty@unsrat.ac.id*
[d)]*niodejane@unsrat.ac.id*
[e)]*diah.kusumawaty@upi.edu*
[f)]*billy.kepel@unsrat.ac.id*
[g)]*souvia@unpad.ac.id*
[h)]*effendiy@uai.ac.id*
[i)]*rinaldi.idroes@unsyiah.ac.id*
[j)]*sefrentumilaar1@gmail.com*
[k)]*shafimahmudfz@gmail.com*
[l)]*talhabmb@bgctub.ac.bd*

The 4th International Conference on Science and Technology (ICST) 2021
AIP Conf. Proc. 2480, 050001-1–050001-10; https://doi.org/10.1063/5.0103885
Published by AIP Publishing. 978-0-7354-4463-8/$30.00

Abstract. Bromelain is a glycoprotein that contains a single carbohydrate moiety group and displays proteolytic activity. Bromelain has long been known to act as an immunomodulator; however, the immunomodulatory activity of its carbohydrate moiety has been very rarely studied and is not well understood. This study aimed to predict the immunomodulatory activity of the carbohydrate moiety of bromelain (CMB) by evaluating it using the PASS server, and the binding affinities between CMB and various pro-inflammatory cytokines were analyzed using a molecular docking approach. This research was conducted by docking the CMB ligand to the following receptor proteins: interleukin-6 (IL-6), IκBβ/nuclear factor (NF)-κB p65 homodimer complex (IκBα/NF-κB p65), tumor necrosis factor-alpha (TNF-α), and IL-1β. The biological potential of CMB was predicted using the online PASS server. The results of the computational analysis indicated that the highest binding energy was identified between CMB and IκBβ/NF-κB p65 (−10.3 kcal/mol), followed by TNF-α (−9.7 kcal/mol), and IL-6 and IL-1β, with values of −8.2 kcal/mol for both. The CMB activity predicted by the Way2Drug PASS server indicated that this molecule is likely to display significant immunostimulant activity. These findings suggested that CMB can be considered a potential lead molecule for further drug development to identify agent that can prevent or cure inflammation and restore balance to the immune system. However, the predictions determined in this study remain to be validated in future in vitro and in vivo studies.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has emphasized the importance of maintaining immune system function as much as possible because no specific drug has yet been developed to treat this disease [1, 2], and vaccines are still being developed to reduce the incidence of this disease outbreak [3, 4]. COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has been associated with a high death rate caused in part by a cytokine storm, which is an imbalance in the immune system that triggers a systemic, hyperinflammatory response [5, 6].

Other viral infections also commonly result in acute respiratory reactions [7]. Viral infections can affect a patient's resistance against secondary bacterial infections, which can often result in a more severe clinical disease course [8]. The mechanisms that underlie bacterial–viral infections are complex and include a multifactorial process that is mediated by interactions between viruses, bacteria, and the host immune system [9]. In exposed patients, disease severity can be caused by an uncontrolled immune system reaction that can damage the patient's vital organs [10].

Indonesia produces agricultural commodities that are in demand by the export market, including pineapple, and 1.8 million tons of pineapple are produced each year, based on reports from 2018, in the form of both fresh and canned fruit. The fruit and stem of pineapple (*Ananas comosus* L.) are known to contain a significant number of nutrients that are beneficial to health, including bromelain. Bromelain is known to act as an immunomodulator, activating natural killers, with demonstrated antiviral activity, and bromelain can be used as a phytotherapeutic agent due to its lack of side effects [11].

COVID-19 is a new disease that results in serious global health problems, and research remains necessary to identify specific preventive and therapeutic regimens capable of addressing the morbidity and mortality associated with this disease. Efforts remain necessary in the face of this disease, including identifying methods for improving and maintaining the immune system. Immunomodulators can be used as preventive or therapeutic strategies to increase or suppress the host immune response. Natural products with immunomodulatory and antioxidant functions are widely used to treat several diseases, including infections caused by viruses [12,13]. Recently, natural products have been in great demand because they are abundant, cheap, and are typically associated with low levels of toxicity compared with synthetic therapeutic agents.

The regular intake of pineapple has the potential to affect immunological markers, reducing the impacts of viral and bacterial infections [14]. Bromelain is a protease compound with high oral bioavailability and minimal side effects and has previously been reported to induce immunostimulatory and anti-inflammatory effects [12,15] and antiviral activity [11]. Bromelain has also been reported to suppress coughing induced by the angiotensin-converting enzyme (ACE) (16) due to its ability to degrade bradykinin [17], a molecule that plays a crucial role in inflammation. Some pro-inflammatory factors that are often examined in in silico studies of immunomodulatory effects include human interleukin-6 (IL-6), the IκBβ/nuclear factor (NF)-κB p65 homodimer complex (IκBα/NF-κB P65), tumor necrosis factor-alpha (TNF-α), and IL-1β. These molecules are inflammatory markers that are typically upregulated when the body experiences a viral or microbial infection, in addition to other inflammatory physiological disorders [18,19].

Bromelain is a glycoprotein that contains a carbohydrate prosthetic group as a distinct single moiety, consisting of mannose, xylose, fucose, and N-acetylglucosamine (GlcNAc) [15,20]. Little research has been performed to examine the effects of CMB as an immunomodulator. Therefore, this study focused on the potential for CMB to serve as an immunomodulator, using a molecular docking approach and the prediction of biological activity using the PASS server.

MATERIALS AND METHODS

Ligand and Receptor Preparation

The ligand used in this study, carbohydrate moiety of bromelain (CMB), was retrieved from PubChem server (https://pubchem.ncbi.nlm.nih.gov/compound/44263865) (Fig. 1). The ligand was downloaded in .sdf file and then converted into .pdb file using Open Babel. After the torque was adjusted by detecting root, then the file was saved in .pdbqt format. Swiss ADME online tool was employed to evaluate ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of CMB.

The receptors used in this study were Human Interleukin-6 (IL-6) (PDB code 1ALU), IkappaBbeta/NF-kappaB p65 homodimer complex (IκBα/NF-κB P65) (PDB code 1OY3), TNF-alpha (TNF-α) (PDB code 2AZ5), and Interleukin-1beta (IL-1β) (PDB code 2NVH). All three-dimensional structures of the receptors were downloaded from the Protein Data Bank (http://www.rcsb.org). BIOVIA Discovery Studio Visualizer 2020 was used to open receptor files. With this software, all water molecules and native ligands were removed, then the clean files were saved in .pdb format. Estimation of the active site at the ligand-binding receptors were identified using the CASTp web server (http://sts.bioe.uic.edu/castp/) [21].

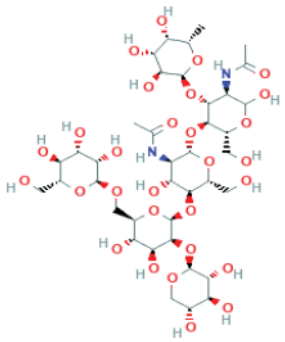


FIGURE 1. The two-dimensional structure of carbohydrate moiety of bromelain (CMB) (Pubchem CID: 44263865)

Receptor-ligand Docking and Visualization

The docking process was executed using AutoDock Vina. The .pdbqt formats for the ligand and receptors were input into AutoDock Vina. Based on the receptor type, the grid point is determined by maintaining the grid spacing at 1 Å. The configuration of Vina was typed into a notepad program and saved as conf.txt. A command prompt was used to run the Vina program. The selection of the best docking pose was performed as an in silico evaluation process. The pose with the most negative Gibbs' free energy binding value is considered the best pose from the molecular docking process. Biovia Discovery Studio 2020 was used to visualize the interaction between the ligand and the receptors. The best pose from AutoDock Vina, as determined by the lowest binding affinity value, was used to estimate the half-maximal inhibitory concentration (IC50) of the ligand using AutoDock 4. The visualization of 3D and 2D structures from the docking calculations was performed using BIOVIA Discovery Studio Visualizer 2020.

Prediction of CMB Immunomodulating Activity

The immunomodulating activity of CMB was evaluated using Way2Drug, an online software for the prediction of biological activity of drug-like organic compounds [22]. The software can be accessed through http://www.pharmaexpert.ru/passonline/.

RESULTS AND DISCUSSIONS

The COVID-19 pandemic has emphasized the importance of maintaining proper immune system function to prevent infectious diseases [23]. Several isolated compounds have been studied as immunomodulators, including bromelain. These potentials immunomodulating compounds can be investigated using computer simulations, such as a molecular docking approach. The CMB in this study was examined because its activities have not been widely explored, despite research that describes bromelain as an immunomodulator [11,24].

For a compound to be considered an appropriate drug candidate, the ADMET properties must be evaluated [25]. The ADMET properties of CMB were evaluated using the SwissADME ser The estimated gastrointestinal (GI) absorption of CMB is low, with no indication of toxicity as it does not inhibit cytochrome P450 (CYP), which is a hemeprotein that plays a key role in the metabolism of drugs and other xenobiotics. When evaluated for drug-likeness, CMB registered three violations of Lipinski's Rule of Five (Ro5), and its bioavailability score was only 0.17, which indicates only a 17% chance for the molecule to cross various biological membranes. The bioavailability of GlcNAc itself was reported as 26% in humans [26]. Bioavailability depends on the molecular mass of a compound and decreases drastically when the mass exceeds 700 Da (27). The CMB molecule is quite large (1026.94 Da) and may also be degraded into smaller molecules in the digestive system if administered orally. However, one study [15] showed that bromelain was stable after 4 hrs in artificial stomach juice as well as in artificial blood.

In the present study, IL-6, IκBα/NF-κB P65, TNF-α, and IL-1β were analyzed for possible interactions with CMB. The binding affinity between CMB and four of these examined receptors is presented in Table 1, including two compounds that were used as comparisons which were believed to have immunomodulatory activity: hypophyllanthin and phyllanthin. These active compounds are derived from the plants *Phyllanthus amarus* and *Phyllanthus urinaria*. These plants have been used as medicinal herbs that exhibit immunomodulatory properties and have been reported to restore the immune system [28]. Yuandani et al. [29] stated that the inhibito activities of these plants are likely due to the high levels of phyllanthin and hypophyllanthin found in these plants. The results of the present study indicated that CMB has a better affinity for the four tested receptors than either of the two control molecules. The best bind affinity was found between CMB and IκBα/NF-κB P65 (−10 kcal/mol), follo d by TNF-α (−9.7 kcal/mol) and IL-1β and IL-6, with va s of −8.2 kcal/mol for each. The binding energies of IL-1β and IL-6 with CMB were higher than those between IL-1β and IL-6 and chlorogenic acid, kaempferol, and indomethacin, where were previously reported [30].

In a review, carbohydrates were shown to be capable of activating both the innate and adaptive immune responses [31,32]. Dias et al. [33] explained that GlcNAc could be used as a simple immunomodulatory strategy for the therapy of inflammatory bowel disease (IBD), which is no xic and does not cause side effects. Guo et al. [34] also r ted that D-mannose increases immunomodulation by inhibiting IL-6 secretion in periodontal ligament stem cells. IL-6 is a multifunctional cytokine that plays a major role in the host de e against foreign compounds. However, the overexpression of IL-6 has a detrimental effect on the body and has been associated with several diseases, such as rheumatoid arthritis [35]. Xylose and mannose are monosaccharide components that contribute to macrophage stimulating activity [36]. Fucose has also been suspected of playing a role as an immunomodulator [37].

TABLE 1. The binding affinity results from positive control ligands and CMB to several receptors that play a role in proinflammation.

Ligan	Binding affininty (kcal/mol) between ligands and receptors			
	Human Interleukin-6 (PDB code 1ALU)	IκBα/ NF-κB P65 (PDB code 1OY3)	TNF-alpha (PDB code 2AZ5)	Interleukin-1β (PDB code 2NVH)
Hypophyllanthin	-6.0	-7.6	-8.1	-7.1
Phyllanthin	-4.6	-5.3	-6.5	-5.0
CMB	-8.2	-10.3	-9.7	-8.2

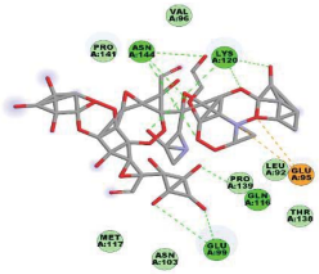
Table 2 shows the types of interactions identified between the ligands and receptors. The number of conventional H-bonds formed between CBM and the tested receptors were nine for IL-6, 14 for IκBα/NF-κB, six for TNF-α, and 12 for IL-1β. Wang et al. [38] and Fatima et al. [39] showed that the active sites in TNF-α were Leu57, Tyr59, Ser60, Leu120, Gly121, Gly122, and Tyr151. Table 2 and Fig. 1 show that the CMB binds with the proposed active site of TNF-α, particularly Tyr59, Leu57, Ser60, Leu120, Gly121, and Tyr151 but not Gly122. The results of previous research [40] showed that the sugar moiety from rutin (SMR) formed H-bonds with TNF-α at Gly121, and the flavanol moiety forms π-amide interactions with Gly-121 and Gly-122 and π- π interaction with Chain A: Tyr59. These findings indicate that CMB binds with an active site, similar to the SMR. The nucleus of polyphenolic flavanols and sugar SMR units form H-bonds with Arg4 in IL-1β [40]. The current study also found that CMB binds to Arg (A4). CMB also binds to amino acid residues that have been shown to bind with indomethacin (Pro91, Tyr68, Lys65, and Glu64), chlorogenic acid (Pro2, Ser43, Lys63, and Lys65), and kaempferol (Asn7, Ser43, Tyr68, Asn66, and Glu64), as reported previously [30].

TABLE 2. Amino acid interactions and the number of hydrogen bonds between CMB and several immunomodulatory receptors. The bold letters indicate the exact interaction at the active site (pocket) of the receptors.

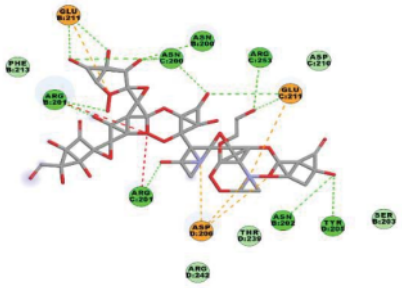
Receptors	Docking Score (Kcal/mol)	Interacting Residues	Distance (Å)	Category	Type
Human Interleukin-6 (PDB ID: 1ALU)	-8.2	Glu(A99)	3.37	H-Bond	Conventional
		Gln(A99)	3.10	H-Bond	Conventional
		Gln(A116)	2.80	H-Bond	Conventional
		Glu(A95)	3.24	Electrostatic	Attractive Charge
		Glu(A95)	3.25	Electrostatic	Attractive Charge
		Asn(A144)	2.41	H-Bond	Conventional
		Asn(A144)	2.48	H-Bond	Conventional
		Asn(A144)	2.77	H-Bond	Conventional
		Lys(A120)	2.31	H-Bond	Conventional
		Lys(A120)	2.91	H-Bond	Conventional
		Lys(A120)	5.42	H-Bond	Conventional
		Met(A117)	-	Electrostatic	Van der Waal's
		Asn(A103)	-	Electrostatic	Van der Waal's
		Thr(A138)	-	Electrostatic	Van der Waal's
		Pro(A139)	-	Electrostatic	Van der Waal's
		Leu(A92)	-	Electrostatic	Van der Waal's
		Pro(A141)	-	Electrostatic	Van der Waal's
		Val(A96)	-	Electrostatic	Van der Waal's

Receptors	Docking Score (Kcal/mol)	Interacting Residues	Distance (Å)	Category	Type
IκBα/ NF-κB P65 (PDB ID: 1OY3).	-10.3	Tyr(D205)	3.02	H-Bond	Conventional
		Asn(B202)	2.24	H-Bond	Conventional
		Glu(C211)	2.23	H-Bond	Conventional
		Glu(C211)	3.18	H-Bond	Conventional
		Asn(C200)	2.62	H-Bond	Conventional
		Asn(C200)	2.03	H-Bond	Conventional
		Asn(C200)	2.96	H-Bond	Conventional
		Glu(B211)	2.98	H-Bond	Conventional
		Glu(B211)	2.84	H-Bond	Conventional
		Arg(B201)	2.65	H-Bond	Conventional
		Arg(B201)	2.87	H-Bond	Conventional
		Arg(C201)	2.84	H-Bond	Conventional
		Arg(C253)	2.88	H-Bond	Conventional
		Asn(B200)	2.90	H-Bond	Conventional
		Glu(C211)	4.31	Electrostatic	Attractive Charge
		Asp(D206)	3.85	Electrostatic	Attractive Charge
		Asp(D206)	4.58	Electrostatic	Attractive Charge
		Asp(D206)	4.78	Electrostatic	Attractive Charge
		Glu(B211)	5.33	Electrostatic	Attractive Charge
		Arg(C201)	5.01	Unfavorable	Positive-Positive
		Arg(B201)	4.12	Unfavorable	Positive-Positive
		Phe(B213)	-	Electrostatic	Van der Waal's
		Arg(D242)	-	Electrostatic	Van der Waal's
		Thr(D239)	-	Electrostatic	Van der Waal's
		Ser(B203)	-	Electrostatic	Van der Waal's
		Asp(C210)	-	Electrostatic	Van der Waal's
TNF-alpha (PDB ID: 2AZ5)	-9.7	**Ser(A60)**	4.28	H-Bond	Conventional
		Leu(B120)	5.52	H-Bond	Conventional
		Gly(A121)	2,84	H-Bond	Conventional
		Ser(B60)	4.27	H-Bond	Conventional
		Tyr(B151)	2.09	H-Bond	Conventional
		Leu(B120)	3.50	H-Bond	Carbon
		Tyr(A59)	4.95	Hydrophobic	Pi-Cation
		Tyr(A59)	4.95	Hydrophobic	Pi-Cation
		Gln(A61)	-	Electrostatic	Van der Waal's
		Leu(B57)	-	Electrostatic	Van der Waal's
		Leu(A120)	-	Electrostatic	Van der Waal's
		Tyr(A119)	-	Electrostatic	Van der Waal's
		Tyr(B59)	-	Electrostatic	Van der Waal's
		Gln(B61)	-	Electrostatic	Van der Waal's
		Tyr(B119)	-	Electrostatic	Van der Waal's
		Gly(B121)	-	Electrostatic	Van der Waal's
		Leu(D157)	-	Electrostatic	Van der Waal's
		Leu(D55)	-	Electrostatic	Van der Waal's
		Tyr(A151)	-	Electrostatic	Van der Waal's

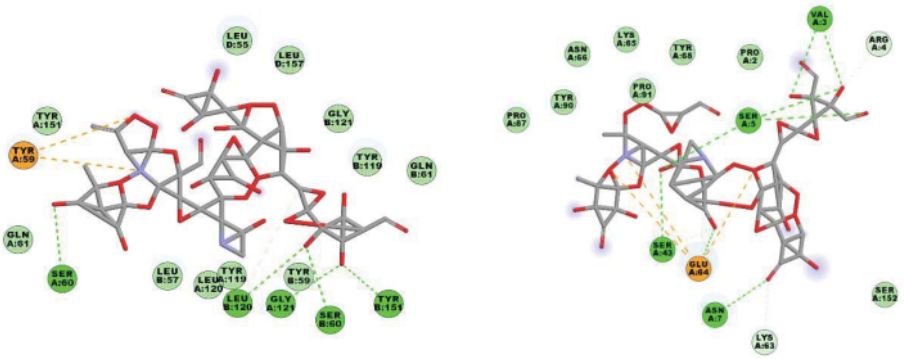
Receptors	Docking Score (Kcal/mol)	Interacting Residues	Distance (Å)	Category	Type
		Val(A3)	3.01	H-Bond	Conventional
		Val(A3)	3.04	H-Bond	Conventional
		Ser(A5)	2.43	H-Bond	Conventional
		Ser(A5)	2.43	H-Bond	Conventional
		Ser(A5)	2.04	H-Bond	Conventional
		Ser(A43)	2.62	H-Bond	Conventional
		Glu(A64)	1.86	H-Bond	Conventional
		Asn(A7)	3.06	H-Bond	Conventional
		Arg(A4)	3.10	Carbon	Carbon
		Ser(A43)	3.74	H-Bond	Carbon
		Lys(A63)	3.01	H-Bond	Carbon
		Glu(A64)	3.22	H-Bond	Carbon
		Glu(A64)	4.38	Electrostatic	Attractive Charge
Interleukin-1 (PDB ID: 2NVH).	-8.2	**Glu(A64)**	4.69	Electrostatic	Attractive Charge
		Glu(A64)	5.26	Electrostatic	Attractive Charge
		Pro(A87)	-	Electrostatic	Van der Waal's
		Tyr(A90)	-	Electrostatic	Van der Waal's
		Asn(A66)	-	Electrostatic	Van der Waal's
		Pro(A91)	-	Electrostatic	Van der Waal's
		Lys(A65)	-	Electrostatic	Van der Waal's
		Tyr(A68)	-	Electrostatic	Van der Waal's
		Pro(A2)	-	Electrostatic	Van der Waal's
		Ser(A152)	-	Electrostatic	Van der Waal's



A



B



C D

FIGURE 2. Visualization of the types of bonds formed and amino acid residues involved between CMB and receptors (A) Human Interleukin-6 (PDB ID: 1ALU), (B) IκBα/NF-κB P65, (C) TNF-alpha, and (D) Interleukin 1β.

Predictions of the immunomodulating abilities of CMB were performed using Way2Drug. The results are shown in Table 3. The criteria used to categorize the immunomodulatory activities of CMB were based on the Pa value as follows: (1) Pa > 0.7, the tested compound has a form and activity analogous to the drug compound; (2) 0.5 < Pa < 0.7, the tested compound has a different shape and is likely to present with reduced activity relative to the drug compound; (3) Pa < 0.5, the tested compound is unlikely to show activity similar to that of the drug compound. The evaluation results showed that CMB has immunostimulant activity.

TABLE 3. Prediction of CMB activity using Way2Drug

Pa	Pi	Activities
0,175	0,014	Cytokine release inhibitor
0,952	0,002	Immunostimulant

The molecular docking results showed that CMB could potentially suppress an immune response, whereas the results from the Way2Drug analysis indicated that CMB was a potential immunostimulant. These findings are in line with previous research [41], which showed that bromelain could simultaneously increase or inhibit T cell responses both in vitro and in vivo. These findings indicate that bromelain has immunomodulatory activity and should be considered as a potential immunomodulatory agent or vaccine adjuvant. By using AutoDock 4, a constant inhibition value of 5.52 μM was obtained for CMB against TNF-α.

CONCLUSION

Based on in silico predictions, CMB has a good binding affinity with the pro-inflammatory cytokine molecules IL-6, IL-1β, TNF-α, and IκBα/NF-κB P65. CMB has a very strong affinity with the binding sites of TNF-α and IL-1β and may represent a new candidate lead compound for the development of novel immunomodulatory agents. However, further assays including in vitro and in vivo verifications of anti-inflammatory abilities of CMB remain necessary to validate the results of this study.

ACKNOWLEDGMENTS

We are grateful to the Ministry of Research and Technology/National Research and Innovation Agency of the Republic of Indonesia for providing research funding through the basic research program for fiscal year 2021. This research is also partially funded by the COVID-19 research and innovation consortium under the ministry of research

and technology/the National Research and Innovation Agency (BRIN) in collaboration with the Indonesia Endowment Fund for Education (LPDP) of the Republic of Indonesia.

REFERENCES

1. A. Wu, Y. Peng, B. Huang, X. Ding, Wang, P. Niu, J. Meng, Z. Zhu, Z. Zhang, J. Wang, J. Sheng, L. Quan, Xia, W. Tan, G. Cheng, T. Jiang, Cell Host Microbe. 27(3), 325–328, (2020).
2. T. E. Tall S. G. Tumilaar, N. J. Niode, Fatimawali, B. J. Kepel, R. Idroes, Y. Effendi, Scientifica 2020, 6307457 (2020).
3. S. H. Hodgson, K. Mansatta, G. Mallett, V. Harris, K. R. W. Emary, A. J. Pollard, Lancet Infect. Dis. 3099(20), 1–10 (2020).
4. T. Tallei, S. Tumilaar, L. Lombogia, A. Adam, S. Sakib, T. Emran, R. Idroes, IOP Conf. Ser. Earth Environ. Sci. (2021) In Press.
5. T. Tallei, R. Maulana, A.Windah, I. Wahongan, S. Tumilaar, Fatimawali, M. Kumaunang, A. Sambul, A. am, R. Idroes, IOP Conf. Ser. Earth Environ. Sci. (2021) In Press.
6. J. Alijotas-Reig, E. Esteve-Valverde, C. Belizna, A. Selva-O'callaghan, J. Pardos-Gea, A. Quintana, A. Autoimmun Rev. 19(7), 102569 (2020).
7. H. Aktürk, M. Sütçü, S. Badur, S. H. Törün, A. Çitak, O. B. Erol, A. Somer, N. Salman, Turk. Pediatr. Ars. 50(4), 217–225 (2015).
8. Trottein, J. F. Alcorn, Front Immunol. 10, 1–3 (2019).
9. H. M. Rowe, V. A. Meliopoulos, A. Iverson, P. Bomme, S. Schultz-Cherry, J. W. Rosch, Nat Microbiol. 4(8), 13 1336 (2019).
10. Felsenstein, J. A. Herbert, P. S. Mcnamara, C. M. Hedrich, Clin. Immunol. 16, 108448 (2020).
11. Rathnavelu, N. B. Alitheen, S. Sohila, S. Kanagesan, R. Ramesh, Biomed. Reports 5(3), 283–288 (2016).
12. N. E. Mohamad, S. Keong Yeap, B. K. Beh, M. F. Romli, H. M. Yusof, Y. W. Kristeen-Teo, S. A. Sharifuddin, K. Long, N. B. Alitheen, J. Sci. Food. Agric. 4(4), 12–19. (2018)
13. A. Reyes-Munguía, M. L. Carrillo-Inungaray, C. Carranza-Álvarez, D. J. Pimentel-González, B. Alvarado- chez, Front Life Sci. 9(2), 90–98 (2016).
14. M. C. Cervo, L. O. Llido, E. B. Barrios, L. N. Panlasigui, J. Nutr. Metab. 2014, 861659 (2014).
15. R. Pavan, S. Jain, Shraddha, A. Kumar, Biotechnol. Res. Int., 2012, 976203 (2012).
16. Z. Esam, J. Med. Res. 6(2), 44–45 (2020).
17. I. M. Tahir, M. Akram, N. Akhter, M. Daniyal, A. Sharif, F.Ajaz, F. S. Khan, R. Asif, Pak. J. Med. Biol. Sc. 2), 40–47 (2017).
18. K. Popko, E. Gorska, A. Stelmaszczyk-Emmel, R. Plywaczewski, A. Stoklosa, D. Gorecka, B. Pyrazk, U. Demkow, Eur. J. Med. Res. 15(Suppl. 2), 120–122 (2010).
19. Giridharan, M. Srinivasan, J. Inflamm. Res. 11, 407–419 (2018).
20. Yasuda, N. Takahashi, T. Murachi, Biochem. 9(1), 25–32 (1970).
21. W. Tian, C. Chen, X. Lei, J. Zhao, J. Liang, Nucleic Acids Res. 46(W1), W363–367 (2018).
22. D. S. Druzhilovskiy, A. V. Rudik, D. A. Filimonov, T. A. Gloriozova, A. A. Lagunin, A. V. Dmitriev, P. V. Pogodin, V. I. Dubovskaya, S. M. Ivanov, O. A. Tarasova, V. M. Bezhentsev, K A. Murtazalieva, M. I. Semin, . Maiorov, A. S. Gaur, G. N Sastry, V. V. Poroikov, Russ. Chem. Bull. 66(10), 1832–1841 (2017).
23. Y. P. N. P. Lagniton, S. Ye, E. Li, R. H. Xu, Int. J. Biol. Sci. 16(10), 1753–1766 (2020).
24. Chobotova, A. B. Vernallis, F. A. A. Majid, Cancer Lett. 290(2), 148–156 (2010).
25. Guan, H. Yang, Y. Cai, L. Sun, P. Di, W. Li, G. Liu, Y. Tang, MedChemComm. 10(1), 148–157 (2019).
26. S. Barclay, C. Tsourounis, G. M. Mccart, Ann. Pharmacother. 32, 574–579 (1998).
27. A. Muheem, F. Shakeel, M. A. Jahangir, M. Anwar, N. Mallick, G. K. Jain, M. Warsi, F. Ahmad, Saudi Pharm. 4(4), 413–428 (2016).
28. I. Jantan, M. A. Haque, M. Ilangkovan, L. Arshad, Front Pharmacol. 10, 878 (2019).

29. Yuandani, M. Ilangkovan, I. Jantan, H. F. Mohamad, K. Husain, A. F. Abdul Razak, Evid. Based Complement Alternat Med. 2013, 603634 (2013).
30. B. Karmakar, P. Talukdar, S. N. Talapatra, Res. J. Life Sci. Bioinfor. Pharm. Chem. Sci. 5(1), 582 (2019).
31. K. Lin, A. M. Kasko, ACS Macro. Lett., 3(7), 652–657 (2014).
32. H. I. Kankia, Biotechnol. Mol. Biol. Rev. 10(1), 1–11 (2015).
33. A. M. Dias, A. Correia, M. S. Pereira, C. R. Almeida, I. Alves, V. Pinto, T. A. Catarino, N. Mendes, M. Leander, M. T. Olivia-Teles, L. Maia, C. Delerue-Matos, N. Taniguchi, M. Lima, I. Pedroto, R. Marcos-Pinto, J. Lago, C. A. Reis, M. Vilanova, S. S. Pinho, Proc. Natl. Acad. Sci. U S A 115(20), 4651–4560 (2018).
34. J. Guo, Y. Hou, L. Song, S. Zhu, F. Lin, Y. Bai, Stem Cells Int. 2018, 7168231 (2018).
35. R. J. Simpson, A. Hammacher, D. K. Smith, J. M. M. Hews, L. D. Ward, Protein Sci. 6(5), 929–955 (1997).
36. M. Yin, Y. Zhang, H. Li, Front. Immunol. 10, 145 (2019).
37. D. J. Marciani, Int Immunopharmacol. 29(2), 908–913 (2015).
38. Z. Wang, Y. Wang, P. Vilekar, S. P. Yang, M. Gupta, M. I. Oh, A. Meek, L. Doyle, L. Villar, A. Brennecke, I. Liyanage, M. Reed, C. Barden, D. F. Weaver, PeerJ 2020(7), 1–21 (2020).
39. A. Fatima, A. B. H. Abdul, R. Abdullah, R. A. Karjiban, V. S. Lee, Int. J. Mol. Sci. 16(2), 2747–2766 (2015).
40. A. Ganeshpurkar, A. Saluja, Indian J. Biochem. Biophys. 56(1), 28–33 (2019).
41. C. R. Engwerda, D. Andrew, A. Ladhams, T. L. Mynott, Cell Immunol. 210(1), 66–75 (200).

Prediction of the activity of carbohydrate moiety of bromelain as immunomodulator using an in silico approach

ORIGINALITY REPORT

20%	15%	17%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Liana Ghasemi, Mahdi Behzad, Ali Khaleghian, Alireza Abbasi, Anita Abedi. "Synthesis and characterization of two new mixed - ligand Cu (II) complexes of a tridentate NN'O type Schiff base ligand and N - donor heterocyclic co - ligands: in - vitro anticancer assay, DNA/Human leukemia/Covid - 19 molecular docking studies and pharmacophore modeling", Applied Organometallic Chemistry, 2022 Publication	1%
2	pubs.aip.org Internet Source	1%
3	Jun-Xia Zheng, Yu-Shui Han, Jin-Cai Wang, Hui Yang et al. "Strigolactones: a plant phytohormone as novel anti-inflammatory agents", MedChemComm, 2018 Publication	1%
4	dspace.iiuc.ac.bd:8080 Internet Source	1%

5	Shigeo Hanada, Mina Pirzadeh, Kyle Y. Carver, Jane C. Deng. "Respiratory Viral Infection-Induced Microbiome Alterations and Secondary Bacterial Pneumonia", Frontiers in Immunology, 2018 Publication	1 %
6	Jiří Prokop, Pavel Anzenbacher, Eva Mrkvicová, Leoš Pavlata et al. "In vivo evaluation of effect of anthocyanin-rich wheat on rat liver microsomal drug-metabolizing cytochromes P450 and on biochemical and antioxidant parameters in rats", Food and Chemical Toxicology, 2018 Publication	1 %
7	www.researchgate.net Internet Source	1 %
8	hydra.hull.ac.uk Internet Source	<1 %
9	drum.lib.umd.edu Internet Source	<1 %
10	iranarze.ir Internet Source	<1 %
11	Itika Varshney, Preeti Chand, Tulika Prasad. "Nano-based Strategies Against COVID-19: Prevention and Therapeutics", ECS Transactions, 2022 Publication	<1 %

12	T R Noviandy, A Maulana, N R Sasmita, R Suhendra et al. "The implementation of K-Means clustering in kovats retention index on gas chromatography", IOP Conference Series: Materials Science and Engineering, 2021 Publication	<1 %
13	Mok Piew Heng, Kae Shin Sim, Kong Wai Tan. "Nickel and zinc complexes of testosterone N4-substituted thiosemicarbazone: Selective cytotoxicity towards human colorectal carcinoma cell line HCT 116 and their cell death mechanisms", Journal of Inorganic Biochemistry, 2020 Publication	<1 %
14	academic.oup.com Internet Source	<1 %
15	biblio.ugent.be Internet Source	<1 %
16	srp-center.iq Internet Source	<1 %
17	Nikhil Aggarwal, Shohei Kitano, Ginette Ru Ying Puah, Sandra Kittelmann, In Young Hwang, Matthew Wook Chang. "Microbiome and Human Health: Current Understanding, Engineering, and Enabling Technologies", Chemical Reviews, 2022 Publication	<1 %

18	onlinelibrary.wiley.com Internet Source	<1 %
19	www.mdpi.com Internet Source	<1 %
20	Dar-chone Chow, Lena Brevnova, Xiao-lin He, Monika M Martick, Alex Bankovich, K.Christopher Garcia. "A structural template for gp130-cytokine signaling assemblies", Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 2002 Publication	<1 %
21	G. S. Suhartati Djarkasi, Lana E. Lalujan, Erny Nurali, Maria F. Sumual. "Antioxidant activity of karimenga (Acorus calamus)", AIP Publishing, 2019 Publication	<1 %
22	rcastoragev2.blob.core.windows.net Internet Source	<1 %
23	voi.id Internet Source	<1 %
24	web.archive.org Internet Source	<1 %
25	www.aimspress.com Internet Source	<1 %
26	coek.info Internet Source	<1 %

27	digilib.unhas.ac.id Internet Source	<1 %
28	ir.unilag.edu.ng Internet Source	<1 %
29	www.medrxiv.org Internet Source	<1 %
30	A. Rajkumar, D. Prithiviraj, P. Chithambaranathan, M. Chenthil. "Manufacturing of Bromelain based PVA support structure for 3D printing", Materials Today: Proceedings, 2022 Publication	<1 %
31	www.nature.com Internet Source	<1 %
32	www.rndsystems.com Internet Source	<1 %
33	core.ac.uk Internet Source	<1 %
34	Keenan, Thomas W., and D. James Morre. "Phospholipid class and fatty acid composition of Golgi apparatus isolated from rat liver and comparison with other cell fractions", Biochemistry, 1970. Publication	<1 %
35	conference.unsyiah.ac.id Internet Source	<1 %

36	downloads.hindawi.com Internet Source	<1 %
37	Muhammad Syafiq Akmal Mohd Fahmi, Puspanjali Swain, Amirah Hani Ramli, Wan Norhamidah Wan Ibrahim et al. "In silico studies, X-ray diffraction analysis and biological investigation of fluorinated pyrrolylated-chalcones in zebrafish epilepsy models", Heliyon, 2023 Publication	<1 %
38	chemrxiv.org Internet Source	<1 %
39	eprints.whiterose.ac.uk Internet Source	<1 %
40	sciendo.com Internet Source	<1 %
41	assets.researchsquare.com Internet Source	<1 %
42	aip.scitation.org Internet Source	<1 %
43	jgeb.springeropen.com Internet Source	<1 %
44	livrepository.liverpool.ac.uk Internet Source	<1 %
45	www.intechopen.com	

	Internet Source	<1%
46	www22.big.or.jp Internet Source	<1%
47	R.C. Hughes. "Glycoproteins as components of cellular membranes", Progress in Biophysics and Molecular Biology, 1973 Publication	<1%
48	pesquisa.bvsalud.org Internet Source	<1%
49	www.growingscience.com Internet Source	<1%
50	www.saisns.com Internet Source	<1%
51	Chen, Fang, David T. Teachey, Edward Pequignot, Noelle Frey, David Porter, Shannon L. Maude, Stephan A. Grupp, Carl H. June, Jan J. Melenhorst, and Simon F. Lacey. "Measuring IL-6 and sIL-6R in serum from patients treated with tocilizumab and/or siltuximab following CAR T cell therapy", Journal of Immunological Methods, 2016. Publication	<1%
52	Wenxue Sun, Hongwei Han, Zhaoyue Wang, Zhongling Wen et al. " Natural naphthoquinones isolated from suppress	<1%

dextran sulfate sodium-induced murine experimental colitis ", Cold Spring Harbor Laboratory, 2019
Publication

53	bpsbioscience.com Internet Source	<1 %
54	doczz.net Internet Source	<1 %
55	journal.hki.or.id Internet Source	<1 %
56	www.pubfacts.com Internet Source	<1 %
57	Abida Parveen, Sultan Zahiruddin, Nidhi Agarwal, Muhammad Akhtar Siddiqui, Shahid Husain Ansari, Sayeed Ahmad. "Modulating effects of the synergistic combination of extracts of herbal drugs on cyclophosphamide-induced immunosuppressed mice", Saudi Journal of Biological Sciences, 2021 Publication	<1 %
58	Jonny K. Fajar, Azharuddin Azharuddin. "The association between interleukin 6 −174G/C gene polymorphism and the risk of osteoporosis: A meta-analysis", Journal of Taibah University Medical Sciences, 2017 Publication	<1 %

59	Mukesh Kumar Awasthi, Nur Izyan Wan Azelee, Aizi Nor Mazila Ramli, Siti Aishah Rashid et al. "Microbial biotechnology approaches for conversion of pineapple waste in to emerging source of healthy food for sustainable environment", International Journal of Food Microbiology, 2022 Publication	<1 %
60	Zahrul Jannat Peya, Bipasha Zaman, M. A. H. Akhand, Nazmul Siddique. "Chapter 3 Autism Spectrum Disorder Detection from EEG Through Hjorth Parameters and Classification Using Neural Network", Springer Science and Business Media LLC, 2023 Publication	<1 %
61	referencecitationanalysis.com Internet Source	<1 %
62	search.bvsalud.org Internet Source	<1 %
63	oist.repo.nii.ac.jp Internet Source	<1 %
64	"NF-κB/Rel Transcription Factor Family", Springer Science and Business Media LLC, 2006 Publication	<1 %
65	J. Shyam Sundar, R. Subramanian, N. Venkateshwaran, R. Jayasree, A. Saravanan.	<1 %

"Preparation and characterization of bromelain based poly-vinyl alcohol fiber", AIP Publishing, 2021
Publication

66 Rinaldi Idroes, Teuku Rizky Noviandy, Aga Maulana, Rivansyah Suhendra et al. "Application of Genetic Algorithm-Multiple Linear Regression and Artificial Neural Network Determinations for Prediction of Kovats Retention Index", International Review on Modelling and Simulations (IREMOS), 2021 <1%
Publication

67 Ziyu Ge, Luming Yang, Fang Xiao, Yani Wu, Tingting Yu, Jing Chen, Jiexin Lin, Yanzhen Zhang. "Graphene Family Nanomaterials: Properties and Potential Applications in Dentistry", International Journal of Biomaterials, 2018 <1%
Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		