Ad-Dawaa' Journal of Pharmaceutical Sciences
ISSN: 2654-7392, E-ISSN: 2654-6973
Vol. 5, No. 2, December 2022, page 94 – 102
DOI: https://doi.org/10.24252/djps.v5i2.32721

HMG-CoA Reductase Inhibitory Activity Potential of Iota-, Kappa-, and Lambda-carrageenan: A Molecular Docking Approach

Arif Setiawansyah[1*], Muhammad Ikhlas Arsul[2], Nur Adliani[3], Leni Wismayani[4]

[1]Faculty of Pharmacy, Universitas Kader Bangsa Palembang, Indonesia
[2]Department of Pharmacy, Universitas Islam Alauiddin Makassar, Indonesia
[3]Department of Pharmacy, Faculty of Science, Institut Teknologi Sumatera, Indonesia
[4]Pharmacy Study Program, Universitas Ngudi Waluyo, Indonesia

**Corresponding author e-mail: arif12.setiawansyah@gmail.com*

ABSTRACT

HMG-CoA reductase is an essential enzyme responsible for the biosynthesis of cholesterol. Hyperactivity of HMG-CoA reductase will increase cholesterol production, leading to the elevation of blood cholesterol levels. Inhibition of HMG-CoA reductase is one way to block cholesterol biosynthesis to lower blood cholesterol levels. This study evaluated the inhibitory potential of iota-, kappa-, and lambda-carrageenan against HMG-CoA reductase. The study was undertaken in silico using a molecular docking approach via Autodock 4.2 assisted by ADT graphical user interface. HMG-CoA reductase co-crystal structure was used as the target, and iota-, kappa-, and lambda-carrageenan as the test ligands. The result revealed that iota- and lambda-carrageenan possess an excellent affinity to HMG-CoA reductase with the free binding energy of -12.44 and -11.87 kcal/mol and Ki value of 0.765 and 2.01 nM, respectively, which is found to be better than Simvastatin and the native ligand. The compounds' chemical properties influenced the molecules' molecular interactions affecting their affinity. The number of SO_4 groups is assumed to affect the HMG-CoA reductase inhibitory activity of iota-, kappa-, and lambda-carrageenan.

KEYWORDS*: iota-, kappa-, and lambda-carrageenan; HMG-CoA reductase; inhibitory activity; molecular docking*

INTRODUCTION

Hypercholesterolemia is a metabolic disease by which the total cholesterol serum level increases above the standard (>200 mg/dL) (Martinez-Hervas & Ascaso, 2019). The elevation of the total cholesterol serum is generally caused by various factors, including high cholesterol food intake and hyperactivity of 3-hydroxy-3methyl-glutaryl-coenzyme A (HMG-CoA) reductase (Cha & Park, 2019; Haines et al., 2013). HMG-CoA reductase is an essential enzyme that has been recognized to contribute to cholesterol synthesis by catalyzing the conversion of HMG-CoA to mevalonate, the precursor of many isoprenoid products (DeBose-Boyd, 2008). Chronic hypercholesterolemia will cause atherosclerosis that clogs blood circulation leading to various cardiovascular-related diseases (Jellinger et al., 2017).

Several drugs have been developed and used to treat hypercholesterolemia by limiting the biosynthesis of cholesterol, one of which is through the inhibition of HMG-CoA reductase (Bansal & Cassagnol, 2022). Many HMG-CoA reductase inhibitors, i.e., Simvastatin, atorvastatin, and rosuvastatin, have been widely used to lower the blood cholesterol level and have been pointed out as the primary prevention of coronary heart disease (Grundy et al., 2019). However, in spite of their evidence in lowering the total cholesterol serum level, various adverse effects have been reported, including muscle-related problems (Koskinas et al., 2007), angioedema (Naz et al., 2018), and liver toxicity (Famularo et al., 2007). Therefore, this increases the need to discover new lipid-lowering candidates working on inhibiting HMG-CoA reductase, which provides high efficacy and safety with minimum toxicity and side effects.

Carrageenan is one of the natural product compounds derived from marine algae that is widely used in pharmaceutical products as a stabilizer, gelling agent, thickener, and emulsifying agent (Amin et al., 2022; Lomolino et al., 2022; Rosmiati et al., 2018). Carrageenan presents in three major types, including iota, kappa, and lambda (Frediansyah, 2021), which provide spacious pharmacological properties such as antioxidant (Mani et al., 2021), antimicrobial (Júnior et al., 2021), immunomodulator (Cicinskas et al., 2020), and antiviral (Jang et al., 2021). Even though they possess various biological activities, there are no reports regarding the anti-hypercholesterolemia of carrageenan. Therefore, this is the preliminary report to evaluate the potential of carrageenan as an anti-hypercholesterolemia candidate through the inhibition of HMG-CoA reductase using a molecular docking approach.

MATERIAL AND METHODS

Materials

This study was implemented by *in silico* computational methods using a unit of ASUS X455LA personal computer equipped with 10 GB of RAM, 256 GB of SSD, Intel® Core™ i3-4030U processor, 2 GB of Intel® HD Graphics VGA and Windows 10 Pro 64-bit (10.0, Build 18362). The software used for the molecular docking-related study were Autodock 4.2, ADT graphical user interface, Biovia Discovery Studio, ChemDraw Pro 12.0 and notepad^{++}.

Protein and ligand preparation

HMG-CoA reductase was used as the macromolecule target with the x-ray diffraction computed structure model retrieved from the Protein Data Bank database (PDB ID: 1HW9) (Istvan & Deisenhofer, 2001). The choosing criteria of the macromolecule were applied, including resolution (≤ 2.5 Å), method (x-ray diffraction), and mutations (nil). The macromolecule was processed in Biovia Discovery Studio to remove all unnecessary

properties, including water, heteroatoms, and ligands. On the other hand, the iota-, kappa-, and lambda-carrageenan two-dimensional structure was first drawn using ChemDraw Ultra 12.0 (Figure 1) and covert to the 3D structure using Chem3D Pro 12.0 and saved in PDB format. The 3D structure was then further processed in ADT graphical user interface by adding the hydrogen atoms and Kollman charge.

Molecular docking study

A molecular docking study was carried out as described by Reynaldi & Setiawansyah (2022) using Autodock 4.2 provided by The Scripps Research Institute, assisted by ADT graphical user interface. The grid box parameters, including dimension (40 x 40 x 40), spacing (0.375 Å), and x, y, and z-axis, were set following the native ligand as the active site (figure 2). The docking process was set as a flexible ligand with a rigid macromolecule and used a Genetic Algorithm (100 runs) as the parameter and a Lamarckian Genetic Algorithm as an output. The study repeated at least five replications.

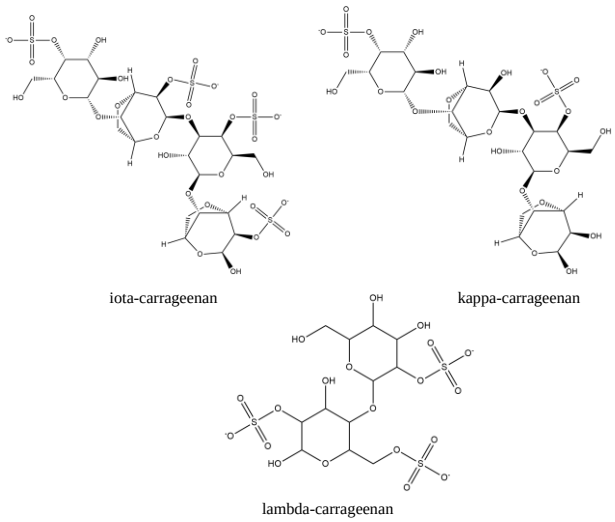


Figure 1. Structure of iota-, kappa-, and lambda-carrageenan

RESULTS AND DISCUSSION

The initial step in this study was validating the docking methods to ensure that all docking parameters meet the criteria for the docking process of the test ligands. The critical point for validating the docking method was the root mean square deviation (RMSD) value obtained from the re-docking process of the native ligands. The RMSD value provides information regarding the similarity of structure conformation of the re-docking ligand with the original native ligand. The lower the RMSD value (getting closer to zero), the more similar the structure conformation between the re-docking ligand and the original native ligand (Aziz et al., 2020). The RMSD value obtained from the re-docking ligand in this study was 1.59 Å. Morris et al. (2009) stated that the acceptable RMSD value for the validation process should be ≤ 2.0 Å. Therefore, the docking parameters in this study were stated as valid.

The validated parameters were further used for the docking study of the test ligands

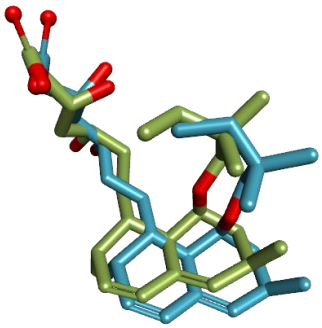


Figure 2. Overlay structure of original native ligand (green) and re-docking ligand (blue)

(iota-, kappa-, and lambda-carrageenan). The assessment of ligands' inhibitory activity against HMG-CoA reductase was justified by distinguishing their binding energy. As illustrated in Figure 3, iota-, kappa-, and lambda-carrageenan possess smaller binding energy than Simvastatin as the positive control. However, only iota- and lambda-carrageenan have smaller than the native ligands. Several studies reported that ligands with smaller binding energy than the native ligand or the positive control are predicted to have a better affinity than the native ligand or the positive control (Muttaqin et al., 2020). The binding strength will be stronger when the binding energy gets smaller. It has therefore been recognized that iota- and lambda-carrageenan have an excellent affinity with HMG-CoA reductase.

Another parameter in assessing the ligands' affinity is justifying their inhibition constant (Ki). The Ki is essential to evaluate molecules' inhibitory activity (iota-, kappa-, and lambda-carrageenan) against HMG-CoA reductase. As described in Table 1, it can be seen that iota- and lambda-carrageenan provide a smaller inhibition constant (Ki) compared to Simvastatin as well as the native ligand. It indicates that iota- and lambda-carrageenan have an excellent affinity with HMG-CoA reductase. The Ki value is proportional to the free binding energy. The lower the Ki value is, the smaller the free binding energy of the molecules (Brooks et al., 2009; Setiawansyah & Gemantari, 2022).

Table 1. Estimated inhibition constant (Ki) of iota-, kappa-, and lambda-carrageenan against HMG-CoA reductase

Ligands	Inhibition Constant (Ki) (nM)
Iota-carrageenan	0.765
Kappa-carrageenan	323.70
Lambda-carrageenan	2.01
Simvastatin	1160
Native ligand	118.58

The affinity of iota-, kappa-, and lambda-carrageenan are directly influenced by their molecular interaction with amino acid

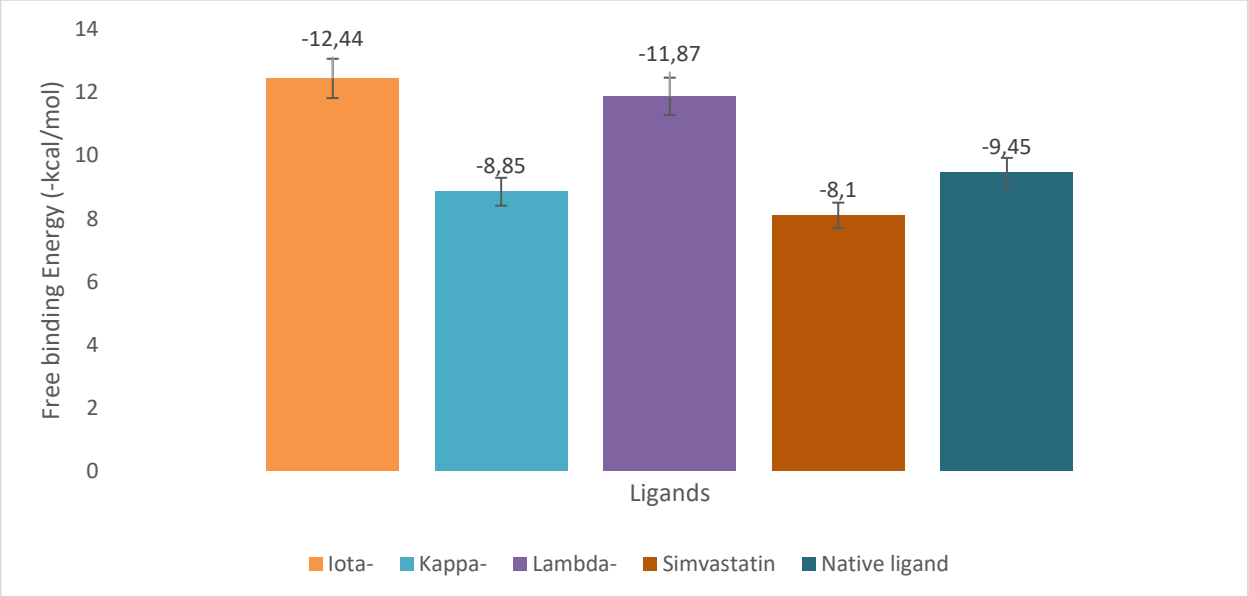
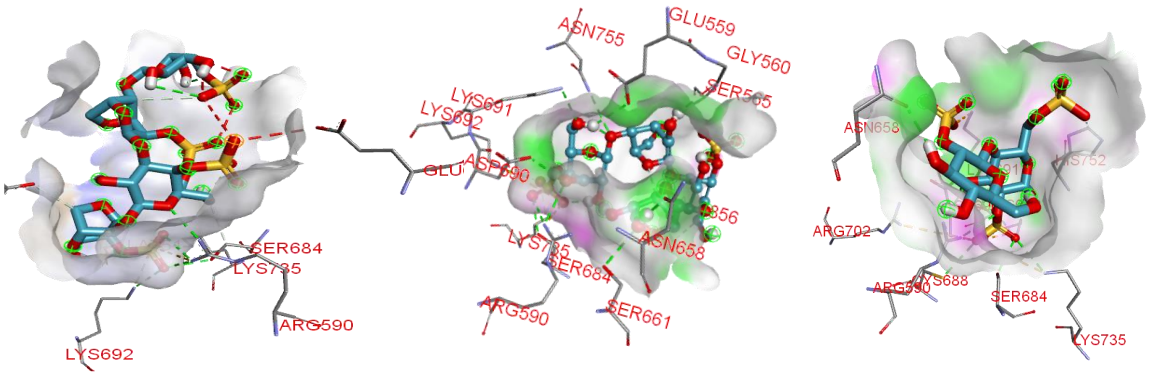


Figure 3. the free binding energy of iota-, kappa-, and lambda-carrageenan against HMG-CoA reductase



(a) (b) (c)

Figure 4. 3D molecular interaction of (a) iota-, (b) kappa-, and (c) lambda-carrageenan with HMG-CoA reductase amino acid residues

residues in the active site of HMG-CoA reductase. The diversity of interactions with the amino acid residues will lead to the difference in the affinity of the iota-, kappa-, and lambda-carrageenan. Figures 4 and 5 illustrate that iota, kappa, and lambda-carrageenan interact with different amino acid residues. This is the primary cause of the affinity of each compound varying significantly.

Iota-, kappa-, and lambda-carrageenan have similarities in structure. However, the number of sulfates (SO_4) group is different, affecting the molecule's affinity against HMG-CoA reductase due to the molecular interactions. As shown in Figure 5d, the native ligand interacts with several amino acid residues in HMG-CoA reductase binding pocket, such as Ser 661, Arg 590, Ser 684, Lys 735, Lys 692, Ala 751, Asp 690, Asn 755, Lys 691 and Leu 853. These amino acid residues are recognized as the critical factor in promoting the inhibitory activity of the ligand. Interacting with these amino acid residues will allow a molecule to be well-bound with the HMG-CoA reductase. There are some differences in the types of interaction from iota-, kappa-, and lambda-carrageenan with the native ligand. The native ligand interacts with the essential amino acid residues via hydrogen bond and pi interaction, whereas iota-, kappa-, and lambda-carrageenan interact via a hydrogen bond and interact via salt-bridge interactions. Therefore, the number of SO_4 groups directly influences the affinity of iota-, kappa-, and lambda-carrageenan. As illustrated in Figure 5a-c, the SO_4 group is responsible for forming hydrogen bonds and salt-bridge. Iota-carrageenan has four SO_4 groups that provide three salt-bridge with one negative-negative and acceptor-acceptor interaction, while lambda-carrageenan possesses three SO_4 groups which provide five salt-bridge. On the other hand, kappa-carrageenan only has two SO_4 groups, which only provide three salt-bridge. The more SO_4 group in the molecule's structure, the more salt-bridge

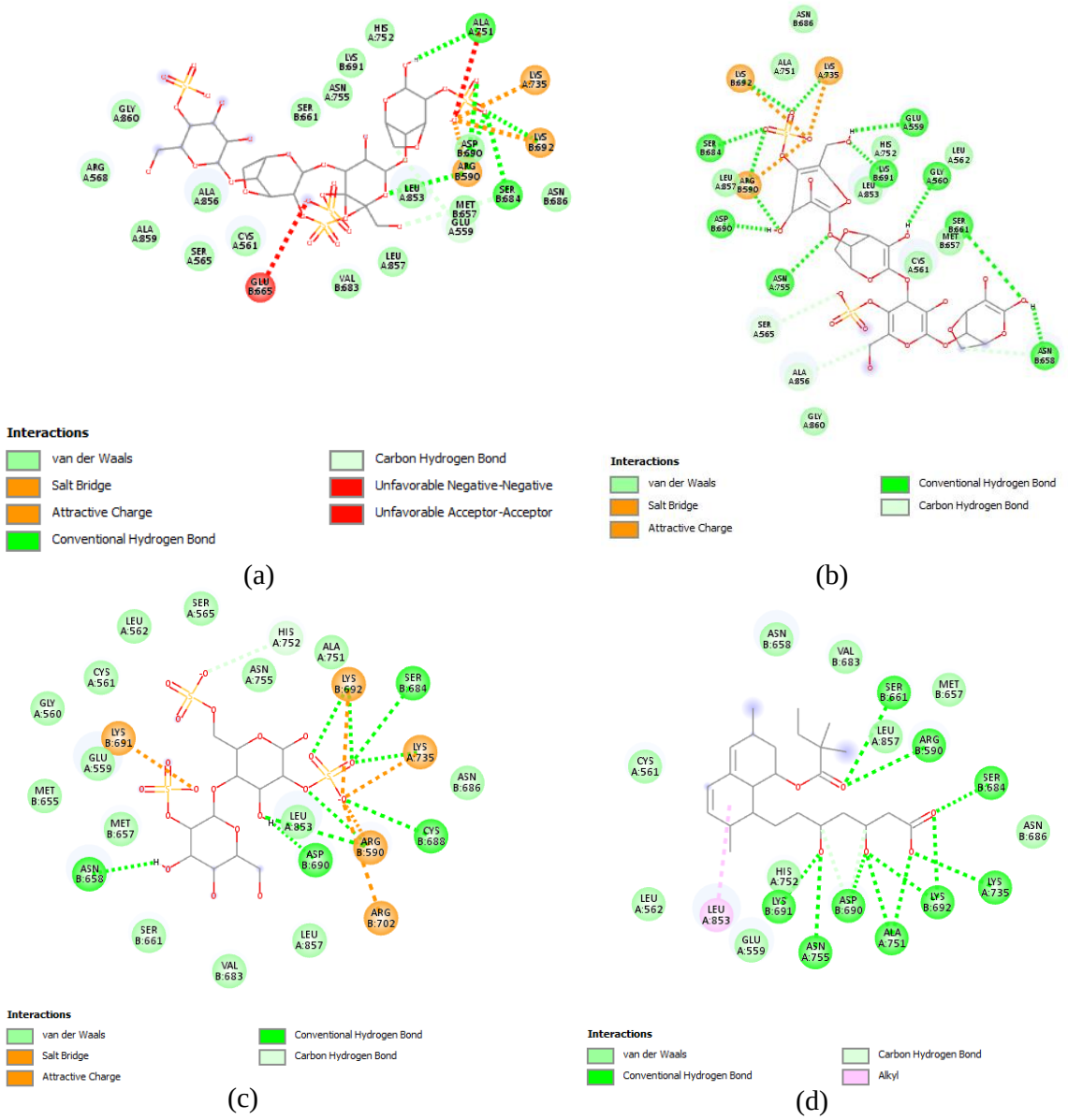


Figure 5. 2D interaction of (a) iota-, (b) kappa-, (c) lambda-carrageenan, and (d) native ligand with HMG-CoA reductase amino acid residues

formation occurred. It is indicated that the salt-bridge formation significantly contributes to the affinity of iota-, kappa-, and lambda-carrageenan against HMG-CoA reductase. The complete molecular interaction of iota-, kappa-, and lambda-carrageenan is summarized in Table 2.

CONCLUSION

Iota-, kappa-, and lambda-carrageenan have been tested for their potential to inhibit HMG-CoA reductase using a molecular docking approach. The number of SO_4 groups in the structure of iota-, kappa-, and lambda-carrageenan is recognized as the primary cause of their affinity distinction due to the salt-bridge formation. Among those compounds, iota- and lambda-carrageenan have lower binding affinity and Ki than Simvastatin and the native ligand. It indicates that iota- and lambda-carrageenan are potentially developed as HMG-CoA

Table 2. Summary of molecular interactions of iota-, kappa-, and lambda-carrageenan with HMG-CoA reductase

Ligands	Hydrogen bond interactions	Non-hydrogen bond interactions
Iota-carrageenan	Ala 751, Ser 684, Asp 690, Met 657, Glu 559, Leu 853.	Lys 735, Lys 692, Arg 590, Glu 665.
Kappa-carrageenan	Lys 692, Lys 735, Glu 559, Lys 691, Gly 560, Ser 661, Asn 658, Ala 856, Asn 755, Asp 690, Arg 590, Ser 684.	Lys 692, Lys 735, Arg 590.
Lambda-carrageenan	His 752, Lys 692, Ser 684, Lys 735, Cys 688, Arg 590, Asp 690, Asn 658.	Lys 691, Lys 692, Lys 735, Arg 590, Arg 702.
Simvastatin	Ala 751, Asn 755, Lys 735, Ser 684, Lys 691, Lys 692, Ala 751.	Leu 853, His 752.
Native ligand	Ser 661, Arg 590, Ser 684, Lys 735, Lys 692, Ala 751, Asp 690, Asn 755, Lys 691.	Glu 853.

reductase inhibitors. However, further investigations are needed to evaluate their potential.

REFERENCES

Amin, P., Riyadi, P. H., Kurniasih, R. A., & Husni, A. (2022). Utilization of κ-carrageenan as stabilizer and thickener of honey pineapple (*Ananas comosus* [L. Merr]) jam. *Food Research*, *6*(2), 93–98. https://doi.org/10.26656/fr.2017.6(2).060

Aziz, S., Adliani, N., & Sukrasno, S. (2020). Penambatan molekul senyawa turunan orizanol terhadap enzim 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reduktase. *Journal of Science and Applicative Technology*, *4*(1), 43. https://doi.org/10.35472/jsat.v4i1.191

Bansal, A. B., & Cassagnol, M. (2022). *HMG-CoA Reductase Inhibitors.* https://pubmed.ncbi.nlm.nih.gov/31194369/

Brooks, B. R., Brooks, C. L. 3rd, Mackerell, A. D. J., Nilsson, L., Petrella, R. J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A. R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Karplus, M. (2009). CHARMM: the biomolecular simulation program. *Journal of Computational Chemistry*, *30*(10), 1545–1614. https://doi.org/10.1002/jcc.21287

Cha, D., & Park, Y. (2019). Association between dietary cholesterol and their food sources and risk for hypercholesterolemia: The 2012-2016 Korea National Health and Nutrition Examination Survey. *Nutrients*, *11*(4). https://doi.org/10.3390/nu11040846

Cicinskas, E., Kalitnik, A. A., Karetin, Y. A., Mohan Ram, M. S. G., Achary, A., & Kravchenko, A. O. (2020). Immunomodulating Properties of Carrageenan from Tichocarpus crinitus. *Inflammation*, *43*(4), 1387–1396. https://doi.org/10.1007/s10753-020-01216-x

DeBose-Boyd, R. A. (2008). Feedback regulation of cholesterol synthesis: sterol-accelerated ubiquitination and degradation of HMG CoA reductase. *Cell Research*, *18*(6), 609–621. https://doi.org/10.1038/cr.2008.61

Famularo, G., Miele, L., Minisola, G., & Grieco, A. (2007). Liver toxicity of rosuvastatin therapy. *World Journal of Gastroenterology*, *13*(8), 1286–1288. https://doi.org/10.3748/wjg.v13.i8.1286

Frediansyah, A. (2021). The antiviral activity of iota-, kappa-, and lambda-carrageenan against COVID-19: A critical review. *Clinical Epidemiology*

and Global Health, *12*(June), 100826. https://doi.org/10.1016/j.cegh.2021.100826

Grundy, S. M., Stone, N. J., Bailey, A. L., Beam, C., Birtcher, K. K., Blumenthal, R. S., Braun, L. T., de Ferranti, S., Faiella-Tommasino, J., Forman, D. E., Goldberg, R., Heidenreich, P. A., Hlatky, M. A., Jones, D. W., Lloyd-Jones, D., Lopez-Pajares, N., Ndumele, C. E., Orringer, C. E., Peralta, C. A., Yeboah, J. (2019). Guideline on the management of blood cholesterol: A report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Circulation*, *139*(25), e1082–e1143. https://doi.org/10.1161/CIR.0000000000000625

Haines, B. E., Wiest, O., & Stauffacher, C. V. (2013). The increasingly complex mechanism of HMG-CoA reductase. *Accounts of Chemical Research*, *46*(11), 2416–2426. https://doi.org/10.1021/ar3003267

Istvan, E. S., & Deisenhofer, J. (2001). Structural mechanism for statin inhibition of HMG-CoA reductase. *Science*, *292*(5519), 1160–1164. https://doi.org/10.1126/science.1059344

Jang, Y., Shin, H., Lee, M. K., Kwon, O. S., Shin, J. S., Kim, Y. il, Kim, C. W., Lee, H. R., & Kim, M. (2021). Antiviral activity of lambda-carrageenan against influenza viruses and severe acute respiratory syndrome coronavirus 2. *Scientific Reports*, *11*(1), 1–12. https://doi.org/10.1038/s41598-020-80896-9

Jellinger, P. S., Handelsman, Y., Rosenblit, P. D., Bloomgarden, Z. T., Fonseca, V. A., Garber, A. J., Grunberger, G., Guerin, C. K., Bell, D. S. H., Mechanick, J. I., Pessah-Pollack, R., Wyne, K., Smith, D., Brinton, E. A., Fazio, S., & Davidson, M. (2017). American Association Of Clinical Endocrinologists and American College of Endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease. *Endocrine Practice : Official Journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists*, *23*(Suppl 2), 1–87. https://doi.org/10.4158/EP171764.APPGL

Júnior, E. H., Gonçalves, A. G., Noseda, M. D., Duarte, M. E. R., Murakami, F. S., & Ducatti, D. R. B. (2021). Semi-synthesis of N-alkyl-kappa-carrageenan derivatives and evaluation of their antibacterial activity. *Carbohydrate Research*, *499*, 108234. https://doi.org/https://doi.org/10.1016/j.carres.2021.108234

Koskinas, K., Tzellos, T. G., Gougoulias, K., Gougoulias, G., Papakonstantinou, C., & Kouvelas, D. (2007). Simvastatin-induced rhabdomyolysis: A case study on clinical-decision making based on the evidence-based medicine approach. *Archives of Medical Science*, *3*(3), 267–271.

Lomolino, G., Vincenzi, S., Zannoni, S., Marangon, M., De Iseppi, A., & Curioni, A. (2022). Emulsifying activity of potato proteins in the presence of k-carrageenan at different pH conditions. *Food Chemistry: X*, *13*(January), 100232. https://doi.org/10.1016/j.fochx.2022.100232

Mani, S. D., Govindan, M., Muthamilarasan, M., & Nagarathnam, R. (2021). A sulfated polysaccharide κ-carrageenan induced antioxidant defense and proteomic changes in chloroplast against leaf spot disease of tomato. *Journal of Applied Phycology*, *33*(4), 2667–2681. https://doi.org/10.1007/s10811-021-02432-0

Martinez-Hervas, S., & Ascaso, J. F. (2019). Hypercholesterolemia. In I. Huhtaniemi & L. Martini (Eds.), *Encyclopedia of Endocrine Diseases (Second Edition)* (Second Edi, pp. 320–326). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-801238-3.65340-0

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Muttaqin, F. Z., Kharisma, D., Asnawi, A., & Kurniawan, F. (2020). Pharmacophore and molecular docking-based virtual screening of B-cell lymphoma 2 (BCL 2) Inhibitor from zinc natural database as anti-small cell lung cancer. *Journal of Drug Delivery and Therapeutics*, *10*(2), 143–147. https://doi.org/https://doi.org/10.22270/jddt.v10i2.3923

Naz, S., Saleem, M. W., & Haider, A. W. (2018). Angioedema; An Unreported Adverse Effect Of Pitavastatin. *Journal of Ayub Medical College, Abbottabad : JAMC*, *30*(4), 603–604.

Reynaldi, M. A., & Setiawansyah, A. (2022). Potensi anti-kanker payudara tanaman songga (*Strychnos lucida* R . Br): Tinjauan interaksi molekuler terhadap reseptor estrogen- α *in silico*. *Sasambo Journal of Pharmacy*, *3*(1). https://doi.org/https://doi.org/10.29303/sjp.v3i1.149

Rosmiati, M., Abdassah, M., & Yohana Chaerunisaa, A. (2018). Effect of carrageenan as gelling agent on tocopherol acetate emulgels. *Indonesian Journal of Pharmaceutical Science and Technology*, *5*(1), 1. https://doi.org/10.24198/ijpst.v5i1.14280

Setiawansyah, A., & Gemantari, B. M. (2022). Potential activity of caryophyllene derivatives as xanthine oxidase inhibitor : An in silico quantitative structure-activity relationship analysis. *Journal of Food and Pharmaceutical Sciences*, *10*(3), 700–708.