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Original Research Article

Momordica charantia Fruit Extract on Cardiac Biomarker Serum Attenuation in Rats and its Bioactive Compound Molecular Docking Against *SIRT-1* Protein

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ABSTRACT

Momordica charantia has various biological activities as antihyperglycemic, anti-inflammatory, and antioxidant. Thisstudy evaluated *Momordica charantia* fruit extract against the LDH and CKMB serum in rats induced with isoproterenol. We also evaluated the molecular docking of bioactive compounds in *Momordica charantia* fruit against the SIRT1 protein. Wistar male rats were divided into four groups: negative control (Na-CMC), positive control (Na-CMC and isoproterenol), and resveratrol group at 9 mg/kg b.wt, and *Momordica charantia* fruit extract at 300 mg/kg b.w with isoproterenol. The interaction between ligand and receptor was evaluated and calculated with molecular docking software. The ligands were docked using the *AutoDock Tools - 1.5.7*. The binding affinity was calculated using the binding free energy (kcal/mol). The results showed that *Momordica charantia* fruit extract at 300 mg/kg b.w could attenuate the LDH (49.37%) and CKMB (44.5%) increase in serum after isoproterenol-induced myocardial infarction in rats. Molecular docking of selected compounds from *Momordica charantia* fruit extract presents that momordicilin has great potential as a SIRT-1 activator with a binding energy of -11.3 kcal/mol. These findings implicate that *Momordica charantia* fruit extract can be developed further as a potential cardioprotective drug.

Keywords: Molecular docking, *Momordica charantia*, LDH, CKMB, SIRT1.

Introduction

The ischemic condition occurs as a heart attack causes heart function to decrease.[1] Atherosclerosis is a triggering factor of heart attacks [2] due to fat accumulation in the arteries and elevated blood clot risk.[3] The use of natural products is still widely chosen by the public to treat and prevent cardiovascular disease,[4] whereas 80% of them in the world use traditional medicine.[3] *Momordica charantia* as a beneficial natural product has been reported with various biological activities, namely antidiabetic,[5] antioxidant,[6] several types of cancer disease inhibitor,[7] anti-inflammatory,[8] and cardiovascular disease preventive properties by reducing cholesterol,[9] hypotensive effects [10], and antiplatelet activity.[11] Rich phytochemicals in fruit and leaves of *Momordica* can promote organ health due to its bioactive compounds.[8] The fruit contains several secondary metabolites, such as steroidal saponins, glucosides, and alkaloids,[12] specifically Momordol, Momordicin I, Momordicilin, and Zeaxhantin.

Sirtuin 1, a protein encoded as the *SIRT-1* gene, is referred to as nicotinamide adenine dinucleotide (NAD+) dependent.[13] *SIRT-1* is located in the nucleus and cytoplasm.[14] *SIRT1* can protect cells from oxidative stress,[15,16] besides decreasing the metabolic abnormalities and neurodegenerative disease as aging-related.[17-19]

As many beneficial activities have been reported in *Momordica charantia*, the affinity of the bioactive compounds in *Momordica charantia* to SIRT1 protein needs further evaluation to discuss.

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This study evaluated the interaction between the bioactive compounds of *Momordica charantia* fruit against the *SIRT1* protein based on the molecular docking and demonstrated whether the administration of the *Momordica charantia* fruit extract could inhibit the increased serum level as a cardiac biomarker *in vivo*. A comparative compound, namely resveratrol as a natural antioxidant with cardioprotective effect in rats[20] was also applied in this study.

Materials and Methods

Evaluation of serum cardiac biomarkers associated with myocardial infarction in rat

Momordica charantia Extraction

Momordica charantia (MC) fruit samples were obtained from Manoko, Bandung City, West Java in October 2021. Plant sample identification was confirmed by the Herbarium of Plant Taxonomic Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, the University of Padjajaran with the number No.54/HB/10/2021. After confirmation, plant samples were prepared for simplicial production. The samples were washed with running water, drained, dried, ground into powder, and stored in a closed container. The 250 g of MC fruits were extracted using 70% ethanol as a solvent with the maceration method. The extraction was repeated three times at a 1:10 ratio, thus the solvent volume was 2.5 L. After the extracts were filtered, the solvent was evaporated using a rotavapor to obtain a thick extract.[3]

Experimental group

Male Wistar rats were obtained from the School of Life Sciences and Technology) Laboratory, Bandung Institute of Technology. The 27 male Wistar rats with an initial weight of 200–220 g were used in the current investigation. For 12 days, all rats were maintained in a typical cage filled with four rats. Before the experimental condition, the animals were maintained at 22°C with steady moisture of 45–55% and a 12-hour light-dark cycle with unrestricted access to meals and tap water. Wistar male rats were divided into four groups, namely negative control (Na-CMC), positive control (Na-CMC and isoproterenol), resveratrol group at 9 mg/kg b.wt., and *Momordica*

charantia fruit extract at 300 mg/kg b.w. All treatment groups were administered with isoproterenol as a myocardial infarction induction, except the negative control treatment group. Resveratrol as a comparative compound and MC fruit extract treatment groups were applied orally for 14 consecutive days. Isoproterenol solution was obtained from 85 mg/kg isoproterenol powder dissolved in 0.9% NaCl and injected intraperitoneally on the 13- and 14th days. After 24 hours of isoproterenol administration, the animal blood was taken intracardiac for cardiac biomarker measurement through sacrifice.[21]

Bioactive Compound Molecular Docking in Momordica charantia Against SIRT-1 Protein

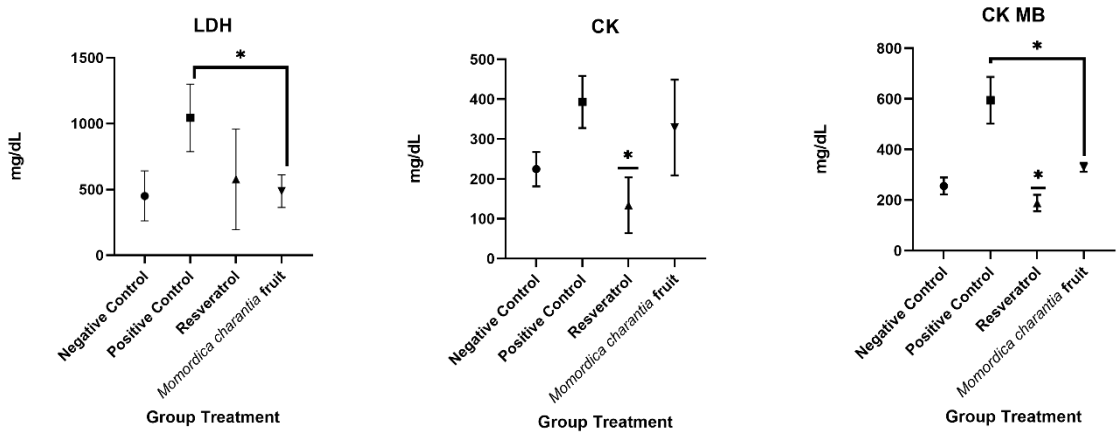
The chemical structure of *SIRT-1* was collected from the *Protein Data Bank* (PDB ID: 4ZZJ; https://www.rcsb.org/structure/4zzj) and bioactive compounds from *Momordica charantia* fruit were prepared using the *ChemDraw Ultra version 12.0.2.1076* (*Cambridge Soft*). Ligand and receptor interaction was evaluated and calculated using molecular docking. Ligands were docked using the *AutoDock Tools - 1.5.7*. Binding affinity was calculated using the binding-free energy (kcal/mol). Molecular docking data were visualized with the *AutoDockTools* software. The type of bond on each macromolecule with ligands is presented using the *Discovery Studio 2021 Client software*.[22]

Results and Discussion

Momordica charantia Fruit Extract on Lactate Dehydrogenase and CKMB Attenuation

Momordica charantia has various biological activities such as antidiabetic, antioxidant[6], several types of cancer disease inhibitor,[7] anti-inflammatory,[8] and cardiovascular disease preventive actor by reducing cholesterol,[9] hypotensive effects,[10] and antiplatelet activity.[11] In our study, we designed myocardial infarction in rats with isoproterenol induction. Isoproterenol, a beta-adrenoceptor agonist, can enhance cardiac output, relax the smooth muscle in the lungs and stomach, and lower peripheral vascular resistance. Isoproterenol is utilized to treat asthma, heart block, bronchospasm, and cardiac arrest.[23] Isoproterenol induction at 85 mg/kg b.w. twice with a delay of 24 hours can produce a myocardial infarction, so the serum biomarker-related myocardial infarction significantly increases, compared to the negative control group (without isoproterenol induction).[24] The biomarker level in serum affected by *Momordica charantia* fruit extract can be seen in **Figure 1**. The administration of *Momordica charantia* fruit extract at 300 mg/kg b.w on the LDH and CKMB serum attenuation was significantly different from the positive control group, while the creatine kinase (CK) parameter had no significant difference. Therefore, these findings indicate that the MC extract has a cardioprotective potential. In the resveratrol treatment group, the CK and CKMB parameters were significantly different, except the LDH parameter. LDH and CKMB are serum levels that increase within injury due to myocardium damage or myocardial infarction.[25]

The measurements of the CK and CKMB enzymes are still useful for Acute Myocardial Infarction (AMI) diagnosis.[26] These findings were consistent with the previous study, which showed that *Momordica charantia* could reduce infarction area in the rat,[27] reduce LDL values in a rat model with high cholesterol diet,[9] inhibit lipid peroxidation, and protect the activity of antioxidant enzymes due to the polysaccharide contents.[28]



(a) (b) (c)

Figure 1. Biomarker levels in serum affected by *Momordica charantia* fruit extract. Data are shown as mean ± SD (n=4). Data were analyzed using a one-way ANOVA test (* means of $p < 0.05$ compared to the positive control). **(a)** LDH, **(b)** CK, and **(c)** CKMB

Molecular Docking Bioactive Compound in Momordica charantia Against SIRT-1 Protein

Heart failure occurs due to insufficient cardiac output in peripheral organs. Heart failure is also known as a leading cause of death cases in worldwide due to myocardial infarction.[29] *SIRT-1* is one of the phosphorylation enzymes that can alleviate oxidative stress.[19] Oxidative stress is a problem in most degenerative diseases. In myocardial infarction, compounds that have a high activity of *SIRT-1* binding are needed to decrease this condition. *Momordica charantia* fruit is known to contain momordol, momordicin I, momordicilin, and zeaxhantin. The molecular docking between these compounds and the *SIRT-1* protein is shown in Table 1, while the interaction between the bioactive compounds of *Momordica charantia* and *SIRT-1* can be visualized in Figure 2. The highest energy binding to *SIRT-1* was found in momordicillin at -11.3 kcal/mol. In zeaxanthin, hydrogen bonds were absent in the SIRT-1 amino acid residue. The common measurement of the ligand-target interaction in drug discovery for further development and optimization is binding affinity. Affinity in pharmacology is described as the number of ligands to occupy 50% of the targets at equilibrium conditions.[30]

Conclusion

Momordica charantia fruit extract at 300 mg/kg b.w. can attenuate the LDH and CKMB serum increase, after isoproterenol-induced myocardial infarction in rats. The molecular docking of selected compounds from *Momordica charantia* fruit extract presents that momordicilin has great potential as a *SIRT-1* activator with a binding energy of -11.3 kcal/mol. These findings implicate that *Momordica charantia* fruit extract can be developed further as a potential cardioprotective drug.

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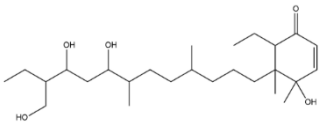
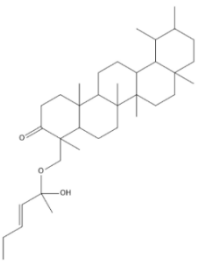
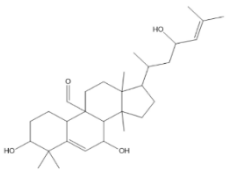
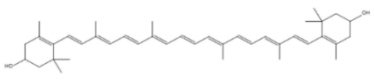
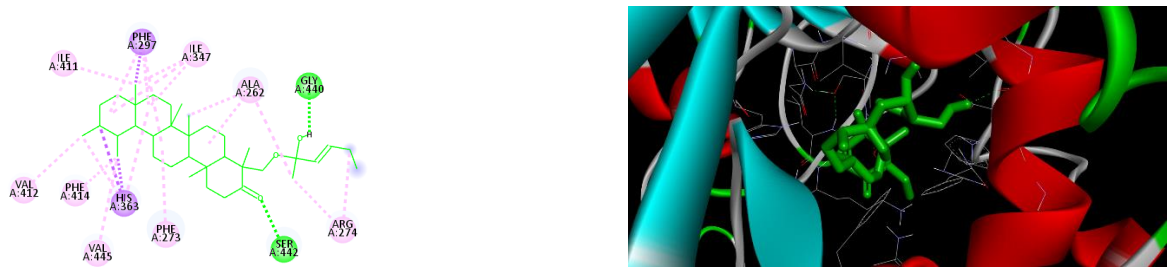
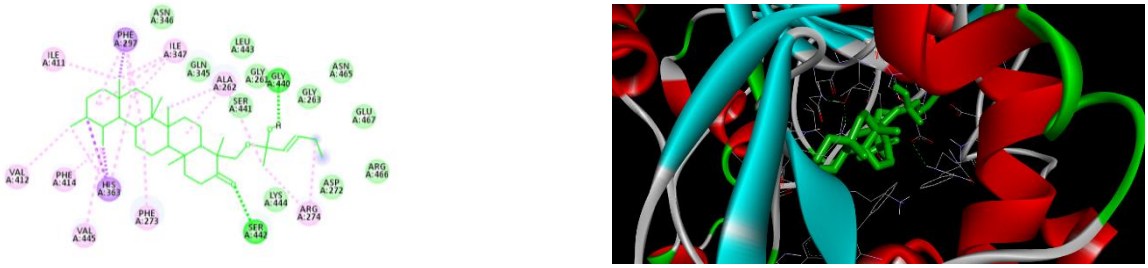
Table 1: Molecular docking

Bioactive compound	Binding Energy (kcal/mol)	Hydrogen Bond Interaction	Non-Hydrogen Interaction
 Momordol	-9.59	Gly 440, Ser 442	Arg 274, Ala 262, Ile 347, Phe 273, Phe 297, His 363, Val 445, Phe 414, Ile 411, Val 412
 Momordicilin	-11.3	Gly 440, Ser 442	Ile 411, Phe 297, Asn 346, Ile 347, Gun 345, Leu 443, Ala 262, Gly 261, Ser 441, Gly 263, Asn 465, Glu 467, Arg 466, Asp 272, Lys 444, Arg 274, Phe 273, His 363, Val 445, Phe 414, Val 412
 Momordicin I	-9.29	Gln 345, Lys 444	Phe 297, Ile 347, Ile 411, His 363, Ala 262, Val 445
 Zeaxhantin	-4.49	-	Arg 274, Lys 444, Val 445, Arg 446, Pro 447, His 423, Asn 417, Glu 416, Leu 418, Gly 415

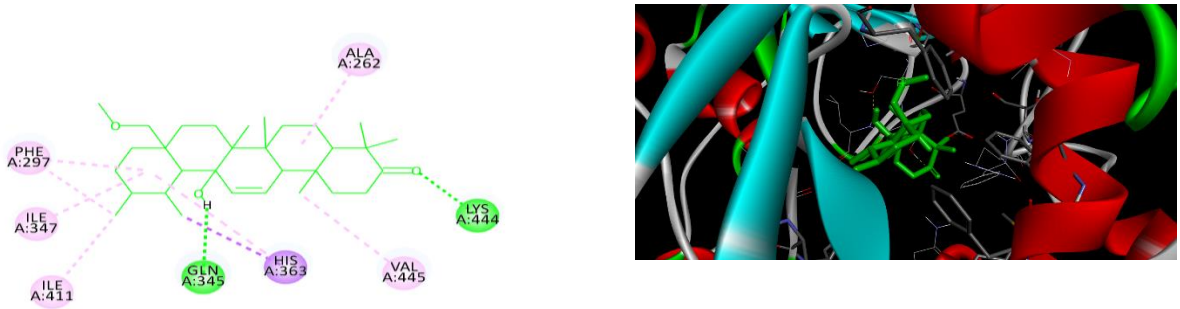
Momordol



Momordicilin



Momordicin I



Zeaxhantin

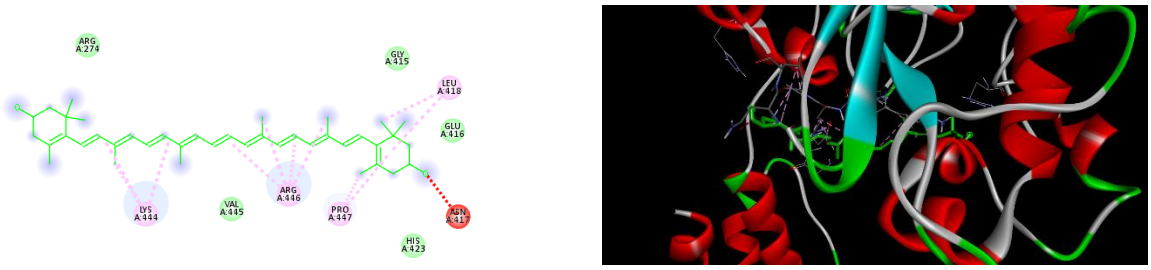


Figure 2: The binding interaction between bioactive compounds of *Momordica charantia* and *SIRT-1* protein.

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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Ethical Statement

This study was approved by the Animal Research Ethics Committee, Sekolah Farmasi, Institut Teknologi Bandung with reference number: No. 05/KEPHP-ITB/03-2021.

References

1. Severino P, D´Amato A, Pucci M. Ischemic Heart Disease and Heart Failure: Role of Coronary Ion Channels. Int J Mol Sci. 2020; 21:3167.
2. Palasubramaniam J, Wang X, Peter K. Myocardial Infarction: From Atherosclerosis to Thrombosis. Arterio, Thromb, and Vasc Biology. 2019; 39:e176–e185.
3. Morakinyo AE, Omoniyu FE, Nzekwe SC. Antioxidant, Anti-hyperlipidemic and Cardioprotective Potentials of Diet Supplemented with *Parkia biglobosa* Roasted Seeds on Isoproterenol-Induced Cardiotoxicity in Wistar rats. Trop J. Nat Prod Res. 2022; 6: 1882–1887.
4. Bachheti RK, Worku LA, Gonfa YH. Prevention and Treatment of Cardiovascular Diseases with Plant Phytochemicals: A Review. Evid-Bas Comp and Alt Med; 2022: e5741198.
5. Effect of Aqueous Extract of *Momordica charantia* on Survival, Locomotive Behaviour and Antioxidant Status of *Drosophila melanogaster*. Trop J. Nat Prod Res. 2021*;* **5**: 178–181.
6. Upadhyay A, Agrahari P, Singh DK. A Review on Salient Pharmacological Features of *Momordica charantia*. Int J. of Pharm. 2015; 11:405-413.
7. Grover JK, Yadav SP. Pharmacological actions and potential uses of Momordica charantia: a review. J Ethnopharmacol. 2004; 93:123-132.
8. Bortolotti M, Mercatelli D, Polito L. *Momordica charantia*, a Nutraceutical Approach for Inflammatory Related Diseases. Front in Pharm. 2019; 10.
9. Innih SO, Eze IG, Omage K. Cardiovascular benefits of *Momordica charantia* in cholesterol-fed Wistar rats. Clin Phyto. 2021; 7: 65.
10. Jandari S, Ghavami A, Ziaei R. Effects of *Momordica charantia* L on blood pressure: a systematic review and meta-analysis of randomized clinical trials. Int J. of Food Prop. 2020; 23:1913-1924.
11. Zhang J, Wang W, Liu T, Wang Y, Wei X, Qi S, Gu B. Effects of *Momordica charantia* exosomes on platelet activation, adhesion, and aggregation. Blood Coagul Fibr. 2022; 33: 372–380.
12. Patel R, Mahobia N, Upwar N, Waseem N, Talaviya H, Patel Z. Analgesic and antipyretic activities of *Momordica charantia* Linn. fruits. J Adv Pharm Technol Res. 2010; 1:415–418.
13. Salminen A, Kaarniranta K, Kauppinen A. Crosstalk between Oxidative Stress and SIRT1: Impact on the Aging Process. Int J. of Mol Sci. 2013; 14:3834–3859.
14. Ren Z, He H, Zuo Z, Xu Z, Wei Z, Deng J. The role of different SIRT1-mediated signaling pathways in toxic injury. Cell Mol Biol Lett; 2019: 24: 36.
15. Singh CK, Chhabra G, Ndiaye MA, Garcia-Peterson LM, Mack NJ, Ahmad N. The Role of Sirtuins in Antioxidant and Redox Signaling. Antioxid Redox Sig. 2018; 28:643–661.
16. Alam F, Syed H, Amjad S, Baig M, Khan TA, Rehman R. Interplay between oxidative stress, SIRT1, reproductive and metabolic functions. Cur Res in Phys. 2021; 4: 119–124.
17. Donmez G, Outeiro TF. SIRT1 and SIRT2: emerging targets in neurodegeneration. EMBO Mol Med. 2013; 5**:**344–352.
18. Manjula R, Anuja K, Alcain FJ. SIRT1 and SIRT2 Activity Control in Neurodegenerative Diseases. Front Pharmacol. 2020; 11:585821.
19. Elibol B, Kilic U. High Levels of SIRT1 Expression as a Protective Mechanism Against Disease-Related Conditions. Front in End. 2018; 9:1-7.
20. Hung LM, Chen JK, Huang SS, Lee RS, Su MJ. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovasc Res. 2000; 47: 549–555.
21. Murugesan, M. Cardioprotective effect of fenugreek on isoproterenol-induced myocardial infarction in rat. Indian J. Pharm. 2011; 5:516-519.
22. Makisake RG, Montolalu RI, Mewengkang HW. Studi In Silico Senyawa Aktif Daun Tagalolo (*Ficus septica* Burm F) sebagai Ligan Uji pada Enzim L-Histidin Decarboxylase. Med Tek H Peri. 2022; 10: 122–126.
23. Abel P, Rorabaugh B. Isoproterenol. in *xPharm: The Comprehensive Pharmacology Reference* (eds. Enna, S. J. & Bylund, D. B.). Elsevier. 2007; 1-7. Doi:10.1016/B978-008055232-3.63729-8.
24. Kurniati NF, Nathania J, Prasesti GK. Cardioprotective Activity of Combination of Soursop Leaves (*Annona Muricata* L.) and Avocado Seeds (*Persea Americana* Mill.) on Isoproterenol-Induced Myocardial Infarction in Rats. Egypt J of Chem. 2022; 65: 173–179.
25. Amani M, Jeddi, Sajad, Ahmadiasl N, Usefzade N, Zaman J. Effect of HEMADO on Level of CK-MB and LDH Enzymes after Ischemia/Reperfusion Injury in Isolated Rat Heart. Bioimpacts. 2013; 3: 101–104.
26. Aydin S, Ugur K, Aydin S, Sahin İ, Yardim M. Biomarkers in acute myocardial infarction: current perspectives. Vasc Health Risk Manag. 2019; 15: 1–10.
27. Czompa A, Gyongyosi A, Szoke K, Bak I, Csepanyi E. Effects of *Momordica charantia* (Bitter Melon) on Ischemic Diabetic Myocardium. Molecules. 2017; 22:1-15.
28. Raish, M. *Momordica charantia* polysaccharides ameliorate oxidative stress, hyperlipidemia, inflammation, and apoptosis during myocardial infarction by inhibiting the NF-κB signaling pathway. Int J. of Bio Macro. 2017; 97:544–551.
29. Matsushima S, Sadoshima J. The role of sirtuins in cardiac disease. Am J Physiol Heart Circ Physiol. 2015; 309: H1375–H1389.
30. Hoare, S. R. J. 1.10 - Kinetics of Drug-Target Binding: A Guide for Drug Discovery. in *Comprehensive Pharmacology* (ed. Kenakin, T.) Elsevier. 2022; 227–271. Doi:10.1016/B978-0-12-820472-6.00011-6.