OPEN ACCESS

Scholars International Journal of Traditional and Complementary Medicine

Abbreviated Key Title: Sch Int J Tradit Complement Med
ISSN 2616-8634 (Print) | ISSN 2617-3891 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Mechanistic Investigation of *Daucus carota* Active Flavonoid against Inhibitory Action on *malonyl Co-A decarboxylase* on Treating Myocardial Ischemia Reperfusion Injury

Ankit Shukla[1]*, Jitender Malik[1], Surendra Pratap Singh[1], Nida Musheer[1]

[1]Faculty of Pharmacy, P.K. University, Shivpuri (M.P.), India

DOI: https://doi.org10.36348/sijtcm.2025.v08i02.003 | **Received:** 18.01.2025 | **Accepted:** 22.02.2025 | **Published:** 25.02.2025

***Corresponding author:** Ankit Shukla
Faculty of Pharmacy, P.K. University, Shivpuri (M.P.), India

Abstract

Background*:** Cardiovascular diseases (CVDs) represent the most prevalent non-communicable diseases worldwide. In 2019, around 17.9 million individuals succumbed to cardiovascular diseases, accounting for 32% of all global fatalities. Mitochondria are essential for maintaining cellular metabolic equilibrium, facilitating cell survival and apoptosis, and generating the majority of cellular energy. Protein–protein interactions (PPIs) play a crucial role in both physiological and pathological processes, with abnormal PPIs linked to numerous disorders, making them prospective pharmacological targets across diverse therapeutic domains. Peptides are highly promising as protein-protein interaction inhibitors due to their capacity to replicate natural interaction patterns and encompass rather extensive interaction regions. Computational methods are extensively employed to accelerate drug discovery by screening prospective lead molecules. ***Purpose*:** Current work was designed to check efficacy of *Daucus carota* flavonoid for cardioprotective activity**.** ***Methodology*:** Scientific validation of the current investigation was done by computational based molecular docking study of lead molecules of *Daucus carota* pulp against ***malonyl Co-A decarboxylase enzyme. ***Result*:** The flavonoid found in *D.carota* has been identified as an effective cardioprotective drug and their lead molecules luteolin and apigenin demonstrating effective binding to the target protein ***malonyl Co-A decarboxylase*** with binding energies of -7.34 and -7.12 kcal/mol, respectively. ***Conclusion*:** The findings indicated that each selected lead chemical for additional investigation shown significant inhibitory activity against ***malonyl Co-A decarboxylase***, hence revealing its cardio protection potential.

Keywords: *Daucus carota*, molecular docking, luteolin and apigenin.

INTRODUCTION

Cardiovascular disease (CVD) encompasses a range of disorders that impact the heart and vascular system. Cardiovascular diseases constitute the predominant cause of mortality in the United States and globally. In 2019, there were 18 million deaths from cardiovascular disease globally, representing 32% of total fatalities. Cardiovascular diseases are projected to result in 25 million fatalities globally by 2030 [1]. In cardiac ischemia, myocardial damage encompasses not only myocyte loss but also dysfunction of coronary endothelial cells. This leads to compromised coronary endothelium-dependent vasorelaxation and nitric oxide (NO) homeostasis, consequently diminishing coronary perfusion and worsening myocardial necrosis [2]. Myocardial infarction (MI), commonly referred to as a heart attack, together with its associated consequences, constitutes the primary cause of mortality globally. The utilization of natural antioxidants is rising as protective agents against various cardiovascular disorders. Bioactive drugs derived from natural sources have become essential in contemporary medicine, mitigating the risks of heart diseases by neutralizing free radical production. Herbal remedies significantly contribute to healthcare for a substantial fraction of the global population and are considered an integral part of the cultural history of diverse tribes. Polyphenols exhibit cardioprotective effects via preventing the oxidation of low-density lipoprotein. The majority of pharmacologically significant medications are sourced from plants. Plant derivatives as pharmaceuticals serve a crucial part in healthcare systems worldwide for both animals and humans. They are utilized not just for disease management but also for the maintenance of optimal health. Medicinal plants have long been utilized for the treatment of ischemic heart conditions. The accumulation of phytochemical, biological, and clinical data throughout the past decade of the 20th century indicated that plant-based herbal treatments are increasingly preferred for the treatment of various ailments [3]. *Daucus carota* L., belonging to the Apiaceae family, consists of 13 subspecies, including one cultivated variety (D. carota L. ssp. sativus (Hoffm.) Arcang.) and 12 wild subspecies. The wild carrot has historically been acknowledged for its antilithic, diuretic, carminative, antibacterial, and anti-inflammatory characteristics, and has been utilized in the treatment of urinary calculi, cystitis, gout, prostatitis, and cancer. Although there is a wealth of literature regarding the phytochemical, pharmacological, and therapeutic assessments of the farmed carrot, there is a paucity of information concerning the wild carrot. Prior research demonstrated that terpenoids and phenolics are two prominent chemical classes, with terpenoids subdivided into monoterpenes (e.g., α-pinene and geranyl acetate), sesquiterpenes (e.g., humulene and carotol), diterpenes (e.g., phytol), triterpenes (e.g., squalene), and tetraterpenes (e.g., α-carotene). Phenolics include phenylpropanoids, flavonoids, and tannins. [4]. The pharmacological studies indicated that the plant exhibited cytotoxic, antioxidant, antidiabetic, antimicrobial, smooth muscle relaxant, hypotensive, intraocular pressure-lowering, gastroprotective, nephroprotective, hepatoprotective, cardioprotective, antidepressant, memory-enhancing, anti-inflammatory, reproductive, wound-healing, and heat-inducing properties, among numerous other effects [5, 6].

Experiment work

Scientific validation of cardioprotective Potential by Molecular docking

Selection of Lead molecule

According to the literature assessment, *D. carota* pulp is a rich source of phytochemicals, including carotenoids, phenolics, polyacetylenes, isocoumarins, and sesquiterpenes. Carrots contain three distinct flavonoids: quercetin, luteolin, and kaempferol, together with apigenin. Numerous phytochemical investigations have been conducted on this plant, resulting in the isolation of various active constituents, including triterpenes, volatile oils, tannins, carbohydrates, steroids, amino acids, glycerides, flavonoids, hydrocarotene, and carotene [7].

Earlier studies and clinical trials on Luteolin [Lut] have concentrated on cancer and inflammation due to its significant anti-tumor and anti-inflammatory flavonoid properties. Since the 1950s, there has been a rise in the volume of reports concerning the cardiovascular effects of Luteolin. Previous studies indicated that Lut affects the heart and blood arteries. Recent breakthroughs in the comprehension of oxidative stress and inflammatory mechanisms within the cardiovascular system indicate that Lut demonstrates significant cardiovascular protective effects through intricate signal transduction pathways and target effectors. High dietary consumption of Lut is associated with a reduced incidence of acute myocardial infarction [8]. Apigenin is believed to provide preventive effects against atherosclerosis formation through its antioxidative and anti-inflammatory properties [9]. So, luteolin and apigenin were selected as lead compound for cardioprotective activity.

Description of lead molecule [10-12]

Description	**Luteolin**	**Apigenin**
Molecular formula	$C_{15}H_{10}O_6$	$C_{15}H_{10}O_5$
Synonym	Luteolin 491-70-3 3',4',5,7-Tetrahydroxyflavone Digitoflavone Luteolol	Apigenin 520-36-5 5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one Versulin Apigenol
Molecular weight	286.24 g/mol	270.24 g/mol
Pharmacology	Antitumor anti-convulsion diabetes control anti-inflammatory neuroprotection anti-oxidation anti-cardiovascular anti-apoptotic	Antioxidant Anti-inflammatory Blood pressure reduction Chemo-preventive

Selection of target receptor

Malonyl CoA decarboxylase

Modifications in cardiac energy metabolism significantly contribute to the elevated prevalence and severity of heart disease globally. These modifications may involve a reduction in ATP generation required to satisfy the heart's elevated energy demands, alongside detrimental shifts in the heart's energy substrate choice. Regarding this last issue, research indicates that a reduction in cardiac efficiency, resulting from an elevation in cardiac fatty acid oxidation and/or an increase in the uncoupling of glycolysis from glucose oxidation, detrimentally affects cardiac function and contributes to heart illness. Therapeutic techniques that regulate these metabolic pathways and enhance cardiac efficiency have advantageous outcomes in the context of heart disease. One method involves elevating cardiac malonyl CoA levels, a crucial inhibitor of mitochondrial fatty acid absorption. This encompasses the inhibition of malonyl CoA decarboxylase (MCD), leading to elevated cardiac malonyl CoA concentrations, reduced cardiac fatty acid oxidation rates, and enhanced cardiac efficiency [13].

Designing of *In-Silico* molecular docking in current investigation

Natural compounds can engage with various cellular target proteins and may be selected as potential therapeutic candidates. There is a necessity to prioritize chemicals that safeguard the cardiovascular system from pathological circumstances. We highlight luteolin and apigenin, which were previously identified as cardioprotective compounds in our investigations *via* multi-level data integration. This inquiry examines the selected compounds for their inhibitory effects on ***malonyl CoA decarboxylase*** to assess cardioprotective effectiveness.

Molecular docking studies

Ligand Preparation:

The 2D structures of apigenin and luteolin were generated using Chem Sketch, and the two-dimensional representations of the synthesized ligands were subsequently transformed into their optimal 3D geometries. The optimized structure was preserved in PDB format for compatibility with AutoDock. The fundamental structures of the synthesized ligand are presented below:

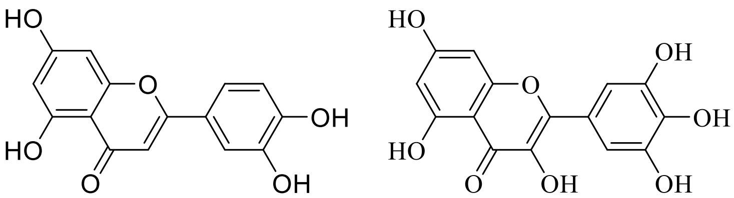


Figure 1: 2D structure of apigenin and luteolin

Preparation of the grid file

The regions of interest utilized by Autodock were delineated by constructing a grid box encompassing the active sites. The grid box is critical in the docking process as it encompasses all amino acids in the active sites essential for binding, excluding those found in the receptor. The grid box contains three thumbwheel widgets that allow for the adjustment of the number of points in the x, y, and z dimensions. The spacing and grid points for all receptors examined in this investigation are presented in Table 1 [15, 16].

Table 1: Grid parameters used in current docking analysis of malonyl COA decarboxylase receptor

S. No	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	Malonyl COA Decarboxylase	40	40	40	0.431	-45.093	56.855	70.648

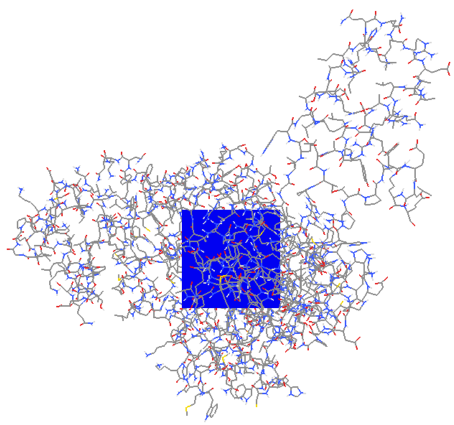


Figure 2: Grid box covering all active sites in malonyl COA decarboxylase receptor

Preparation of the docking file

All computations were conducted with Autodock 4.2 as the docking tool. The visualization and other programs required for docking investigations were conducted using Pymol, Chimera, DS Visualizer, and MMP Plus [17-19].

Docking Study

Crystal structure

The crystal structure of the malonyl CoA decarboxylase receptor protein has been retrieved from the Protein Data Bank portal. All essential information pertaining to the receptor's structure was documented in the Protein Data Bank [20-22]. The intricate ligand was isolated utilizing Chimera software for all target receptors.

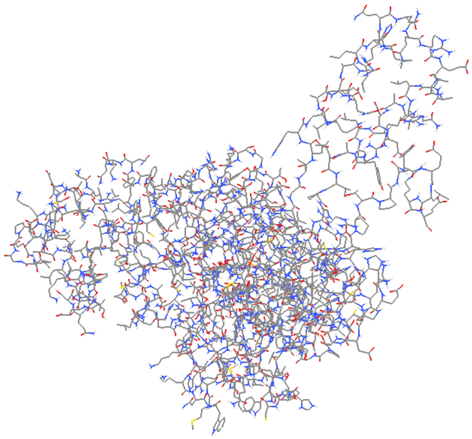


Figure 3: Crystal structure of malonyl COA decarboxylase receptor (PDB ID-4f0x)

Processing of Protein

All downloaded receptor proteins possess a single chain, specifically chain A, which has been designated for experimental purposes, with the complex ligand having been removed. The bound ligand was isolated from the macromolecular complex utilizing Chimera software. [23-26].

Molecular Docking Simulation Studies

Docking of ligands such as apigenin and luteolin to the malonyl-CoA decarboxylase receptor was conducted using Autodock. All bonds of each ligand were maintained in a flexible state, however no residues in the receptor were rendered flexible [27-29].

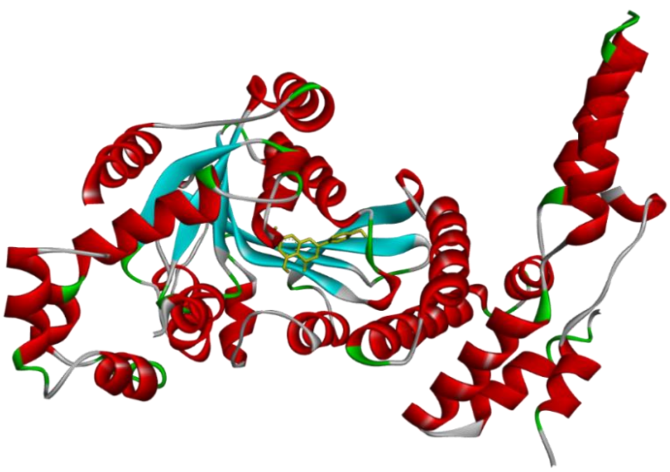


Figure 4: Binding mode of apigenin within the active site of malonyl COA decarboxylase receptor

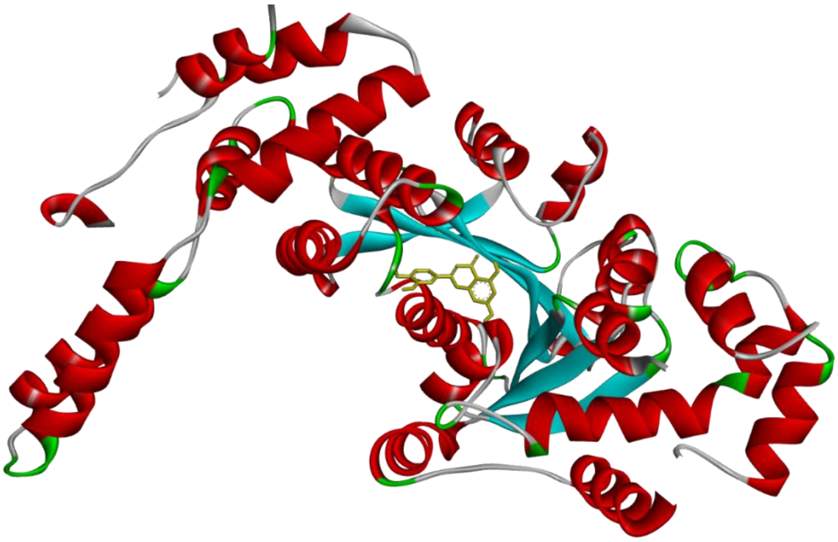


Figure 5: Binding mode of luteolin within the active site of malonyl COA decarboxylase receptor

Toxicity & ADME-T Studies

The ligand compounds, namely apigenin and luteolin, were analyzed using the online tool OSIRIS to predict the presence of any hazardous groups and to evaluate their ADME-T characteristics [30].

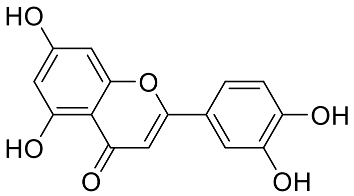
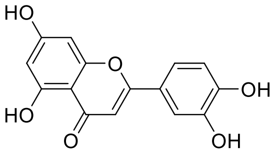
RESULT AND DISCUSSION

The results of the current investigation indicated that the selected lead molecules serve as effective cardioprotective agents, binding to the target protein ***malonyl Co-A decarboxylase*** with binding energies of -7.34, -7.16, and -7.12 kcal/mol for luteolin and apigenin, respectively. The Ki values were determined to be 12.38 and 12.017 for luteolin and apigenin, respectively. The IC50 values were obtained using the ki method, yielding results of 0.085 for luteolin and 0.087 for apigenin, respectively. The outcome was recorded in Table 2. The binding mechanism of the selected lead compounds is illustrated in Figures 4 and 5. The two-dimensional and three-dimensional interactions of the selected chemical are illustrated in Figures 6-9. The affinity of lead compounds for the receptor was determined to be relatively comparable. The interaction of luteolin and apigenin with the active site of ***malonyl CoA decarboxylase*** is illustrated as follows:

Compound	Conventional Hydrogen bounding	Pi-alkyl	Pi-Pi	Week Vander's interaction
Luteloin	Leu293 Leu 298 Leu303 Glu302 Ile 294 His 423	Val 419	Phen422	Val 218 Ser 292 Val 251 Val 301 GLn 299 Gly 300 Ser 329
Apigenin	Leu293 Leu303 Leu298 Glu302 His 423 Ile 291	Val419	Phe422	Val 218 Ser 292 Val 251 Val301 GLn 299 Gly 299 Gly 300

The interaction results indicated that both lead molecules attach at comparable positions by typical hydrogen, pi-alkyl, and pi-pi interactions, demonstrating a synergistic effect of both compounds from *D. carota* in exerting cardioprotective activity. The pharmacokinetic profile indicates a favourable pharmacokinetic profile; however, it also presents significant hazardous consequences, including mutagenicity, tumorigenicity, and reproductive toxicity. The pharmacokinetic and toxicity profiling data of ligands such as luteolin and apigenin are presented in Figures 10-11 and Tables 3-5. All ligand compounds have demonstrated promising docking scores theoretically.

Table 2: Results of docking of ligands like apigenin and luteolin against malonyl COA decarboxylase receptor

S. No	Compound Name	Structure	Binding energy	Ki value	IC50
1	Apigenin		-7.34	12.38	0.085
2	Luteolin		-7.12	12.017	0.087

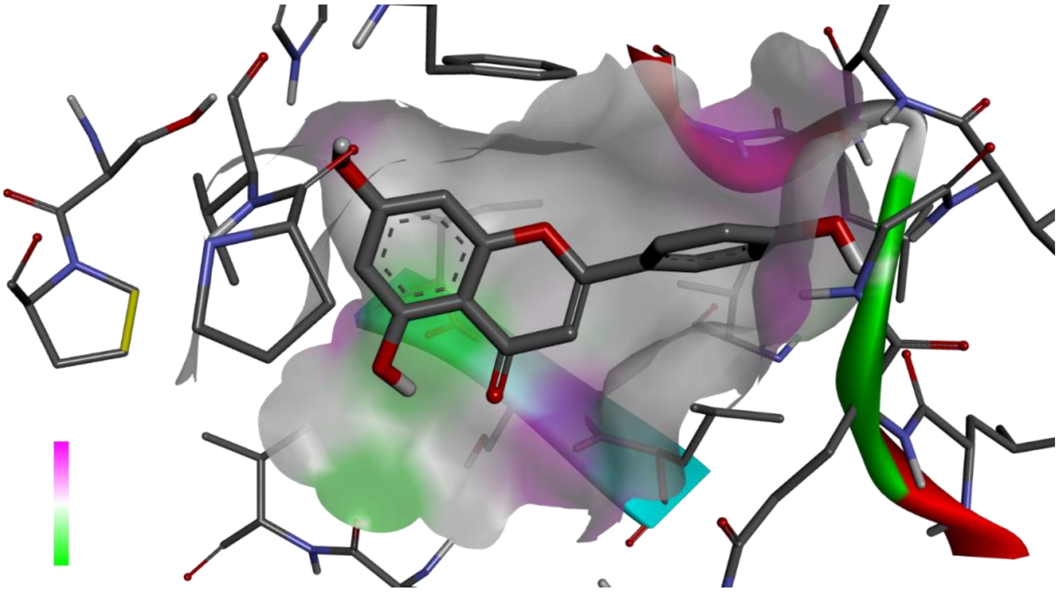


Figure 6: Three-dimensional binding mode of apigenin within the active site of malonyl COA decarboxylase receptor

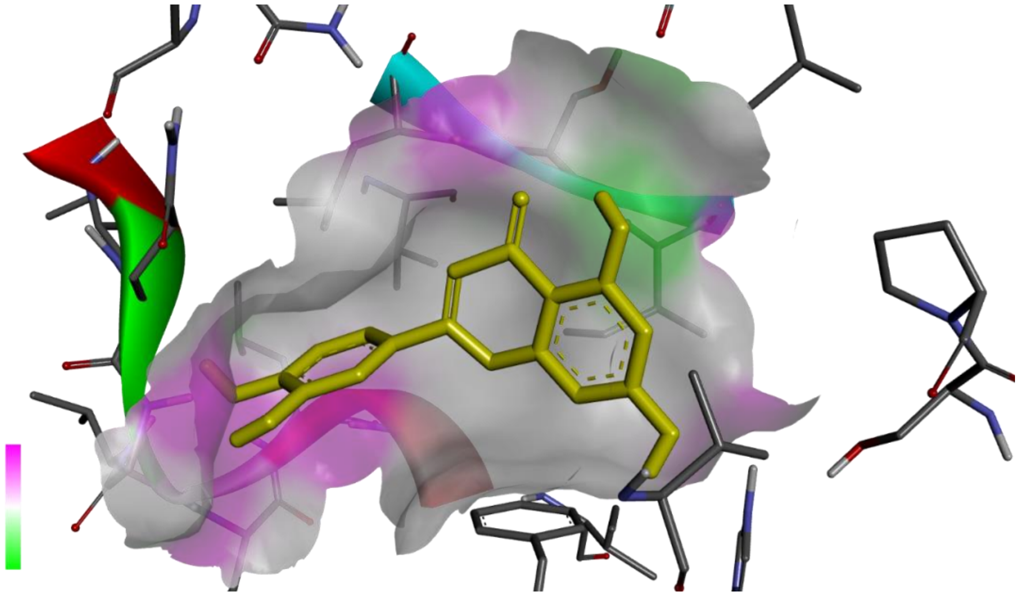


Figure 7: Three-dimensional binding mode of luteolin within the active site of malonyl COA decarboxylase receptor

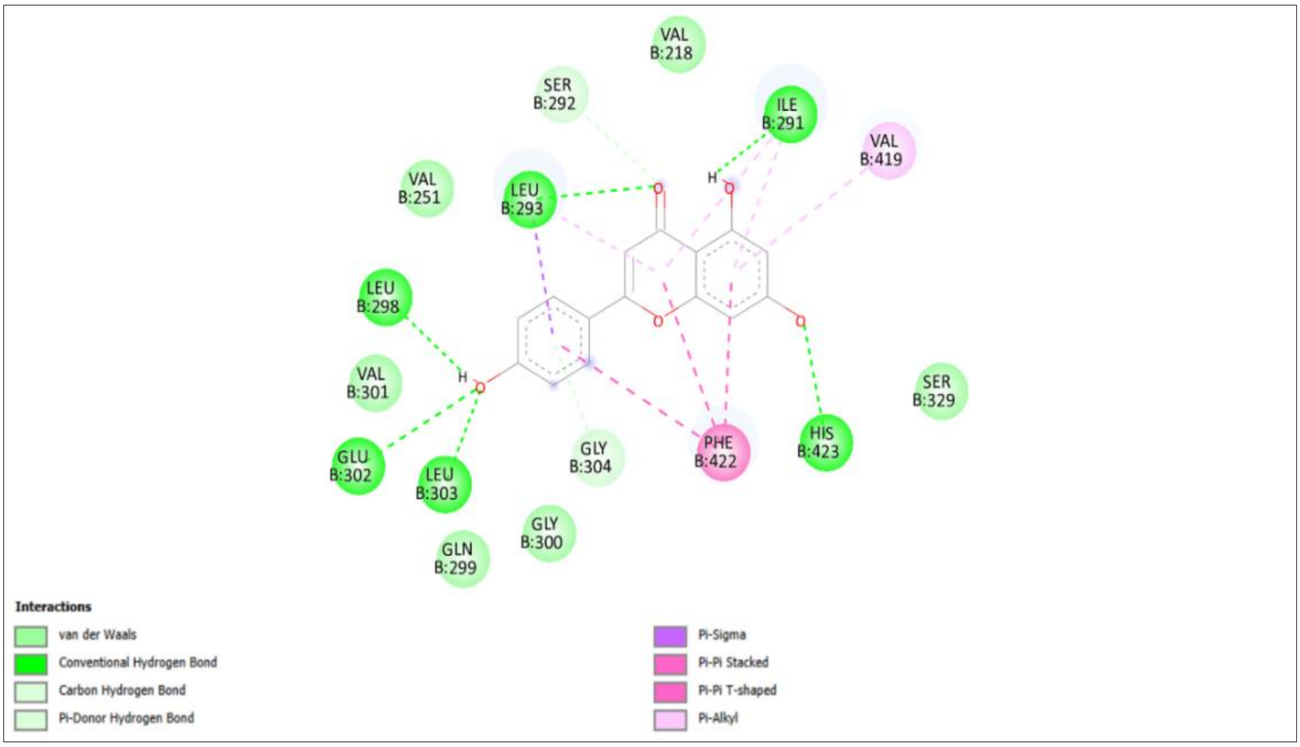


Figure 8: Two-dimensional binding mode of apigenin within the active site ofmalonyl COA decarboxylase receptor

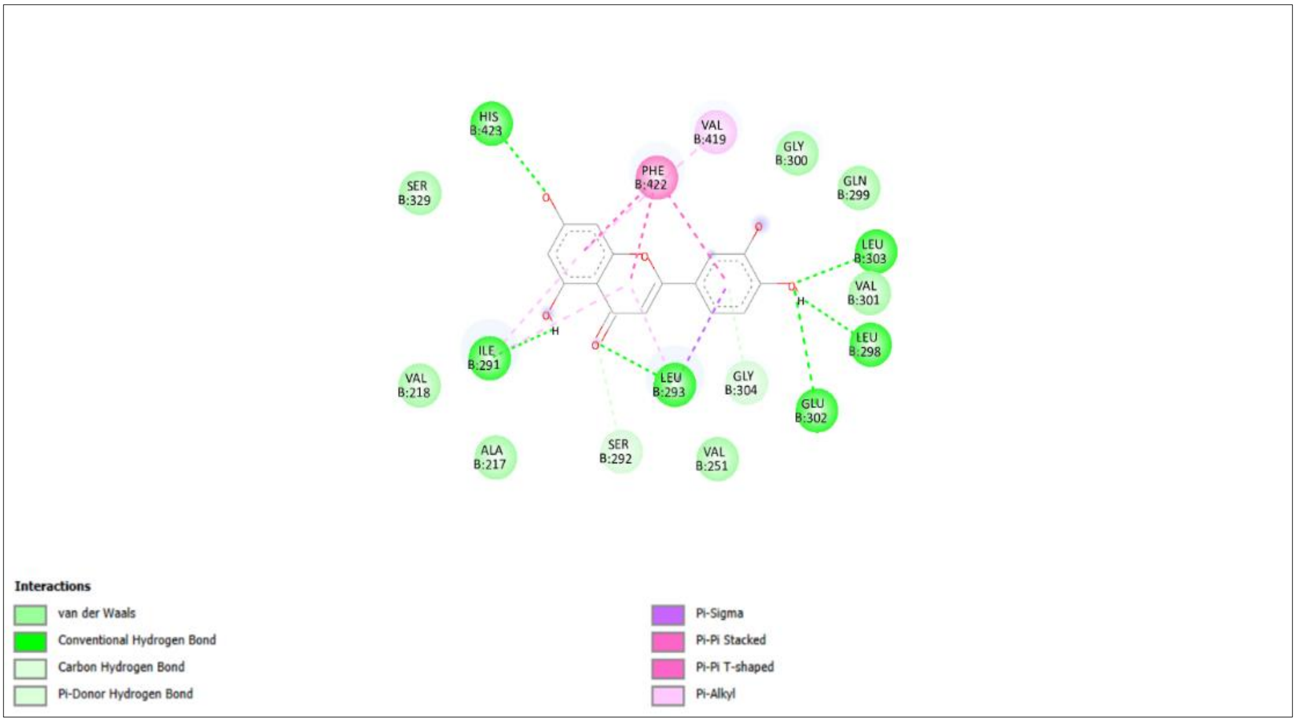


Figure 9: Two-dimensional binding mode of luteolin within the active site ofmalonyl COA decarboxylase receptor

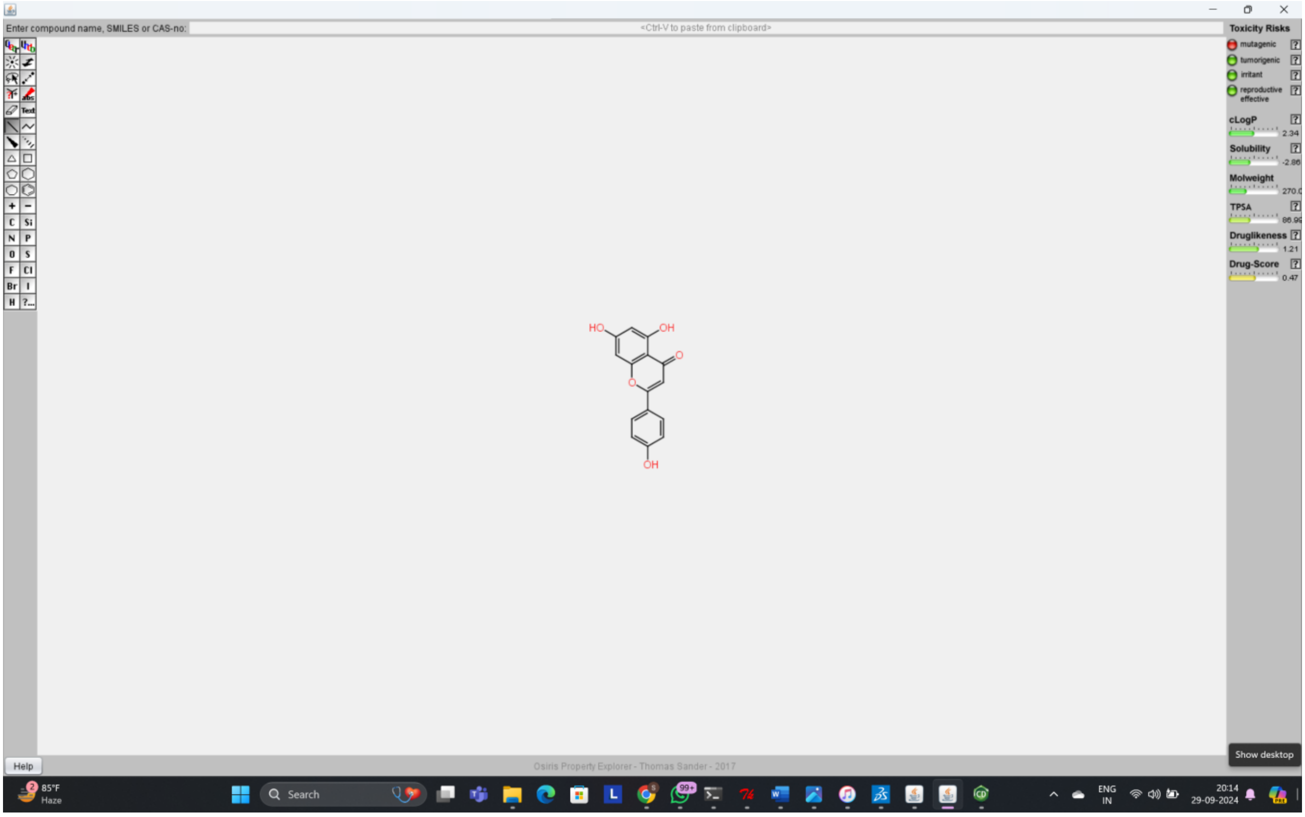


Figure 10: Pharmacokinetic and toxicity profiling of apigenin

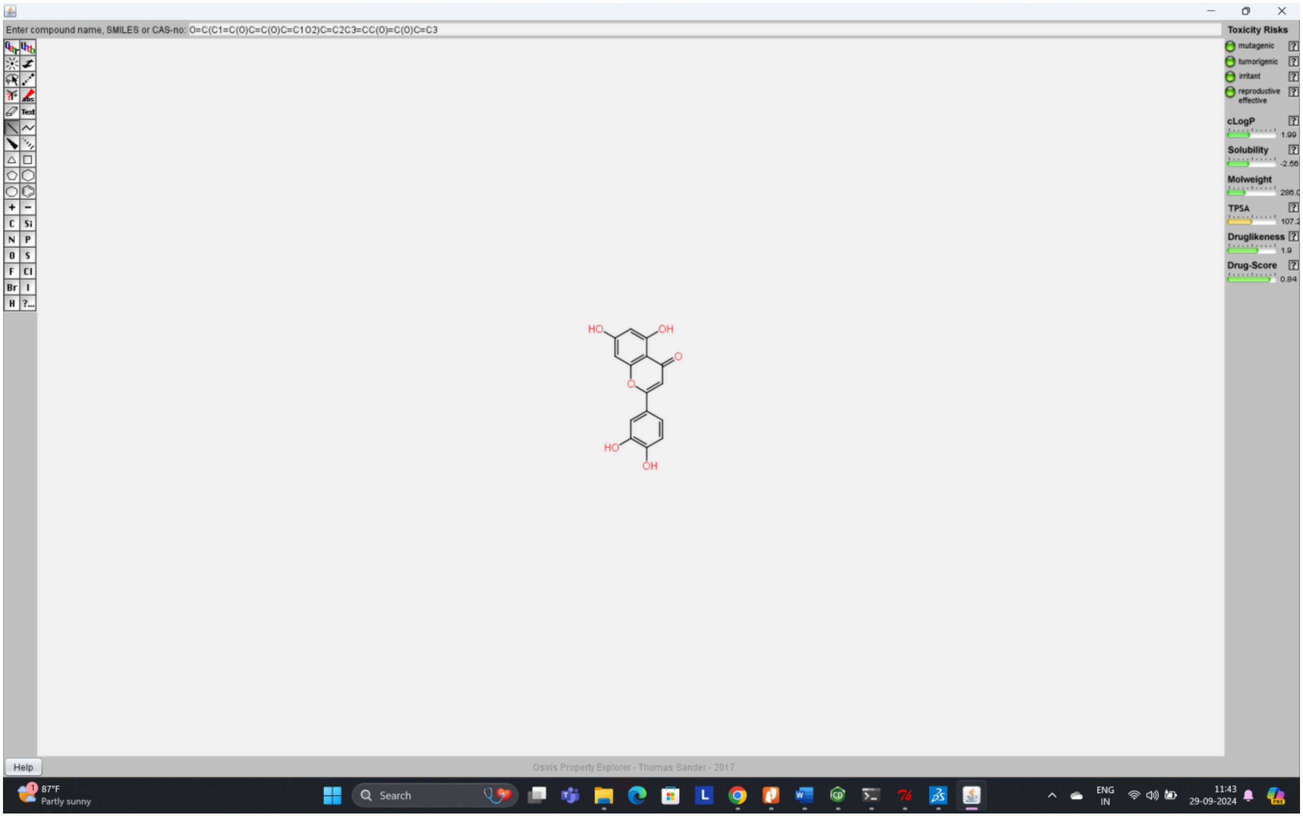


Figure 11: Pharmacokinetic and toxicity profiling of luteolin.

Table 3: Pharmacokinetic Profiling of lead molecules

Compound	ADMET			
	Mutagenic	Tumorigenic	Irritant	Reproductive effectivity
Luteolin	NO	NO	NO	No
Apigenin	NO	NO	NO	No

Table 4: Lipinski Properties of lead molecules

Compound	*cLogP*	Solubility	Mol.wt.	TPSA	Drug likeness	Drug score
Luteolin	1	-2.56	286	107.2	-0.19	0.14
Apigenin	2.34	-2	270	95	-0.21	0.47

Table 5: Drug likeness of lead molecules

Compound	Lipinski rule of five	H bond donar(<5)	H bond acceptor (<10)
Luteolin	Yes	4	6
Apigenin	Yes	3	5

CONCLUSION

Luteolin and apigenin, active flavonoids present in *D. carota* aqueous extract, bind to malonyl-CoA decarboxylase (MCD) through hydrogen, π-alkyl, and π-π interactions, resulting in the inhibition of MCD and a reduction in fatty acid oxidation due to elevated malonyl-CoA levels. Elevated levels of malonyl CoA inhibit fatty acid entry into mitochondria, augment triglyceride production, and enhance antioxidant activity, hence providing protection to the heart against ischemia/reperfusion injury. The current experiment's results demonstrate that both lead drugs, Luteolin and Apigenin, display similar inhibitory effects on malonyl Co-A decarboxylase and provide synergistic cardioprotective potential. Regular intake of *D. carota* juice may provide protective and preventative advantages against cardiovascular diseases.

REFERENCE

1. Zerihun, M., & Qvit, N. (2023). Selective inhibitors targeting Fis1/Mid51 protein-protein interactions protect against hypoxia-induced damage in cardiomyocytes. *Front Pharmacol*, 14, 1275370.
2. Allaoui, H., Rached, N., Marrakchi, N., Cherif, A., Mosbah, A., & Messadi, E. (2022). In Silico Study of the Mechanisms Underlying the Action of the Snake Natriuretic-Like Peptide Lebetin 2 during Cardiac Ischemia. *Toxins (Basel)*, *14*(11), 787.
3. Shah, S. M. A., Akram, M., Riaz, M., Munir, N., & Rasool, G. (2019). Cardioprotective potential of plant-derived molecules: a scientific and medicinal approach. *Dose-response*, *17*(2), 1559325819852243.
4. Ismail, J., Shebaby, W. N., Daher, J., Boulos, J. C., Taleb, R., Daher, C. F., & Mroueh, M. (2023). The wild Carrot (Daucus carota): a phytochemical and pharmacological review. *Plants*, *13*(1), 93.
5. Deshmukh, S. P. (2021). A Review: Pharmacological Actions of Daucus carota. *Ijppr Human*, *21*(2), 302-314.
6. Zaini, R., Clench, M. R., & Maitre, C. L. (2011). Bioactive Chemicals from Carrot (Daucus carota) Juice Extracts for the Treatment of Leukemia. *J of Medicinal Food*, 14, 1303-1312.
7. Mehnaz, G. N., Latif, A., Ashiq, K., Shah, S. A., Batool, A., & Faiz, S. (2021). Review Article Pharmacological and phytochemical overview of Daucus carota L. (Blac carrot). *Int. J. Biosci*, *19*(2), 160-169. http://www.innspub.net.
8. Luo. Target Effectorsin Cardio-Protective Mechanisms of Luteolin. Frontiers in Pharmacology | www.frontiersin.org 1 October 2017 | Volume 8 | Article 692.
9. Thomas, S. D., Jha, N. K., Jha, S. K., Sadek, B., & Ojha, S. (2023). Pharmacological and molecular insight on the cardioprotective role of apigenin. *Nutrients*, *15*(2), 385.
10. /pubchem.ncbi.nlm.nih.gov
11. pubchem.ncbi.nlm.nih.gov/compound/luteolin
12. Kumar, V., & Kundu, S. (2022). Studies on apigenin and its biological and pharmacological activity in brain disorders. *Advanced Pharmaceutical Bulletin*, *12*(4), 645-648.
13. Fillmore, N., & Lopaschuk, G. D. (2014). Malonyl CoA: a promising target for the treatment of cardiac disease. *IUBMB life*, *66*(3), 139-146.
14. ACD/Structure Elucidator, version 2018.1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2019.
15. Soni, H., Mishra, S., Mishra, R. K., & Mishra, S. R. (2022). Silibin as potent inhibitor of COVID-19 main protease: in-silico docking approach. *Journal of Molecular Pharmaceuticals and Regulatory Affairs*, *4*(1), 1-7.
16. Malik, J. K., Soni, H., Sharma, S., & Sarankar, S. (2020). Hydroxychloroquine as potent inhibitor of COVID-19 main protease: Grid based docking approach. *Eurasian Journal of Medicine and Oncology*, *4*(3), 219-226.
17. Soni, H., Gautam, D., Sharma, S., & Malik, J. (2020). Rifampicin as potent inhibitor of COVID-19 main protease: In-silico docking approach. *Saudi Journal of Medical and Pharmaceutical Sciences*, *6*(9), 588-593.

18. Sander, T., Freyss, J., von Korff, M., Reich, J. R., & Rufener, C. (2009). OSIRIS, an entirely in-house developed drug discovery informatics system. *Journal of chemical information and modeling*, *49*(2), 232-246.
19. Kciuk, M., Mujwar, S., Szymanowska, A., Marciniak, B., Bukowski, K., Mojzych, M., & Kontek, R. (2022). Preparation of Novel Pyrazolo [4, 3-e] tetrazolo [1, 5-b][1, 2, 4] triazine Sulfonamides and Their Experimental and Computational Biological Studies. *International Journal of Molecular Sciences*, *23*(11), 5892.
20. Kciuk, M., Gielecińska, A., Mujwar, S., Mojzych, M., Marciniak, B., Drozda, R., &Kontek, R. (2022). Targeting carbonic anhydrase IX and XII isoforms with small molecule inhibitors and monoclonal antibodies. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *37*(1), 1278-1298.
21. 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.
22. Mujwar, S., & Pardasani, K. R. (2015). Prediction of Riboswitch as a Potential Drug Target for Infectious Diseases: An Insilico Case Study of Anthrax *Journal of Medical Imaging and Health Informatics*, *5*(5), 7-16.
23. Mujwar, S., & Pardasani, K. (2015). Prediction of riboswitch as a potential drug target and design of its optimal inhibitors for Mycobacterium tuberculosis.*International Journal of Computational Biology and Drug Design*, *8*(4), 326-347.
24. Shah, K., Mujwar, S., Gupta, J. K., Shrivastava, S. K., & Mishra, P. (2019). Molecular Docking and In Silico Cogitation Validate Mefenamic Acid Prodrugs as Human Cyclooxygenase-2 Inhibitor. *Assay Drug Dev Technol*, *17*(6), 285-291.
25. Khantham, C., Yooin, W., Sringarm, K., Sommano, S. R., Jiranusornkul, S., Carmona, F. D., ... & Ruksiriwanich, W. (2021). Effects on steroid 5-alpha reductase gene expression of Thai rice bran extracts and molecular dynamics study on SRD5A2. *Biology*, *10*(4), 319.
26. Malik, J., Jhariya, D., Ahirwar, P., Sharma, S., Upadhyay, S., & Soni, H. (2024). Mechanistic insight anti-arthritis efficacy of bio-actives of Moringa oleifera: In-silico molecular docking. *Journal of Pharmacognosy and Phytochemistry*, *13*(1), 44-48.
27. Kciuk, M., Mujwar, S., Rani, I., Munjal, K., Gielecińska, A., Kontek, R., & Shah, K., 2022. Computational bioprospecting guggulsterone against ADP ribose phosphatase of SARS-CoV-2. *Molecules*, *27*(23), p.8287.
28. Soni, S., Malik, J. K., Sarankar, S. K., & Soni, H. (2019). Rutin as a potent inhibitor of dihydrofolate reductase: A computational design and docking. *EAS J. Pharm. Pharmacol*, *1*(6), 130-134.
29. Himesh, S. (2024). Computational Modeling for Designing of Syringic acid against mTOR1: As Potent Anticancer Agents. *Journal of Pharmacognosy and Phytochemistry*, *13*(5), 359-363.
30. Thomas Sander, Idorsia Pharmaceuticals Ltd, Hegenheimermattweg 91, 4123 Allschwil, Switzerland, Email: thomas.sanderidorsia.com.