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Original Research Article

In Silico Study of Green Tea and Green Coffee Compounds Inhibiting Cardiac Fibrosis in Metabolic Syndrome Via Dual Inhibition FGF23/FGFR4

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ABSTRACT

Cardiac fibrosis can occur due to various causes, including metabolic syndrome. Increased expression of FGF23, which can activate the cardiac fibrosis pathway via FGFR4, is often observed in metabolic syndrome. Meanwhile, inhibitor therapies targeting FGF23 and FGFR4 for cardiac fibrosis are minimal. Therefore, this study used green tea and coffee compounds to determine their potential as specific inhibitors of FGF23 and FGFR4 to inhibit cardiac fibrosis signaling using bioinformatics methods. Six compounds derived from green tea and coffee—cafestol, CGA, ECG, EGC, EGCG, and Kahweol—were evaluated through ADMET screening, membrane permeability prediction, toxicity prediction, molecular docking, and molecular dynamics simulations. The analysis revealed that all six compounds could pass through the phospholipid membrane, with cafestol having the lowest energy transfer value. Molecular docking and molecular dynamics analyses of FGF23 showed that cafestol and kahweol compounds had better binding affinity values (-6.2 and -5.9 kcal/mol) than the control and showed stable ligand stability and movement (~3 Å). Meanwhile, all compounds had good binding affinity and succeeded in occupying more active sites on FGFR4, with EGC, EGCG, and kahweol compounds showing the most stable stability and movement compared to other compounds against the native ligand (~3 Å). This study demonstrates that the six compounds derived from green tea and coffee have strong potential as specific inhibitors of FGF23 and FGFR4 to inhibit cardiac fibrosis signaling in metabolic syndrome conditions.

Keywords: Cardiac fibrosis, Fibroblast Growth Factor Receptor 4, Fibroblast Growth Factor 23, Green Coffee, Green Tea

Introduction

Fibrosis refers to a clinical condition marked by the increased disposition of extracellular matrix (ECM) proteins in an organ, which is mostly a marker related to the failure of an organ's reparative processes. Fibrosis is one of the main causes of organ dysfunction in various diseases, such as heart failure. [1] Cardiac fibrosis can occur due to various causes, one of which is metabolic syndrome, which, according to reports from Global Burden Disease, continues to increase in prevalence by 82%. [2] Various clinical conditions, such as dyslipidemia, insulin resistance, and an increased potential for mineral metabolism disorders cause imbalances in the mineral levels in the blood.[3]

An imbalance in mineral levels in the blood increases Fibroblast Growth Factor 23 (FGF23) levels, which balances phosphate levels in the blood serum by forming a complex with α-klotho. [3] α-klotho is a co-receptor of FGF23, which can strengthen the affinity of FGF23 to bind to Fibroblast Growth Factor Receptor 4 (FGFR4).

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Binding of the FGF23-α-klotho complex to FGFR4 activates the renin-angiotensin-aldosterone system (RAAS), which can then induce Left Ventricular Hypertrophy (LVH). [4] Apart from inducing LVH, local activation of RAAS can induce the production of various pro-fibrotic, pro-inflammatory, and pro-hypertrophic molecules in cardiac cells, which can accelerate the process of developing cardiac fibrosis.[5] Activation of FGF23 via FGFR4 in cardiac myocytes also stimulates phospholipase Cγ (PLCγ) and calcium-neuron/nuclear factor of activated T cells (NFAT) signaling. The stimulation of these two pathways is also one of the main mechanisms of cardiac fibrosis.[6] The lack of therapies targeting FGF23 and FGFR4 means that treatment options targeting FGF23 or FGFR4 are limited. Previous research has only explained the existence of the small molecule SSR128129E as an allosteric inhibitor of FGFR signaling. Based on the results of this research, it is known that oral selective inhibitors of FGFR need to be developed to expand treatment therapies that target FGF Receptor signaling.[7]

Tea and coffee are the two most widely consumed beverages globally, largely due to their rich content of bioactive compounds that benefit the body, including the cardiovascular system. Tea and coffee are also rich in polyphenol compounds such as catechins—including epicatechin gallate (ECG), epigallocatechin gallate (EGCG), and epigallocatechin (EGC)—which are widely found in tea and coffee and are known to have good activity in the body. In addition, other active compounds, such as chlorogenic acid (CGA), which is widely found in green coffee, are known to have good antioxidant potential.[8] Therefore, this study explains the potential of active compounds derived from green tea and green coffee as

selective inhibitors of FGF23 and FGFR4 to inhibit the activation of cardiac fibrosis caused by FGF23 via FGFR4 with *in silico* studies.

Materials and Methods

Data Mining

The crystallized structures of human FGF23 protein (PDB ID: 2P39) and FGFR4 (PDB ID: 6JPE) were retrieved from the RSCB Protein Data Bank (https://www.rcsb.org/). The bioactive compounds from green coffee and green tea used in this study were selected based on previous research.[9] A total of six active compounds were selected, including three compounds from the catechin group obtained from tea, namely epigallocatechin (EGC) (CID:72277), epicatechin gallate (ECG) (CID:107905), and Epigallocatechin Gallate (EGCG) (CID:65064); and three compounds from green coffee, namely Chlorogenic Acid (CGA) (CID:1794427), cafestol (CID:108052), and Kahweol (CID:114778). Metformin (CID:4091) was used as a drug control based on previous research, which showed that the use of metformin as a drug control reduced the expression of several genes in cases of cardiac fibrosis.[10] Protein models were acquired in .pdb file format, whereas the structures of the ligands were retrieved in .sdf format.

Pharmacokinetics and Drug-likeness Prediction

All selected ligands were evaluated for their drug-likeness analysis using the SwissADME web server (http://www.swissadme.ch/)[11]. Pharmacokinetic analysis was conducted to determine and evaluate a component's ability and physical-chemical characteristics to act as a drug, which was later carried out based on the Five Rules of Lipinski[12]. The parameters to be evaluated included molecular weight, LogP value, and number of H-bonds. Donor, H-bond acceptor, GI Absorption, and Bioavailability Score.

Membrane Permeability Prediction and Toxicity Evaluation

Each compound used in this study will also be predicted for toxicity using the Pro-Tox II webserver (https://tox-new.charite.de/protox_II/)[13]. Toxicity analysis determined the compound's potential to induce hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, and cardiotoxicity. Each compound was also tested for predicting membrane permeability using the PerMM webserver (https://permm.phar.umich.edu/server)[14]. This analysis aimed to assess the compounds' ability to penetrate cellular membranes, thereby allowing the prediction of their absorption potential within the gastrointestinal tract.

Redocking Validation Protocol and Molecular Docking

Redocking was performed to validate the docking protocol and determine its effectiveness in reproducing the original binding pose of the ligand as observed in the crystallographic complex. This step was essential to ensure the reliability and precision of the docking settings used. The 3D structures of the target proteins (2P39 and 6JPE) with native ligands were retrieved from the Protein Data Bank (https://www.rcsb.org/). Prior to docking, the protein structures underwent preparation by eliminating water molecules and native ligands. The extracted native ligands were then re-docked using Pyrx software. The redocked pose was visualized and compared with the crystallographic conformation using PyMOL, ensuring that key interactions and binding modes were maintained. The root-mean-square deviation (RMSD) between the docked and original crystallographic structures was computed to evaluate the accuracy of the docking procedure. An RMSD value ≤ 2.0 Å was considered acceptable and indicative of a reliable docking protocol.

Following validation, protein structures were refined using Discovery Studio 2021 by eliminating residual water molecules and native ligands. Ligands preparation was conducted using Open Babel, integrated within PyRx 8.0, for energy minimization and conversion into .pdb format files. Molecular docking was subsequently performed with AutoDock Vina, embedded within PyRx 8.0. The active site of FGF23 was targeted based on prior studies, identifying critical amino acid residues (SER105, GLN54, HIS168, THR68, GLY63, and TYR107) within the Klotho-binding domain, with the docking grid centered at coordinates X: 8.2345, Y: 7.0405, Z: 10.1002 and dimensions of X: 29.4705 Å, Y: 13.8872 Å, and Z: 23.0256 Å.[15] Previous research has demonstrated that forming the FGF23-Klotho complex enhances the binding affinity of FGF23 for FGFR4.[4] Similarly, molecular docking for FGFR4 was carried out focusing on its active site, guided by prior studies showing that several kinase inhibitors target key residues, including LEU473, GLU475, ALA501, GLU551, CYS552, ALA553, ALA554, GLY556, ASN557, ARG616, LEU619, and ALA629. The docking grid for FGFR4 was centered at X: 45.5785, Y: -2.5473, Z: 81.9010 with dimensions X: 20.9295 Å, Y: 23.3823 Å, and Z: 18.1991 Å.[12] Post-docking analysis and visualization of protein-ligand complexes were performed using Discovery Studio, specifically identifying ligand-residue interactions and evaluating the structural conformations.

Molecular Dynamics

Molecular dynamics (MD) simulations are extensively utilized in drug discovery research to investigate the structural properties and dynamic interactions of protein-ligand complexes at the microscopic scale. This study conducted MD simulations using YASARA (Yet Another Scientific Artificial Reality Application) software, version 25.1.13 (2024), developed by YASARA Biosciences GmbH, Austria, employing the AMBER14 force field applied. The system was simulated to mimic physiological cellular environments closely, maintaining a temperature of 310°K, pH 7.4, atmospheric pressure of 1 atm, water density of 0.997 g/mL, and a NaCl ion concentration of 0.9% over a simulation period of 20 nanoseconds.[17] The macro scripts used included md_run to execute the simulations, md_analyze to assess the RMSD (Root-Mean-Square Deviation), and md_analyzeres to evaluate the RMSF (Root-Mean-Square Fluctuation).

Results and Discussion

Pharmacokinetics and Drug-Likeness Prediction

Almost all six green tea and coffee compounds met the Lipinski Rules of Five criteria, with the exception of EGCG, which exhibited two violations –possessing more than 5 Hydrogen Bond Donors and more than 10 Hydrogen Bond Acceptors (HBA) (Table 1). Several compounds showed good Bioavailability Scores with high GI Absorption capabilities, except for several compounds such as CGA, ECG, and EGCG. This information can be used as an initial screening for its development as a drug so that it can have a maximum effect.

The ADMET analysis showed that almost all compounds showed a pharmacokinetic profile under the Five Rules of Lipinski, except for EGCG. Several compounds showed good Bioavailability Scores with high GI Absorption capabilities, except for several compounds such as CGA, ECG, and EGCG. This result can be used as an initial screening for its development as a drug so that it can have a maximum effect. Parameters that also need to be considered when determining an active compound as a drug candidate include hydrogen bond donors and acceptors. The quantity of hydrogen bond donors and acceptors significantly affects the compound's physico-chemical properties and is closely related to its efficacy during drug development. A compound with less than five donor hydrogen bonds of less than 5 shows excellent polarity, making the compound have better permeation and absorption capabilities.[18]

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Table 1: Pharmacokinetics and Drug-Likeness Characterization screening results based on Lipinski Rule of Five

Compound	CID	Formula	MW (g/mol)	MlogP	HBA	HBD	GI Absorption	Bioavailability Score
Metformin	4091	$C_4H_{11}N_5$	129.16	-0.56	2	3	High	0.55
Cafestol	108502	$C_{20}H_{28}O_3$	316.43	2.89	3	2	High	0.55
CGA	1794427	$C_{16}H_{18}O_9$	354.31	-1.05	9	6	Low	0.11
Kahweol	114778	$C_{20}H_{26}O_3$	314.42	2.80	3	2	High	0.55
ECG	107905	$C_{22}H_{18}O_{10}$	442.37	0.05	10	7	Low	0.55
EGC	72277	$C_{15}H_{14}O_7$	306.27	-0.29	7	6	High	0.55
EGCG	65064	$C_{22}H_{18}O_{11}$	458.37	-0.44	11	8	Low	0.17

Membrane Permeability Prediction and Toxicity Evaluation

Membrane permeability prediction was performed for the six selected compounds to determine their potential to penetrate the phospholipid membrane. The prediction results showed that all green tea and coffee compounds could penetrate the phospholipid membrane. Among the six compounds, cafestol showed the lowest energy transfer value, whereas EGCG demonstrated the highest in penetrating the phospholipid membrane (Fig. 1).

In addition to their ability to penetrate membranes, the selected compounds were subjected to toxicity analysis to determine the potential adverse effects in inducing hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, and cardiotoxicity to ensure their safety as drugs. The analysis showed that all compounds had low potential to cause toxicity to the liver, neurons, and cardiovascular system (Fig. 2). Several compounds, such as CGA, ECG, EGC, and EGCG, have shown moderate potential to be toxic to nephrons. These results also indicate that all compounds tended to have moderate toxicity in inducing the respiratory system. LD50 analysis of each compound showed that most compounds had a toxicity level of 4, which means that the compound was slightly toxic. This analysis can be used for initial screening and to determine the pharmacokinetic properties of each compound to predict its effects on the body when used as a drug.

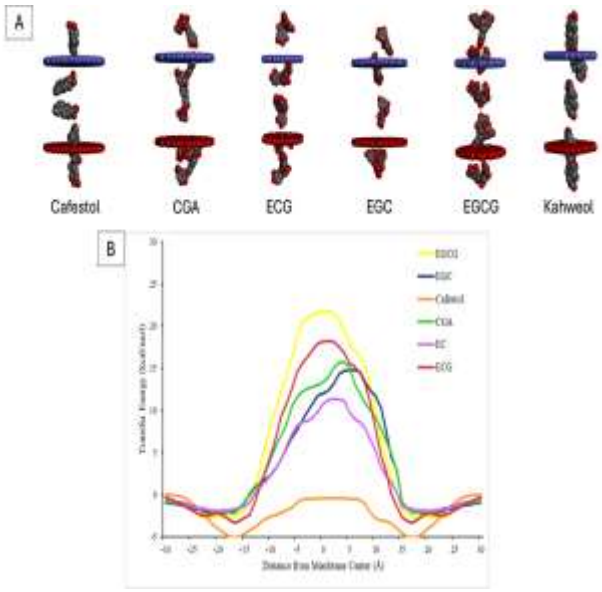

Figure 1: Membrane permeability prediction for six compounds from green tea and green coffee, A) Schematic representation of compounds penetrating phospholipid membranes, B) Graph of the minimum energy required by a compound to penetrate the phospholipid membrane.

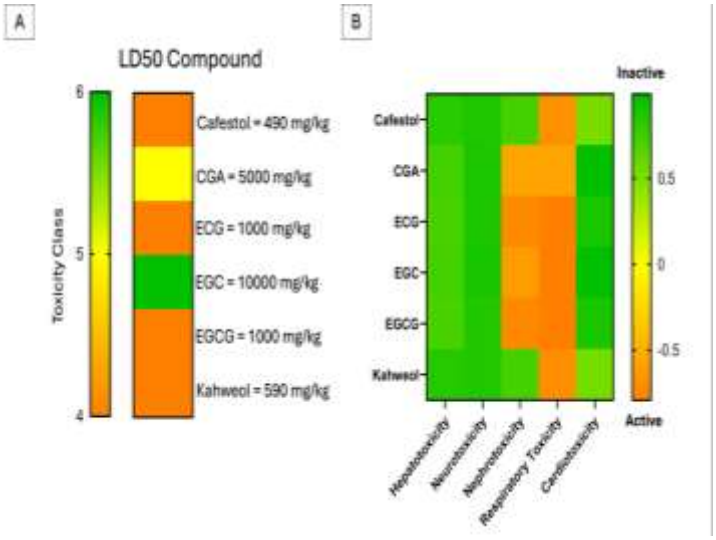

Figure 2: Toxicity evaluation from six compounds from green tea and green coffee.

Redocking Validation

Redocking the native ligand to FGF23 yielded a binding affinity of -5.6 kcal/mol, whereas redocking to FGFR4 produced a binding affinity of -10.5 kcal/mol. The redocking process was validated by aligning the native and redocked ligands using PyMOL software. The alignment for FGF23 demonstrated an RMSD value of 0.875 Å (Fig 3a). In contrast, the alignment for FGFR4 exhibited an RMSD value of 0.142 Å (Fig. 3b). Both redocking results produced RMSD values below 2 Å, confirming that the docking protocol employed in this study was accurate and reliable.

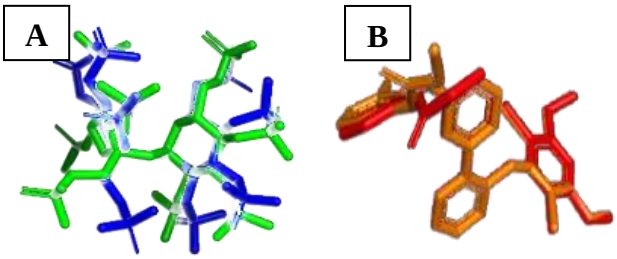

Figure 3: Re-docking of the native ligand pre- and post-docking, (A) FGF23 and (B) FGFR4

Interaction Between FGF23 and Bioactive Compound

The docking results of FGF23 with all compounds are shown in Table 2. As a control, metformin showed a low binding affinity value (-3.3 kcal/mol). The docking results also show two hydrogen bonds at the amino acid residues ILE102 (2.78 Å and 2.29 Å) and VAL61 (1.91 Å). The VAL61 residue also formed a

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Carbon Hydrogen Bond with a distance of 3.57 Å. Another Carbon Hydrogen Bond was also formed on the HIS60 amino acid residue, and there was an electrostatic interaction on the ASP97 amino acid residue. The docking results also showed two van der Waals interactions between the amino acid residues ASN101 and PHE103.

The docking results of FGF23 with six active compounds from green tea and green coffee showed that cafestol derived from green coffee had the best binding affinity value (-6.2 kcal/mol) compared to other compounds (Table 2). Cafestol interacted with ARG160 by forming hydrogen bonds with a distance of 3.05 Å. Cafestol also forms several other bonds, such as alkyl and Pi-Alkyl on PRO165 and ILE164, as well as a T-shaped Pi-Pi interaction on the amino acid residue HIS168. Cafestol also forms two van der Waals interactions with amino acid residues GLN67 and GLU163. Another coffee compound, CGA, shows a binding affinity value of -5.9 kcal/mol by showing the presence of two hydrogen bonds formed at the amino acid residues GLN67 (2.95 Å and 2.96 Å) and GLU163 (2.31 Å). The amino acid residue GLN67 also showed an unfavorable acceptor-acceptor interaction with the CGA. In addition to hydrogen bonds, there are several other bonds, such as three-carbon hydrogen bonds in the amino acid residues HIS52, THR68, and PRO165. There were also Pi interactions consisting of Pi-Alkyl on the ILE164 amino acid residue and T-shaped Pi-Pi on the HIS168 amino acid residue (Fig. 4).

In CGA, several van der Waals interactions occur at amino acid residues SER29, HIS66, THR68, and ILE69 (Fig. 4). For the Kahweol compound, docking results presented that the compound had a binding affinity value of -5.9 kcal/mol. However, Kahweol showed poor interactions because the docking results showed that there was only an alkyl interaction on the amino acid residue ILE164, and there were two unfavorable donor-donor and acceptor-acceptor interactions on the amino acid residues ARG160 and GLU163. The kahweol docking results also showed that there were several van der Waals interactions between the amino acid residues HIS52, HIS66, GLN67, PRO165, and HIS168 (Fig. 4). For tea compounds, all compounds have similar binding affinity values, namely, -5.9 kcal/mol, -5.9 kcal/mol, and -5.8 kcal/mol respectively for ECG, EGC, and EGCG. For ECG, docking results showed that there was one hydrogen bond at the amino acid residue GLU163 (2,19 Å), one Pi-alkyl interaction at the amino acid residue ILE164, one Carbon Hydrogen Bond at the amino acid residue HIS168, and two unfavorable donor-donor interactions at the amino acid residues GLN67 and ILE69. The docking results of ECG compounds also showed that several van der Waals interactions formed on the amino acid residues HIS52, HIS66, THR68, TYR70, ARG160, and PRO16.

The molecular docking results from this study also indicated that the compounds from green tea and green coffee had better binding affinity values than metformin as a control. The location of metformin interaction shows a position that is not at all in the active site of the FGF23-α-klotho binding domain. This finding indicates that metformin does not directly target FGF23. This observation indicates that metformin does not directly target FGF23. This result is consistent with previous research, which explains that metformin does not show activity directly on FGF23. However, metformin influences FGF23 indirectly through FGF21 by increasing circulating FGF21 from activating the AMPK-dependent pathway.[19]

Table 2: Molecular docking results of control compounds and bioactive compounds from green tea and green coffee with FGF23 and FGFR4

Compound	Binding Affinity (kcal/mol)	
	FGF23	FGFR4
Metformin (control)	-3.3	-4.5
LY2874455 (control)	-	-8.0
Cafestol	-6.2	-8.2
CGA	-5.9	-7.8
Kahweol	-5.9	-8.0
ECG	-5.9	-8.8
EGC	-5.9	-7.6
EGCG	-5.8	-8.7

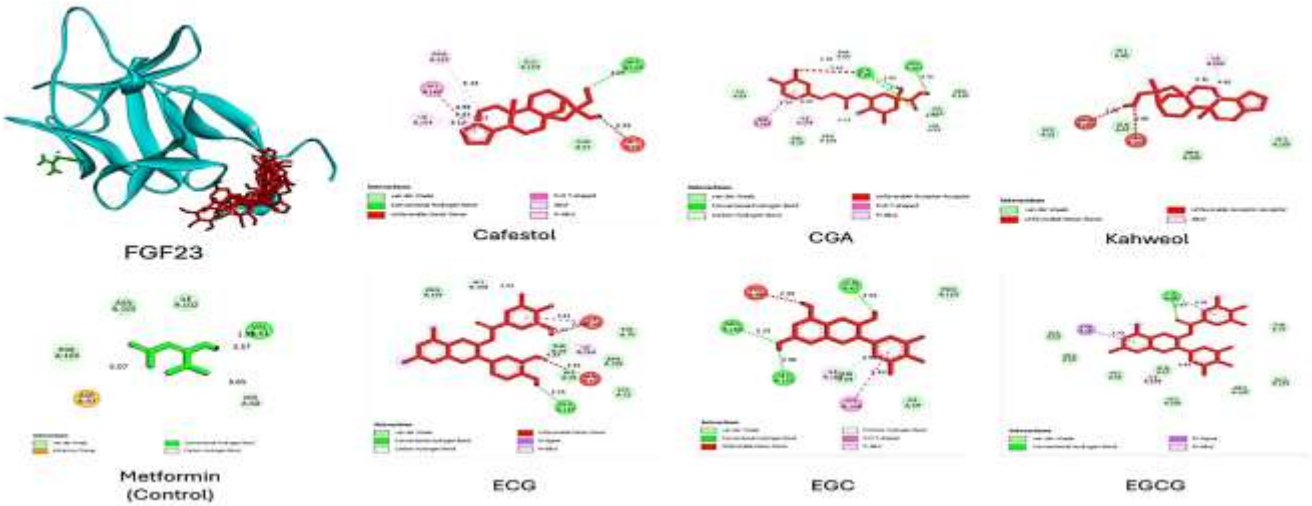

Figure 4: Visualization of 2D and 3D interactions of FGF23 and compounds

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Interaction Between FGFR4 and Bioactive Compound

The docking results of FGFR4 with all compounds are shown in Table 2. LY2874455, a small molecule inhibitor of FGFR4, showed a binding affinity value of -8.0 kcal/mol. Furthermore, metformin as a control showed a binding affinity value below that of the native ligand (-4.5 kcal/mol). Docking results showed that metformin forms salt-bridge interactions at the amino acid residues GLU520 and ASP630. ASP63O could also form hydrogen bonds (4.06 Å). Several interactions were also observed at amino acid residues, such as VAL481, LYS503, MET524, ILE534, VAL548, VAL550, PHE631, GLY632, and LEU633. These results indicated that metformin is not a potent inhibitor of FGFR4 (Fig. 5).

For docking analysis with the six compounds, the results showed that the six compounds had binding affinity values close to or even better than the binding affinity of the native ligand. Compounds from green coffee show excellent binding affinity values, namely -8.2 kcal/mol for cafestol, -7.8 kcal/mol for CGA, and -8.0 for Kahweol. Cafestol binds to four residues from the same active site occupied by the native FGFR4 inhibitor, LEU473, ALA501, ALA553, and LEU619. A hydrogen bond is established at residue ALA501 (2.31 Å); Alkyl and Pi-alkyl interactions are the others. In CGA, five hydrogen bonds (LYS503, GLU520, ARG616, and ASP630), one Pi-Sigma interaction (LEU619), and one alkyl interaction (VAL550) were formed (Fig. 5). At least three amino acid residues—ASN557, ARG616, and LEU619—were identified as corresponding to the active site of FGFR4. In the case of Kahweol, five residues—LEU473, ALA501, CYS552, ALA553, and LEU619—were identified as interacting with the active site, with a hydrogen bond formed at the residue ALA553 with a distance of 1.99 Å (Fig. 5). For compounds in green tea, it is known that ECG has a better binding affinity value compared to the native ligand (-8.8 kcal/mol), followed by EGCG (-8.7 kcal/mol), and EGC (-7.6 kcal/mol) (Table 2) The results of docking FGFR4 with ECG show that eight residues correspond to the active site (LEU473, ALA501, CYS552, ALA553, ASN557, ARG616, LEU619, and ALA629) (Fig. 5). Of the eight residues, residues LEU473 and ALA553 succeeded in forming hydrogen bonds with distances of 2.91 Å and 2.32 Å respectively. There are also interactions formed on ASN557, an unfavorable donor-donor that could affect the stability of the compound's interaction with FGFR4. The interaction between ECG and FGFR4 is considered the most stable and is close to the native ligand of FGFR4. Docking results showed that EGCG can bind to five residues, which are the active sites of FGFR4 (Fig. 5). These five residues consist of two hydrogen bonds (LEU473 and ALA553) and three alkyl interactions (ALA501, LEU619, and ALA629). The kahweol compound also contains at least five residues that are the active site of FGFR4, which consists of one hydrogen bond (ALA553) and four others are Alkyl and Pi-Alkyl interactions (LEU473, ALA501, CYS552, and LEU619). Several compounds appear to show more interactions with the active site residues of FGFR4, supporting the strong potential of these compounds to interact with FGFR4, especially at the binding site of the FGF23-α-Klotho complex.

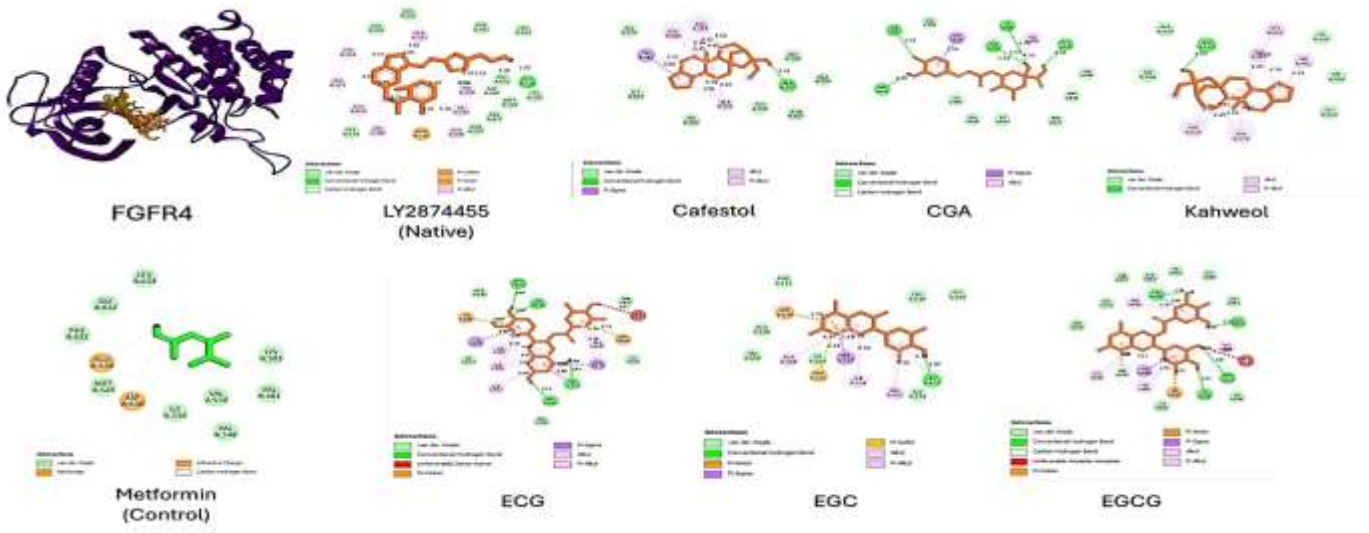

Figure 5: Visualization of 2D and 3D interactions of FGFR4 and compounds

Molecular Dnamics Simulation

Root Mean Square Deviation (RMSD)

Molecular Dynamics (MD) analysis showed that when compared with metformin as a control, from the six compounds from tea and coffee used, EGC showed the best RMSD value compared to metformin as a control (Fig. 6a). The FGF23-EGC complex also showed good stability as indicated by the constant RMSD value of approximately 3 Å. Cafestol also showed an RMSD value similar to metformin as a control (around 3 Å). For the other compounds, the RMSD results were not very good, as indicated by the high fluctuations that occurred during the simulation. However, the ligand RMSD data for FGF23-EGC and FGF23-cafestol demonstrated superior stability compared to other compounds (Fig. 6c).

Meanwhile, the MD analysis results of bioactive compounds with FGFR4 showed that metformin exhibited a stability profile comparable to that of the native ligand. The stability formed still shows a good value of approximately 3 Å, even though several fluctuations cause the RMSD value to increase to 3.5 Å at some point. Meanwhile, the stability of the three tea compounds, ECG, EGC, EGCG, and Kahweol, showed good stability with RMSD values that tended to remain constant below 3 Å until the simulation ended (Fig. 6b). The RMSD results also showed that the movement of these compounds as ligands was better than that of the native inhibitor compounds used. The RMSD value of ligand movement of EGC, EGCG, and Kahweol compounds was much lower and more stable than that of the control (Fig. 6d).

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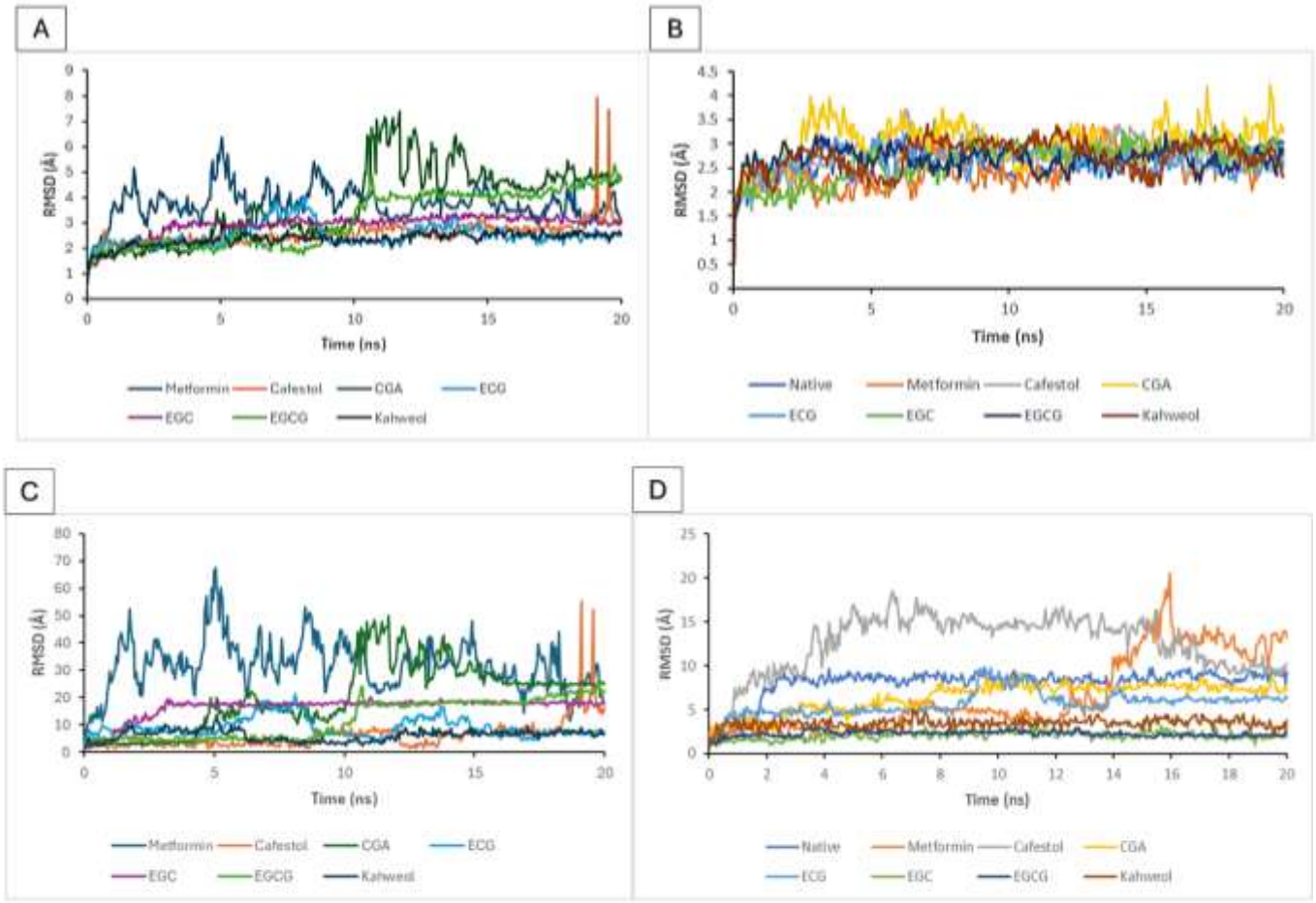

Figure 6: RMSD analysis from protein-ligand complex, a) RMSDAll FGF23, b) RMSDAll FGFR4, c) RMSDLigMove FGF23, and d) RMSDLigMove FGFR4

Root Mean Square Fluctuation

RMSF analysis of the FGF23 protein complex with bioactive compounds showed similar plots for all compounds and was relatively stable with RMSF values ranging from 3 Å. Kahweol showed a slight increase in fluctuation at residue number 48 (4.1 Å) and cafestol at amino acid residues number 79 and 140 with RMSF values of 4.0 Å (Fig. 7a.). These results indicate that the amino acid residues of FGF23 are relatively stable when interacting with compounds from green tea and coffee. Meanwhile, the RMSF results for the bioactive compound complex with FGFR4 showed a relatively similar plot; namely, almost all amino acid residues of FGFR4 have RMSF values ranging from 3-4 Å, but amino acid residues 571-581 show quite high fluctuations (above 3 Å) which explains why that these amino acid residues are very volatile and unstable in the formation of bonds or interactions with ligand compounds (Fig. 7b).

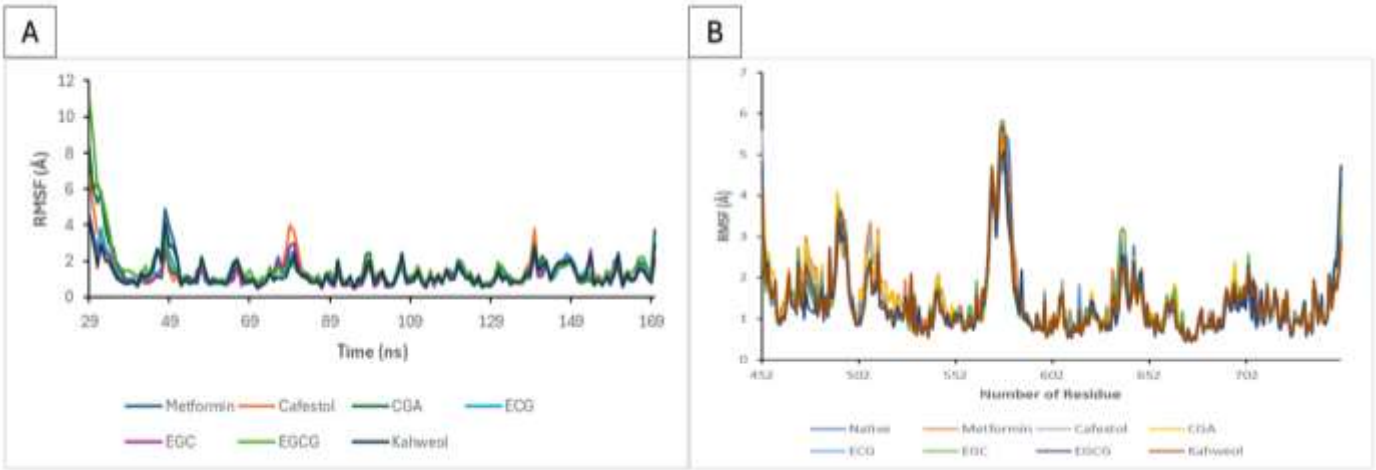

Figure 7: RMSF plot of bioactive compound from green tea and coffee with protein target, a) FGF23 and b) FGFR4.

Molecular dynamics simulations also show data supporting these findings, as results indicate that compounds such as EGC, Cafestol, and Kahweol show better stability and lower residue fluctuation than drug controls or previously identified small-molecule inhibitors. MD analysis is a computational analysis used to evaluate the stability of interactions within the docked ligand-receptor complex. This phenomenon is because the atoms of biomolecules move constantly, where molecular function and intermolecular interactions in the body depend on the molecules' dynamics.[20] The bond in a complex can be said to be stable if it has an RMSD value below 3 Å.[21,22] In addition, the RMSF value indicates the fluctuation of each amino acid residue of the target protein from the ligand. The RMSF value is related to the RMSD value, where higher fluctuation of amino acid residues can contribute to forming a high RMSD plot, as RMSD measures global fluctuations of a protein-ligand complex.[23]

Recently, the use of herbs as therapeutic agents for fibrosis has been widely developed. In addition to green tea and coffee used in this study, research conducted by Suryono et al. showed that leaf extract from *Moringa oleifera* improved cardiac fibrosis by regulating the expression levels of TGF-β1 and Galectin-3 in diabetic rat models.[24] In addition, in a different case, research by Firdausi et al.[25] using *Elephantopus scaber* showed that it improved pulmonary fibrosis. This study investigated compounds derived from green tea and green coffee, highlighting their potential as inhibitors in preventing the activation of cardiac fibrosis signaling pathways. The results offer important perspectives on the potential application of herbal sources as specific FGF23/FGFR4 inhibitor candidates, particularly given the limited availability of inhibitors targeting this pathway. Nevertheless, additional in vitro or in vivo validation is necessary to substantiate these findings and support the development of specific FGF23/FGFR4 inhibitors to mitigate cardiac fibrosis.

Conclusion

In conclusion, six compounds from green tea and coffee showed good potential as inhibitors of FGF23 or FGFR4 to inhibit cardiac fibrosis signaling caused by metabolic syndrome. Compounds from green tea and green coffee were proven to occupy the klotho binding domain on FGF23 and the active site of the FGF23-FGFR4 binding site with good binding affinity and stability values. These results provide initial information on the potential of active compounds from green tea and green coffee as direct inhibitors of FGF23 or FGFR4 when there is a lack of drugs or direct inhibitors that target these proteins in cases of cardiac fibrosis. However, in vitro or in vivo studies that directly target these proteins are needed to confirm the exact effects of these compounds as cardiac fibrosis therapy options.

Conflict of Interest

The author`s declare no conflicts 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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