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In Silico Identification of *Heterotrigona itama* Propolis Compounds as GC-C Agonists and LGR5 Inhibitors for Colorectal Cancer Chemoprevention

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Abstract

Abnormal regulation of LGR5 receptors in the Wnt pathway and loss of Guanylyl Cyclase C (GCC) expression have been implicated in the pathogenesis of colorectal cancer, rendering them promising therapeutic targets for further exploration. With the growing focus on natural product-based drug development, *Heterotrigona itama* propolis emerges as a potential source of bioactive compounds. This *in silico* study aims to analyze the potential of active compounds from stingless bee propolis as GCC receptor agonists and LGR5 receptor inhibitors and to identify the compounds with the most favorable binding affinity. The results revealed that eleven propolis compounds, including CAPE, Quercetin, Chrysin, 9-octadecenoic acid, Cardol, Cardanol, and Anacardic acid, could bind to both GC-C and LGR5 target receptors. Among these, cardanol had the highest binding affinity for GCC (-7.3 kcal/mol), while Anacardic acid showed the best binding affinity for LGR5 (-6.5 kcal/mol). Overall, the *Heterotrigona itama* propolis compounds exhibited a stronger binding affinity for LGR5 than for GCC. The substance has good action in binding to the receptor, suggesting that it may be used as treatment to prevent the progression of colorectal cancer. These findings provide a foundational basis for further studies to validate the pharmacological potential of propolis for colorectal cancer treatment.

Keywords: cancer, colorectal, LGR5, GCC, propolis.

INTRODUCTION

Cancer is a progressive degenerative disease and a major public health challenge in the 21st century. It is defined by genetic and epigenetic alterations that result in unregulated cellular proliferation, tissue infiltration, and metastasis (Asaduddin, *et al*., 2025; Rathva & Desai, 2020). According to WHO data from 2022, cancer accounts for 19.98 million new cases and 9.74 million deaths globally, with lung, breast, and colorectal cancer (CRC) being the most prevalent. Asia bears a significant burden, representing 56.1% of global cancer deaths, and this number continues to rise. In Indonesia, 408,661 new cancer cases and 242,988 deaths were recorded in 2022, with CRC ranking as the fourth most common cancer (6.7 per 100,000 population) and responsible for 9.5% of all cancer-related deaths (WHO, 2025).

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The pathological process of colorectal cancer frequently entails the deregulation of the Wnt/β-catenin signaling system, essential for regulating cell proliferation and migration (Parasido, *et al*., 2024). Key targets within this pathway include LGR5, a seven-transmembrane receptor and a marker of intestinal stem cells, and Guanylyl Cyclase C (GCC), a transmembrane receptor that regulates fluid and electrolyte balance through its interaction with guanylin and uroguanylin peptides. Mutations in the APC/β-catenin/TCF axis disrupt normal peptide expression and promote early tumorigenesis, whereas activation of GCC by its natural ligands (Blomain, *et al*., 2020; Horst, *et al*., 2019; Piroozkhah, *et al*., 2023; Waldman & Camilleri, 2018).

Natural products, such as those derived from the stingless bee (*Heterotrigona itama*), offer promising potential for anticancer drug development. The primary products include honey (15.4%), pollen (20.9%), and propolis (63.7%) (Arung, *et al*., 2021). Propolis contains over 300 compounds, including CAPE, quercetin, and chrysin, which exhibit anticancer, anti-inflammatory, and antioxidant activities (Kustiawan, *et al*., 2022). It has been shown that the cardol molecule induces apoptosis in human cancer cell lines (SW620). Cardol significantly increased the activity of caspase-3 and caspase-9, two important enzymes in the apoptosis pathway (Kustiawan, *et al*., 2017).

Although numerous studies have reported the pharmacological potential of propolis and its anticancer activity, most investigations have focused on general cytotoxic effects or broad biological activities. Limited studies have explored the specific molecular interactions between individual propolis derived compounds and key receptors involved in colorectal cancer progression, such as GCC and LGR5. Furthermore, the potential role of stingless bee propolis, particularly from *Heterotrigona itama*, in targeting these receptors remains insufficiently explored. Addressing this gap is important to better understand the mechanistic basis underlying the chemopreventive potential of propolis.

Therefore, the present study aims to identify and evaluate the binding interactions of selected bioactive compounds from *Heterotrigona itama* propolis with GCC and LGR5 receptors using molecular docking analysis, in order to provide a preliminary mechanistic understanding of their potential role in colorectal cancer chemoprevention.

MATERIALS AND METHODS

Methods

This study employed an *in silico* molecular docking approach to analyze interactions between bioactive compounds from *Heterotrigona itama* propolis and the target proteins LGR5 and GCC. The methodology was adapted from Yuniati, *et al*., (2023) with modifications.

Materials and Equipment

The research utilized the 3D structures of seven propolis-derived compounds: Caffeic Acid Phenethyl Ester (CAPE), Quercetin, Chrysin, 9-Octadecenoic acid, Cardol, Cardanol, and Anacardic acid. These ligand structures were retrieved in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The 3D crystal structures of the target proteins, GCC (PDB ID: 8GHP) and LGR5 (PDB ID: 4UFR), were downloaded in ".pdb" format from the Protein Data Bank (RCSB) (http://www.rcsb.org). All computational work was performed on an Acer TravelMate P214 laptop equipped with an 11th Gen Intel® Core™ i7-1165G7 processor, 8 GB RAM, a 64-bit Windows 10 Pro, and utilizing the software PyRx 0.8 (with AutoDock Vina and Open Babel), BIOVA Discovery Studio Visualizer, and Avogadro. Avogadro was used solely for ligand geometry optimization and energy minimization prior to docking. This step ensures chemically reasonable, low-energy ligand conformations and improves docking reliability.

Protein Preparation

The Protein, PDB ID (8GHP) for GCC and PDB ID (4UFR) for LGR5, prepared using BIOVA Studio Visualizer. The protein was stripped of all water molecules and natural ligands. Hydrogen atoms are added to the protein structures to correct for valence and protonation states at physiological pH. The prepared proteins were saved in ".pdb" format for subsequent docking studies.

Ligand Preparation

The 3D structures of the seven bioactive compounds were prepared using the PyRx 0.8 software. The Open Babel tool within PyRx was used to import the ligand files. Each ligand underwent energy minimization to optimize its geometry and obtain a stable conformational state. Subsequently, the minimized ligands were converted into the ".pdbqt" format, which includes atomic partial charges and torsional flexibility data required for docking with AutoDock Vina.

Validation

The native ligand (co-crystallized with each protein) was reintroduced into its respective prepared protein structure. GCC native ligands: guanylin and uroguanylin (co-crystallized reference ligand used for validation: NAG, as provided in the PDB structure). LGR5 native ligand: R-spondin (R-spondin2). That native ligands were then docked back into its original binding site using AutoDock Vina within PyRx. The natural ligand's original crystallographic pose and the docked pose were compared using the Root Mean Square Deviation (RMSD). According to established criteria (Jain & Nicholls, 2008), a docking protocol is considered valid if the RMSD value is ≤3.0 Å, confirming the software's ability to reproduce the experimental binding mode.

Docking of Ligands and Proteins

The molecular docking process was executed using PyRx 0.8 (Vina Wizard) software. A docking grid was defined to encompass the known active site of each target protein. All seven prepared ligands were docked individually against both GCC and LGR5 proteins. The software automatically generated and ranked the resulting ligand-protein complexes based on their calculated binding affinity (in kcal/mol). The conformation with the most negative binding affinity (lowest energy) for each compound was selected for further analysis and saved in ".pdb" format.

Bond Visualization and Analysis

The selected docked complexes were visualized and analyzed using BIOVA Discovery Studio Visualizer. The interactions between each ligand and the amino acid residues inside the protein's binding pocket were shown in both 2D and 3D using the program. The specific types of molecular interactions such as hydrogen bonds, hydrophobic interactions, van der Waals forces, and ionic bonds were identified and documented (Gul, *et al*., 2023) ionic bonds were identified and documented (Gul, *et al*., 2023). The primary data for analysis were the binding affinity values (in kcal/mol). The binding affinity represents the strength of the interaction, where a more negative value indicates a stronger and thermodynamically more favorable binding. The ligands were ranked and compared based on these values to identify the compounds with the highest predicted binding potency for each target protein (LGR5 and GCC). No further statistical testing was applied, as the comparative analysis was based directly on the computational energy scores. Agonist and antagonist interaction was used in a predictive and hypothesis generating context. Therefore, receptor activation was predicted as potential agonist interaction and inhibitory action as potential antagonist.

Lipinski's Rule of Five Prediction

The prediction based on Lipinski's Rule of Five was conducted to identify potential bioactive compounds derived from stingless bee propolis and

to evaluate their physicochemical characteristics using the web-based platform Mcule property calculator (https://mcule.com/apps/property-calculator). Lipinski's Rule of Five describes several important physicochemical parameters that are commonly used to estimate the oral bioavailability of a compound. According to this rule, an ideal candidate for oral drugs generally has a molecular weight below 500 Da, a partition coefficient (log*P*) of less than 5, no more than five hydrogen bond donors, and fewer than ten hydrogen bond acceptors. Compounds that exceed more than one of these thresholds are generally considered less suitable for oral drug development (Lipinski, 2004).

RESULTS

Bioactive compounds from stingless bee propolis were evaluated in silico molecular docking technique against the LGR5 and GCC receptors. This process was obtained seven propolis compounds to analysis included CAPE, Quercetin, Chrysin, 9-Octadecenoic acid, Cardol, Cardanol, and Anacardic acid. The docking simulations were performed using rigid protein structures without explicit solvent modeling, and that these factors may influence binding predictions.

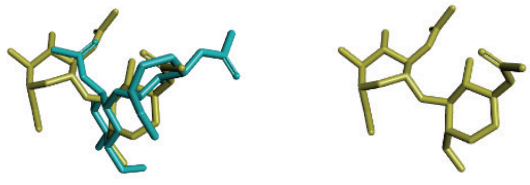
Validation of docking

The docking protocol was validated by redocking the native ligand into its respective prepared protein structure. The study evaluated the accuracy of the docking method using the RMSD between the redocked pose and the crystallographic conformation. The RMSD values were within the acceptable threshold of ≤3.0 Å. This result confirms that the protocol can reproduce the general binding mode of the native ligand.

For the GCC receptor, the native ligand NAG was redocked using grid coordinates centered at X = 11.32, Y = −57.76, Z = 64.65 Å with a grid box dimension of 25 × 26 × 26 Å. The redocking process produced a binding affinity of −6.1 kcal/mol. Figure 1a shows the superimposition of the native and redocked ligands with a similar binding orientation in the active site. Perfect spatial overlap was not observed. This condition is expected due to the limitations of molecular docking such as the use of rigid receptor models and simplified scoring functions. Figure 1B confirms that the ligand occupies the correct binding pocket.

For the LGR5 receptor, the native ligand R-spondin2 was redocked at X=−0.89, Y=38.78, Z=90.14 Å with a grid box size of 26×31×31 Å. The calculated binding affinity was −5.4 kcal/mol. Figure 2a shows that the redocked pose maintains a comparable binding orientation with minor positional deviations. Figure 2b shows that the ligand interacts with key amino acid residues including Trp168, His216, and Thr192 through hydrogen bonds and hydrophobic interactions.

Overall, the protocol shows consistent performance across both receptors. The results indicate that the docking setup is reliable for predicting ligand and receptor interactions. Therefore, it is suitable for further docking analysis of propolis derived compounds.



A B

Figure 1. A. Native NAG 3D structure (yellow) and redocking NAG (blue); B. Native NAG 3D structure.

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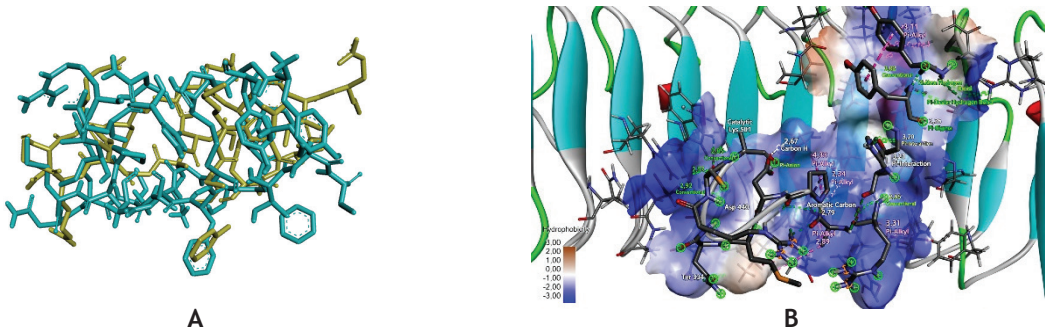

A B

Figure 2. Validation of molecular docking on the LGR5 receptor using the native ligand R-spondin2. A. 3D structure of native R-spondin (yellow) and R-spondin redocking (blue); B. Binding Interaction of R-spondin2 on LGR5.

Drug-Likeness Analysis (Lipinski's Rule of Five)

Lipinski's Rule of Five was used to analyze drug-likeness. The CAPE and Chrysin compounds met all five rules (100% compliance). The other compounds Cardol, Cardanol, Quercetin, Anacardic acid, and 9-Octadecenoic acid met four of the five rules (75% compliance), primarily due to high Log*P* values (Table 1).

Table 1. Lipinski's rule of five analysis for propolis compounds.

Propolis compound	TP SA	MR	Molecular weight	HBD	HBA1	log*P*	Lipinski Rule of Five
Cardol	40.46	102.752	320.50934	2.0	2.0	6.7315	75.0%
Cardanol	20.23	99.307	298.4623	1.0	1.0	6.3539	75.0%
CAPE	66.76	80.772	284.306554	2.0	4.0	2.8969	100.0%
Quercetin	131.36	78.035	302.2357	5.0	6.0	1.988	75.0%
Anacardic acid	57.53	107.6883	348.51944	2.0	3.0	6.7241	75.0%
9-Octadecenoic acid	37.3	89.9378	282.46136	1.0	2.0	6.1085	75.0%
Chrysin	70.67	71.966	254.2375	2.0	3.0	2.8712	100.0%

Molecular Docking Affinity and Interactions

The molecular docking results revealed varying binding affinities of the propolis compounds for the two target receptors. For the GCC receptor, Cardanol presented the strongest binding affinity (-7.3 kcal/mol), then succeeded by Cardol (-6.9 kcal/mol) and Anacardic acid (-6.8 kcal/mol). The detailed affinity values for all compounds are presented in Table 2. Key interactions for the top-binding compounds involved hydrogen bonds and hydrophobic interactions with critical residues such as Tyr126.

Table 2. Docking results of propolis compounds with the GCC receptor.

Ligand (Propolis)	Binding Affinity (kcal/mol)	RMSD/ub	RMSD/lb
Cardanol	**-7.3**	0	0
Cardol	-6.9	0	0
Anacardic acid	-6.8	0	0
Quercetin	-6.2	0	0
CAPE	-6.1	0	0
9-Octadecenoic acid	-5.8	0	0
Chrysin	-5.3	0	0

For the LGR5 receptor, Anacardic acid showed the highest binding affinity (-6.5 kcal/mol), succeeded by Cardol (-6.3 kcal/mol) and Cardanol (-5.9 kcal/mol). The complete results are shown in Table 3.

Table 3. Docking results of propolis compounds with the LGR5 receptor.

Ligand (Propolis)	Binding Affinity (kcal/mol)	RMSD/ub	RMSD/lb
Anacardic acid	**-6.5**	0	0
Cardol	-6.3	0	0
Cardanol	-5.9	0	0
CAPE	-5.6	0	0
Quercetin	-5.0	0	0
Chrysin	-5.0	0	0
9-Octadecenoic acid	-4.6	0	0

The specific types of molecular interactions with conventional hydrogen bonds, carbon-hydrogen bonds, hydrophobic π-alkyl/π-π interactions between each ligand and the key amino acid residues of GCC and LGR5 are detailed in Table 4 and Table 5, respectively. Visualization of the binding poses in 2D and 3D for all compounds with both receptors is provided in Figure 1 and Figure 2.

Among the tested compounds, cardanol exhibited the highest binding affinity for GCC at -7.3 kcal/mol. Additionally, certain propolis compounds, particularly quercetin and chrysin, demonstrate similar interaction patterns to the native ligand, notably involving residues Tyr126 and Leu119.

As shown in the interaction (Figure 3), green lines represent hydrogen bonds between the compound and the amino acid residues. Pink lines indicate hydrophobic interactions, including π-π stacking and π-alkyl contacts. Orange lines signify electrostatic interactions, such as anionic bonds.

Anacardic acid exhibited the strongest binding affinity toward the target (-6.5 kcal/mol), followed by cardol (-6.3 kcal/mol). Additionally, compounds such as CAPE, quercetin, chrysin, and 9-octadecenoic acid demonstrated interactions with key amino acid residues (*e.g.*, Thr192, His216, and Trp168) that were comparable to those observed for the native ligand, suggesting a similar binding mode.

Table 4. Interaction analysis of propolis compounds with GC-C receptor.

Ligand	Hydrogen Conventional	Carbon-Hydrogen	Hydrophobic			π cation/ anion
			π sigma	π-alkyl/ alkyl	π-π	
Cardanol	84 Tyr 89 Ile 91 Asn 111 Asn	N/A	N/A	N/A	126 Tyr	107 Arg
Cardol	130 Gln	N/A	N/A	N/A	126 Tyr	N/A
Anacardic acid	89 Ile 139 Tyr	107 Arg 127 Ser	N/A	40 Leu	N/A	107 Arg
Quercetin	91 Asn 126 Tyr	N/A	N/A	40 Leu 89 Ile 107 Arg 119 Leu	N/A	107 Arg
CAPE	N/A	N/A	N/A	40 Leu 107 Arg 126 Tyr	N/A	N/A
9-Octadecenoic acid	107 Arg 111 Asn 130 Gln	N/A	N/A	40 Leu 89 Ile 115 Met 119 Leu 126 Tyr	N/A	N/A
Chrysin	91 Asn 126 Tyr	87 Gly	N/A	83 Met 107 Arg 126 Tyr	N/A	N/A

In the interaction (Figure 4), green lines denote hydrogen bonds, and pink lines represent hydrophobic interactions, specifically π-π stacking and π-alkyl contacts.

Key interactions identified include hydrogen bonding and hydrophobic contacts with critical residues, such as Tyr126 for GCC and Trp168, Thr192, and His216 for LGR5. Additionally, these residues have a role in native ligand binding as well. This interaction profile suggests the compounds function as a potential agonist for GCC and as an antagonist for LGR5. It could help restore tumor suppressor signaling and inhibit the activation of the oncogenic Wnt pathway, respectively.

DISCUSSION

To the best of our knowledge, this study is among the first to systematically evaluate the interaction of bioactive compounds derived from *Heterotrigona itama* propolis with two important colorectal cancer related targets, GCC and LGR5, using a molecular docking approach. While propolis has been widely reported to possess anticancer properties, information regarding the specific compounds responsible for targeting these receptors remains limited.

This study used molecular docking to evaluate seven stingless bee propolis compounds. These compounds were tested as modulators for two receptors in colorectal cancer pathogenesis. The receptors included the tumor suppressor GCC and the cancer stem cell marker LGR5. This performed a drug-likeness analysis based on Lipinski's Rule of Five. The analysis provides early insights into the potential oral bioavailability of the compounds. CAPE and chrysin complied with all five rules. Other compounds including cardol, cardanol, quercetin, anacardic acid, and 9-octadecenoic acid adhered to four criteria. This results in an 80 percent

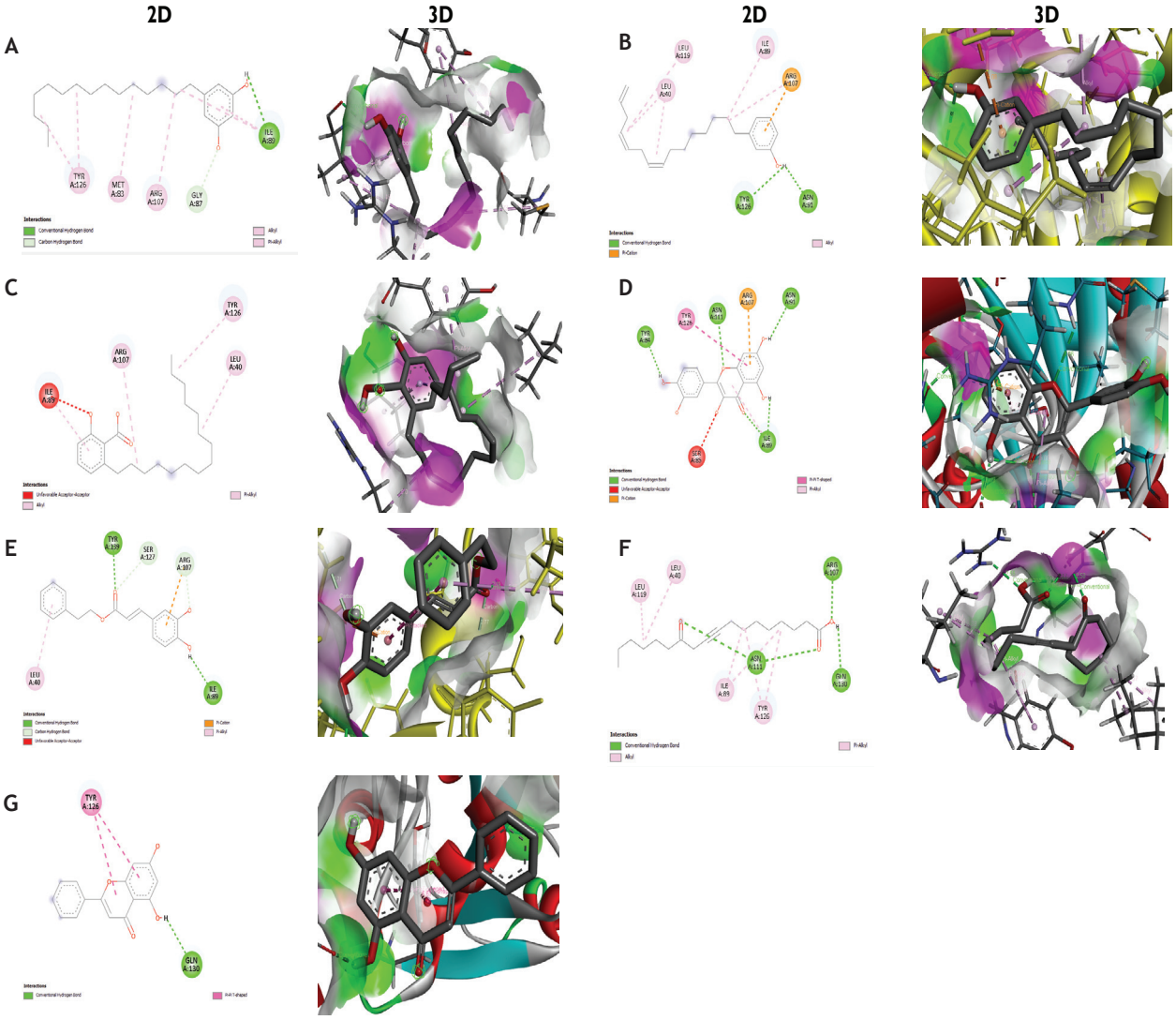

Figure 3. Molecular interaction of propolis compounds with GCC receptor: A. Cardol; B. Cardanol; C. Anacardic acid; D. Quercetin; E. CAPE; F. 9-Octadecenoic acid; G. Chrysin (2D and 3D representations).

compliance rate. Meeting four out of five criteria is generally sufficient for initial drug-likeness evaluations.

Molecular docking was utilized to evaluate seven stingless bee propolis compounds as potential modulators of GCC (a tumor suppressor) and LGR5 (a cancer stem cell marker), both of which are critical in colorectal cancer (CRC) pathogenesis. Based on Lipinski's Rule of Five, the drug-likeness analysis, provides preliminary insight into the potential oral bioavailability of these compounds. While CAPE and Chrysin fully complied with all rules, the other compounds, including the high-affinity Cardanol and Anacardic acid, showed high Log*P* values, indicating lipophilicity that might affect solubility. This does not preclude their therapeutic potential but highlights a consideration for future formulation strategies. Compounds that satisfy all five criteria of Lipinski's Rule of Five are exemplified by CAPE and propolis-derived chrysin, demonstrating 100% compliance. Several other propolis compounds cardol, cardanol, quercetin, anacardic acid, and

Table 5. Interaction analysis of propolis compounds with the LGR5 receptor.

Ligand	Hydrogen Conventional	Carbon-hydrogen	Hydrophobic			π cation/anion
			π sigma	π-alkyl/ alkyl	π-π	
Cardanol	49 Arg 69 Ser	N/A	N/A	117 Lys 118 Val	N/A	93 Glu
Cardol	N/A	192 Thr	N/A	190 Ala	168 Trp 216 His	N/A
Anacardic acid	216 His	166 His	190 Ala	190 Ala 213 Val 214 Val	N/A	N/A
Quercetin	N/A	N/A	N/A	166 His 168 Trp 190 Ala 213 Val 214 Val	168 Trp	N/A
CAPE	167 Leu 216 His	192 Thr	N/A	190 Ala 213 Val 214 Val	168 Trp 190 Ala	N/A
9-Octadecenoic acid	192 Thr 216 His	N/A	N/A	168 Trp 190 Ala	N/A	N/A
Chrysin	167 leu	N/A	N/A	166 His 190 Ala 213 Val 214 Val	168 Trp	N/A

9-octadecenoic acid adhere to four of the five rules, equivalent to a 75% compliance rate. High affinity compounds like cardanol and anacardic acid showed high Log*P* values. This suggests high lipophilicity which might impact solubility. These findings do not eliminate their therapeutic potential. However, they do highlight a need for specific formulation strategies in the future. Lipinski's Rule of Five was used as a qualitative tool to prefer if a compound is suitable for oral formulation. It predicts solubility and membrane permeability. A successful bioactive compound typically has a low molecular weight and moderate lipophilicity (Li, *et al*., 2024; Roskoski, 2019).

Although the similarity of amino acid residues to the native ligand is an important consideration, the primary parameter for assessing a compound's potential is a more negative binding affinity value. The absence of residue similarity does not necessarily indicate an incorrect result. The lowest energy binding conformation can result in different interaction patterns, and stable interactions can occur outside the primary active site. Therefore, the analysis focused on the lowest binding energy configuration as the best predictor of potential biological activity (Gul, *et al*., 2023; Zothantluanga & Chetia, 2022).

GCC is a tumor suppressor receptor in the intestine. Its normal activation by native ligands (guanylin or uroguanylin) triggers cGMP signaling that inhibits proliferation, sequesters β-catenin, and maintains genomic stability. In colorectal cancer, the expression of these native ligands is lost, causing receptor dysregulation and cellular hyperproliferation (Husairi, *et al*., 2020; Kraft, *et al*., 2017; Piroozkhah, *et al*., 2023). Molecular docking results showed that cardanol had a stronger binding affinity (-7.8 and -7.3 kcal/mol) than the native ligand uroguanylin (-5.3 kcal/mol). This suggests cardanol has the potential to act as a GCC agonist, mimicking the function of the lost native ligand. It interacts with key residues (such as Tyr126) at the active site, which can stabilize the receptor conformation and trigger intracellular

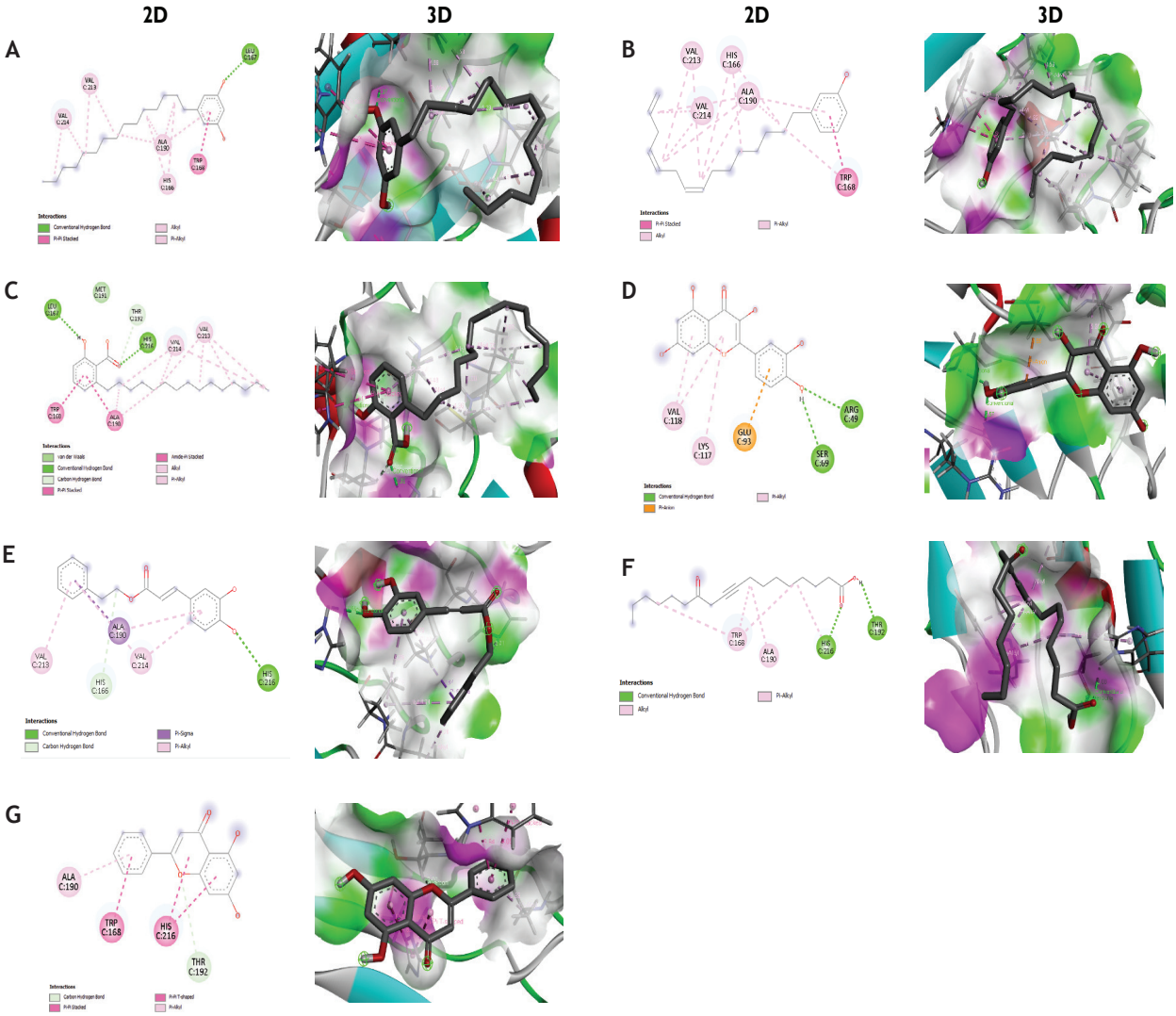

Figure 4. Molecular interaction of propolis compounds with LGR5 receptor: A. Cardol; B. Cardanol; C. Anacardic acid; D. Quercetin; E. CAPE; F. 9-Octadecenoic acid; G. Chrysin (2D and 3D representations).

cGMP signaling. This activation is expected to inhibit cancer cell proliferation, prevent β-catenin accumulation, and promote apoptosis (Piroozkhah, *et al*., 2023; Waldman & Camilleri, 2018).

LGR5 is a cancer stem cell marker that amplifies Wnt/β-catenin signaling, promoting tumor initiation, drug resistance, and recurrence. Overexpression of LGR5 or hyperactivation of its R-spondin ligand leads to β-catenin accumulation and transcription of pro-proliferative genes (such as Cyclin D1) (He, *et al*., 2023; Parasido, *et al*., 2024; Tang, *et al*., 2022; Zhou, *et al*., 2017). Anacardic acid exhibits a strong binding affinity for LGR5 (-6.5 kcal/mol), surpassing that of its native ligand, R-spondin (-5.4 kcal/mol). It binds at the R-spondin binding site, interacting with key residues including

Trp168, Thr192, and His216. This indicates anacardic acid has the potential to be an LGR5 antagonist. By competing for the R-spondin binding site, it can block the formation of the LGR5-R-spondin complex. This is expected to disrupt the amplification of abnormal Wnt signaling, suppress the transcription of pro-tumorigenic genes, reduce cancer stem cell-like properties, increase sensitivity to apoptosis, and ultimately inhibit tumor proliferation and recurrence (He, *et al*., 2023; Parasido, *et al*., 2024; Sawaguchi, *et al*., 2024).

Cardanol exhibited superior binding to GCC, supported by numerous hydrogen and hydrophobic bonds. The similarity in amino acid residues between the test ligand and the native ligand suggests that cardanol binds at a specific site, effectively replacing the lost native ligand (Zothantluanga & Chetia, 2022). These results indicate a strong potential for cardanol to act as a GCC agonist. For the LGR5 receptor, anacardic acid showed stronger binding, which is supported by a high number of hydrophobic and hydrogen bonds. Notably, there is overlap in the amino acid residues involved compared to the native ligand. This finding demonstrates that anacardic acid can specifically bind to the receptor's native ligand-binding site, thereby likely inhibiting the binding of the endogenous ligand R-spondin. Cardanol is predicted to effectively activate GCC-mediated antitumor pathways due to its strong binding at the GCC active site. Conversely, anacardic acid is predicted to inhibit LGR5-induced oncogenic signaling in colorectal cancer more effectively via competitive binding to the receptor.

The primary implication of these findings is the identification of specific natural compounds with high predictive binding to key CRC targets, supporting the rationale for further investigation of stingless bee propolis in cancer chemoprevention. The predictions are based on static protein structures and computational scoring functions, which may not fully capture the dynamics of ligand-receptor interactions in a biological system. Furthermore, the analysis does not account for metabolic stability, toxicity, or cellular uptake. The promising binding affinities observed require empirical validation.

In the present study, molecular docking analysis was specifically conducted at functionally relevant binding sites to better predict the potential biological roles of the tested compounds. For GCC, all compounds were docked at the native ligand-binding site corresponding to the guanylin/uroguanylin interaction region. Compounds that demonstrated favorable binding energies and formed interactions with key amino acid residues involved in receptor activation are therefore predicted to act as potential GCC activators. This approach is supported by the principle that ligand binding at the endogenous activation site may mimic the physiological function of native agonists, thereby promoting downstream signaling pathways associated with intestinal homeostasis and tumor suppression.

Similarly, docking simulations targeting the LGR5 receptor were performed at the R-spondin interaction interface, which plays a critical role in the activation of Wnt signaling. Compounds that bind within this region and exhibit stable interactions with residues involved in native ligand recognition are predicted to interfere with R-spondin binding. Such competitive interactions may result in the inhibition of LGR5-mediated signaling, suggesting a potential antagonistic effect on pathways associated with cancer stem cell maintenance and colorectal cancer progression.

However, it is important to emphasize that these predictions are based solely on computational docking analyses and do not directly confirm functional activity. Therefore, further experimental validation is required to substantiate these findings. In particular, *in vitro* assays such as cyclic guanosine monophosphate (cGMP) assays for GCC activation and Wnt signaling assays for LGR5 inhibition are necessary to confirm the predicted agonistic and antagonistic effects, respectively.

CONCLUSION

This study provides molecular insight into the interaction between bioactive compounds of *Heterotrigona itama* propolis and two colorectal cancer-related receptors, GCC and LGR5, using an *in silico* molecular docking approach. Among the identified compounds, cardanol exhibited the strongest predicted binding affinity toward GCC with a binding energy of −7.3 kcal/mol, whereas anacardic acid demonstrated the highest affinity for the LGR5 receptor (−6.5 kcal/mol). These interactions suggest that specific constituents of *H. itama* propolis may have the potential to modulate molecular targets involved in colorectal cancer progression.

Importantly, this study highlights the potential of stingless bee propolis as a promising source of bioactive compounds for targeting colorectal cancer-associated receptors. The findings also provide a preliminary scientific basis for further pharmacological investigation of propolis-derived compounds. Nevertheless, because the present study is limited to computational prediction, further validation through molecular dynamics simulations, *in vitro* biological assays, and *in vivo* studies is required to confirm the stability, efficacy, and therapeutic relevance of these interactions.

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