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Molecular docking analysis of acetogenin and procyanidin, components of soursop (*Annona muricata Linn.*) seed, as potential anti-cervical cancer agents

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Abstract: Cervical cancer is one of the most prevalent cancers among women. This study aimed to investigate the molecular interactions of acetogenin and procyanidin, compounds found in soursop (*Annona muricata Linn.*) seed extract, as potential anti-cervical cancer agents using a molecular docking approach. The software tools used included Biovia Discovery Studio® 2024, Autodock Tools 1.5.6, Avogadro, pkCSM, PubChem, Notepad++, and Molview. Molecular docking analysis focused on the interaction of these compounds with the human vaccinia-related kinase 2 (VRK-2) protein (PDB ID: 5UU1). The native ligand-5UU1 protein complex exhibited two hydrogen bonds, a binding free energy of -8.84 kcal/mol, and an inhibition constant of 331.88 nM. In comparison, acetogenin formed three hydrogen bonds with 5UU1, achieving a binding free energy of -7.33 kcal/mol and an inhibition constant of 4.21 nM. Similarly, procyanidin also formed three hydrogen bonds, with a binding free energy of -2.99 kcal/mol and an inhibition constant of 6.38 nM. The results indicate that both acetogenin and procyanidin have potential as anti-cervical cancer agents, with acetogenin demonstrating stronger binding affinity and inhibition potential compared to procyanidin.

Keywords: acetogenin, anti-cervical cancer, procyanidin, soursop seed, *Annona muricata* Linn

Introduction

Cervical cancer is one of the most prevalent cancers affecting women worldwide and represents the second leading cause of cancer-related deaths among women in Indonesia, following breast cancer. A primary factor contributing to cervical cancer is infection with the Human Papillomavirus (HPV) [1]. Standard treatments, including radiation, surgery, and chemotherapy, often come with significant side effects and variable success rates, highlighting the urgent need for safer and more effective therapeutic alternatives [2].

This study employs a molecular docking approach to investigate the potential of acetogenin and procyanidin molecules from soursop seed extract as anti-cervical cancer agents. Molecular docking is a computational technique that predicts interactions between bioactive compounds (ligands) and biological targets, such as proteins [3].

The study aims to identify bioactive compounds from soursop seed extract with strong binding affinities to biological targets associated with cervical cancer progression. Through molecular docking analysis, the study evaluates these compounds to determine those with the highest potential as therapeutic agents. Additionally, it examines the molecular interactions between the active compounds and their target proteins to provide insights into their mechanisms of action [4].

Methods

Software and tools

The molecular docking process was conducted using two laptops with the following specifications. Personal computer 1: Intel® Celeron® N4020 Processor CPU @ 1.10GHz (2 CPUs), 4.0 GB RAM, running Windows 10 Home Single Language 64-bit (Build 19042). Personal computer 2: 12th Gen Intel® Core™ i5-1235U CPU @ 1.30 GHz, 16.0 GB RAM, running Windows 11 Home Single Language 64-bit.

The software tools utilized included Biovia Discovery Studio® 2024, Autodock Tools 1.5.6, Avogadro, pkCSM, PubChem, Notepad++, and Molview.

Materials

The 3D structure of the 5UU1 protein was obtained from the Protein Data Bank website (https://www.rcsb.org/). The three-dimensional structures of acetogenin and procyanidin were downloaded from Molview (https://molview.org/) in .mol format and converted into .pdb format using Biovia Discovery Studio®.

Molecular docking

The molecular docking process began with the preparation of the human vaccinia-related kinases protein (PDB ID 5UU1), which involved removing water molecules, residues, and native ligands using Autodock Tools 1.5.6. This step ensured that only the protein and native ligands remained in the **.pdb** file, ready for docking analysis [5]. The compounds used in this study—acetogenin and procyanidin extracted from soursop seeds—were prepared by downloading their three-dimensional structures from the Molview website. These structures were further processed and evaluated for their physicochemical properties using Lipinski's Rule of Five.

The docking method was validated to ensure reliability by analyzing the Root Mean Square Deviation (RMSD) value using Autodock Tools. The method was deemed valid if the RMSD value was ≤ 2 Å, confirming the accuracy of the docking setup [5]. Following validation, a grid box was configured to position the ligand appropriately within the binding site while allowing free movement. The parameters were saved in .gpf format (dock.gpf), and the docking process was conducted in two stages. First, the "Run AutoGrid" option was used to prepare the grid file, and subsequently, the "Run AutoDock" option was executed to complete the docking process. Both stages involved configuring files to the required format before running the analysis, which automatically processed the data until completion.

Once the docking process was completed, the results were analyzed using Notepad++®. The ligand conformation with the smallest binding energy was identified as the best candidate, as it represented the most favorable interaction with the receptor protein. Finally, the ligand-protein complex was visualized using Biovia Discovery Studio®, which allowed for a detailed examination of the molecular interactions between the ligand and the active binding site of the 5UU1 protein.

Results

The structure of the human vaccinia-related kinase 2 (VRK-2) receptor was obtained from the Protein Data Bank (PDB ID: 5UU1). To validate the molecular docking method, the native ligand was re-docked into the 5UU1 protein, yielding an RMSD value of 0.650 Å. Since an RMSD value ≤ 2 Å is considered valid, the method was deemed reliable for further analysis [8]. RMSD (Root Mean Square Deviation) measures the structural similarity between two conformations, with lower values indicating that the new ligand binding site is closer to the crystallographic ligand site [9].

The tested ligands in this study were secondary metabolites from soursop seeds (*Annona muricata Linn.*). The 3D structures of acetogenin and procyanidin were obtained from PubChem and converted into .pdb format for molecular docking. Grid parameter files (GPF) and docking parameter files (DPF) were prepared prior to the docking process, with grid coordinates set at X: 0.539, Y: -18.004, and Z: -13.408 for the VRK-2 receptor.

Key parameters evaluated included the binding free energy (ΔG), inhibition constant (Ki), and amino acid residues involved in ligand-protein interactions. The binding free energy and inhibition constant were used to assess the affinity of the compounds for the receptor. Higher affinity was indicated by lower ΔG values and Ki. Specifically, a lower Ki value corresponds to more negative ΔG, reflecting stronger ligand binding to the receptor [10].

Docking results were analyzed and visualized to determine ligand-receptor interactions. The pharmacokinetic properties of acetogenin and procyanidin were evaluated using Lipinski's Rule of Five to assess their potential as oral drugs. According to this rule, drug molecules must have a molecular weight ≤500 g/mol, a log P value ≤5, hydrogen bond donors ≤5, and hydrogen bond acceptors ≤10 [11]. While procyanidin met these criteria with a molecular weight of 350.29 g/mol, acetogenin, with a molecular weight of 606.92 g/mol, exceeded the threshold, indicating potential limitations in its drug-like properties. Molecular weight is critical as it affects a drug's ability to penetrate biological membranes, with smaller molecules exhibiting better membrane permeability [12].

The figures below illustrate the 3D structures of the protein target and test compounds, as well as the docking results:

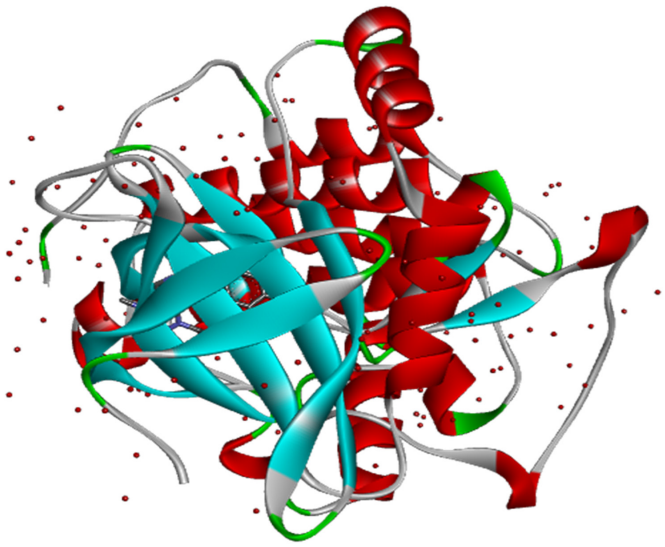

Figure 1. 3D structure of the protein target (PDB ID: 5UU1).

Table 1. Physicochemical properties of soursop seed extract compounds acetogenin and procyanidin

Metabolites	Molecule weight (g/mol)	Log P	HBA	HBD	Violation	GI Absorption	Information
Acetogenin	606.92	7.55	6	2	No; 2 violations: MLOGP>4.15	LOW	Do not meet the requirement
Procyanidin	350.29	2.31	7	1	Yes; 0 violation: MLOGP>4.15	LOW	Meet the requirement

Table 2. Box size, grid box, RMSD, and binding free energy of native ligand

PDB ID	Box Size	Grid Box	RMSD	Free binding energy (kcal/mol)
5UU1	58 x 42 x 42	X: 0.539 Y: -18.004 Z: -13.408	0.650 Å	-8.84

Table 3. Results of molecular docking of procyanidin, acetogenin, and native ligand

No	Ligand	ΔG (kcal/mol)	Constant of inhibition (nM)
1	Procyanidin	-2.99	6.38
2	Acetogenin	-7.33	4.21
3	Native ligand	-8.84	331.88

Tabel 4. Amino acid residues of molecular docking of procyanidin, acetogenin, and native ligand

No.	Ligand	Number of hydrogen bond	Amino acid residues
1	Procyanidin	3 (ASN A:50, ARG A:57, HIS A:58)	ASN A:50, ARG A:57, HIS A:58, LYS A:51, GLU A:53, PRO A:48
2	Acetogenin	3 (LEU A:124, ARG A:123, ASP A:127)	LEU A:124, ARG A:123, ASP A:127, LYS A:61, ALA A:185, VAL A:59, MET A:121, ILE A:43, TYR A:176, ILE A:126
3	Native ligand	2 (LYS A:61, LEU A:124)	LYS A:61, LEU A:124, MET A:121, ALA A:185, VAL A:59, LEU A:173, ILE A:43, ILE A:35

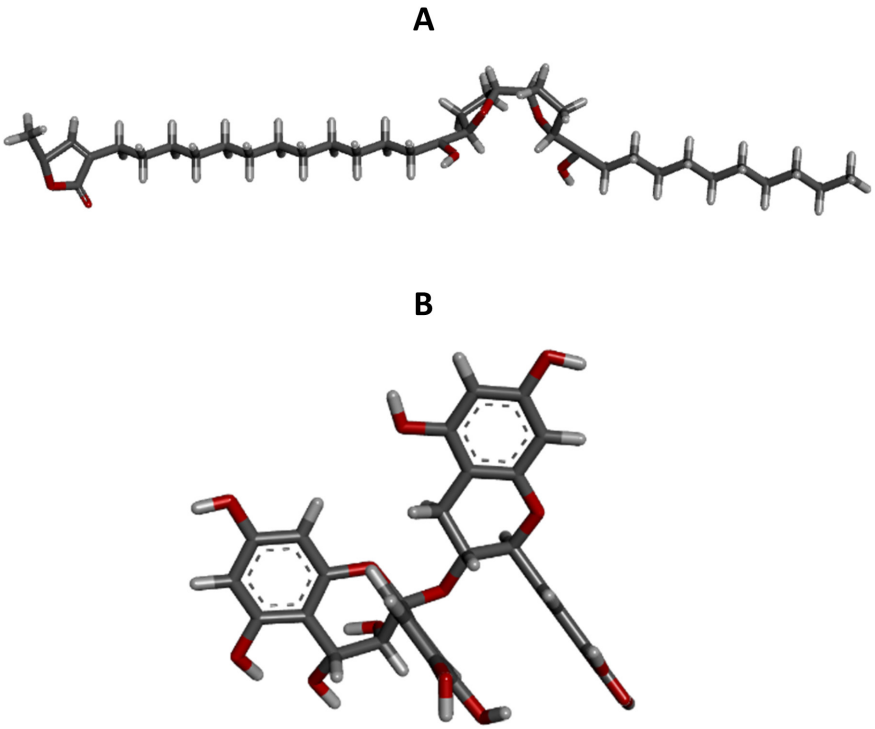

Figure 2. Geometry-optimized structures of test compounds: (a) acetogenin and (b) procyanidin.

Discussion

Lipophilicity and hydrophobicity, as indicated by log P values, reflect the solubility of compounds in fats, oils, lipids, and nonpolar solvents. These properties are critical for oral drugs, as they must traverse the lipid bilayer of the intestinal epithelium. Drugs require an optimal level of hydrophobicity to penetrate this lipid barrier without becoming irreversibly embedded, which could prevent their release and potentially lead to toxicity [13]. In this study, acetogenin exhibited a log P value of 7.55, classifying it as highly hydrophobic, which suggests a tendency to persist in the bloodstream and potentially cause toxicity. In contrast, procyanidin had a log P value of 2.31, meeting the requirement of log P ≤ 5, indicating its suitability for oral delivery.

Hydrogen bond donors and acceptors also influence the pharmacokinetic behavior of drug molecules, as these parameters affect properties like solubility, melting point, and boiling point [14]. Acetogenin and procyanidin both met the criteria for hydrogen bond donors (≤5), with acetogenin having two and procyanidin having one. Similarly, their hydrogen bond acceptors (acetogenin: six, procyanidin: seven) satisfied the threshold of ≤10 [15]. However, the gastrointestinal absorption of acetogenin was limited due to its high molecular weight (606.92 g/mol), which exceeded Lipinski's Rule of Five limit of ≤500 g/mol. Conversely, procyanidin, with a molecular weight of 350.29 g/mol, fulfilled these criteria, making it a promising candidate for oral formulations.

The molecular docking results showed that acetogenin had the lowest binding free energy (-7.33 kcal/mol) among the tested compounds, indicating a more stable interaction with the 5UU1 protein compared to procyanidin (-2.99 kcal/mol). However, the native ligand of the 5UU1 protein complex exhibited the most stable binding free energy (-8.80 kcal/mol). The inhibition constant for acetogenin was 4.21 nM, suggesting strong receptor inhibition, whereas procyanidin had an inhibition constant of 6.38 nM. These values demonstrate that while acetogenin interacts more stably with the receptor, procyanidin also exhibits notable affinity and inhibition potential.

Hydrogen bonding analysis revealed that both acetogenin and procyanidin formed three hydrogen bonds with the 5UU1 receptor, compared to two for the native ligand. Specifically, acetogenin interacted with LEU124, ARG123, and ASP127 residues, while procyanidin bonded with ASN50, ARG57, and HIS58. These hydrogen bonds contribute to the stability of the ligand-receptor complex, as they enhance specificity

and affinity [18, 19]. In addition to hydrogen bonds, hydrophobic interactions were observed, playing a role in ligand stability at the receptor binding site. The interaction sites for all tested compounds overlapped with those of the native ligand, indicating a shared active binding site on the 5UU1 protein [24].

The interactions between each test compound and the 5UU1 protein target were analyzed through docking scores and visualizations. The binding energy values and inhibition constants, obtained from the detailed **.dlg** files generated by AutoDock, were used to evaluate the stability and efficacy of the ligand-receptor interactions. Lower binding energy values indicate greater stability of the bond formed between the ligand and the receptor, while lower inhibition constants reflect more effective inhibition of receptor activity.

Visualization using Biovia Discovery Studio® software revealed the number of hydrogen bonds and amino acid residues involved in the ligand-receptor interactions [17]. Among the tested compounds, the acetogenin-5UU1 complex exhibited the lowest binding free energy value (-7.33 kcal/mol) and an inhibition constant of 4.21 nM. In comparison, the procyanidin-5UU1 complex had a binding free energy value of -2.99 kcal/mol and an inhibition constant of 6.38 nM. The native ligand-5UU1 complex had the lowest binding free energy value (-8.80 kcal/mol) and an inhibition constant of 334.10 nM. These results indicate that acetogenin forms a more stable interaction with the target receptor than procyanidin, although it is less stable than the native ligand. Based on these findings, acetogenin shows potential as a candidate drug for cervical cancer treatment in molecular docking studies.

Hydrogen bonding analysis further supported these observations. The native ligand formed two hydrogen bonds with the 5UU1 protein, while both acetogenin and procyanidin formed three hydrogen bonds. Acetogenin interacted with LEU124, ARG123, and ASP127, while procyanidin formed hydrogen bonds with ASN50, ARG57, and HIS58. Hydrogen bonds, which occur between positively charged hydrogen atoms and electronegative atoms such as oxygen or nitrogen, contribute significantly to the stability and specificity of ligand-receptor complexes [18–20]. An increase in the number of hydrogen bonds enhances the stability of these interactions [21].

In addition to hydrogen bonding, hydrophobic interactions were observed, which play a crucial

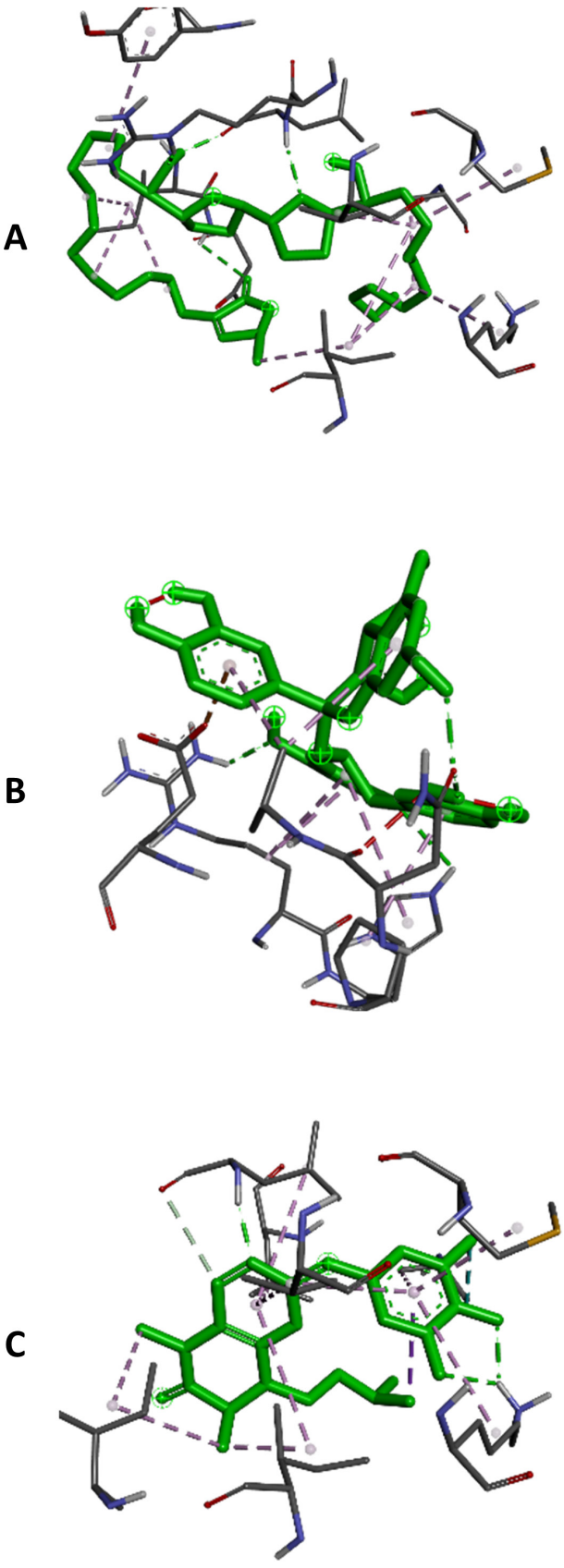

Figure 3. 3D structures of ligands and receptor: (a) acetogenin, (b) procyanidin, and (c) native ligand.

role in maintaining the stability of ligand-receptor complexes at the binding site. These interactions reduce nonpolar residue exposure to water and facilitate ligand stabilization. Other non-binding interactions, such as van der Waals forces, molecular shape complementarity, and hydrophobic effects, also influence ligand specificity and binding affinity [22, 23].

The molecular docking analysis showed that procyanidin formed three hydrogen bonds with ASN A:50, ARG A:57, and HIS A:58 and involved additional residues such as LYS A:51, GLU A:53, and PRO A:48. Acetogenin also formed three hydrogen bonds with LEU A:124, ARG A:123, and ASP A:127 and interacted with residues including LYS A:61, ALA A:185, VAL A:59, MET A:121, and ILE A:43. The native ligand formed two hydrogen bonds with LYS A:61 and LEU A:124 and interacted with additional residues such as MET A:121, VAL A:59, and ALA A:185. The shared binding residues between the test compounds and the native ligand suggest that the active binding site on the 5UU1 receptor is consistent across ligands [24].

Conclusion

While acetogenin demonstrated stronger binding and inhibition properties, its pharmacokinetic limitations, including excessive hydrophobicity and high molecular weight, restrict its suitability as an oral drug. Procyanidin, despite lower binding energy, fulfills Lipinski's criteria, suggesting better absorption and potential for oral formulations. Further in vitro and in vivo studies are recommended to confirm the pharmacological activity and therapeutic potential of both compounds for cervical cancer treatment.

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Author contributions

Conceptualization, AHS, NCP, WNA; Methodology, NCP, RFF, TF, EDM, GMBK, MAN, PV, WNA; Investigation, WNA, AHS; Writing – Original Draft, NCP, RFF, TF, EDM, GMBK, MAN, PV, AHS; Writing – Review & Editing, AHS

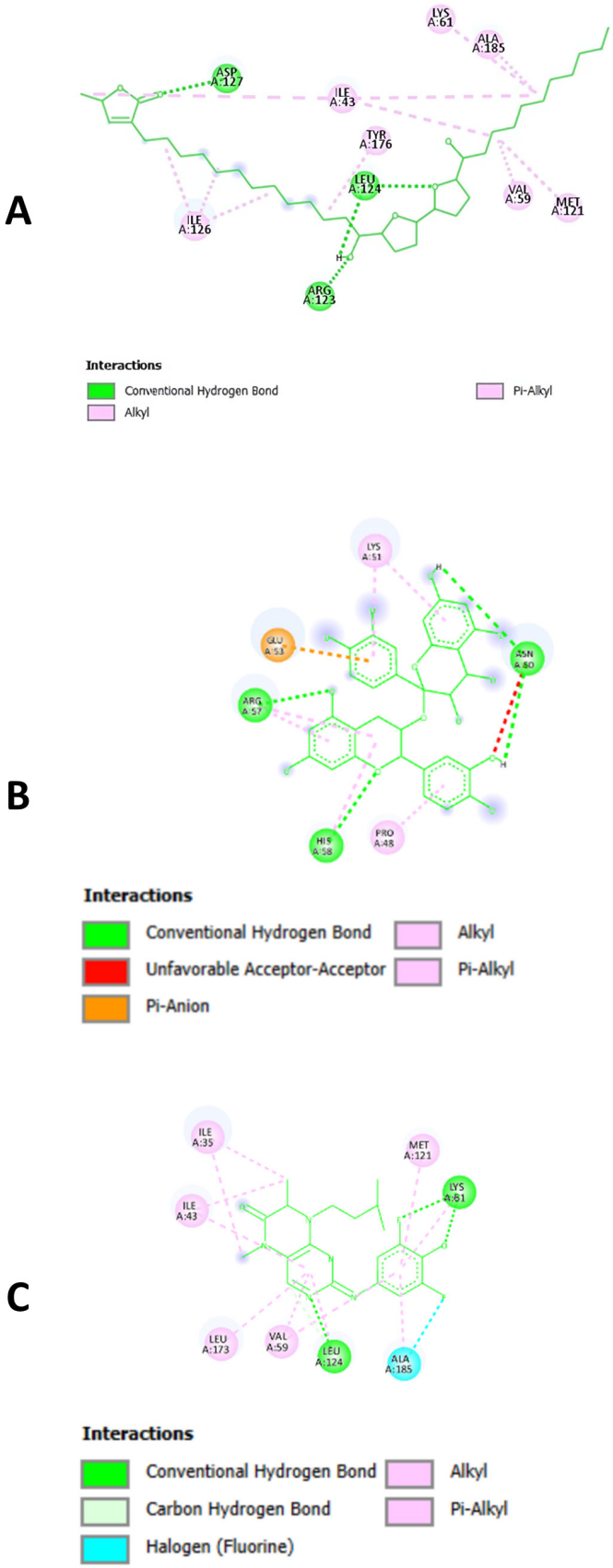

Figure 4. 2D docking visualization of ligand-receptor interactions: (a) acetogenin, (b) procyanidin, and (c) native ligand.

Declaration of interest

The authors declare that none of them has any conflict of interest with any private, public or academic party related to the information contained in this manuscript

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