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# In Silico Study of Acetogenin Compounds from Soursop (*Annona muricata*) Leaves as Sodium-Glucose Cotransporter-2 (SGLT2) Inhibitors

## Studi In Silico Senyawa Acetogenin dari Daun Sirsak (Annona muricata) Sebagai Inhibitor Sodium-Glucose Cotransporter-2 (SGLT2)

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***ABSTRACT***

*Acetogenin derived from soursop (Annona muricata) leaves are known to have antidiabetic and anticancer activities. Nevertheless, there has been no study related to the compounds found in A. muricata leaves, such as acetogenin, as SGLT2 inhibitors. This research aims to investigate the activity of acetogenin compounds as SGLT2 inhibitors while maintaining low selectivity against SGLT1 using molecular docking methods using Molegro Virtual Docker (MVD). Based on the Rerank score, five acetogenin compounds, namely muricin H, annonacin A, annopentocin B, murihexocin C, and corossolone, are predicted to be SGLT2 inhibitors with better selectivity compared to empagliflozin. Among these five compounds, muricin H and corossolone exhibit the most similarity in interaction with amino acid residues in the SGLT2 A-chain compared to empagliflozin. In silico ADMET analysis results indicate that both compounds have absorption, distribution, and metabolism capabilities, similar to empagliflozin. However, it should be noted that both compounds are more toxic, with muricin H predicted to have hepatotoxic properties.*

***Keywords*****:** *Annona muricata, SGLT2, antidiabetic, acetogenin, molecular docking*

**ABSTRAK**

Acetogenin yang berasal dari daun sirsak (*Annona muricata*) diketahui memiliki aktivitas antidiabetik dan antikanker. Meski demikian, belum ada penelitian terkait senyawa yang terdapat pada daun *A. muricata* tersebut sebagai inhibitor SGLT2. Penelitian ini bertujuan untuk mengetahui aktivitas senyawa acetogenin sebagai inhibitor SGLT2 namun dengan selektivitas yang rendah terhadap SGLT1 secara *in silico* menggunakan *Molegro Virtual Docker* (MVD). Berdasarkan hasil analisis penambatan molekuler tersebut didapatkan *Rerank Score* lima senyawa acetogenin yaitu muricin H, annonacin A, annopentocin B, murihexocin C, dan corossolone diprediksi merupakan inhibitor SGLT2 dengan selektivitas yang lebih baik dibandingkan empagliflozin. Di antara kelima senyawa tersebut, muricin H dan corossolone menunjukkan kemiripan interaksi dengan residu asam amino pada rantai A SGLT2 dibandingkan dengan empagliflozin. Hasil analisis ADMET secara *in silico* menunjukkan bahwa kedua senyawa tersebut memiliki kemiripan dalam kemampuan absorpsi, distribusi, dan metabolisme dengan empagliflozin. Namun demikian, kedua senyawa tersebut diprediksi bersifat lebih toksik, dengan muricin H diperkirakan memiliki sifat hepatotoksik

**Kata kunci:** *Annona muricata, SGLT2, antidiabetes, acetogenin, penambatan molekuler*

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## INTRODUCTION

Several studies indicate that individuals with type 2 diabetes may have a higher risk of developing breast cancer, particularly after menopause, and vice versa (Jafari et al., 2021; Lipscombe et al., 2013; Wang et al., 2020). Type 2 diabetes is often associated with insulin resistance, which affects hormone levels in the body. Elevated levels of insulin and insulin-like growth factor (IGF) have the potential to influence the growth of breast cells. (Christopoulos et al., 2015). Diabetes is also frequently associated with chronic inflammation, which can contribute to the development of breast cancer. Additionally, individuals with type 2 diabetes tend to experience obesity, further increasing the risk of breast cancer (Kim & Scherer, 2021).

Soursop leaf extract (*Annona muricata*) is known to have multiple therapeutic potentials, including anticancer and antidiabetic properties (Mutakin et al., 2022). *A. muricata* leaves contain active compounds, such as acetogenin groups, which possess antioxidant and anti-inflammatory properties. (Coria-Téllez et al., 2018). This activity helps combat cellular damage that triggers breast cancer (Eketunde, 2020). Several studies indicate that *A. muricata* leaf extract can also inhibit cell proliferation and induce apoptosis, which are critical steps in controlling breast cancer progression (Hadisaputri et al., 2021; J. Y. Kim et al., 2018). Additionally, *A. muricata* leaves have shown the potential to inhibit angiogenesis and block the nutrient supply to breast cancer cells (Syed Najmuddin et al., 2016). *A. muricata* leaf extract is also known to enhance the sensitivity of breast cancer cells to chemotherapy (Salsabila et al., 2021). This allows for lower doses of chemotherapy to achieve the same effect, thereby reducing the negative impact of the treatment.

Methanol extract of *A. muricata* leaves is known to inhibit α-glucosidase activity, while dichloromethane extract is more effective at inhibiting α-amylase activity (Agu et al., 2019). In an in vivo study, methanol extract of *A. muricata* leaves was found to improve metabolic parameters such as reducing total cholesterol (TC), triglycerides (TG), LDL, and VLDL, while increasing HDL levels and the advanced atherogenic index (AAI) in streptozotocin-induced diabetic rats (Adeyemi et al., 2008). Furthermore, a clinical study demonstrated a positive adjuvant effect of lowering blood sugar levels in type 2 diabetes patients who were administered a combination of glibenclamide and ethanol extract of *A. muricata* leaves, compared to those given glibenclamide alone (Arroyo J et al., 2009).

Sodium-glucose cotransporter-2 (SGLT2) inhibitors are a new class of oral antihyperglycemic agents approved for the treatment of diabetes mellitus. SGLT2 inhibitors work by reducing glucose reabsorption by the renal tubules, thereby lowering blood glucose levels without stimulating insulin release (Hsia et al., 2017). This transporter is an ideal target for diabetes treatment because it is responsible for approximately 90% of glucose reabsorption (Shubrook et al., 2015; Triplitt & Cornell, 2015). Nevertheless, SGLT1 is responsible for the remaining 10% of glucose reabsorption. SGLT2 inhibitors have also been shown to exhibit anticancer effects in several malignancies, including breast, liver, pancreatic, thyroid, prostate, and lung cancers (Basak et al., 2023). In MCF-7 breast cancer cells, SGLT2 expression was detected using RT-PCR and immunohistochemistry, and ipragliflozin, an SGLT2 inhibitor, at concentrations of 1-50 μM significantly and dose-dependently suppressed MCF-7 cell growth. Ipragliflozin is thought to induce hyperpolarization of the MCF-7 cell membrane and inhibit glucose absorption into the cells (Komatsu et al., 2020). Inhibition of glucose absorption into cancer cells means that the cancer cells will experience a nutrient deficiency, which can inhibit their proliferation.

Based on the explanations above, the activity of compounds derived from soursop (*A. muricata*) leaf extract as SGLT2 inhibitors is intriguing for further research. This is to ascertain whether the antidiabetic and breast cancer inhibitory activities of soursop leaf extract can be

linked to its compound's activity as an SGLT2 inhibitor. Additionally, this research aims to discover new SGLT2 inhibitors derived from plants. The Food and Drug Administration (FDA) has issued warnings regarding the use of SGLT2 inhibitors such as canagliflozin and dapagliflozin, which have been reported to cause acute kidney injury (Center for Drug Evaluation and Research, 2023). Canagliflozin is also known as an SGLT1 inhibitor, and inhibition of SGLT1 can lead to gastrointestinal complications, such as severe diarrhea (Shubrook et al., 2015; Sokolov et al., 2020). Therefore, high selectivity towards SGLT2 may be more pharmacologically beneficial.

In this study, testing of metabolite compound activity from soursop leaf (*A. muricata*) as an SGLT2 inhibitor was performed in silico using Molegro Virtual Docker (MVD). The metabolite compounds tested from soursop leaves belong to the acetogenin group. An analysis of compound content in *A. muricata* mentions approximately 47 acetogenin compounds present in its leaves (Abdul Wahab et al., 2018). These compounds are known to have anticancer activity, including against MCF-7 cells (Jacobo-Herrera et al., 2019). Acetogenins are the only lactone compound group found in soursop leaves, predominantly soluble in acetone and methanol (López-Romero et al., 2022). n this study, candidate SGLT2 inhibitor compounds of acetogenin origin produced in silico with MVD were then subjected to molecular docking tests against SGLT1 to assess their selectivity towards SGLT2. In silico absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis of SGLT2 inhibitor candidates was conducted using pkCSM. pkCSM is an in silico screening approach for predicting five classes of pharmacokinetic properties: absorption, distribution, metabolism, excretion, and toxicity (ADMET). These predictions are based on graph-based signatures by encoding distance patterns between atoms used to represent small molecules and training predictive models (Pires et al., 2015).

## METHOD

### Materials

The 3D structures or conformations of 47 acetogenin compounds (Abdul Wahab et al., 2018), were searched in the PubChem database (S. Kim et al., 2023) and saved in Structure Data File (SDF) format. Energy minimization of the 3D structures was performed using Datawarrior (Sander et al., 2015). Crystal structures of SGLT2 (PDB ID: 7VSI) and SGLT1 (PDB ID: 7WMV) were obtained from the RCSB PDB database (www.pdb.org) and saved in Protein Data Bank (PDB) file format. In silico testing of acetogenin compounds as SGLT2 and SGLT1 inhibitors was conducted using Molegro Virtual Docker (MVD) (Free trial). The computer specifications used were Windows 11 Pro, Intel(R) Core (TM) i7-8665U CPU @ 1.90 GHz - 2.11 GHz, with 16.0 GB RAM.

**Procedures** (Bitencourt-Ferreira & de Azevedo, 2019)

### Preparation of protein and ligan

The crystal structures of proteins downloaded from the RCSB PDB database were imported into Molegro Virtual Docker (MVD). For the purpose of molecular docking, all water molecules were removed and non-ideal amino acid residues were repaired. Ligand preparation was conducted using Datawarrior to find the most stable conformation or the one with the lowest energy (Merck Molecular Force Field, MMFF94) value.

### Detection of SGLT2 *cavity*

The ligand binding cavity or potential binding site of SGLT2 (PDB ID: 7VSI) was predicted using MVD. A cavity with a volume of 1018.37 $Å^3$ and a surface area of 2237.44 $Å^2$ was predicted

as the cavity occupied by 7R3 (empagliflozin) as the native ligand in chain A of the SGLT2 crystal structure (7VSI). This binding site was defined within a constraint sphere with a radius of 20 and centered at X: 42.00, Y: 59.78, Z: 52.51. The MolDock grid score was set with a grid resolution of 0.30. The determination of the binding site of SGLT1 (7WMV) was also conducted using MVD based on the template of the native ligand (LX2761) present in its crystal structure.

**Analysis of Ligand-Protein Interactions with Molecular Docking**

Molecular docking was performed using MVD on all 3D molecules of acetogenin compounds from *A. muricata* leaves against the protein crystal structures. Prior to this, a re-docking process of the 3D molecule empagliflozin (7R3) against 7VSI and the ligand LX2761 (1YI) against 7WMV was conducted on the protein binding site to validate the docking method used. A Root Mean Square Deviation (RMSD) value of < 2 Å was set as the validity criterion for the method (Cole, Murray, Nissink, Taylor, & Taylor, 2005). Molecular docking was then carried out on the 3D structures of acetogenin against the protein crystal structures. The parameters measured in the docking process included the energy values involved, such as MolDock Score, Rerank Score, and Hbond. To measure the strength of the ligand-receptor protein binding, the commonly used parameter is the Rerank Score (Molegro ApS., 2011).

**In Silico ADMET Analysis Using pkCSM** (Pires et al., 2015)

ADMET analysis was conducted on acetogenin compounds predicted to be high-selectivity SGLT2 inhibitor candidates. High selectivity means that the ligand interacts weaklier with SGLT1 compared to its native ligand. The strength of the ligand-protein interaction was determined based on the Rerank Score (Molegro ApS, 2011).

## RESULTS AND DISCUSSION

**Preparation of protein and ligan**

In the PubChem database, 27 out of the 47 acetogenin compounds reported by Abdul Wahab et al. (2018) were found. The 3D structures of these 27 compounds were downloaded and their energies were minimized using MMFF94. The native ligand (7R3) in the crystal structure of SGLT2 (7VSI) and the energy-minimized 3D conformations of the 27 acetogenin compounds were imported into MVD. SGLT2 has two protein chains, with the native ligand located on chain A (7VSI [A]). The 3D structures of the native ligand and protein 7VSI [A], as well as one example of a prepared acetogenin ligand (annonacin A), are shown in Figure 1.

**Detection of SGLT2 cavity**

The cavity in protein 7VSI [A] occupied by the native ligand (empagliflozin/7R3) can be seen in Figure 2a. There are five cavities in 7VSI [A], one of which is occupied by the native ligand. The interaction between the native ligand and the binding site in the protein cavity of 7VSI [A] is shown in Figure 2b. Figure 2b shows that the cavity volume is larger compared to the native ligand. However, when compared to the size of one of the tested acetogenin compounds (annonacin A), the difference in size between the protein cavity and annonacin A is not as significant as the difference when compared to the native ligand.

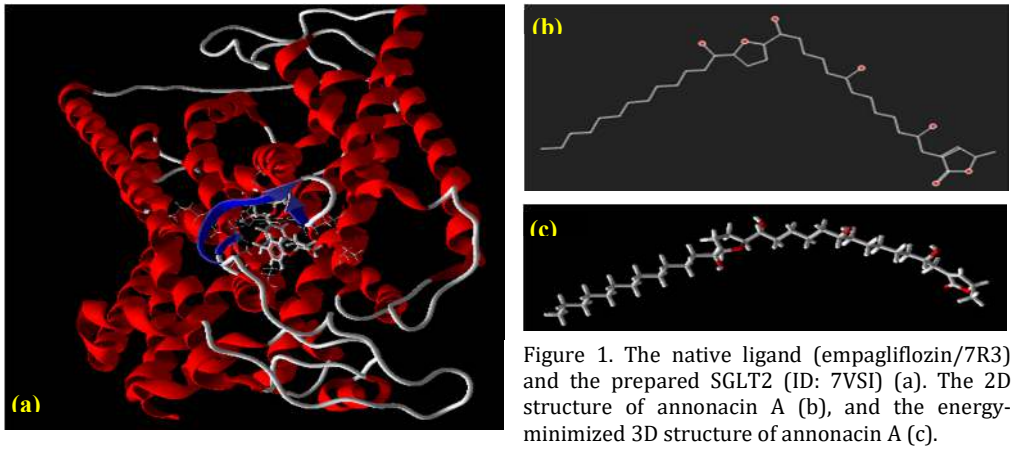


Figure 1. The native ligand (empagliflozin/7R3) and the prepared SGLT2 (ID: 7VSI) (a). The 2D structure of annonacin A (b), and the energy-minimized 3D structure of annonacin A (c).

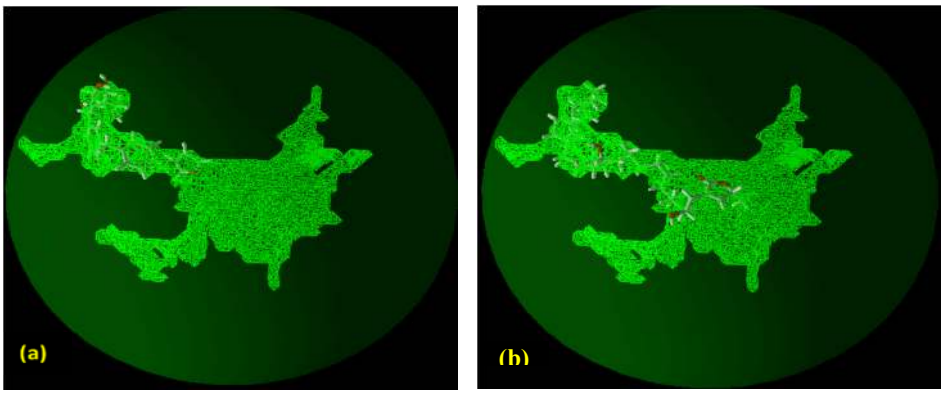


Figure 2. Interaction between the binding site of the protein cavity 7VSI [A] with the native ligand (a), and with annonacin A (b).

**Analysis of Ligand-Protein Interactions with Molecular Docking**

The validation results of the interaction between 7VSI [A] and the native ligand (empagliflozin), and between 7WMV and the native ligand (LX2761), are shown in Figure 3 and Table 1. The LX2761 ligand is located in one of the cavities on the A chain of the 7WMV protein. Based on the redocking process of the native ligands with proteins 7VSI [A] and 7WMV [A], it was observed that many hydrogen bonds and steric-electrostatic interactions occurred at the same amino acid residues. The RMSD values from the redocking process of the native ligands with proteins 7VSI [A] and 7WMV [A] were below 2 Å, indicating that the molecular docking method used is valid. After validating the molecular docking method, the next step involved docking 27 acetogenin compounds with protein 7VSI [A]. The results of the molecular docking are presented in Table 2.

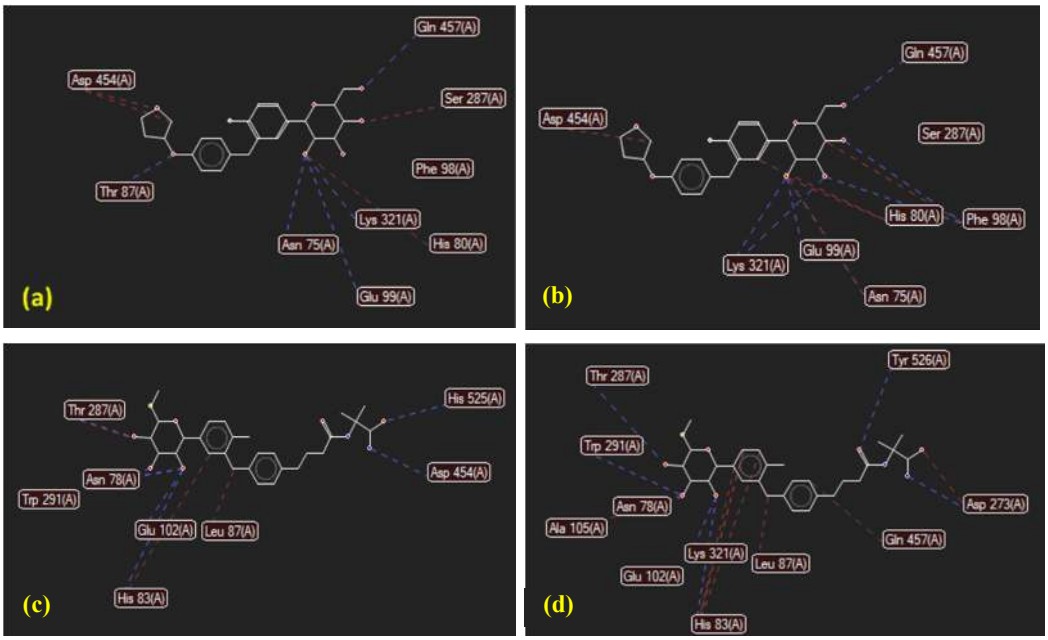


Figure 3. Interactions of SGLT2 (7VSI [A]) with the native ligand (empagliflozin/7R3) in its crystal structure (a) and redocking results (b). Interactions of SGLT1 (7WMV [A]) with the native ligand (LX2761/1YI) in its crystal structure (c) and redocking results (d). Blue dashed lines represent hydrogen bonds, and red lines indicate steric-electrostatic interactions.

Based on the molecular docking results of acetogenin compounds against 7VSI [A] (chain A of SGLT2) as shown in Table 2, seven acetogenin compounds have lower Rerank Scores compared to empagliflozin (native ligand of 7VSI [A]). Subsequently, molecular docking was performed on these seven compounds against SGLT1 (7WMV [A]). The results of molecular docking can be seen in Table 3. Table 3 shows that muricin H, annonacin A, annopentocin B, murihexocin C, and corossolone have higher Rerank Scores (less negative) compared to empagliflozin. This indicates that these compounds interact less stably with 7WMV [A], making them potential SGLT2 inhibitors that are more selective than empagliflozin. The interactions of muricin H, annonacin A, annopentocin B, murihexocin C, and corossolone with 7VSI [A] can be seen in Figure 4 and Table 4.

Table 2. Results of molecular docking of acetogenin compounds against 7VSI [A] (chain A of SGLT2)

| Name | MolDock Score | Rerank Score | HBond |
|---|---|---|---|
| [00]muricin H | -216.999 | -167.622 | -11.76 |
| [00]annonacin A | -206.455 | -159.984 | -7.327 |
| [00]murihexocin C | -205.28 | -157.195 | -2.42609 |
| [00]annopentocin B | -192.931 | -156.578 | -8.90259 |
| [00]corossolone | -210.607 | -154.077 | -11.9450 |
| [00]annocatacin B | -200.385 | -150.579 | -5.89565 |
| [00]muricapentocin | -195.023 | -147.171 | -7.80722 |

| Name | MolDock Score | Rerank Score | HBond |
|---|---|---|---|
| [02]empagliflozin | -169.098 | -147.119 | -8.28999 |
| [02]aromin | -187.254 | -147.084 | -1.84273 |
| [00]muricin I | -193.050 | -145.027 | -5.51648 |
| [00]annohexocin | -181.214 | -138.351 | -7.03262 |
| [01]annopentocin A | -179.984 | -137.446 | -11.9109 |
| [01]annomuricin A | -182.463 | -137.277 | -2.84719 |
| [02]muricatalin | -170.262 | -136.661 | -10.3831 |
| [00]muricatocin B | -175.093 | -136.341 | -8.60114 |
| [02]annomuricin C | -174.932 | -135.510 | -9.42108 |
| [00]muricoreacin B | -178.341 | -134.411 | -11.3479 |
| [02]isoannonacin | -176.919 | -131.715 | -1.74628 |
| [01]annocatalin | -167.262 | -130.070 | -4.94534 |
| [03]cis-solamin | -160.310 | -127.925 | -3.97226 |
| [02]annopentocin C | -165.333 | -123.292 | -7.19578 |
| [02]annomuricin E | -161.557 | -122.052 | -11.9893 |
| [03]muricatalicin | -164.649 | -118.071 | -13.0942 |
| [03]gigantecin | -164.446 | -117.268 | -4.72738 |
| [03]annomutacin | -170.698 | -116.847 | -7.63225 |
| [02]annonacinone | -135.646 | -92.9987 | -7.64199 |
| [01]cis-corossolone | -116.264 | -85.9066 | -1.26184 |

Table 3. Results of molecular docking of acetogenin compounds against 7WMVI [A] (chain A of SGLT1)

| Name | MolDock Score | Rerank Score | HBond |
|---|---|---|---|
| [01]annocatacin B | -203.510 | -156.510 | -4.86313 |
| [00]1YI_701 [A] | -175.836 | -156.237 | -11.3838 |
| [00]muricapentocin | -210.681 | -149.859 | -5.76509 |
| [00]empagliflozin | -172.491 | -146.300 | -9.67234 |
| [00]annonacin A | -180.286 | -139.678 | -7.06384 |
| [02]annopentocin B | -166.440 | -138.724 | -5.5577 |
| [04]muricin H | -171.465 | -131.758 | -9.17102 |
| [02]murihexocin C | -159.557 | -118.997 | -6.53717 |
| [00]corossolone | -153.635 | -116.481 | -3.74573 |

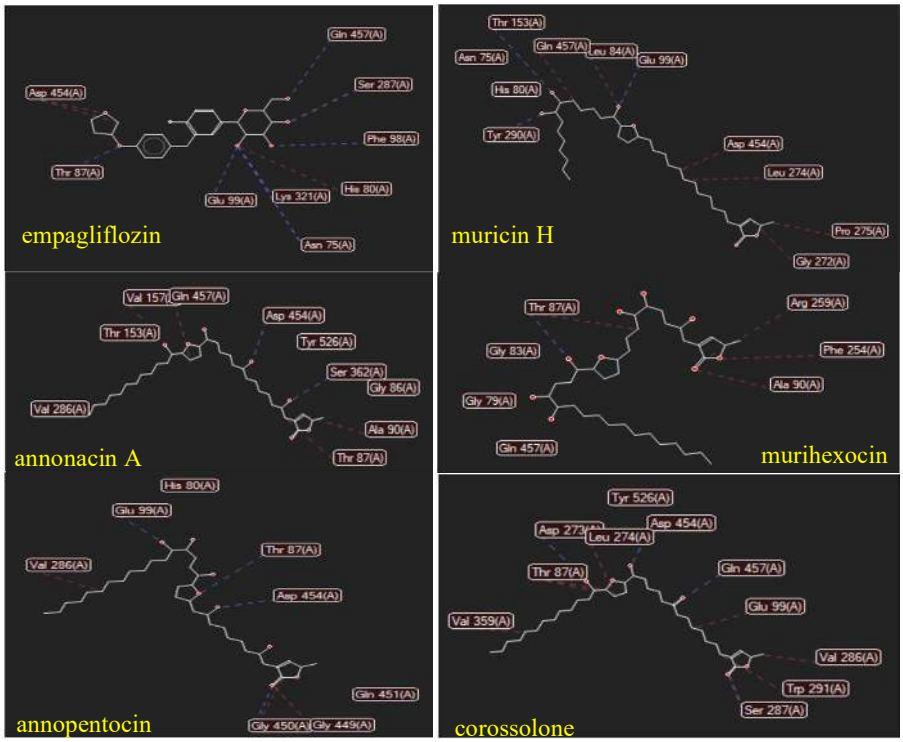


Figure 4. Interactions of five acetogenin compounds and the native ligand (empagliflozin) with SGLT2 (7VSI [A]). Blue dashed lines represent hydrogen bonds, and red lines indicate steric-electrostatic interactions.

In Figure 4, it can be seen that empagliflozin as the native ligand interacts with 7VSI [A] through five hydrogen bonds and four steric-electrostatic interactions. Table 4 shows that muricin H and corossolone interact with the same five amino acid residues of 7VSI [A] as empagliflozin. This indicates that muricin H and corossolone are compounds that exhibit the most similar interactions with SGLT2 (7VSI [A]) compared to empagliflozin. Thus, both compounds are the most promising acetogenin compounds from *A. muricata* leaves as candidates for SGLT2 inhibitors with high selectivity. ADMET analysis of muricin H and corossolone was then conducted using pkCSM.

Table 4. Interactions of acetogenin compounds with amino acid residues of 7VSI [A]

| Interaction of amino acids | Empagli-flozin | Muricin H | Annonacin A | Murihe-xocin C | Annopentocin B | Corosso-lone |
|---|---|---|---|---|---|---|
| Asn 75(A) | □ | □ | - | - | - | - |
| His 80(A) | □ | □ | - | - | □ | - |
| Thr 87(A) | □ | - | □ | □ | □ | □ |
| Phe 98(A) | □ | - | - | - | - | - |
| Glu 99(A) | □ | □ | - | - | □ | □ |
| Ser 287(A) | □ | - | - | - | - | □ |
| Lys 321(A) | □ | - | - | - | - | - |
| Asp 454(A) | □ | □ | □ | - | □ | □ |
| Gln 457(A) | □ | □ | □ | □ | - | □ |

**Notes: □ (H bond interaction); □ (steric-electrostatic interaction)**

Table 5 shows the ADMET predictions for empagliflozin, muricin H, and corossolone. Muricin H exhibits the lowest solubility in water, while empagliflozin shows the highest. All three compounds are predicted as substrates of P-glycoprotein, as well as inhibitors of P-glycoprotein I and II. The distribution of all three compounds is predicted to be less permeable in tissues. However, corossolone shows better permeability across the blood-brain barrier and central nervous system (CNS) compared to empagliflozin and muricin H. Regarding metabolism, all three compounds are predicted to be substrates of the CYP3A4 isoform and not inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4, indicating no potential inhibition of detoxification by cytochrome P450. Total clearance (CLTOT) of muricin H and corossolone is predicted to be better than empagliflozin. However, based on toxicity parameters such as LD50 and Oral Rat Chronic Toxicity (LOAEL), both acetogenin compounds are predicted to be more toxic than empagliflozin. Additionally, muricin H is predicted to exhibit hepatotoxic properties.

MVD recommends using Rerank Score for evaluating molecular docking results. Rerank Score is a measure of binding affinity that is a linear combination of the energy released from ligand-protein interactions (Einter) and the internal energy of the ligand (Eintra) (Molegro ApS., 2011) By incorporating the internal energy of the ligand (bond torsion, sp2-sp2 bond, hydrogen bond, van der Waals interactions, and electrostatic interactions), Rerank Score enhances the accuracy of ligand pose selection. A more negative Rerank Score indicates a more stable binding formed between the ligand and the receptor. A lower Rerank Score can also be associated with increased compound/ligand activity. The activity coefficient can be directly linked to Gibbs free energy or the energy released from ligand-receptor interactions (Ingenmey et al., 2019).

Table 5. Results of ADMET analysis of empagliflozin, muricin H, and corossolone using pkCSM

| **Properties** | **Predicted values** | | | **Criteria** |
|---|---|---|---|---|
| | **Empagliflozin** | **Muricin H** | **Corossolone** | |
| **Absoprtion** | | | | |
| Water solubility | -3.56 | -5.313 | -4.997 | log mol/L << (less solubility) |
| Caco2 permeability | (-0.019x10 $^{-6}$) | (0.501x10 $^{-6}$) | (0.529x10 $^{-6}$) | Papp < 8x10 $^{-6}$ cm/s (low) |
| Intestinal absorption (human) | 55.879 | 76.423 | 87.9 | % Diserap > 30% (high) |
| Skin Permeability | -2.744 | -2.732 | -2.724 | log Kp < -2.5 (high) |
| P-glycoprotein substrate | Yes | Yes | Yes | -- |
| P-glycoprotein I inhibitor | Yes | Yes | Yes | -- |
| P-glycoprotein II inhibitor | Yes | Yes | Yes | -- |
| **Distribution** | | | | |
| VDss (human) | -0.387 | -0.718 | -0.816 | log L/kg <-0.15 (low) |
| *Fraction unbound* | 0.073 | 0.047 | 0.039 | -- |
| BBB permeability | -1.101 | -1.433 | -0.542 | log BB<-1 (low) |
| CNS permeability | -3.515 | -3.132 | -2.975 | log PS <-3.0 (low) |
| **Metabolism** | | | | |
| CYP2D6 substrate | No | No | No | -- |
| CYP3A4 substrate | Yes | Yes | Yes | -- |
| CYP1A2 inhibitor | No | No | No | -- |

| Properties | Predicted values | | | Criteria |
|---|---|---|---|---|
| | Empagliflozin | Muricin H | Corossolone | |
| **Absoprtion** | | | | |
| CYP2C19 inhibitor | No | No | No | -- |
| CYP2C9 inhibitor | No | No | No | -- |
| CYP2D6 inhibitor | No | No | No | -- |
| CYP3A4 inhibitor | No | No | No | -- |
| **Excretion** | | | | |
| Total Clearance | 0.404 | 1.791 | 1.740 | -- |
| Renal OCT2 substrate | No | No | No | -- |
| **Toxicity** | | | | |
| AMES toxicity | No | No | No | (not mutagenic) |
| Max. tolerated dose (human) | 0.250 | -0.095 | 0.103 | log mg/kg/day <0.477 (low) |
| hERG I inhibitor | No | No | No | -- |
| hERG II inhibitor | Yes | No | No | -- |
| Oral Rat Acute Toxicity (LD50) | 2.554 | 1.978 | 1.917 | LD50 >> (less toxic) |
| Oral Rat Chronic Toxicity (LOAEL) | 3.51 | 0.244 | 1.009 | LOAEL >> (less toxic) |
| Hepatotoxicity | No | Yes | No | -- |
| Skin Sensitisation | No | No | No | -- |
| T.Pyriformis toxicity | 0.286 | 0.296 | 0.291 | log μg/L > -0.5 (toxic) |
| *Minnow toxicity* | 0.168 | -3.006 | -3.349 | log mM <-0.3 (toxic) |

The results of this in silico study identified seven acetogenin compounds predicted to be better SGLT2 inhibitors compared to empagliflozin. Empagliflozin is an FDA-approved SGLT2 inhibitor (Fala, 2015). SGLT2 inhibitors function by inhibiting SGLT2 in the proximal convoluted tubule (PCT) to prevent glucose reabsorption, where approximately 97% of glucose is normally reabsorbed (Kalra, 2014; Vallon, 2011). Hyperglycemic conditions are known to induce structural changes in the PCT, including thickening due to increased reabsorption capacity (Vallon & Thomson, 2012; Yara M Michelacci, 2015). A study also reported that dose-dependent administration of ethanol extract of *A. muricata* leaves in alloxan-induced mice improved the thickening observed in the left and right kidney PCT (Handayani et al., 2022).

Unlike SGLT2, which is specifically expressed in PCT cells, SGLT1 expression is distributed more widely in tissues such as the intestine, heart, and central nervous system (Song et al., 2016). Inhibiting SGLT1, either alone or in combination with SGLT2 inhibition, has beneficial effects on glycemic control in diabetic patients. However, the inactivation of SGLT1 in genetic models suggests that inhibiting this transporter may have adverse effects on various tissues (Tsimihodimos el al., 2018). Molecular docking results on the A chain of SGLT1 with seven acetogenin compounds indicate that five of them—muricin H, annonacin A, annopentocin B, murihexocin C, and corossolone—interact less stably compared to empagliflozin and LX2761, which are native ligands for SGLT1. Thus, these five acetogenin compounds are predicted to have a higher selectivity for SGLT2 compared to empagliflozin. Empagliflozin is known as the most selective SGLT2 inhibitor in its class (Anker & Butler, 2018).

Muricin H and corossolone are potential SGLT2 inhibitors that exhibit the most similar interactions to SGLT2 compared to empagliflozin. Therefore, these two acetogenin compounds are expected to have a similar mechanism of action as empagliflozin. Although both compounds

are predicted to be more toxic than empagliflozin, based on their elimination parameters, muricin H and corossolone have higher total clearance (CLTOT) compared to empagliflozin. Higher total clearance (CLTOT) indicates faster elimination of both compounds, which may potentially compensate for their toxicity. However, total clearance is also related to the bioavailability of the drug, so high total clearance may also affect the efficacy of both compounds as SGLT2 inhibitors.

## CONCLUSION

Molecular docking results of acetogenin compounds from soursop leaves (*A. muricata*) against SGLT2 and SGLT1 indicate that muricin H, annonacin A, annopentocin B, murihexocin C, and corossolone are predicted as SGLT2 inhibitors with better selectivity compared to empagliflozin. Muricin H and corossolone show the highest similarity in interactions with SGLT2 amino acid residues compared to empagliflozin, but both are predicted to be more toxic. Based on these findings, further research on the anti-diabetic and anti-cancer activities of soursop leaves (*A. muricata*) can evaluate their mechanisms of action in vitro and in vivo, focusing on their potential as SGLT2 inhibitors considering their selectivity and toxicity effects.

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