

Structure Based *Molecular Docking* Reveals Bioactive Saponin as Potential Inhibitor of Rumen Protozoal *Endo-1,4 β -Xylanase*

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Abstract: Enteric methane from ruminants contributes approximately 30% of global methane emissions, necessitating effective mitigation strategies. Rumen protozoa, as primary hosts for methanogenic archaea, represent a key target, with inhibition of their fibrolytic enzymes particularly *endo-1,4- β -xylanase* emerging as a promising approach. This study investigated the inhibitory potential of eleven saponin compounds identified from *Albizia chinensis* leaf powder via LC-MS analysis against rumen protozoa *endo-1,4- β -xylanase* (*Epidinium ecaudatum*, UniProt: B7FBK8) using *molecular docking*. The protein's 3D structure was retrieved from UniProt, ligand structures from PubChem, and blind *docking* was performed using PyRx (AutoDock Vina). Protein-ligand interactions were visualized using BIOVIA Discovery Studio 2019. Results demonstrated that Albiziasaponin B exhibited the highest binding affinity (−10.541 kcal/mol) with 12 hydrogen bonds, followed by Albiziasaponin A (−10.496 kcal/mol) and Albiziatrisside A (−10.424 kcal/mol). Notably, hydrogen bond geometry quality proved more critical than quantity; Acacioside A showed reduced affinity despite 11 hydrogen bonds due to unfavorable donor-donor interactions at ARG135. Hydrophobic and Van der Waals interactions contributed significantly to complex stability. These findings suggest saponins from *Albizia chinensis* as promising plant-based feed additive candidates for sustainable enteric methane mitigation, warranting further in vitro and in vivo validation.

Keywords: Enteric Methane Mitigation; *Endo-1,4 β -Xylanase*; *Molecular Docking*; Rumen Protozoa; Saponin.

Introduction

The global food system produces around 16-18 billion tons of CO₂ equivalent per year, equivalent to 30-35% of total global anthropogenic emissions (Tubiello et al., 2022). Within this system, the livestock sector contributes a dominant 8-18% of global emissions (Crippa et al., 2021). This figure places livestock as the third largest emitter in the world after China and the United States (Barthelmie, 2022). The main gas driving this impact is methane, which is 80 times more potent than CO₂ in the ozone layer and dominates emissions in the meat and dairy sectors (Olczak et al., 2023). Food system emissions have continued to increase by 7-17% since the 1990s, driven by changes in consumption patterns and the growth of global supply chains (Y. Li et al., 2023).

Enteric methane emissions from ruminant livestock account for 30% of global methane emissions and $\pm$ 5-6% of total global greenhouse gases (Black et al., 2021), when calculated over a 20 year time horizon, this contribution jumps to 36% of greenhouse gas emissions in the food system because methane has a global warming potential 80-84 times that of CO₂ over two decades (Arndt et al., 2022). Reducing methane emissions from ruminants is a quick and effective step to curb the rate of medium-term warming (Reisinger et al., 2021). In the 1.5°C scenario, these emissions need to be reduced by 11-30% by 2030 and 24-47% by 2050 compared to the 2010 baseline, but full adoption of this strategy is not enough to achieve the 2050 target (Arndt et al., 2022). This is exacerbated by the increasing demand for livestock products in lower-middle-income countries, while emissions are falling by

less than 1% per year, which is not enough to offset the growth in global animal protein consumption (Beauchemin et al., 2020). Rapid emissions reductions with a 1.5°C target in the livestock sector are therefore almost certain to be unattainable.

Rumen protozoa act as the primary H₂ source and host for methanogens, so that approximately 37% of rumen CH₄ can be directly attributed to methanogens associated with rumen protozoa (Pereira et al., 2022). Consistent defaunation can reduce methane gas emissions by approximately 11-12% accompanied by an increase in propionate and a decrease in the acetate:propionate ratio due to H₂ conversion (Z. Li et al., 2018), but the defaunation process will reduce fiber digestibility due to the loss of protozoan fibrolitic enzymes, including *endo-1,4-β-xylanase* (GH10/GH11) expressed in the protozoan metatranscriptome (Williams et al., 2020). Plant compounds such as saponins and tannins can induce the defaunation process while also directly inhibiting methanogens, with a potential reduction in methane gas of around 11-20%, but the effects of defaunation depend on chemical structure, dosage, and diet composition (Ramos-Morales et al., 2017), to design selective and specific inhibitors, *molecular docking* analysis can be used to predict binding and binding energy to target proteins from protozoa and methanogens before proceeding to in vitro and even in vivo testing (Kim et al., 2025).

Various strategies for mitigating enteric methane in ruminants, such as feed manipulation, rumen additives, and genetic selection, are generally grouped into animal management, ration formulation, and rumen microbiome manipulation (Beauchemin et al., 2022). The most effective additives currently available, 3-NOP and Asparagopsis, can reduce CH₄ by 30-90%, but they are less realistic in extensive development systems in developing countries (Honan et al., 2022), but there are secondary metabolites found in tropical plants such as saponins, tannins, and other essential oils that are suitable for small scale farmers in developing countries and can be integrated into direct grazing (Ku-Vera et al., 2020). The main mechanism of saponin compounds is to deflate and suppress the protozoa population, thereby reducing the association of protozoa with methanogens, which can shift fermentation to the propionate pathway and increase microbial protein synthesis (Ramos-Morales et al., 2017).

Efforts to overcome these limitations can be developed through chemical modification of saponin compounds for anti-protozoan effects, as well as enzymatic transformation of saponins as evidence of reducing CH₄ proportions (Zhang et al., 2025). However, the interaction of saponins with enzymes from protozoa at the molecular level have not yet been studied, and structural approaches to rumen protozoan enzyme targets such as *endo-1,4-β-xylanase* have never been investigated, therefore this analysis opens a significant research gap.

This study aims to identify bioactive saponin compounds from *Albizia chinensis* plants that have the potential to inhibit rumen protozoa *endo-1,4-β-xylanase* (El-Haddad et al., 2026). Molecular interaction and binding affinity analysis between saponins and selected proteins was performed using *molecular docking* (Moussa et al., 2025). Key characteristics in the interaction included bond type, amino acid residues, and the contribution of hydrogen bonds and hydrophobic interactions. The study is expected to demonstrate the inhibitory potential of saponin compounds with rumen protozoa *endo-1,4-β-xylanase* protein at the molecular level and produce an antiprotozoal effect derived from saponins (Ramos-Morales et al., 2017). This approach opens up opportunities for the development of feed additives, precision feed design based on saponins from sustainable local tropical plants, and is in line with the process of mitigating methane emissions from ruminants in developing countries (Ku-Vera et al., 2020).

Materials and Methods

Research Procedures

Target Protein Selection and Preparation

This study used in silico *molecular docking* analysis to analyze the interaction of bioactive saponin compounds with enzymes derived from rumen protozoa, that is *Endo-1,4-β-xylanase* (B7FBK8) from *Epidinium ecaudatum* **Figure 1**. The protein structure was downloaded from the UniProt website (<https://www.uniprot.org/>).

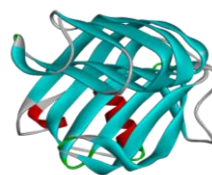


Figure 1. Structure of B7FBK8 (*Endo-1,4-β-xylanase*)

Selection and Preparation of Bioactive Ligands

Saponin compounds were obtained from *Liquid Chromatography Mass Spectrometry* (LCMS) analysis of sengon leaf powder (*Albizia chinensis*), that is Vitalboside A, Musennin, Desglucomusennin, Concinnoside D, Concinnoside A, Albiziatriside A, Albiziasaponin A, Albiziasaponin B, Albiziasaponin D, Albiziasaponin E, and Acacioside A. The three-dimensional (3D) structures of the ligands were downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) in SDF format. If the 3D structure was not available, the Avogadro software was used by entering the corresponding SMILES notation.

Molecular Docking

The prepared ligands will be loaded into PyRx via Open Babel (Trott et al., 2010; O'Boyle et al., 2011), minimized in energy throughout the ligands, and converted into PDBQT format for *docking* analysis. Protein preparation is performed using PyMOL v3.1.3.1 to remove water molecules and then loaded into AutoDock Tools on the PyRx software before the *docking* process, while visualization of molecular interactions uses BIOVIA Discovery Studio 2019 Client (Susilo et al., 2023). The grid dimension used was blind *docking*, where the center coordinates are $X = -6.8042$, $Y = -1.4666$, and $Z = 3.6778$, the box dimensions were $X = 47.3609$, $Y = 36.3899$, and $Z = 53.8667$ and exhaustiveness was set to 8.

Protein-Ligand Interaction Analysis and Visualisation

The *docking* poses were visualised in two and three dimensions using BIOVIA Discovery Studio Visualizer 2019. Interactions were characterised by the number and distribution of hydrogen bonds, interacting amino-acid residues, hydrophobic contacts, van der Waals interactions, pi-related interactions, and unfavourable donor-donor or acceptor-acceptor contacts. Where available, interaction distances were recorded to support interpretation of ligand orientation and contact geometry (Susilo et al., 2023).

Physicochemical and ADMET Prediction

The eleven compounds were further evaluated using ADMET-AI and pkCSM. ADMET-AI was used to obtain the molecular

descriptors reported in Table 3, including molecular weight, lipophilicity (Log P), hydrogen-bond acceptors and donors, Lipinski-rule descriptors, quantitative estimate of drug-likeness (QED), stereocentres, and topological polar surface area (TPSA) (Swanson et al., 2024). The pkCSM platform was used to obtain the predicted absorption, distribution, metabolism, excretion, and toxicity endpoints presented in Table 4, including Caco-2 permeability, blood-brain barrier permeability, CYP2D6 and CYP3A4 substrate status, total clearance, AMES toxicity, hepatotoxicity, and skin sensitisation. These outputs were treated as computational predictions rather than experimentally measured safety or pharmacokinetic outcomes.

Data Analysis

The data were analysed descriptively and comparatively across the eleven ligands. AutoDock Vina binding-affinity scores (kcal/mol) were tabulated and ranked, with more negative scores interpreted as more favourable predicted binding within the same *docking* protocol. The number of hydrogen bonds and the types and locations of amino-acid interactions were compared alongside the two- and three-dimensional visualisations. Physicochemical descriptors and ADMET predictions were summarised by compound and interpreted using the criteria displayed in Tables 3 and 4. No inferential statistical test was applied because the manuscript reports a computational screening rather than independent biological replicates. *Docking* scores and ADMET predictions were not interpreted as direct evidence of enzyme inhibition, antiprotozoal efficacy, methane reduction, or biological safety.

Result and Discussion

Interpretation of Compound Interactions Based on the Number of Hydrogen Bonds

Molecular docking results for 11 saponin compounds obtained binding affinity values and the number of hydrogen bonds formed. These results were obtained from simulations using a validated *molecular docking* method. Table 1 presents complete data on both parameters to facilitate comparative analysis between compounds.

The results of the analysis indicate that compounds can be grouped based on the number of hydrogen bonds and their binding affinity

values. The analysis shows that Albiziasaponin B has the highest number of hydrogen bonds, that is 12, and a binding affinity of -10.541, indicating that it is a compound with very stable interaction potential. Meanwhile, the compounds Albiziatrionoside A and Concinnoside D have only 2-3 hydrogen bonds but high affinity values of -10.424 and -10.260, respectively. *Molecular docking* results show that compounds with minimal hydrogen bonds can occur through hydrophobic interactions (Lestari *et al.*, 2023), while Acacioside A with 11 hydrogen bonds has a lower affinity value (-9.379) compared to the compound Albiziasaponin A with only 9 hydrogen bonds and an affinity value of -10.496. This indicates that the position and quality of hydrogen bonds are more significant than their quantity.

Table 1. Results of hydrogen bond and binding affinity analysis

Compound Name	Total Hydrogen Bond	Binding Affinity
Acacioside A	11	-9.379
Albiziasaponin A	9	-10.496
Albiziasaponin B	12	-10.541
Albiziasaponin D	5	-9.974
Albiziasaponin E	9	-10.059
Albiziatrionoside A	2	-10.424
Concinnoside A	2	-8.977
Concinnoside D	3	-10.260
Desglucimusennin	8	-8.958
Musennin	4	-9.960
Vitalboside A	4	-9.114

Compounds with low affinity, such as Concinnoside A (-8.977), Vitalboside A (-9.114), and Desglucimusennin (-8.958), despite variations in the number of hydrogen bonds. Low affinity may be due to limited interaction with the active site or steric hindrance, but interestingly, the Musennin compound with 4 hydrogen bonds recorded an affinity value of -9.960, which is still below other compounds with a similar number of hydrogen bonds. Overall, the data confirms that the binding affinity value is the result of the accumulation of various non-covalent interactions, not just the formation of a single factor, that is hydrogen bonds (Purnomo, 2019).

Visualization of Molecular Interactions of Test Compounds in Protein-Ligand Complexes

Molecular interaction visualization analysis was performed to understand the orientation and position of test compounds on the active site of the target protein. Two-dimensional (2D) visualization presents the types of interactions formed, such as hydrogen bonds, hydrophobic interactions, and Van der Waals interactions with amino residues. Meanwhile, three-dimensional (3D) visualization shows the spatial orientation of the compound and its depth of penetration into the protein pocket. The combination of these two forms of visualization allows for a comprehensive interpretation of the stability of the complex formed. The visualization data for the interactions of these 11 compounds can be seen in Figure 2 two dimensions and Figure 3 three dimensions.

Based on the visualization of the *molecular docking* interactions generated, compounds can be grouped according to the hydrogen bonds formed with the target amino acid residues. The first group consists of compounds that form numerous hydrogen bonds scattered across their active sites, that is Albiziasaponin B, Albiziasaponin A, and Acacioside A. The 2D and 3D visualizations show that Albiziasaponin B forms extensive hydrogen bonds with residues on its active site, indicating its highly stable interaction potential with a binding affinity value of -10.541. The compound Albiziasaponin A shows similar interactions with an even distribution of hydrogen bonds on the active side of the residue, and its affinity value is strongly supported with a value of -10.496. The compound Acacioside A, with 11 hydrogen bonds, shows several bonds that are less than optimal geometrically, resulting in a lower affinity value of -9.379 than the other compounds, but it has a high hydrogen value. This confirms that the quality and geometric position of hydrogen bonds significantly determine the stability of the binding (Susilo *et al.*, 2023).

The second group consists of compounds with limited hydrogen bonds but still exhibit favorable orientation, such as Albiziatrionoside A, Concinnoside D, and Musennin. The 3D visualization of Albiziatrionoside A shows that this compound produces two hydrogen bonds, but its molecular orientation penetrates deep into the hydrophobic pocket of the protein, therefore this

compound still has a high affinity value of -10.424. The compound Concinnoside D with 3 hydrogen bonds shows Van der Waals interactions with the surrounding residues, contributing to an optimal affinity value of -10.260. The compounds Concinnoside A and Desglucimusennin show less complementary interactions on the active site, as seen from several functional groups that do not interact

optimally. The 3D visualization of the Vitalboside A compound shows steric hindrance that limits its flexibility in adapting to the protein pocket. Overall, this visual interpretation shows that binding affinity is the result of the integrity of various factors, including hydrogen bond geometry, hydrophobic interactions, and molecular shape compatibility (Lestari et al., 2023).

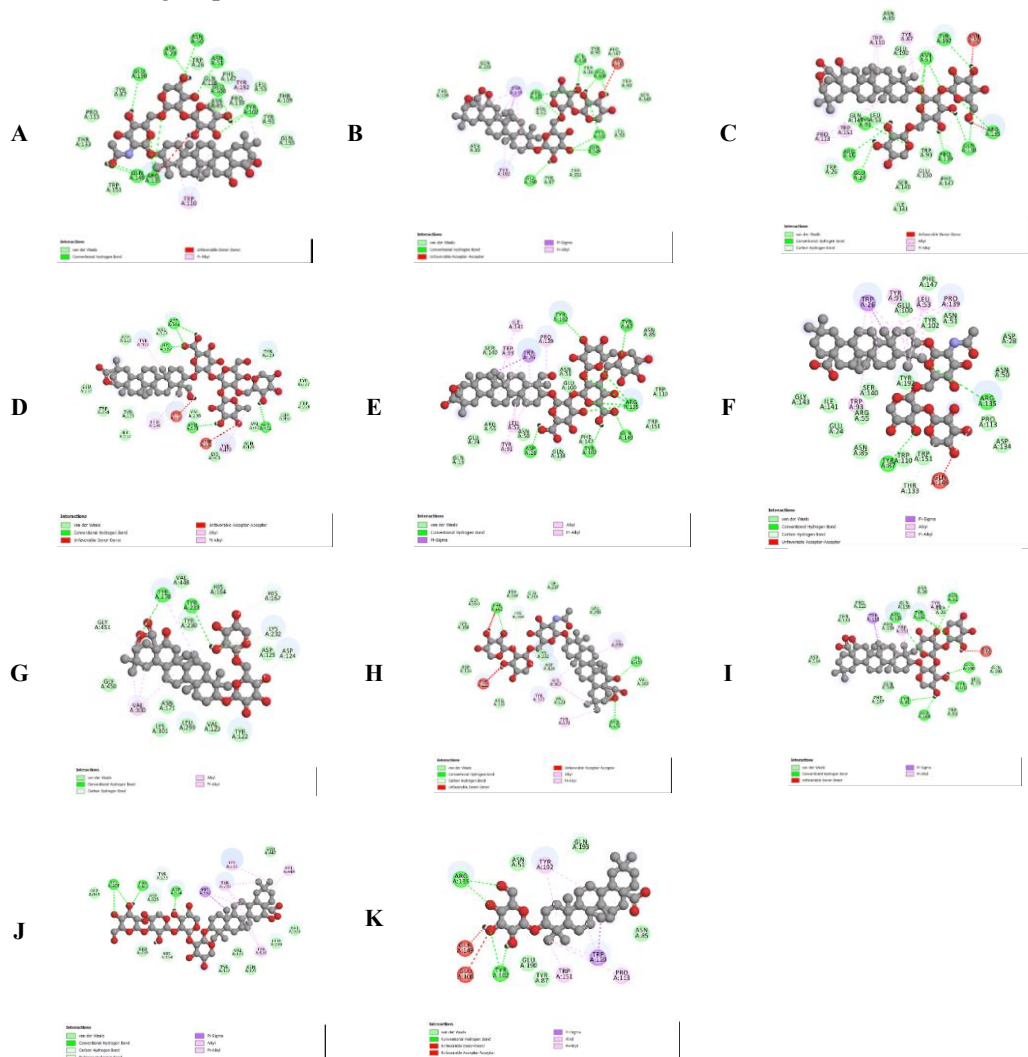


Figure 2. 2D Interactions of Ligand-Protein Complexes. (A) Acacioside A (B) Albiziasaponin A (C) Albiziasaponin B (D) Albiziasaponin D (E) Albiziasaponin E (F) Albiziatrioside A (G) Concinnoside A (H) Concinnoside D (I) Desglucimusennin (J) Musennin (K) Vitalboside A

This study successfully identified the potential of eleven saponin compounds from *Albizia chinensis* as inhibitors of rumen protozoa *endo-1,4- β -xylanase* through a *molecular docking* approach. The main finding shows that Albiziasaponin B has the highest binding affinity (-10,541 kcal/mol) with 12 hydrogen bonds, making it the most potential inhibitor candidate among all the compounds tested. These results directly address the research objective of

identifying bioactive saponin compounds that can inhibit rumen protozoa enzymes as a strategy for mitigating enteric methane. Albiziatrioside A and Concinnoside D also showed impressive performance with affinity values of -10.424 and -10.260, respectively, despite having only 2-3 hydrogen bonds, indicating that hydrophobic and Van der Waals interactions play a crucial role in binding stability (Lestari et al., 2023). Conversely, Desglucimusennin and

Concinnoside A recorded the lowest affinities (-8.958 and -8.977), confirming that the quantity of hydrogen bonds alone does not guarantee high binding affinity.

The findings of this study are in line with the report by (Ramos-Morales et al., 2017), which states that saponin compounds can induce rumen protozoa defaunation. However, this study provides novelty at the molecular level by identifying a specific enzyme target, that is *endo-1,4-β-xylanase*. Kim et al., (2025) confirmed that *molecular docking* analysis can predict binding and binding energy to target proteins before in vitro testing is conducted, and this study proved that eleven saponin compounds showed varying binding affinities to the enzyme. Unlike the study by Honan et al. (2022), which reported that synthetic additives such as 3-NOP can reduce methane by 30-90%, the saponin-based approach from tropical plants offers a more affordable and sustainable solution for farmers in developing countries (Ku-Vera et al., 2020). The novelty of this study lies in the fact that no previous research has examined the interaction between saponins and rumen protozoa *endo-1,4-β-xylanase* enzymes at the molecular level, as mentioned in the research gap analysis in the introduction.

The theoretical implications of this study reinforce the understanding that protozoan enzyme inhibition can be achieved through saponin compounds with diverse binding mechanisms. The theory of ligand-protein interactions, which has focused on hydrogen bonds as the main determinant of stability, needs to be revised, given that compounds such as Albiziatrinoside A with only 2 hydrogen bonds are able to achieve higher affinity than Acacioside A with 11 hydrogen bonds. Practically, this finding opens up opportunities for the development of saponin-based feed additives from *Albizia chinensis* that can be integrated into forage for livestock in developing countries. Williams et al. (2020) stated that *endo-1,4-β-xylanase* is expressed in protozoan metatranscriptomics, so inhibition of this enzyme has the potential to suppress protozoan populations without completely eliminating them as in total defaunation. From a policy perspective, the results of this study support the methane emission mitigation strategy mandated in the 1.5°C scenario, which requires an 11-30% reduction in emissions by 2030 (Arndt et al., 2022).

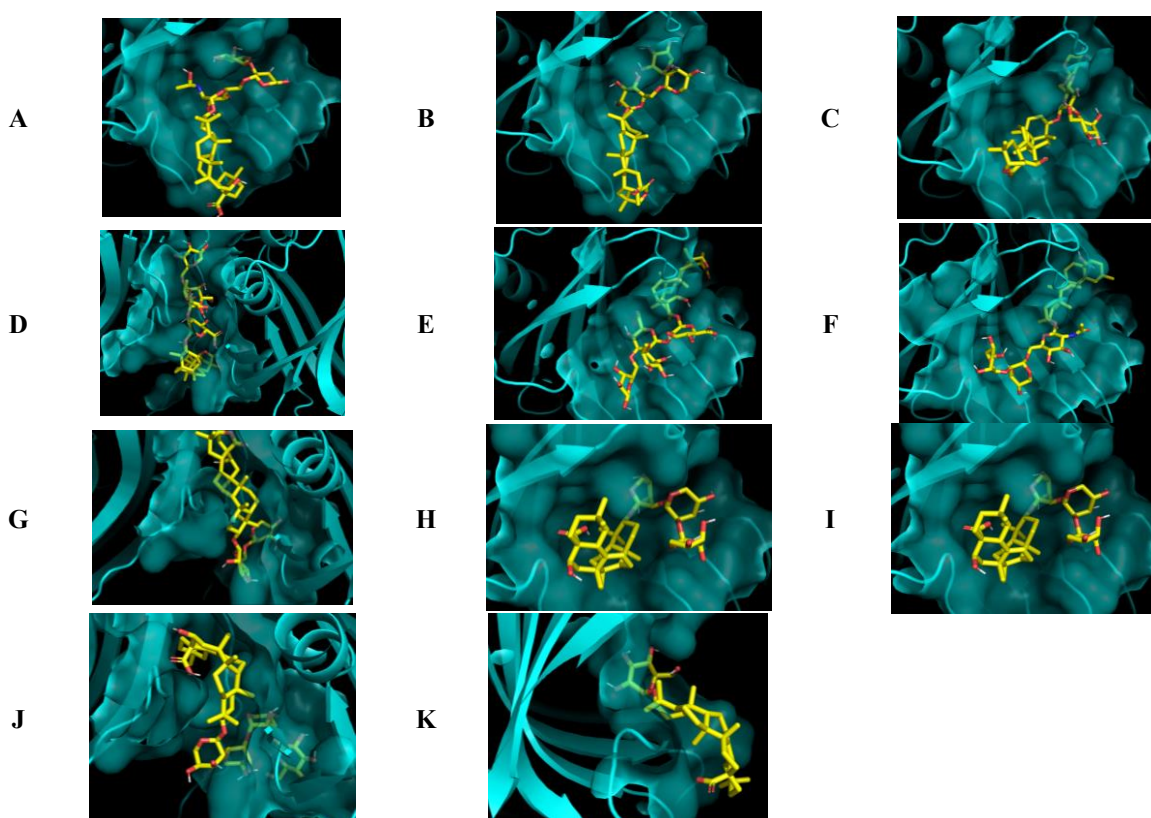


Figure 3. 3D Interactions of Ligand-Protein Complexes. (A) Acacioside A (B) Albiziasaponin A (C) Albiziasaponin B (D) Albiziasaponin D (E) Albiziasaponin E (F) Albiziatrinoside A (G) Concinnoside A (H) Concinnoside D (I) Desglucimusennin (J) Musennin (K) Vitalboside A

An unexpected finding in this study was the low affinity of Acacioside A (-9.379) despite having 11 hydrogen bonds, which was lower than that of Albiziasaponin A with 9 hydrogen bonds (-10.496). Further analysis revealed the presence of unfavorable Donor-Donor interactions at the ARG135 residue, which explains this phenomenon. This confirms Purnomo (2019) statement that binding affinity is the result of the accumulation of various non-covalent interactions, not just the single factor of hydrogen bonds. Another surprise is the performance of Musennin with 4 hydrogen bonds, which achieved an affinity of -9.960, higher than Vitalboside A (-9.114) with the same number of hydrogen bonds, indicating that the quality of interactions is more decisive than the quantity.

Analysis of Compound Interactions with Target Amino Acid Residues

Molecular interactions between test compounds and amino acid residues on the active site of the target protein can be analyzed in detail to understand the binding mechanism. This analysis identifies various types of interactions, such as hydrogen bonds, hydrophobic interactions, Van der Waals interactions, and Pi-Alkyl or Pi-Sigma interactions. Hydrogen bonds play an important role in the stability of the compound's orientation, while hydrophobic and Van der Waals interactions contribute to the overall binding affinity. Unfavorable interactions can also be identified to evaluate possible steric hindrance or electrostatic repulsion. Data related to the amino acid residues produced and the types of interactions in 11 saponin compounds are presented in Table 2.

Table 2. Amino acid residues involved in the interaction of the test compound with the target protein

Compound	Amino Acid Residues			
	Hydrogen Bonds	Hydrophobic Bonds	Van Der Waals Bonds	Others
Acacioside A	GLN149 (2), ARG135 (2), TYR102 (2), GLU100, ASN51, ASN50, ASP28, GLU190	-	TRP151, THR133, PRO113, TYR87, TRP26, GLN138, PHE147, ASN85, PRO139, LEU53, THR109, TYR91, GLN193	Unfavorable Donor-Donor: ARG135 Pi-Alkyl: TRP110
Albiziasaponin A	GLU190, GLN149, TYR102 (2), GLU100 (2), GLN138, ARG135 (2)	-	GLN193, THR109, ASN85, TYR87, TRP151, LEU53, SER140, TRP93, PHE147, TYR91, TRP26, ASN51	Unfavorable Acceptor-Acceptor: PRO139 Pi-Sigma: TRP110 Pi-Alkyl: TYR192, TRP110
Albiziasaponin B	TYR91 (2), ARG55, GLU24, PRO139, GLN138 (2), ARG135, TYR192, ASN51 (3)	GLU100, PRO139	ASN85, GLU190, PHE147, TRP93, SER140, ILE141, LEU53, GLN149, TRP26	Unfavorable Donor-Donor: ASN50 Alkyl dan Pi-Alkyl: RTP110, TYR87, TRP151, PRO113
Albiziasaponin D	ASN171, GLY451, ASP124 (2), HIS167	-	ASP125, VAL123, TRY230, TRY227, TRP228, GLY450, VAL300, GLN174, LYS301, VAL298, ILE237, TYR233, TRP234, GLU235	Unfavorable Donor-Donor: LYS232 Unfavorable Acceptor-Acceptor: ASN302 Alkyl dan Pi-Alkyl: TYR122, LEU299, TYR170
Albiziasaponin E	ASP28, TYR102, GLN149, ARG135 (4), TYR87, TYR192	-	SER140, ASN51, GLU100, ASN85, TRP110, TRP151, PHE147, GLN138, ASN50, ARG55, GLU24, GLN15	Pi-Sigma: TRP26 Alkyl dan Pi-Alkyl: ILE141, TRP93, PRO139, LEU53, TYR91

Compound	Amino Acid Residues				
	Hydrogen Bonds	Hydrophobic Bonds	Van Der Waals Bonds		Others
Albiziatrinoside A	TYR87, ARG135	ARG135, THR133	GLU100, TYR102, ASP28, PRO113, TRP151, ASN85, SER140, GLU24, GLY143	PHE147, ASN51, ASN50, ASP134, TRP150, TYR192, ARG55, ILE141,	Unfavorable Acceptor-Acceptor: GLN149 Pi-Sigma: TRP26 Alkyl dan Pi-Alkyl: TYR92, TRP26, LEU53, PRO139, TRP93
Concinnoside A	TYR170, TYR233	HIS167, LYS232, ASP124, GLY451	GLY450, LYS301, VAL123, ASP125, TYR230, VAL448	ASN171, LEU299, TYR122, HIS164,	Alkyl dan Pi-Alkyl: VAL300
Concinnoside D	THR162, LYS301, ASN171	HIS164, ASP124	VAL300, ARG231, ASP125, GLY161, GLU235, VAL298	VAL123, LYS232, LYS158, TRP234, ILE237,	Unfavorable Donor-Donor: TYR233 Unfavorable Acceptor-Acceptor: TYR233 Alkyl dan Pi-Alkyl: LEU299, TYR170, HIS167, TYR122
Desglucimusennin	TYR91, GLU100, TYR102, GLU190, ASN51, TYR192 (2), ARG135	-	ASP134, THR133, PRO113, PRO139, GLN138, ASP28, TRP26, GLN193, LEU53, TRP93, GLN149, PHE147	THR133, PRO139, ASP28, GLN193, TRP93,	Unfavorable Donor-Donor: ASN50 Pi-Sigma: TRP110 Pi-Alkyl: TYR87, TYR192, TRP151, TRP110
Musennin	LYS158 (2), THR162, ASP124	TYR233, ASP124, HIS164	GLY161, GLU447, VAL300, LEU299, ASN171, VAL123, TYR122, GLU235	GLU447, LEU299, VAL123,	Pi-Sigma: HIS167 Alkyl dan Pi-Alkyl: TYR170, VAL448, LYS232, TYR230, HIS167
Vitalboside A	ARG135 (2), TYR102 (2)	-	GLU190, ASN85, ASN51	TYR87, GLN193,	Unfavorable Donor-Donor : GLN149 Unfavorable Acceptor-Acceptor : GLU100 Pi-Sigma : TRP110 Alkyl dan Pi-Alkyl : TRP151, PRO113, TRP110, TYR192

Based on the amino acid interaction data, compounds can be grouped according to their complexity and the dominant type of interaction. The first group consists of compounds that form a large number and variety of hydrogen bonds, that is Acacioside A, Albiziasaponin A, Albiziasaponin B, and Albiziasaponin E. The compound Albiziasaponin B produces the most optimal interaction with 11 hydrogen bonds found in the residues TYR91, ARG55, GLU24, PRO139, GLN138, ARG135, TYR192 and ASN51, as well as hydrophobic interactions with GLU100 and PRO139. Albiziasaponin A and E form 8 and 9 hydrogen bonds, respectively, with

the dominant residues ARG135 and TYR102 having high affinity values of -10.496 and -10.059. The compound Acacioside A forms 11 hydrogen bonds but has an unfavorable Donor-Donor bond with ARG135, which shows a lower affinity of -9.379 despite the high number of hydrogen bonds (Purnomo, 2019). The presence of Pi-Alkyl interactions with TRP110 and TRP151 in these compounds will strengthen the stability of the complex with hydrophobic contributions.

The second group includes compounds with a limited number of hydrogen bonds but which make optimal use of hydrophobic and Van

der Waals interactions, such as Albiziatrioside A, Concinnoside D, and Musennin. The compound Albiziatrioside A can form two hydrogen bonds with TYR87 and ARG135 but is supported by hydrophobic interactions with ARG135 and THR133, as well as 17 Van der Waals bonds and an affinity value of -10.424. The Concinnoside D compound forms three hydrogen bonds with THR162, LYS301, and ASN171, as well as hydrophobic interactions with HIS164 and ASP124, plus Pi-Alkyl interactions, and has an affinity value of -10.260. The Musennin compound shows 4 hydrogen bonds and hydrophobic interactions involving TYR233, ASP124, and HIS164, while the Concinnoside A and Vutalboside A compounds have less optimal interactions with a dominance of Van der Waals forces and minimal strong hydrogen bonds. The Desglucimusennin compound has 7 hydrogen bonds but has the lowest affinity value of -8.958 due to unfavorable Donor-Donor interactions at ASN50. Overall, all results confirm that there is an optimal combination of high-quality hydrogen bonds, hydrophobic interactions, and minimal unfavorable interactions in the analysis of these compounds (Lestari et al., 2023).

The strength of this research methodology lies in the use of an *in silico* approach that enables rapid screening of eleven saponin compounds against specific target proteins prior to laboratory testing. The use of standardized software such as PyRx for *molecular docking* and BIOVIA Discovery Studio for interaction visualization ensures the reproducibility of results (Susilo et al., 2023). However, this study has a major limitation in that it has not been validated experimentally *in vitro* or *in vivo* against *in silico* findings. In addition, the use of blind *docking* without determining specific active sites can result in variations in binding orientation that may not fully represent physiological conditions. The protein structures used are from the UniProt database without considering the conformational flexibility of enzymes in the actual rumen environment.

The knowledge gap identified in this study opens up several avenues for future research. First, *in vitro* testing using recombinant *endo-1,4-β-xylanase* enzymes is needed to validate the computationally predicted binding affinity. Second, further research is needed to explore the synergistic effects of combining several saponin compounds, given that these compounds work collectively in plant extracts.

Third, *molecular dynamics* studies are needed to understand the stability of protein-ligand complexes over longer time periods. Fourth, testing in rumen cultures or experimental animals is needed to confirm the actual defaunation and methane reduction effects, as recommended (Beauchemin et al., 2022). Finally, modifications to the chemical structure of selected saponins can be made to optimize affinity and selectivity of inhibition against protozoan enzymes.

Druglikeness Evaluation and Pharmacokinetic Predictions use ADMET AI and pkCSM

The physicochemical and druglikeness parameters of compounds using ADMET AI in **Table 3**. These parameters molecular weight, lipophilicity (Log P), hydrogen bond dynamics and lipinski rule of five. Based on **Table 3** shows the physicochemical results for the 11 compounds analyzed using the ADMET AI tool. All compounds have molecular weights above the threshold of 500 Da, with the highest value reaching 1115.23 Da. Lipophilicity characteristics (Log P) range from hydrophilic to highly lipophilic. Several compounds exhibit acceptor and hydrogen bonding values that exceed general standard criteria. The molecular structures are also highly complex, with some compounds having up to 30 functional groups.

The relatively low druglikeness score (QED) poses a challenge in developing this compound as an oral drug. The very high TPSA value of up to 377.04 also predicts low permeability of the compounds through biological membranes. Lipinski's rules indicate violations of the criteria for molecular weight and hydrogen bonding in all samples, except for the compound Vitalboside A, which has the highest Log P value and a molecular weight close to the standard. Overall, these data indicate that the analyzed compounds require structural optimization to enhance their pharmacological potential. The results of physicochemical analysis using ADMET AI of all eleven compounds showed molecular weight values that exceeded the Lipinski limit (≤ 500 Da) with results between 618.85 Da to 1115.23 Da so that all compounds were classified as macromolecules that have the potential to inhibit oral absorption (Daina et al., 2017). The Log P value of most compounds was within the accepted range (-1.24 to 5.06), the criteria for the number of hydrogen bond donors (> 5) and

acceptors (> 10) in all compounds except Vitalboside A, so that these compounds did not meet the Lipinski Five rule as a whole (Lipinski *et al.*, 1997). A low Quantitative Estimate of Druglikeness (QED) value of around 0.006 to

0.22 requires further structural optimization, such as glycoside modification or compound fragmentation so that the pharmacological potential as an oral drug candidate can be further increased (Bickerton *et al.*, 2012).

Table 3. Physicochemical of eleven compounds using ADMET AI

Criteria	Acaci oside A	Albizia saponin A	Albizias aponin B	Albizi asapo nin D	Albizi asapo nin E	Albizi atriosi de A	Conci nnosi de A	Conci nnosid e D	Desgluc omusen nin	Musei nin	Vitalb oside A
Molecu lar Weight (≤ 500 Da)	940.13	897.07	927.09	1101.24	1115.23	924.14	764.95	938.12	869.06	1031.20	618.85
Log P (≤ 5)	1.10	0.41	-0.23	-1.24	-1.15	2.13	1.94	0.55	1.59	-0.58	5.06
Hydrog en Bond Accepto r (≤ 10)	16	17	18	22	22	15	13	17	15	20	7
Hydrog en Bond Donor (≤ 5)	10	9	10	12	12	9	7	9	9	12	5
Lipinski Rule	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00
Quantit ative Estimat e of Druglik eness (QED)	0.11	0.10	0.09	0.06	0.06	0.12	0.12	0.09	0.13	0.10	0.22
Stereo Centers	22.00	23.00	24.00	30.00	30.00	21.00	19.00	23.00	21.00	26.00	13.00
Topolog ical Polar Surface Area (TPSA)	283.62	263.75	283.98	359.97	377.04	263.39	204.83	272.62	254.52	333.67	136.68

Table 4 summarizes the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles of the eleven compounds as predicted by the pkCSM platform. The evaluation encompasses critical pharmacological parameters, including Caco-2 cell permeability, blood-brain barrier (BBB) penetration, cytochrome P450 enzymatic substrate interactions, and toxicological assessments such as AMES toxicity and hepatotoxicity.

Table 4 shows the ADMET profiles of the 11 compounds analyzed using the pkCSM

platform. All compounds exhibited negative Caco-2 permeability values, indicating low absorption capacity in the small intestine. Regarding metabolism, none of the compounds were substrates for the CYP2D6 enzyme, but several compounds, such as Acacioside A and Musennin, were identified as substrates for the CYP3A4 enzyme. In the toxicity evaluation process, the results were positive, with the compounds found to be free of mutagenic risk according to the AMES test. Other parameters indicated that none of the compounds caused skin sensitization. Meanwhile, hepatotoxic potential

was detected in several compounds, such as Acacioside A, Albiziatrionoside A, and Concinnoside D. Regarding total clearance, several compounds showed low to moderate

ranges in all samples analyzed. Thus, these data provide a comprehensive overview of the pharmacokinetic profile and initial safety profile for all these compounds.

Table 4. ADMET evaluation of eleven compounds using pkCSM

Criteria	Acacioside A	Albiziasaponin A	Albiziasaponin B	Albiziasaponin D	Albiziasaponin E	Albiziatrionoside A	Concinnoside A	Concinnoside D	Desglucomusennin	Musennin	Vitalboside A
Caco-2 > 0.90 (log Papp in 10 ⁻⁶ cm/s)	-0.6	-0.856	-0.871	-0.743	-0.997	-0.567	-0.592	-0.738	-0.668	-0.792	-0.438
BBB permeability (+log BB > 0.30 and -log BB < -1.00)	-1.804	-1.736	-1.975	-2.275	-2.441	-1.653	-1.36	-1.713	-1.818	-2.364	-0.94
CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 substrate	Yes	No	No	No	No	Yes	No	Yes	Yes	No	Yes
Total clearance (log mL/min/Kg)	-0.138	-0.132	-0.65	-0.065	-0.197	-0.172	-0.1	-0.193	-0.127	-0.105	-0.043
AMES toxicity	No	No	No	No	No	No	No	No	No	No	No
Hepatotoxicity	Yes	No	No	No	No	Yes	No	Yes	No	No	No
Skin Sensitization	No	No	No	No	No	No	No	No	No	No	No

The following table shows that the negative Caco-2 permeability values range from -0.438 to -0.997 Log Papp, indicating that the compound has low intestinal absorption and is potentially ineffective if administered orally (Paixão et al., 2010). Blood-brain barrier (BBB) penetration shows that all compounds are below -1.00 log BB, in the compounds Albiziasaponin E (-2.441) and Musennin (-2.364) which indicates that both compounds cannot penetrate the central nervous system so that the risk of neurotoxic effects is considered minimal (Yang et al., 2019). In the metabolic aspect, there were no compounds that were CYP2D6 substrates, but five compounds, that is Acacioside A, Albiziatrionoside A, Concinnoside D, Desglucomusennin, and Vitalboside A, were detected as CYP3A4 substrates, indicating the potential for drug-drug interactions when combined with compounds that inhibit or induce the enzyme (Manikandan and Nagini, 2018).

Conclusion

Molecular docking analysis identified eleven saponin compounds from sengon leaves (*Albizia chinensis*) as potential inhibitors of

rumen protozoa *endo-1,4-β-xylanase*. Albiziasaponin B showed the strongest binding affinity (-10.541 kcal/mol) with 12 hydrogen bonds, followed by Albiziasaponin A, Albiziatrionoside A, and Concinnoside D. Binding stability was influenced more by the quality and geometry of hydrogen bonds than by their quantity, while hydrophobic and Van der Waals interactions also contributed substantially to ligand affinity. The interaction patterns indicate that saponins from *A. chinensis* possess strong potential as natural defaunation agents and methane mitigation compounds in ruminant systems.

The identified compounds demonstrated favorable safety profiles despite limited intestinal permeability and low compliance with Lipinski's rules due to their high molecular weight and polarity. These results support the development of sustainable saponin based phytogenic feed additives derived from tropical agroforestry resources to improve rumen efficiency and reduce enteric methane emissions. Further validation through *molecular dynamics*, *in vitro* enzyme assays, and *in vivo* rumen studies is required to confirm biological activity and practical efficacy under physiological conditions.

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Reference

- Arndt, C., Hristov, A. N., Price, W. J., McClelland, S. C., Pelaez, A. M., Cueva, S. F., Oh, J., Dijkstra, J., Bannink, A., Bayat, A. R., Crompton, L. A., Eugène, M. A., Enahoro, D., Kebreab, E., Kreuzer, M., McGee, M., Martin, C., Newbold, C. J., Reynolds, C. K., ... Yu, Z. (2022). Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5°C target by 2030 but not 2050. *Proceedings of the National Academy of Sciences*, 119(20), e2111294119.
<https://doi.org/10.1073/pnas.2111294119>
- Barthelmie, R. (2022). Impact of dietary meat and animal products on GHG footprints: The UK and the US. *Climate*, 10(3), 43.
<https://doi.org/10.3390/cli10030043>
- Beauchemin, K. A., Ungerfeld, E. M., Abdalla, A. L., Alvarez, C., Arndt, C., Becquet, P., Benchaar, C., Berndt, A., Mauricio, R. M., McAllister, T. A., Oyhantçabal, W., Salami, S. A., Shalloo, L., Sun, Y., Tricarico, J., Uwizeye, A., De Camillis, C., Bernoux, M., Robinson, T., & Kebreab, E. (2022). Invited review: Current enteric methane mitigation options. *Journal of Dairy Science*, 105(12), 9297–9326.
<https://doi.org/10.3168/jds.2022-22091>
- Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J., & Wang, M. (2020). Review: Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. *Animal*, 14(S1), s2–s16.
<https://doi.org/10.1017/S1751731119003100>
- Bickerton, G. R., Paolini, G. V., Besnard, J., Muresan, S., & Hopkins, A. L. (2012). Quantifying the chemical beauty of drugs. *Nature Chemistry*, 4(2), 90–98.
<https://doi.org/10.1038/nchem.1243>
- Black, J. L., Davison, T. M., & Box, I. (2021). Methane emissions from ruminants in Australia: Mitigation potential and applicability of mitigation strategies. *Animals*, 11(4), 951.
<https://doi.org/10.3390/ani11040951>
- Campbell, S., Greenwood, M., Prior, S., Shearer, T., Walkem, K., Young, S., Bywaters, D., & Walker, K. (2020). Purposive sampling: Complex or simple? Research case examples. *Journal of Research in Nursing*, 25(8), 652–661.
<https://doi.org/10.1177/1744987120927206>
- Crippa, M., Solazzo, E., Guizzardi, D., Monforti-Ferrario, F., Tubiello, F. N., & Leip, A. (2021). Food systems are responsible for a third of global anthropogenic GHG emissions. *Nature Food*, 2, 198–209.
<https://doi.org/10.1038/s43016-021-00225-9>
- Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7, 42717.
<https://doi.org/10.1038/srep42717>
- El-Haddad, A. E., Abd El-Al, M. S., Teleb, M., & Farag, M. A. (2026). Antidiabetic action mechanisms of saponins via gut digestive enzymes inhibition and microbiota interaction, a comprehensive review and in silico docking to improve efficacy. *European Journal of Medicinal Chemistry*, 302, 118282.
<https://doi.org/10.1016/j.ejmech.2025.118282>
- Honan, M., Feng, X., Tricarico, J. M., & Kebreab, E. (2022). Feed additives as a strategic approach to reduce enteric methane production in cattle: Modes of action, effectiveness and safety. *Animal Production Science*, 62(14), 1303–1317.
<https://doi.org/10.1071/AN20295>
- Kim, H., Shim, H., Ranganath, A., He, S., Stevenson, G., & Allen, J. E. (2025). Protein-ligand binding affinity prediction using multi-instance learning with docking structures. *Frontiers in Pharmacology*, 15, 1518875.
<https://doi.org/10.3389/fphar.2024.1518875>
- Ku-Vera, J. C., Jiménez-Ocampo, R., Valencia-Salazar, S. S., Montoya-Flores, M. D., Molina-Botero, I. C., Arango, J., Gómez-Bravo, C. A., Aguilar-Pérez, C. F., & Solorio-Sánchez, F. J. (2020). Role of secondary plant metabolites on enteric

- methane mitigation in ruminants. *Frontiers in Veterinary Science*, 7, 584. <https://doi.org/10.3389/fvets.2020.00584>
- Lestari, I. N., Renada, E., Juliana, D., Iswara, K., Sukmara, S., & Physalis, C. (2023). *Studi Penambatan Molekuler Senyawa Antidiabetes pada Reseptor PPAR- γ* . 3(1), 1–9. <https://doi.org/10.47653/farm.v8i1.533>
- Li, Y., Zhong, H., Shan, Y., Hang, Y., Wang, D., Zhou, Y., & Hubacek, K. (2023). Changes in global food consumption increase GHG emissions despite efficiency gains along global supply chains. *Nature Food*, 4, 483–495. <https://doi.org/10.1038/s43016-023-00768-z>
- Li, Z., Deng, Q., Liu, Y., Yan, T., Li, F., Cao, Y., & Yao, J. (2018). Dynamics of methanogenesis, ruminal fermentation and fiber digestibility in ruminants following elimination of protozoa: A meta-analysis. *Journal of Animal Science and Biotechnology*, 9, 89. <https://doi.org/10.1186/s40104-018-0305-6>
- Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 23(1–3), 3–25. [https://doi.org/10.1016/S0169-409X\(96\)00423-1](https://doi.org/10.1016/S0169-409X(96)00423-1)
- Manikandan, P., & Nagini, S. (2018). Cytochrome P450 structure, function and clinical significance: A review. *Current Drug Targets*, 19(1), 38–54. <https://doi.org/10.2174/1389450118666170125144557>
- Moussa, A., Alanzi, A., Luo, J., Wang, J., Cheang, W., & Xu, B. (2025). Role of saponins from *Platycodon grandiflorum* in Alzheimer's disease: DFT, molecular docking, and simulation studies in key enzymes. *Molecules*, 30(8), 1812. <https://doi.org/10.3390/molecules30081812>
- O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, 3, 33. <https://doi.org/10.1186/1758-2946-3-33>
- Olczak, M., Piebalgs, A., & Balcombe, P. (2023). A global review of methane policies reveals that only 13% of emissions are covered with unclear effectiveness. *One Earth*, 6(5), 519–535. <https://doi.org/10.1016/j.oneear.2023.04.009>
- Paixão, P., Gouveia, L. F., & Morais, J. A. G. (2010). Prediction of the in vitro permeability determined in Caco-2 cells by using artificial neural networks. *European Journal of Pharmaceutical Sciences*, 41(1), 107–117. <https://doi.org/10.1016/j.ejps.2010.05.014>
- Pereira, A., Dapkevicius, M. de L. N. E., & Borba, A. (2022). Alternative pathways for hydrogen sink originated from the ruminal fermentation of carbohydrates: Which microorganisms are involved in lowering methane emission?. *Animal Microbiome*, 4, 5. <https://doi.org/10.1186/s42523-021-00153-w>
- Purnomo, H. (2019). *Hubungan kuantitatif struktur dan aktivitas aplikasi CADD, LBDD: QSAR desain molekul antiinflamasi dan desain molekul antikanker kolon*. Penerbit Andi.
- Ramos-Morales, E., de la Fuente, G., Duval, S., Wehrli, C., Bouillon, M., Lahmann, M., Preskett, D., Bragança, R., & Newbold, C. J. (2017). Antiprotozoal effect of saponins in the rumen can be enhanced by chemical modifications in their structure. *Frontiers in Microbiology*, 8, 399. <https://doi.org/10.3389/fmicb.2017.00399>
- Ramos-Morales, E., de la Fuente, G., Nash, R. J., Bragança, R., Duval, S., Bouillon, M. E., Lahmann, M., & Newbold, C. J. (2017). Improving the antiprotozoal effect of saponins in the rumen by combination with glycosidase inhibiting iminosugars or by modification of their chemical structure. *PLOS ONE*, 12(9), e0184517. <https://doi.org/10.1371/journal.pone.0184517>
- Reisinger, A., Clark, H., Cowie, A. L., Emmet-Booth, J., Fischer, C. G., Herrero, M., Howden, M., & Leahy, S. (2021). How necessary and feasible are reductions of methane emissions from livestock to support stringent temperature goals?. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and*

- Engineering Sciences*, 379, 20200452.
<https://doi.org/10.1098/rsta.2020.0452>
- Susilo, A., Cahyati, M., Pranowo, D., Nurjannah, Masyithoh, D., Al Awwaly, K. U., & Hermanto, F. K. (2023). *Simulasi molekuler pada bidang pangan fungsional: Prinsip dasar dan tutorial*. Universitas Brawijaya Press.
- Swanson, K., Walther, P., Leitz, J., Mukherjee, S., Wu, J. C., Shivnaraine, R. V., & Zou, J. (2024). ADMET-AI: A machine learning ADMET platform for evaluation of large-scale chemical libraries. *Bioinformatics*, 40(7), btae416.
<https://doi.org/10.1093/bioinformatics/btae416>
- Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461.
<https://doi.org/10.1002/jcc.21334>
- Tubiello, F. N., Karl, K., Flammini, A., Gütschow, J., Obli-Laryea, G., Conchedda, G., Pan, X., Qi, S. Y., Heiðarsdóttir, H. H., Wanner, N., Quadrelli, R., Souza, L. R., Benoit, P., Hayek, M., Sandalow, D., Contreras, E. M., Rosenzweig, C., Moncayo, J. R., Conforti, P., & Torero, M. (2022). Pre- and post-production processes increasingly dominate greenhouse gas emissions from agri-food systems. *Earth System Science Data*, 14(4), 1795–1809.
<https://doi.org/10.5194/essd-14-1795-2022>
- Williams, C. L., Thomas, B. J., McEwan, N. R., Stevens, R., Creevey, C. J., & Huws, S. A. (2020). Rumen protozoa play a significant role in fungal predation and plant carbohydrate breakdown. *Frontiers in Microbiology*, 11, 720.
<https://doi.org/10.3389/fmicb.2020.00720>
- Yang, H., Lou, C., Sun, L., Li, J., Cai, Y., Wang, Z., Li, W., Liu, G., & Tang, Y. (2019). admetSAR 2.0: Web-service for prediction and optimization of chemical ADMET properties. *Bioinformatics*, 35(6), 1067–1069.
<https://doi.org/10.1093/bioinformatics/bty707>
- Zhang, R., Bao, X., Shi, X., Jin, S., Meng, Y., Li, Z., Du, Z., & Yan, X. (2025). In vitro evaluation of enzymatically transformed alfalfa saponins on methane reduction, rumen microbes and metabolomics in goats. *Animals*, 15(11), 1516.
<https://doi.org/10.3390/ani15111516>