



## *In silico* Study of Ruminant Feed Wafer Formulation with Empon-Empon as Health Support Food

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### ABSTRACT

The innovation of feed wafer formulation represents a promising strategy to enhance nutritional efficiency and promote sustainability within the livestock sector. Spices, classified as herbal plants, have long been utilized in traditional Indonesian medicine. *Empon-empon*, a traditional herbal mixture, typically comprises various rhizomes such as turmeric (*Curcuma longa*), aromatic ginger (*Kaempferia galanga*), and *temulawak* (*Curcuma xanthorrhiza*). The bioactive compounds contained in these plants possess well-documented antioxidant, anti-inflammatory, and antimicrobial activities, and are anticipated to support the health and productivity of ruminant animals. This study employed molecular docking analysis to assess the interactions between bioactive constituents from these three rhizomes and specific target proteins involved in ruminant physiological processes. The selected compounds were identified based on previous GC-MS analyses, and their three-dimensional structures were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The structural data for the target proteins were obtained from the RCSB Protein Data Bank (<https://www.rcsb.org/>). Furthermore, the drug-likeness of the compounds was evaluated according to Lipinski's Rule of Five. Among the tested compounds, *naringenin* and *dihydrocurcumin*—both derived from *Curcuma* species—demonstrated the strongest binding affinities, each recording a value of  $-10.00$  kcal/mol against the Testis-Specific Androgen Binding Protein and the Glucagon Receptor. Additionally, *Bis-Demethoxycurcumin*, a compound isolated from turmeric, exhibited a binding affinity of  $-9.2$  kcal/mol with Cathepsin L protein. These high binding affinities suggest that the compounds have significant potential as inhibitors of key proteins, thereby offering long-term benefits for livestock health through modulation of immune responses, metabolic pathways, and reproductive functions.

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**Keywords:** *In silico*, Turmeric, Aromatic ginger, Curcuma.

### Introduction

The livestock industry plays a pivotal role in enhancing feed nutrition, thereby optimizing production efficiency and ensuring global food security. In 2020, 328 million tons of meat were produced worldwide.<sup>1</sup> This number is projected to reach 373 million tons by 2030, making effective nutritional strategies urgent. Animal feed significantly influences the nutritional efficiency of meat production, particularly through the quality and composition of feed nutrients.<sup>2</sup> Optimizing feed nutrition is essential not only for improving livestock productivity but also for mitigating the environmental impact of the livestock sector, which contributes approximately 12% of total global anthropogenic greenhouse gas emissions.<sup>3</sup> Therefore, efficient feed management is key to increasing production efficiency while reducing environmental impact.

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One promising innovation for optimizing feed nutrition is the development of animal feed wafers.<sup>4</sup> This technology enhances nutritional value, facilitates storage and transportation, and maximizes the utilization of agricultural waste, which in Indonesia alone reaches 230 million tons per year, with only 30-40% being utilized as animal feed.<sup>5</sup> Feed is fundamental in ruminant farming, influencing digestive efficiency, nutrient absorption, and overall animal health. In addition to meeting basic nutritional requirements, feed must also support the health and well-being of livestock.<sup>6</sup> Feed wafers, as a compressed form of feed, enable the optimal utilization of various feed ingredients, including agricultural waste. The increasing concern regarding the use of synthetic additives, particularly after the ban on Antibiotic Growth Promoters (AGP) due to harmful residues, has led to a growing interest in natural alternatives.<sup>7</sup> Many studies have highlighted the potential of *empon-empon* as a natural feed supplement that enhances livestock health and productivity. *Empon-empon* is a group of herbal plants that have long been used in traditional medicine in Indonesia.<sup>8</sup> These plants have various health benefits and are often used as herbal ingredients. *Empon-empon* usually consists of several types of rhizomes, such as turmeric (*Curcuma longa*), aromatic ginger (*Kaempferia galanga*), and curcuma (*Curcuma xanthorrhiza*).<sup>9</sup> Turmeric (*Curcuma longa*) is a plant that belongs to the Zingiberaceae family. It is utilized as a spice and in conventional medicine. The primary active compound in turmeric is curcumin, which gives the plant its characteristic yellow colour and possesses strong anti-inflammatory and antioxidant properties.<sup>10</sup> Additionally, turmeric contains essential oils, carbohydrates, proteins, amino acids, flavonoids, glycosides, steroids, alkaloids, and tannins.<sup>11,12</sup> Traditionally, turmeric has been used to treat

various health conditions, including indigestion, inflammation, and burns.<sup>13</sup>

Aromatic ginger (*Kaempferia galanga*), another rhizome plant from the Zingiberaceae family, has long been utilized in traditional Asia medicine. Its bioactive compounds, particularly ethyl *p*-methoxycinnamate and ethyl-cinnamate, exhibit anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory properties.<sup>14,15</sup> Previous studies have demonstrated that aromatic ginger extract improves growth and health performance in broiler chickens.<sup>16</sup> However, its potential as a feed supplement for ruminants, particularly in wafer form, remains underexplored. Further research is required to assess its impact on various ruminant body systems.

Curcuma (*Curcuma xanthorrhiza*), another member of the Zingiberaceae family, is abundant in tropical regions and contains beneficial secondary metabolites such as starch, curcumin, and essential oils, which include compounds like tricyclene, borneol, and xanthorrhizol.<sup>17,18</sup> Curcuma has been traditionally used to treat digestive disorders, jaundice, leucorrhea, and cardiovascular diseases while also serving as an immune booster.<sup>19</sup> It is commonly processed into herbal medicine, supplements, and traditional remedies.

Conversely, a multitude of proteins have been demonstrated to enhance the functioning of the physiological systems in ruminants. Angiotensin-Converting Enzyme (ACE) is associated with blood pressure regulation and vascular function,<sup>20</sup> which are crucial for nutrient transport and metabolic efficiency in livestock. Cathepsin L is involved in protein degradation and immune response, contributing to optimal digestion and pathogen defense.<sup>21</sup> Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) are key elements in inflammatory pathways,<sup>22</sup> making them relevant targets for evaluating the anti-inflammatory potential of bioactive compounds. Ephrin type A Receptor 2 is linked to cellular communication and tissue development,<sup>23</sup> which may impact overall growth and health. Glucagon Receptor and Insulin are essential regulators of glucose metabolism and energy balance in ruminants, affecting weight gain and feed efficiency.<sup>24</sup> Tyrosine Protein Kinase JAK1 and JAK2 are key mediators in immune signaling pathways, making them relevant to evaluating immunomodulatory effects.<sup>25</sup> Testis-Specific Androgen-Binding Protein is crucial for reproductive health, influencing fertility and breeding performance.<sup>26,27</sup> Estrogen Receptor Alpha plays a role in reproductive and metabolic functions, influencing hormone balance and development.<sup>28</sup>

This study employs the molecular docking method to analyze the interaction between bioactive compounds from turmeric, aromatic ginger, and curcuma with specific target proteins that play vital roles in various ruminant body systems. Molecular docking is a widely used structure-based *in silico* method in drug discovery. This approach predicts molecular interactions, resembling a lock-and-key mechanism, by simulating how bioactive compounds bind to specific proteins.<sup>29</sup> It allows the identification of new compounds with therapeutic potential by evaluating ligand-target interaction at the molecular level. Molecular docking provides insights into the molecular recognition process and structure-activity relationship (SAR) without requiring knowledge of other target modulators' chemical structure.<sup>30</sup> The selected target proteins are those associated with digestion, metabolism, immune function, and overall health, ensuring that the proposed feed formulation has a scientifically supported basis.

The novelty of this research lies in its application of molecular docking to evaluate the potential of empon-empon-based feed wafers in improving ruminant health through targeted protein interactions. Unlike previous studies that primarily focused on poultry, this study aims to provide a comprehensive understanding of how turmeric, aromatic ginger, and curcuma interact with key proteins in ruminants. By integrating natural bioactive compounds into feed wafers, this study aims to contribute to improved food production efficiency, reduced environmental impact, and the development of safer and more effective animal feed.

## Materials and Methods

### Tools and Materials

The instruments utilized in this investigation encompass a laptop (HP AMD Athlon Gold 3150U Processor with Radeon Graphics, 2.40 GHz, 4.00 GB RAM) for the execution of computational analyses. The software utilized includes: The National Center for Biotechnology Information (NCBI) offers two resources for retrieving ligands: PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and the RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank) (<https://www.rcsb.org/>).<sup>28,29</sup> Protein retrieval using PyRx v0.9.8 for molecular docking, AutoDockTools v1.5.7 for protein and ligand preparation, PyMOL v2.5 for molecular visualization, and Discovery Studio v2021 for interaction analysis.

The materials used include 3D structures of bioactive compounds from Curcuma (*Curcuma xanthorrhiza*), Aromatic ginger (*Kaempferia galanga*), and Turmeric (*Curcuma longa*)<sup>12,15,18</sup> obtained from PubChem database. The target proteins used in this study were downloaded from the RCSB PDB database. The selected proteins and their respective PDB IDs are: Estrogen Receptor Alpha (1ERE), Testis-Specific Androgen-Binding Protein (1LHO), Tyrosine-Protein Kinase JAK1 (6GGH), Tyrosine-Protein Kinase JAK2 (6VGL), Cyclooxygenase-1 (COX-1) (6Y3C), Cyclooxygenase-2 (COX-2) (5F19), Ephrin Type-A Receptor 2 (7K7J), Angiotensin-Converting Enzyme (ACE) (PO86), Insulin-Like Growth Factor I Receptor (1ZT3), Glucagon Receptor (7LCJ), and Cathepsin L (3H8B).

### Bioactive compound preparation

The active constituents of empon-empon were optimized using PyRx software to enhance ligand flexibility and achieve the lowest possible binding energy upon interaction with the target protein. Target prediction analyze the interaction between chemical compounds and macromolecules in the body. Target prediction was performed using the SwissTargetPrediction website (<http://www.swisstargetprediction.ch/>). In addition, Lipinski's Rule of Five (RO5) analysis was also performed to predict whether the compound has chemical and physical properties that allow oral administration, using SwissADME.<sup>30,31,32</sup>

### Target protein preparation

Following a thorough analysis and selection of the most suitable receptor for the intended activity, the target proteins were obtained from the RCSB PDB (<https://www.rcsb.org/>). The target proteins that were obtained were subsequently sterilized using AutoDock software in order to disassociate the protein structure from the native ligand and other undesirable molecules.<sup>33</sup>

### Molecular docking and visualization

Molecular docking used PyRx software to determine the interaction between the active compounds of empon-empon with the target proteins. The results of this *in silico* study were presented in form of binding affinity. Binding affinity is defined as the magnitude of the ligand's capacity to bind to the receptor in kilocalories per mole. As the binding affinity value decreases, the degree of affinity between the receptor and ligand increases.<sup>34,35</sup> PyMOL and Discovery Studio v2021 were used to determine the type of bond between empon-empon compounds and target proteins. Bonds that can be formed include hydrogen bonds, hydrophobic bonds, and electrostatic bonds. The strength of bonds formed between the compounds and target proteins is directly related to the diversity of said bonds and the ensuing cellular activities.<sup>36</sup>

## Results and Discussion

### Drug-likeness prediction from compound of empon-empon

In the field of pharmaceutical sciences, not all chemical compounds are suitable for development as drugs. Therefore, it is essential to evaluate their physicochemical characteristics for drug-likeness, often guided by Lipinski's Rule of Five (RO5). This rule serves to assess whether a ligand exhibits hydrophilic or hydrophobic properties, which in turn affects its capacity to traverse cell membranes via passive diffusion can be seen in Table 1.

**Table 1:** Predicted druglikeness of compound of empon-empon based on Lipinski's rule of five

Ligand	Mass (<500) g/mol	HBA (<10)	HBD (<5)	LogP (<5)	Molar refractivity (40-130)
Curdione	236.35	2	0	2.81	72.03
Curcuminone	234.33	2	0	2.79	69.66
Demethoxycurcumin	338.35	5	2	2.78	96.31
Dehydrocurdione	234.33	2	0	2.71	71.56
Calebin A	384.38	7	2	3.33	103.39
Isoprocucumenol	234.33	2	1	2.56	70.44
a Atlantone	218.33	1	0	3.24	70.88
Letestuiainin B	370.40	6	2	2.87	102.48
Bisacumol	218.33	1	1	2.87	70.71
Procucumenol	234.33	2	1	2.56	70.44
Zedoarondiol	252.35	3	2	2.36	72.12
Iso-Velleral	216.32	1	0	3.12	69.75
Letestuiainin A	340.37	5	3	2.90	96.89
Bisdemethoxycurcumin	308.33	4	2	1.75	89.82
Tryptophan	204.23	3	3	0.94	57.36
Curcumlactone	236.35	2	0	3.02	70.84
Xanthorrhizol	218.33	1	1	3.32	71.57
Zedoarol	246.30	3	1	2.59	68.89
Dihydrocurcumin	370.40	6	5	2.87	102.48
Curcumin	368.38	6	2	3.27	102.82
Naringenin	272.25	5	3	1.75	71.57
Curcumenolactone A	248.32	3	0	2.64	68.63
Gweicurculactone	228.29	2	0	2.90	68.96
3-Carene-2,5-dione	164.20	2	0	1.83	45.62
3-Caren-5-one	150.22	1	0	2.21	45.42

The logP value, which ranges between -0.4 and 5, indicates a compound's partition coefficient between lipophilic and aqueous environments. Compounds with molecular weights greater than 500 Da typically face difficulty in crossing cellular membranes. Moreover, a

higher logP value reflects increased hydrophobicity, which may correlate with elevated toxicity due to prolonged retention in the lipid bilayer and extensive systemic distribution, ultimately diminishing the compound's selectivity toward its target enzyme.

Hydrogen bond donor must be below 5 and 10 for hydrogen bond acceptors. Finally, molar refractivity from compounds has a value between 40-130.<sup>30-32</sup>

#### *Molecular docking simulation*

Molecular docking is used to identify new drug candidates, optimize existing compounds, understand the interaction of drug candidate compounds with receptors, propose structural hypotheses about how ligands inhibit their targets, and perform virtual screening of

compounds.<sup>34</sup> The molecular docking process produces data from binding affinity (BA). Binding affinity refers to the measurement of a ligand's capacity to interact with its corresponding receptor, expressed in units of kcal/mol. A lower binding affinity value indicates a stronger interaction between the ligand and the receptor, signifying higher binding strength or affinity.<sup>35</sup> The binding affinity values obtained from the docking process between the ligands and target receptors can be seen in Table 2.

**Table 2:** Results of molecular docking simulation between empon-empon compounds and target proteins

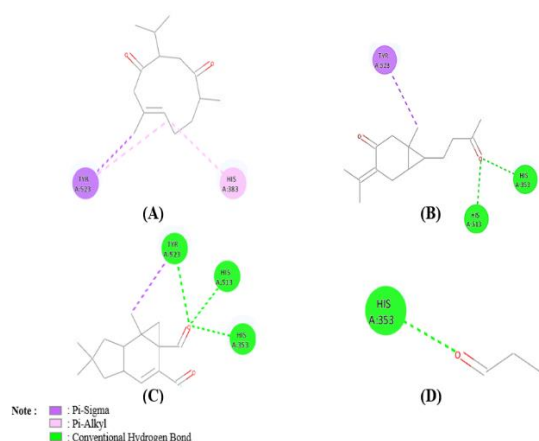
Target Receptor	Compound	Herb	BA (kcal/mol)
Angiotensin Converting Enzyme	Curdione	Turmeric	-7.4
	Curcuminone	Turmeric	-7.4
	Iso-Velleral	Curcuma	-7.1
	Bis Demethoxycurcumin	Turmeric	-9.2
Cathepsin L	Letestuiainin A	Curcuma	-9.1
	Demethoxycurcumin	Turmeric	-8.2
	Dehydrocurdione	Turmeric	-6.9
	Bisdemethoxycurcumin	Curcuma	-7.4
Cyclooxygenase-1	Demethoxycurcumin	Turmeric	-7.4
	Letestuiainin A	Curcuma	-7.4
	Calebin A	Turmeric	-7.3
	Zedoarol	Curcuma	-9.0
Cyclooxygenase-2	Isoprocucumenol	Turmeric	-8.6
	a Atlantone	Turmeric	-8.4
	Curcumalactone	Curcuma	-8.3
	Xanthorrhizol	Curcuma	-7.6
Ephrin type A Receptor 2	Demethoxycurcumin	Turmeric	-6.0
	Calebin A	Turmeric	-5.6
	Tryptophan	Curcuma	-4.7
	Letestuiainin B	Turmeric	-4.6
Glucagon Receptor	Dihydrocurcumin	Curcuma	-4.6
	Dihydrocurcumin	Curcuma	-10.0
	Letestuiainin A	Curcuma	-9.9
	Curcumin	Curcuma	-9.7
Insulin	Bisacumol	Turmeric	-8.8
	Quercetine	Turmeric	-6.0
	Naringenin	Curcuma	-5.7
	Xanthorrhizol	Curcuma	-5.3
Tyrosine protein kinase JAK1	Curcumenolactone A	Curcuma	-8.1
	Gweicurculactone	Curcuma	-7.9
	Procucumenol	Turmeric	-7.9
	Isoprocucumenol	Turmeric	-7.9
Tyrosine protein kinase JAK2	Zedoarondiol	Turmeric	-7.5
	Zedoarol	Curcuma	-8.0

Target Receptor	Compound	Herb	BA (kcal/mol)
Testis Specific Androgen Binding Protein	Curcumenolactone A	Curcuma	-7.9
	Isoprocurcumenol	Turmeric	-7.8
	Gweicurculactone	Curcuma	-7.5
	Procurcumenol	Turmeric	-7.5
	Naringenin	Curcuma	-10.0
	Zedoarondiol	Turmeric	-8.6
	a Atlantone	Turmeric	-7.7
Estrogen receptor alpha	3-Carene-2,5-dione	Aromatic ginger	-7.2
	Curcumenolactone A	Curcuma	-8.4
	Naringenin	Curcuma	-2
	Xanthorrhizol	Curcuma	-7.4
	Bisdemethoxycurcumin	Curcuma	-7.3
	Bis Demethoxycurcumin	Turmeric	-7.1

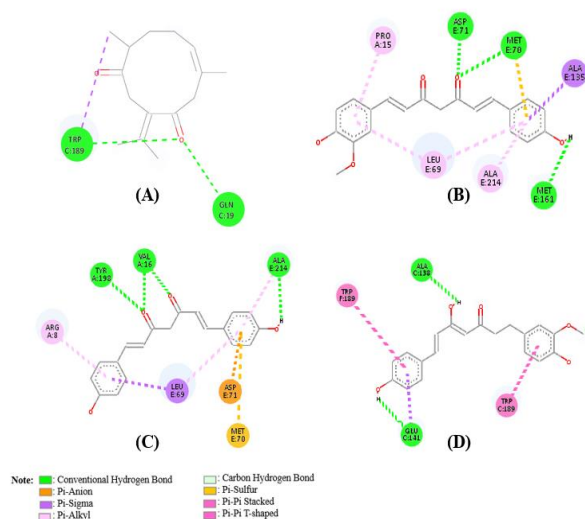
BA = Binding Affinity

### Visualization and interaction

Visualization was conducted to observe the interactions between the active compounds and target proteins as identified through molecular docking analysis. This process illustrates key binding sites, including hydrogen bonding, hydrophobic contacts, and electrostatic interactions within the protein's active region.<sup>36</sup> Docking simulations are utilized to estimate both the binding affinity and orientation of active compounds, offering valuable insights into their potential molecular mechanisms and effectiveness in modulating target protein activity. The interaction is carried out to understand the structure-activity relationship and guide further drug design and optimization efforts.

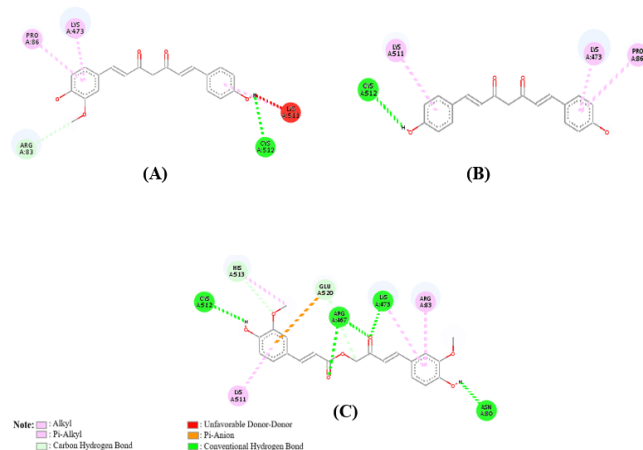


**Figure 1:** Visualization of Angiotensin Converting Enzyme Docking Results with compounds curdione (A), Curcuminone (B), Iso-Velleral (C), Propanal (D)

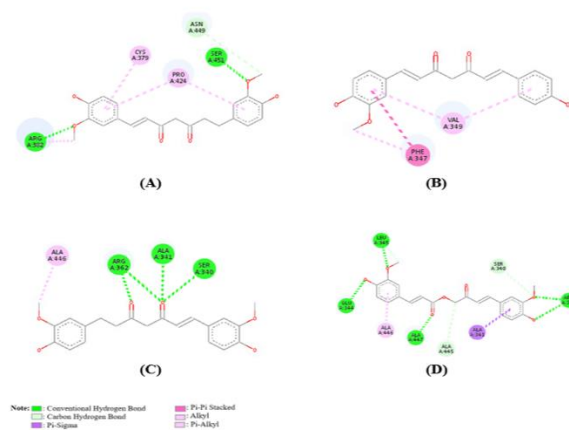


**Figure 2:** Visualization of Cathepsin L Docking Results with compounds Dehydrocordione (A), Demethoxycurcumin (B), Bis demethoxycurcumin (C), Letestuianin (D)

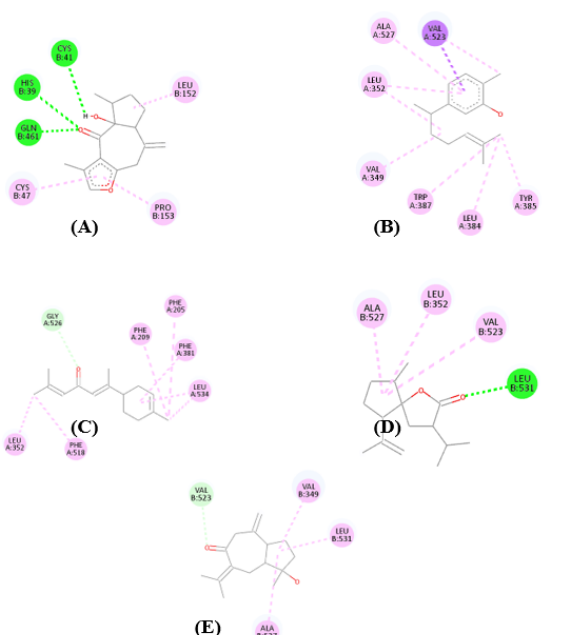
Empon-empon is a traditional medicine consisting of Turmeric (*Curcuma longa*), Aromatic ginger (*Kaempferia galanga*), and Curcuma (*Curcuma xanthorrhiza*). Molecular docking was applied to assess the potential of ginger-based feed wafers in enhancing the health of ruminant livestock through specific interactions with target proteins. A total of eleven selected proteins are involved in supporting various physiological functions that contribute to improved performance in ruminants. Among these, the angiotensin-converting enzyme (ACE) is known for its role in regulating blood pressure and maintaining vascular homeostasis.<sup>20</sup> The presence of a compound that acts as a ligand that can interact with ACE (Figure 1) is expected to function as an inhibitor and prevents cardiovascular diseases such as hypertension in ruminant animals.<sup>40</sup> Curdione and Curcuminone, which are compounds from turmeric, have a binding affinity value of -7.4 kcal/mol with several hydrogen and alkyl bonds formed.



**Figure 3:** Visualization of Cyclooxygenase 1 Docking Results with Curcumin compounds Bis demethoxycurcumin (A), Demethoxycurcumin (B), Calebin A (C)

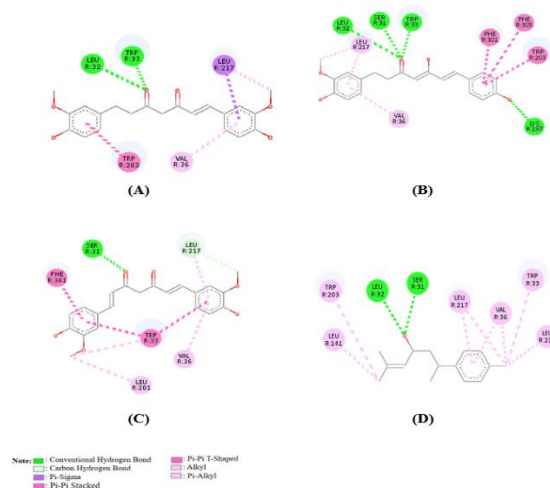


**Figure 5:** Visualization of Docking Results of Ephrin type A Receptor 2 with compounds Dihydrocurcumin (A), Demethoxycurcumin (B), Letestuanin (C), Celebin A (D)

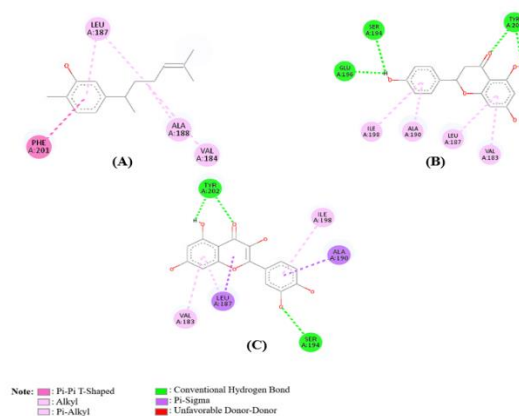


**Figure 4:** Visualization of Cyclooxygenase 2 Docking Results with compounds Zedoarol (A), Xanthorrhizol (B), a Atlantone (C), Curcumalactone (D), Isoprocurcumenol (E)

Cathepsin L (Figure 2) is a cysteine protease enzyme that plays a role in intracellular degradation of proteins and is involved in important processes such as antigen processing, tumor cell invasion, and extracellular matrix remodeling.<sup>21</sup> Several studies have shown the potential of Cathepsin L in diagnosing fasciolosis in cattle, buffalo, and sheep with high sensitivity and specificity.<sup>41</sup> With the binding of the Bis Demethoxycurcumin compound, which has a high binding affinity value of -9.2 kcal/mol and hydrogen bonds at the amino acids TYR198, VAL16, and ALA214, it is expected that it can be an inhibitor of this protein Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) are enzymes involved in the synthesis of prostaglandins, which play a



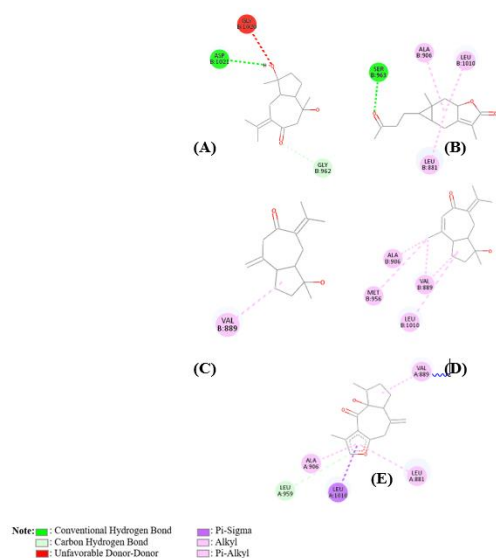
**Figure 6:** Visualization of Docking Results of Glucagon Receptor with Compounds Dihydrocurcumin (A), Letestuanin A (B), Curcumin (C), Bisacumol (D)



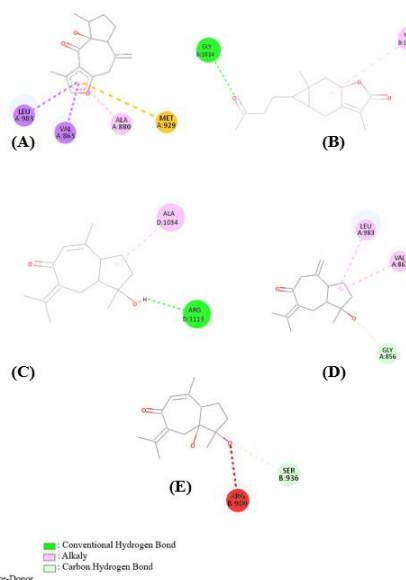
**Figure 7:** Visualization of Insulin Docking Results with Compounds xanthorrhizol (A), naringenin (B), quercetine (C)



crucial role in alleviating inflammation and pain responses within the body.<sup>22,42</sup> The compound Zedoarol, which is from turmeric, had the highest binding affinity value of -9.0 kcal/mol with hydrogen, hydrophobic, and alkyl bonds. It has great potential to activate signaling of the enzyme cyclooxygenase (Figure 3 and 4).



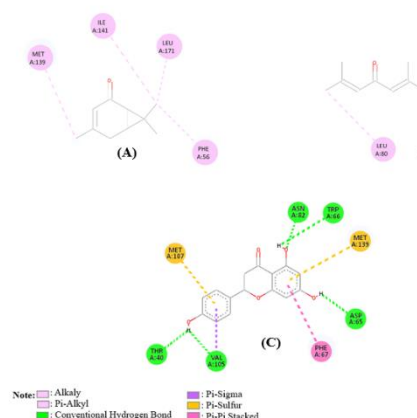
**Figure 8:** Visualization of Docking Results of Tyrosine protein kinase JAK1 with Compounds Zedoarondiol (A), Curcumenolactone A (B), Isoprocucumenol (C), Procucumenol (D), Zedoarol (E)



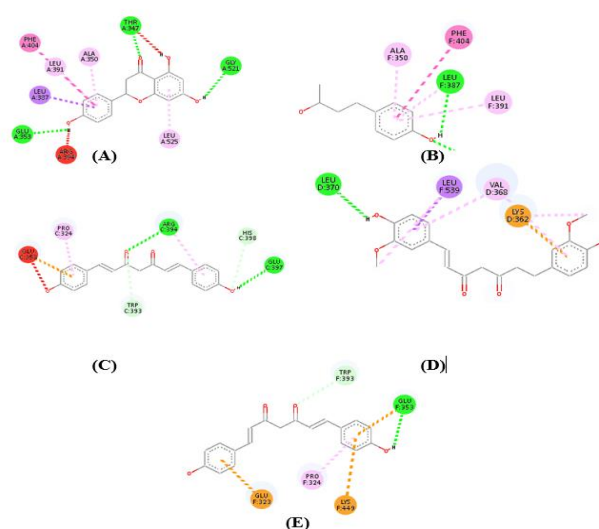
**Figure 9:** Visualization of Docking Results Tyrosine protein kinase JAK2 with Compounds Zedoarol (A), Curcumenolactone A (B), procucumenol (C), Isoprocucumenol (D), procucumadiol (E)

Ephrin type A receptor 2 (Figure 5) is a member of the tyrosine kinase receptor family that regulates multiple cellular functions, including cell migration, adhesion, proliferation, and angiogenesis. This receptor is commonly overexpressed in various cancer types, and its elevated activity has been linked to tumor development and metastatic spread.<sup>23</sup>

With the compound Demethoxycurcumin, which has a binding affinity value of -6.0, it is hoped that it can inhibit the function of this receptor, thereby reducing the invasion and growth of cancer cells. Glucagon Receptors and insulin are essential regulators of glucose metabolism and energy balance in ruminants, affecting weight gain and feed efficiency.<sup>24</sup> The inhibition of glucagon receptors is crucial in the treatment of type 2 diabetes, as it helps regulate blood glucose levels. Similarly, the activation of insulin receptors is essential for maintaining glucose homeostasis in the body, both of which are closely associated with the pathophysiology of type 2 diabetes.<sup>43</sup> Therefore, the Dihydrocurcumin compound, which has the highest binding affinity value of -10.0 kcal/mol, is expected to inhibit glucagon receptors and activate insulin receptors (Figure 6 and 7).



**Figure 10:** Visualization of Docking Results of Testis Specific Androgen Binding Protein with Compounds 3-Caren-5-one (A), a Atlantone (B), Naringenin (C)



**Figure 11:** Visualization of Docking Results Estrogen receptor alpha with Compounds naringenin (A), rhodo (B), Bisdemethoxycurcumin (C), Dihydrocurcumin (D), Bis Demethoxycurcumin (E)

Tyrosine Protein Kinases JAK1 and JAK2 are key mediators in immune signaling pathways, making them relevant to evaluating immunomodulatory effects.<sup>25</sup> The curcumenolactone A compound has a binding affinity value of -8.1 kcal/mol on JAK1 (Figure 8) and -7.9 kcal/mol on JAK2 (Figure 9). This compound shows potential in inhibiting the protein responsible for activating the Signal Transducer and Activator of Transcription (STAT) pathway, which is implicated in the development of cancer and autoimmune disorders. Therefore, it is expected to contribute to enhancing immune responses and regulating metabolic processes in ruminant livestock, particularly in response to infections and metabolic stress.<sup>44</sup> Testis-Specific Androgen-Binding Protein and Estrogen Receptor Alpha are crucial for reproductive health, influencing fertility, breeding performance metabolic functions, and influencing hormone balance.<sup>26,28</sup> Naringenin had the highest binding affinity value of -10.0 kcal/mol on Testis-Specific Androgen-Binding Protein (Figure 10). Meanwhile, in the Estrogen Receptor Alpha, the compound with the highest binding affinity value was curcumenolactone A at -8.4 kcal/mol (Figure 11). These compounds are expected to help ruminant livestock by reducing the risk of fertility disorders, hence improving their reproductive health.

Based on the results of the present *in silico* study, several compounds from the empon-empon extract show promising potential for use as components of animal feed wafers to optimize the health of ruminant livestock. The interaction between these compounds and selected target proteins illustrates the multifunctional potential of the extract in supporting various aspects of livestock health.

## Conclusion

Several compounds from the empon-empon extract in feed wafer interact with target proteins that play a role in various aspects of health, such as blood pressure regulation, protein degradation, inflammation reduction, glucose metabolism regulation, and reproduction. The compounds from Curcuma, namely naringenin and dihydrocurcumin, have the highest potential, with a binding affinity value of -10.00 kcal/mol with testis-specific androgen binding protein and the Glucagon receptor. Meanwhile, the compound from turmeric, namely Bis Demethoxycurcumin, has a binding affinity value of -9.2 kcal/mol with Cathepsin L protein. The high binding affinity of these compounds indicates their ability as potential inhibitors for various important proteins, which contribute to the long-term health of livestock through the regulation of immunomodulation, metabolism, and reproductive function.

## Conflict of Interest

The authors declare no conflicts of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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## References

- Shukla AD and S Y. Food Processing Technology with Future Impacts, Challenge, and Policy for Sustainable Bioeconomy. In: *Bioeconomy for Sustainability*. Springer; 2024:349-374. doi:10.1007/978-981-97-1837-5\_15
- Day L, Cakebread JA, Loveday SM. Food Proteins from Animals and Plants: Differences in the Nutritional and Functional

- Properties. Trends Food Sci Technol. 2022; 119:428-442. doi:10.1016/j.tifs.2021.12.020
- Kholif AE, Anele UY, Patra AK, Varadyova Z. The Use of Phyto-genic Feed Additives to Enhance Productivity and Health in Ruminants. Front Vet Sci. 2021; 8:685262. doi:10.3389/fvets.2021.685262
- Kamra DN, Agarwal N, Sakthivel PC, Chaudhary LC. Garlic as a Rumen Modifier for Eco-Friendly and Economic Livestock Production. J Appl Anim Res. 2012; 40(2):90-96. doi:10.1080/09712119.2011.607764
- Mehrabi Z, Gill M, Wijk MV, Herrero M, Ramankutty N. Livestock Policy for Sustainable Development. Nat Food. 2020; 1(3):160-165. doi:10.1038/s43016-020-0042-9
- Ezeji-for TIN, Enebaue UE, Ogueke C. Waste to Wealth-Value Recovery from Agro-Food Processing Wastes Using Biotechnology: A Review. Br Biotechnol J. 2014; 4(4):418. doi:10.9734/BBJ/2014/7017.
- Rafiq K, Tofazzal HM, Ahmed R, Hasan MM, Islam R, Hossen MI, Islam MR. Role of Different Growth Enhancers as Alternative to In-Feed Antibiotics in Poultry Industry. Front Vet Sci. 2022; 8:794588. doi:10.3389/fvets.2021.794588
- Mardiyanto MB, Foresty RS, Arlysia V, Chorunissa ZFN, Nugroho GD, Yasa A, Setyawan AD. Plants as Herbal Medicine at Nguter Traditional Market, Sukoharjo, Central Java, Indonesia. Asian J Ethnobiol. 2023; 6(1):65-74. doi:10.13057/asianjethnobiol/y060108
- Mazidah, Zahrina R, Tamara ASS. Jamu Empon-Empon the Preservation and Protection of Indigenous Knowledge as a Community District of Central Java Province Kebumen. T Soc Sci. 2017; 12(2):258-261. doi:10.3923/sscience.2017.258.261
- Memarzia A, Khazdair MR, Behrouz S, Gholamnezhad Z, Jafarnezhad M, Saadat S, Boskabady MH. Experimental and Clinical Reports on Anti-Inflammatory, Antioxidant, and Immunomodulatory Effects of *Curcuma longa* and Curcumin, An Updated and Comprehensive Review. BioFactors. 2021; 47(3):311-350. doi:10.1002/biof.1716
- Anyaku IC, Igwilo IO, Ezekwesili CN. Nutritional Profile and Health Promoting Properties of *Curcuma longa* (Linn) (Turmeric). Int J Res Pub Rev. 2023; 4(11):2731-2740.
- Arivoli S, Tennyson S, Divya S, Rani S, Marin G. GC-MS Analysis of Bioactive Compounds of *Curcuma longa* Linnaeus (Zingiberaceae) Rhizome Extract. J Pharmacogn Phytochem. 2019; 8(6):49-52.
- Sherly EA, Kumar P, Tripathi A, Tiwari S, Bari P. Role of Five Medicinal Plants (Giloy/Guduchi, Garlic, Tulsi, Turmeric and Ginger) in Human Immune System. Int J Innov Sci Res Technol. 2023; 8(3):197-205.
- Muzzazinah M, Yunus A, Rinanto Y, Suherlan Y, Ramli M, Putri DS, Nabila SJ. Profile of Chemical Compounds and Potency of Galangal (*Kaempferia galanga* L.) Essential Oils from Kemuning Village, Karanganyar District, Central Java, Indonesia. Biodiversitas J Biol Divers. 2024; 25(4):1386-1393. doi:10.13057/biodiv/d250406
- Das R and Ranindra KN. Ethnomedicinal Uses, Phytochemical Analysis and Antibacterial Activity of *Kaempferia galanga* L. Rhizome. Asian J Microbiol Biotechnol Environ Sci. 2023; 25(2):321-326.
- Sari TV, Harahap RIH, Hasanah U. The Efficacy of Healthy Drinks Based on *Kaempferia galanga*, Ginger and Garlic as Phytobiotics on Fat Content of Breast, Percentage of Abdominal Fat and Broiler Carcass. In: *E3S Web of Conferences*. Vol 332. EDP Sciences; 2021:01004. doi:10.1051/e3sconf/202133201004
- Septama AW, Tasfiyati AN, Kristiana R, Jaisi A. Chemical Profiles of Essential Oil from Javanese Turmeric (*Curcuma xanthorrhiza* Roxb.), Evaluation of Its Antibacterial and Antibiofilm Activities Against Selected Clinical Isolates. South Afr J Bot. 2022; 146:728-734. doi:10.1016/j.sajb.2021.12.017
- Klau ME, Rohaeti E, Rafi M, Artika IM, Ambarsari L, Nurcholis W. Metabolite Profiling of *Curcuma xanthorrhiza* Varieties Grown in Different Regions Using UHPLC-Q-Orbitrap-HRMS and



- Chemometrics Analysis. Biointerface Res Appl Chem. 2023; 13(1):1-13. doi:10.33263/BRIAC131.026
19. Fuloria S, Mehta J, Chandel A, Sekar M, Rani NNIM, Begum MY, Fuloria NK. A Comprehensive Review on the Therapeutic Potential of *Curcuma longa* Linn. in Relation to Its Major Active Constituent Curcumin. Front Pharmacol. 2022; 13:820806. doi:10.3389/fphar.2022.820806
  20. Giani JF, Veiras LC, Shen JZ, Bernstein EA, Cao D, Okwan DD, Bernstein KE. Novel Roles of the Renal Angiotensin-Converting Enzyme. Mol Cell Endocrinol. 2021; 529:111257. doi:10.1016/j.mce.2021.111257
  21. Anes E, Pires D, Mandal M, Azevedo-Pereira JM. Spatial Localization of Cathepsins: Implications in Immune Activation and Resolution During Infections. Front Immunol. 2022; 13:955407. doi:10.3389/fimmu.2022.955407
  22. Vishwakarma RK and Negi DS. The Development of COX-1 and COX-2 Inhibitors: A Review. Int J Pharm Sci Res. 2020; 11(8):3544. doi:10.13040/IJPSR.0975-8232.11(8):3544-3555
  23. Nehal M, Khatoon J, Akhtar S, Khan MKA. Exploring the Potential of EphA2 Receptor Signaling Pathway: A Comprehensive Review in Cancer Treatment. Mol Biol Rep. 2024; 51(1):337. doi:10.1007/s11033-024-09298-8
  24. Allen MS. Control of Feed Intake by Hepatic Oxidation in Ruminant Animals: Integration of Homeostasis and Homeorhesis. Anim. 2020; 14(1):s55-s64. doi:10.1017/S1751731119003215
  25. Bose S, Banerjee S, Mondal A, Chakraborty U, Pumarol J, Croley CR, Bishayee A. Targeting the JAK/STAT Signaling Pathway Using Phytocompounds for Cancer Prevention and Therapy. Cells. 2020; 9(6):1451. doi:10.3390/cells9061451
  26. Dong S, Chen C, Zhang J, Gao Y, Zeng X, Zhang X. Testicular Aging, Male Fertility and Beyond. Front Endocrinol (Lausanne). 2022; 13:1012119. doi:10.3389/fendo.2022.1012119
  27. Sears SM and Hewett SJ. Influence of Glutamate and GABA Transport on Brain Excitatory/Inhibitory Balance. Exp Biol Med (Maywood). 2021; 246(9):1069-1083. doi:10.1177/1535370221989263
  28. Tripathi M, Yen PM, Singh BK. Estrogen-Related Receptor Alpha: An Under-Appreciated Potential Target for the Treatment of Metabolic Diseases. Int J Mol Sci. 2020; 21(5):1645. doi:10.3390/ijms21051645
  29. Pinzi L and Rastelli G. Molecular Docking: Shifting Paradigms in Drug Discovery. Int J Mol Sci. 2019; 20(18):4331. doi:10.3390/ijms20184331
  30. Goel KK, Thapliyal S, Kharb R, Joshi G, Negi A, Kumar B. Imidazoles as Serotonin Receptor Modulators for Treatment of Depression: Structural Insights and Structure-Activity Relationship Studies. Pharmaceutics. 2023; 15(9):2208. doi:10.3390/pharmaceutics15092208
  31. Kim S and Bolton EE. PubChem: A Large-Scale Public Chemical Database for Drug Discovery. In: Open Access Databases and Datasets for Drug Discovery. Wiley; 2024:39-66.
  32. Goodsell DS, Zardecki C, Di Costanzo L, Duarte JM, Hudson BP, Persikova L, Burley SK. RCSB Protein Data Bank: Enabling Biomedical Research and Drug Discovery. Protein Sci. 2020; 29(1):52-65. doi:10.1002/pro.3730
  33. Tjitda PJ, Nitban FO, Parikesit AA, Bessi MI, Wahyuningsih TD. *In Silico* Investigation of Tropical Natural Product for Wild-Type and Quadrupole Mutant PfDHFR Inhibitors as Antimalarial Candidates. Trop J Nat Prod Res. 2024; 8(2):6208-6217. doi:10.26538/tjnpr/v8i2.18
  34. Farahdina AA, Ardiyanti SA, Endharti AT, Fitri LE, Widodo N. LC-MS Chemical Profiling, *In Silico* Docking Studies to Unravel the Therapeutic Potential of *Streptomyces hygroscopicus* as a Source of Antimalarial Bioactive Compounds. Trop J Nat Prod Res. 2024; 8(3):6736-6743. doi:10.26538/tjnpr/v8i3.38
  35. Afnani MR, Emilia NF, Damayanti AE, Rabbani CN, Purnama ER. Potency of Active Compounds Extract of Soursop Leaves (*Annona muricata*) as a Candidate for Cervical Cancer Drug *In Silico*. E3S Web Conf. 2024; 513:03005.
  36. Ham SM, Nursanti O, Aisyah RT. Molecular Docking and Toxicity Studies of Nerve Agents Against Acetylcholinesterase (AChE). J Recept Signal Transduct Res. 2023; 43(5):115-122. doi:10.1051/e3sconf/202451303005
  37. Paggi JM, Pandit A, Dror RO. The Art and Science of Molecular Docking. Annu Rev Biochem. 2024; 93:389-410. doi:10.1146/annurev-biochem-030222-120000
  38. Raval K and Ganatra T. Basics, Types and Applications of Molecular Docking: A Review. IP Int J Compr Adv Pharmacol. 2022; 7(1):12-16. doi:10.18231/j.ijcaap.2022.003
  39. Vidal-Limon A, Aguilar-Toalá JE, Liceaga AM. Integration of Molecular Docking Analysis and Molecular Dynamics Simulations for Studying Food Proteins and Bioactive Peptides. J Agric Food Chem. 2022; 70(4):934-943. doi:10.1021/acs.jafc.1c06110
  40. Khurana V and Goswami B. Angiotensin Converting Enzyme (ACE). Clin Chim Acta. 2022; 524:113-122. doi:10.1016/j.cca.2021.10.029
  41. Corrales JL, Cwiklinski K, Verissimo CDM, Dorey A, Lalor R, Jewhurst H, Dalton JP. Diagnosis of Sheep Fasciolosis Caused by *Fasciola hepatica* Using Cathepsin L Enzyme-Linked Immunosorbent Assays (ELISA). Vet Parasitol. 2021; 298:109517. doi:10.1016/j.vetpar.2021.109517
  42. Hashemi GN, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 in Cancer: A Review. J Cell Physiol. 2019; 234(5):5683-5699. doi:10.1002/jcp.27411
  43. Galsgaard KD, Winther-Sørensen M, Pedersen J, Kjeldsen SA, Rosenkilde MM, Albrechtsen NJW, Holst JJ. Glucose and Amino Acid Metabolism in Mice Depend Mutually on Glucagon and Insulin Receptor Signaling. Am J Physiol Endocrinol Metab. 2019; 316(4):660-673.
  44. Krueger JG, McInnes IB, Blauvelt A. Tyrosine Kinase 2 and Janus Kinase-Signal Transducer and Activator of Transcription Signaling and Inhibition in Plaque Psoriasis. J Am Acad Dermatol. 2022; 86(1):148-157. doi:10.1016/j.jaad.2021.06.869