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In silico's study of Ketepeng leaf (*Cassia alata* L.) as inhibitor of aspartic protease from *Boophillus microplus* as a natural acaricide of ticks in cattle

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ABSTRACT

The objective of this study was to evaluate the potential of *Cassia alata L*. leaf extract as an anti-tick agent through molecular docking analysis against the aspartic protease enzyme from Ixodes ricinus (IrCD1). The study aimed to identify natural compounds that could serve as safer alternatives to synthetic acaricides for controlling Boophilus microplus, a major ectoparasite affecting cattle.The research utilized an in silico approach, employing molecular docking techniques to assess the binding affinity of 14 secondary metabolites from Cassia alata leaves against IrCD1. The receptor structure (PDB ID: 5N7Q) was obtained from the Protein Data Bank, while ligand structures were sourced from PubChem and KNApSAcK. The docking process was performed using YASARA Structure, with binding free energy (ΔG) and dissociation constant (Kd) values analyzed for ligand-receptor interactions. Additionally, the bioavailability of the selected compounds was assessed using Lipinski's Rule of Five. The results identified four key metabolites—chrobisiamone A, chrysophanol, quercetin, and rhein—as the most promising inhibitors, with binding free energy values lower than the native ligand, pepstatin. Chrobisiamone A demonstrated the strongest interaction with IrCD1, indicating its potential as a potent acaricidal compound. The study also found that these compounds exhibit strong hydrogen bonding and hydrophobic interactions at the receptor's active site, enhancing their inhibitory potential. In conclusion, the findings support the potential development of organic acaricides derived from Cassia alata L. leaves, with chrobisiamone A emerging as a promising candidate. Further in vitro and in vivo studies are needed to validate its efficacy and safety in tick control applications.

Keywords: Boophillus microplus, Cassia allata, Cattle ectoparasite, In silico, Molecular docking

INTRODUCTION

The infestation of *Boophilus microplus* ticks on cattle in Indonesia is relatively high, at around 46.9%. These ticks are obligate blood-sucking ectoparasites found primarily on vertebrates, especially mammals. These highly resilient ticks act as vectors for pathogens such as *Babesia bigemina* and *B. bovis,* which cause bovine babesiosis. This disease has not only economic implications but also leads clinical symptoms, including anemia, fever, and multiple organ failure (Spickler *et al*., 2022). The most significant economic consequence of tick infestation is the decline in milk quality (Jain *et al*., 2020). Ticks can transmit a variety of pathogens, including bacteria, rickettsia, protozoa, and viruses, to cattle, as well as cause additional blood-feeding-related diseases as well as cause additional blood -feeding-related diseases such as anaplasmosis and theileriosis (Kristina and Setiyono, 2020).

Boophilus microplus ticks require blood for a process facilitataed a process facilitated by gut enzymes. After feeding, the process the process of hemoglobinolysis occurs, activating the endopeptidase cathepsin L (lrCL1). This phase is crucial for the physiological processes of the tick. The presence of lrCL1 inhibitors offers potential solutions for tick-related issues, aiding in the development of acaricides (Reyes *et al.,* 2020). Tick infestations remain a significant problem on traditional and large-scale cattle farms.

Efforts to control ticks include spraying, dipping cattle in insecticides, and using acaricides, which target ticks and mites. However, continuous use of these chemicals can lead to resistance, toxicity, and residue accumulation in milk. Therefore, exploring herbal-based acaricides as alternatives is important. Research on *Cassia alata L.* has shown its potential as an anti-tick agent through in silico studies. This lays the groundwork for developing safe, organic acaricide alternatives, as the leaves contain compounds with antibacterial, antifungal, and microbe growth-inhibiting properties (Asmah *et al*., 2020).

The first stage of drug development utilizes *in silico* testing, a computational method to study protein-ligand interactions (Purwono *et al*., 2024). Aspartic protease enzymes such as cathepsin D (IrCD1, PDB: 5N7Q) from *Ixodes ricinus* are essential for the organism's survival and play a role in digesting host hemoglobin (Hánová *et al.,* 2018). IrCD1 will act as a receptor in this research, with a secondary metabolite from *Cassia alata* leaves serving as the test ligand. The study aims to evaluate the potential of *Cassia alata* leaf extract as an anti-tick agent by inhibiting aspartic protease enzymes, aiding in pharmaceutical development.

MATERIALS AND METHODS

This study used the aspartic protease enzyme from Ixodes ricinus Cathepsin D1 (lrCD1) as a receptor that was obtained from the Protein Data Bank (PDB ID 5N7Q); also 14 metabolites from *Cassia alata* leaves sourced from the KNApSAcK website (Afendi *et al.,* 2012). The enzyme contains natural ligands at its active site (ASP36 and ASP227), functioning as inhibitors, notably pepstatin A (Hánová *et al*., 2018).

Research Procedure

The 3D structure of the enzyme (PDB code 5N7Q) was downloaded from RCSB PDB in PDB format (https://www.rcsb.org/). Ligands for evaluation were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. Using YASARA Structure, unnecessary components such as–natural ligands and water molecules were removed, and hydrogen atoms were added. The modified structure was saved in PDB format. The Ligands were optimized using YASARA Structure. The natural ligand was separated from the receptor protein and saved in *.pdb format. A grid box size ranging from 0.25 to 7 Å was used for binding validation, with intervals of 0.25 Å, employing the AMBER14 force field for 100 repetitions. The grid box with the lowest binding energy and Kd values was analyzed for RMSD and selected for tethering.

For molecular docking, the prepared protein and test ligand were inserted into the YASARA Structure, with the protein locked and the AMBER14 force field applied for 50 tethering repetitions. 2D interaction visualization was carried out using Discovery Studio Visualizer to determine the type of interaction that occurred 3D visualization was performed using the PyMOL program.

Data Analysis

The analysis and evaluation of the test ligands included calculating ΔG values, dissociation constant, visualization of the interaction between ligands and receptor, analysis of the percentage of binding site similarity (%BSS), and Lipinski's rule of five (Ro5) (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski).

RESULTS AND DISCUSSION

The molecular docking method employed is redocking approach, which involves reattaching the natural ligand pepstatin to its receptor to determine the appropriate grid box size. We selected a grid box size of 2.75 Å, as this size yields the most negative free energy value (ΔG). This grid box was used for the 13 test ligands derived from the metabolites of the *Cassia alata* leaf.

The binding affinity predictions aim to identify the most stable binding conformation between the target protein and the test ligands at the receptor's active site (Ikhlas *et al*., 2023). Based on the molecular docking result, four test ligands with the most negative ΔG values and the lowest dissociation constants value were selected. The best five ligands were Lueolin 3-methyl ether 7-mannosyl-(1>2)alloside, chrobisiamone A, quercetin, chrysophanol, and rhein. The results of the interaction between protein and ligands are presented in Table 1.

The relationship between ΔG and the dissociation constant is directly proportional. As the ΔG value becomes increasingly hostile, the corresponding dissociation constant tends to be more negative. Free binding energy or binding affinity reflects the strength of the bond between the target protein and test ligands (Purwono *et al.*, 2024). A lower ΔG value indicates that less energy is required for binding, leading to more substantial and more efficient interactions (Shen *et al.,* 2020). The dissociation constant represents the point at which all hydrogen bonds in a protein-ligand interaction can be permanently broken.

The molecular interaction analysis indicates that five test ligands have more negative ΔG values than the native ligand, suggesting stronger binding affinity (Aritonang *et al.,* 2024). While these test ligands show potential, further analysis is needed to evaluate their safety. The bioavailability analysis of all test ligands was performed using Lipinski's Rule of Five, with results presented in Table 2.

One ligand of the best five test ligands does not fill the requirement for a bioavailability test in developing oral drug preparation. The ligands are luteolin 3'-methyl ether 7-mannosyl-(1→2)-alloside do not comply with Lipinski's rule because the two criteria failed can still be considered a viable drug candidate, as a compound is only deemed to violate the rule if it fails more than two of the requirements (Lipinski, 2004).

Lipinski's Rule of Five is a crucial guideline for assessing a compound's drug-likeness, particularly concerning its metabolism in the body (Lipinski, 2004). The five rules are as follows: (1) molecular weight should be under 500 Da, (2) the log P value should be below 5, (3) there should be fewer than five hydrogen bond donors, (4) there should be fewer than 10 hydrogen bond acceptors, and (5) molar refractivity should fall between 40 and 130. Molecular weight influences a compound's diffusion through cell membranes; higher weights hinder this process (Klara *et al*., 2023). The log P value indicates solubility in fat versus water, with higher values suggesting increased hydrophobicity, slowing elimination, and reducing protein-ligand selectivity. Very negative log P values may hinder a compound's ability to cross the lipid bilayer (Kilo *et al.*, 2019).

The number of hydrogen bond acceptors and donors affects absorption energy (Syahputra *et al*., 2014). More acceptors and donors can decrease a compound's effectiveness due to the higher energy required for binding. Additionally, molar refractivity indicates a compound's steric properties and absorption potential (Marilia *et al*., 2021; Chedik *et al.,* 2017).

Furthermore, according to the molecule interaction between ligands and receptors aspartic protease, the visualization is shown below in Figure 1 – 5. Figure 1 presents a visualization between native ligand pepstatin and receptor target (ΔG -8.248 Kcal/mol). Visualization ligand test Chrobisiamone A was present in Figure 2 with ΔG -9,355 Kcal/mol. The interaction molecule between quercetin and receptor is shown in Figure 3 (ΔG – 8.643 Kcal/mol). Test ligand chrysophanol (ΔG - 8.633 kcal/mol) has a higher binding affinity than the native ligand. It binds to

Table 1. Results of Molecule Interaction between Protein and Ligands dDerived *Cassia alata L.*

No	Compounds	(ΔG) (kkal/mol)	Dissoc. Constant (μM)
1	*Luteolin 3'-methyl ether 7-mannosyl-(1->2)-alloside*	-9.544	5.33×10^5
2	*Chrobisiamone A*	-9.355	7.34×10^5
3	*Quercetin*	-8.643	2.44×10^6
4	*Chrysophanol*	-8.633	2.48×10^6
5	*Rhein*	-8.632	2.49×10^6
6	*Kaempferol*	-8.575	2.74×10^6
7	*Chrysoeriol*	-8.497	3.12×10^6
8	*Rhamnetin 3-mannosyl-(1->2)-alloside*	-8.382	3.79×10^6
-	*Pepstatin**	-8.248	4.75×10^6
9	*Emodin*	-8.120	5.90×10^6
10	*1-Triacontanol*	-6.482	9.36×10^7
11	*Palmitic acid*	-6.277	1.32×10^8
12	*Octadecanoic acid*	-6.265	1.35×10^8

The compound* is a natural co-crystal ligand that binds to the receptor target. The most effective ligands exhibit higher binding affinity, characterized by a more negative ΔG and lower energy requirements for spontaneous binding between the receptor and the ligand.

Table 2. Result of Selection by Lipinksy Rules of Ligands Derived *Cassia alata L.*

No	Metabolite	Criteria of Ro5				
		Molar mass (Dalton)	Log P	Hydrogen donor bonding	Acceptor Hydrogen bonding	Molar refractivity
1	*Luteolin 3'-methyl ether 7-mannosyl-(1->2)-alloside*	624	-2.27	9	16	142.72
2	*Chrobisiamone A*	464	3.33	2	8	120.82
3	*Quercetin*	302	2.01	5	7	74.05
4	*Chrysophanol*	254	2.18	2	4	67.81
5	*Rhein*	284	1.57	3	6	70.04
6	*Kaempferol*	286	2.31	4	6	72.39
7	*Chrysoeriol*	300	2.42	3	6	77.37
8	*Rhamnetin 3-mannosyl-(1->2)-alloside*	640	-2.6	10	17	143.79

The analysis cannot proceed if there are more than two parameters, and the gray-colored ligands indicate values that do not comply with Lipinski's principles.

ASP36 through hydrogen bonding and to ASP227 via pi-anion bonding. Additional hydrogen bonds occur at GLY38 and SER81, while other residues interact hydrophobically (Figure 4).

The Rhein test ligand (ΔG -8.632 kcal/mol) shows a higher binding energy than the natural ligand. This ligand interacts with the active site of ASP36 primarily through van der Waals forces (hydrophobic interactions) and engages with ASP227 via pi-anion bonding, which is also hydrophobic. Hydrogen bonding occurs with the residues GLY80 and SER81, while the remaining residues interact mainly through hydrophobic forces. A visualization of the Rhein test ligand and the aspartic protease protein is illustrated in Figure 5.

Interactions between ligand target receptors can involve hydrogen bonds and hydrophobic bonds. Hydrophobic interactions occur between non-polar molecules, excluding water, and are essential for the stability of the ligand binding to its receptor (Klara et al., 2022). These interactions typically have low energy values, ranging

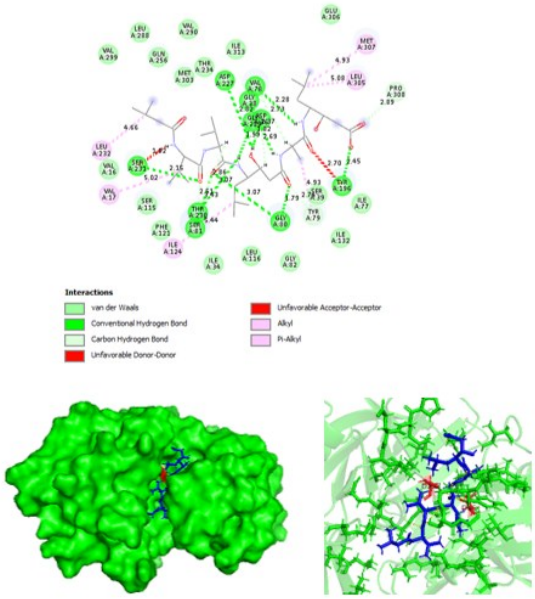

Figure 1. The 2D and 3D visualization illustrates the interaction between the pepstatin molecule, a natural ligand, and its receptor target. Notably, hydrogen interactions at the active site of the ligand engage the amino acid ASP36 and ASP227. These hydrogen bonds are depicted with green dashed lines and represent the most significant interaction for stabilizing the interaction between the two molecules.

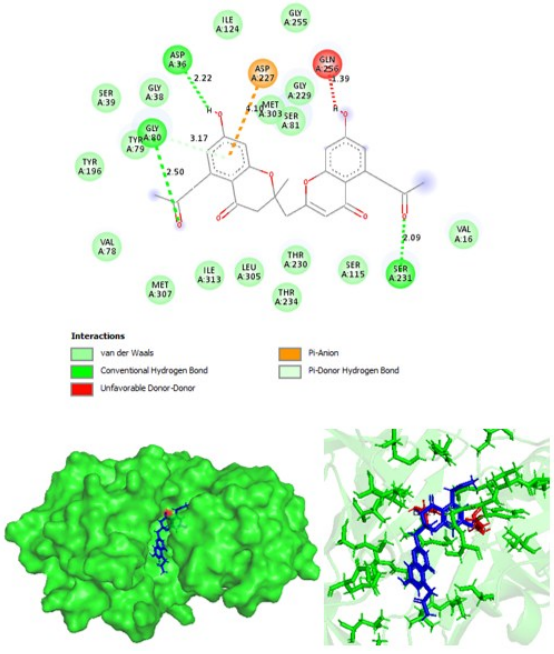

Figure 2. 2D and 3D visualization of the interaction between molecule chrobisiamone and receptor target. Hydrogen binding is shown with green dashed lines at active site ASP36.

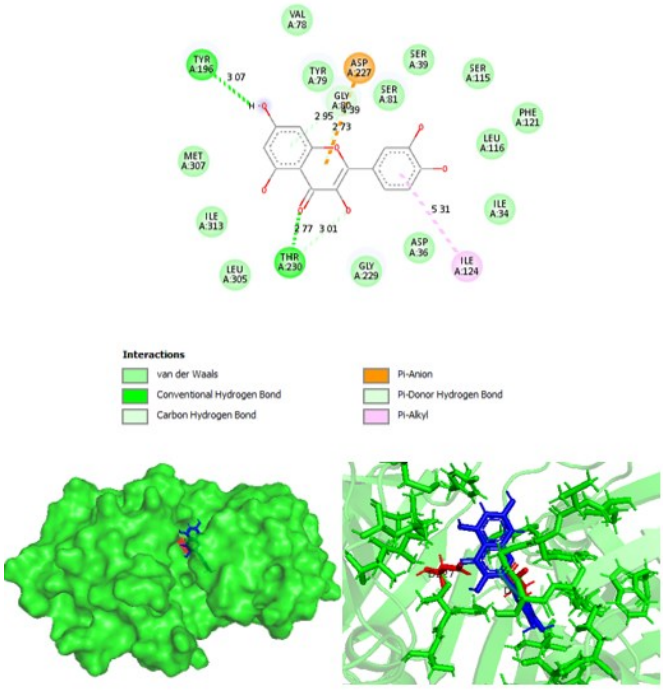

Figure 3. 2D and 3D visualization of the interaction between molecule quercetine and receptor target. Hydrophobic interaction was shown at amino acid residues ASP36 and ASP227.

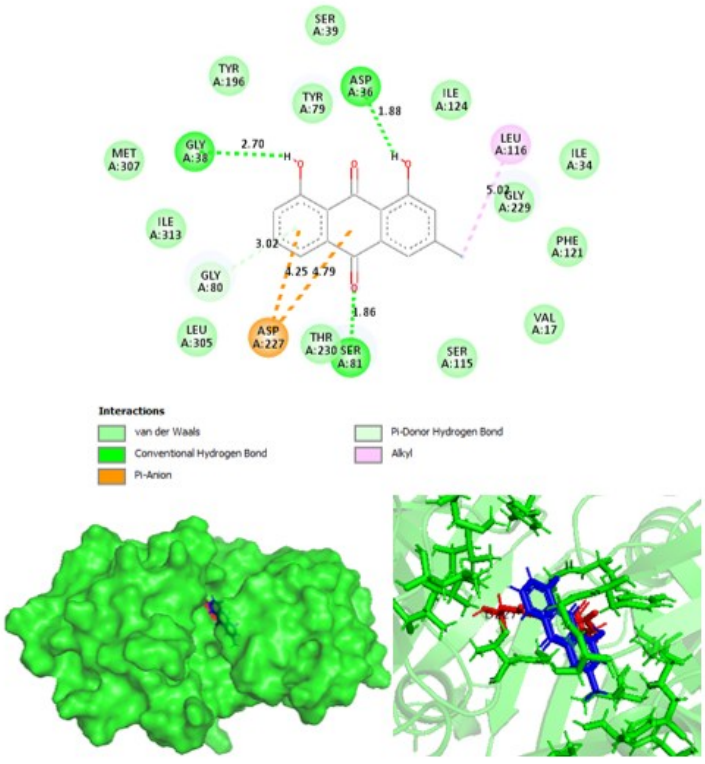

Figure 4. 2D and 3D visualization of chrysophanol test ligand interaction 2D and 3D visualization of the interaction between molecule chrysophanol and receptor target. Hydrogen binding is shown with green dashed lines at active site ASP36 with the shortest distance 1.88 amstrong

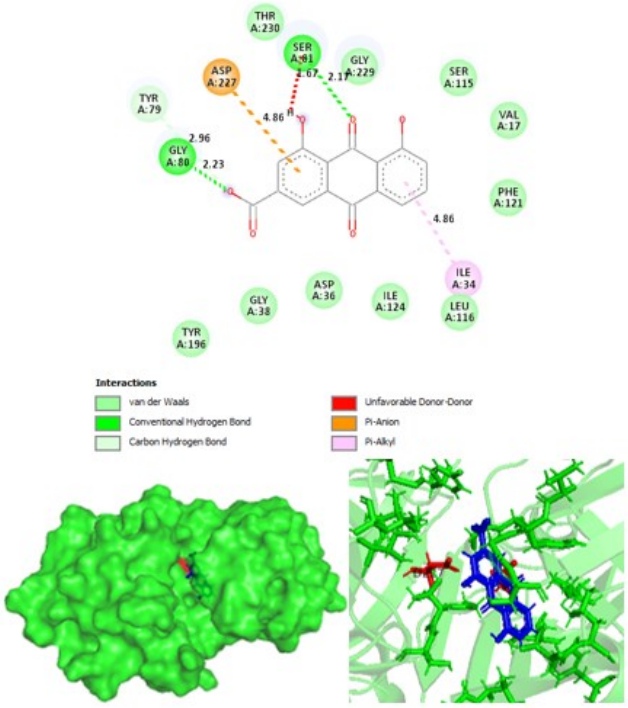

Figure 5. 2D and 3D visualization of the interaction between molecule rhein and receptor target. Hydrophobic interaction was shown at amino acid residues ASP36 and ASP227.

from 0.1 to 0.2 kJ/mol.

Hydrophobic bonds can be classified into van der Waals interactions and other types, such as pi-sigma, alkyl, pi-alkyl, pi-anion, and pi-sulfur bonds, which, while weak, help stabilize ligand-receptor binding (Yahmin *et al*., 2019). This study focuses on the pi-anion bond, a favorable interaction between an electron-deficient aromatic system (π-acid) and an anion, with energy values between 4 and 16 kcal/mol (Sakinah *et al*., 2023). ligand-target protein interactions can involve hydrogen bonds or hydrophobic bonds. Hydrophobic interactions occur between non-polar molecules, excluding water, and are crucial for ligand stability to its receptor (Vitasari *et al*., 2022). These interactions generally have weak energy values, ranging from 0.1 to 0.2 kJ/mol.

Hydrogen bonding is a crucial interaction in determining the ΔG value. This type of bond has a high bond energy, allowing it to form even when the distance between the ligand and the receptor is relatively large. The closer and more numerous the hydrogen bonds formed, the stronger the ΔG will be, making it more effective as an inhibitor (Yahmin *et al.,* 2019).

Specific ligands compete with pepstatin as effective inhibitors of IrCD1. Chrobisiamone A and chrysophanol are critical, as they form additional hydrogen bonds at the active site (ASP36). Moreover, the other four ligands demonstrate strong potential, showcasing more favorable ΔG and dissociation constant values than their natural counterparts.

The inhibitory potential of the aspartic protease enzyme (IrCD1) extracted from *Cassia alata L.* leaves shows promise for further development, particularly regarding binding free energy parameters. Research by Reyes *et al.,* (2020) indicates that ticks lacking this enzyme experience a reduced blood volume intake and a shortened lifespan, which could halt their development. In female ticks, the absence of this enzyme also contributes to a decrease in body weight, thereby limiting blood consumption. This enzyme is crucial during the slow feeding phase, which occurs between days 2 to 6 after the ticks attach to a host. After six days, the ticks typically detach from the host in a dead state (Franta *et al*., 2011).

The larval stage consumes the host's blood until engorged, thereafter molting into a nymph. Nymphs also feed on blood and subsequently molt into adult ticks after satiation. The life cycle of B. microplus varies from 6 weeks to three years, with the potential to yield five generations annually, contingent upon environmental factors (Hadi and Soviana, 2010).

Larvae develop into adults during the parasitic stage on a host. When engorged females fall to the ground to lay eggs, it marks the non-parasitic stage. Adult female ticks can consume up to 0.5 ml of blood. After hatching, larvae crawl to grass edges to attach to passing animals and can survive outside a host for 4 to 5 months, depending on environmental conditions (Mehlhorn *et al*., 2010).

Molecular docking assesses ligand-receptor interactions by evaluating the test ligand chrobisiamone A through affinity binding energy. Chrobisiamone A exhibits a higher affinity energy than the control ligand pepstatin. It is a heterocyclic organic compound with a pyran ring and belongs to the flavonoid group, similar to flavanones (Sysak *et al*., 2023).

Another promising inhibitor is chrysophanol, an anthraquinone glycoside known for its anticancer properties and ability to regulate cell proliferation, apoptosis, and migration. It also suppresses oncogenic activity via the KITENIN/ErbB4 target (Varli *et al*., 2024).

Quercetin is a flavonoid abundant in plant leaves and fruits, known for its renal protective, antioxidant, and anti-inflammatory properties (Madyastuti *et al*., 2015). Studies indicate its promising antiprotozoal potential against Giardia lamblia when combined with chitin (Albogami, 2023). Rhein, an anthraquinone, effectively addresses renal fibrosis in chronic kidney disease (CKD) patients by activating the autophagy pathway (Yu *et al.,* 2017).

Kaempferol is a secondary metabolite in the flavonoid group. Previous studies have demonstrated that kaempferol inhibits LPS-induced airway fibrosis, which can trigger Epithelial-Mesenchymal Transition (EMT). This process is influenced by several factors, including EMT itself, oxidative stress, inflammation, and the extracellular matrix (ECM) (Wang *et al*., 2023).

Chrysoeriol, a flavonoid and secondary metabolite, is known for its antioxidant, anti-apoptotic, and anti-inflammatory properties. These benefits indicate that chrysoeriol may help protect testicular tissue in animals from damage caused by polyethylene microplastics (PE-MP) (Ijaz *et al.,* 2023). Using an in silico approach, specifically the affinity energy value parameter, all ligands demonstrated better values compared to their natural counterparts. The ligand chrobisiamone exhibited the highest affinity energy value, suggesting it could be developed as a safer organic ascaricide with significant potential.

CONCLUSION

Based on *in silico* studies, the leaves of *Cassia alata L.* have been identified as potent inhibitors of the aspartic protease enzyme from *Ixodes ricinus* (IrCD1). Compounds such as chrobisiamone A, chrysophanol, quercetin, and rhein demonstrate significant potential due to their binding free energy (ΔG) and interactions with the receptor. The development of organic acaricides from *Cassia alata* leaves shows great promise, particularly through the mechanism of chrobisiamone A as an inhibitor of cattle ectoparasites.

REFERENCES

Afendi, F. M., T. Okada, M. Yamazaki, A. Hirai-Morita, Y. Nakamura, K. Nakamura, S. Ikeda, H. Takahashi, M. Altaf-Ul-Amin, L. K. Darusman, K. Saitoa, and S. Kanaya. 2012. KNApSAcK family databases: integrated metabolite-plant species databases for multifaceted plant research. Plant Cell Physiol. 53(2): 1-12. https://doi.org/10.1093/pcp/pcr165.

Albogami, B. 2023. Evaluation of the antiparasitic, antihepatotoxicity, and antioxidant efficacy of quercetin and chitosan, either alone or in combination, against infection induced by Giardia lamblia in male rats. Life 13(2): 2316. https://doi.org/10.3390/life13122316.

Anidya, D. K., R. M. Purwono, D. Andrianto, and N. T. Kusumawati. 2023. Aktivitas antibakteri senyawa aktif ekstrak jintan hitam (Nigella sativa) terhadap bakteri MRSA secara in silico. J. Vet. Biomed. 1(2): 92–102. https://doi.org/10.29244/

jvetbiomed.1.2.92-102.

Aritonang, E. A., G. Gunanti, R. M. Purwono, and S. Supriyono. 2024. Systemic anti-inflammatory activity of American cockroach (Periplaneta americana) extract: A molecular docking study. Media Kedokt. Hewan 35(2): 105–112. https://doi.org/10.20473/mkh.v35i2.2024.105-112.

Asmah, N., H. Halimatussakdiah, and U. Amna. 2020. Analisa kandungan senyawa metabolit sekunder ekstrak daun ketepeng cina (*Cassia alata L.*) dari Bireum Bayeun, Aceh Timur. Quimica J. Kimia Sains Terapan 2 (2): 7–10.

Chedik, L., D. Mias-Lucquin, A. Bruyere, and O. Fardel. 2017. In silico prediction for intestinal absorption and brain penetration of chemical pesticides in humans. Int. J. Environ. Res. Public Health 14(6): 708-724.

ECDC (European Centre for Disease Prevention and Control). 2014. Ixodes ricinus - Factsheet for experts. [Diakses 8 Mei 2024]. https://www.ecdc.europa.eu/en/disease-vectors/facts/tick-factsheets/ixodes-ricinus.

Faqih, K., Y. Yahmin, and S. Suharti. 2019. Skrining turunan flavonoid sebagai kandidat inhibitor protease nsP2 dari virus chikungunya menggunakan molecular docking. JC-T (J. Cis-Trans) 3(1): 34–44. https://doi.org/10.17977/um0260v3i12019p034.

Franta, Z., D. Sojka, H. Frantova, J. Dvoral, M. Horn, J. Srba, P. Talacko, M. Mares, E. Schneider, C. S. Craik, J. H. McKerrow, C. R. Caffrey, and P. Kopacek. 2011. IrCL1 - the haemoglobinolytic cathepsin L of the hard tick, Ixodes ricinus. Int. J. Parasitol. 41 (12): 1253-1262. https://doi.org/10.1016/j.ijpara.2011.06.006.

Hadi, U. K. and S. Soviana. 2010. Ektoparasit: Pengenalan, Identifikasi, dan Pengendaliannya. Ed ke-3. Bogor (ID): IPB Press.

Hánová, I., J. Brynda, R. Houštecká, N. Alam, D. Sojka, P. Kopáček, L. Marešová, J. Vondrášek, M. Horn, O. Schueler-Furman, and M. Mareš. 2018. Novel structural mechanism of allosteric regulation of aspartic peptidases via an evolutionarily conserved exosite. Cell Chem. Biol. 25(3): 318–329. https://doi.org/10.1016/j.chembiol.2018.01.001.

Ikhlas, E. N., L. R. Rizkuloh, and R. Mardianingrum. 2023. Analisa in silico senyawa biji lada hitam (Piper nigrum L.) terhadap aktivitas antioksidan. JURRIKES 2 (2): 301–322. https://doi.org/10.55606/jurrikes.v2i2.1815.

Jain, P., I. Satapathy, and R. K. Pandey. 2020. Rhipicephalus microplus: A parasite threatening cattle health and consequences of herbal acaricides for upliftment of livelihood of cattle rearing communities in Chhattisgarh. Biocatal. Agric. Biotechnol. 26: 101611. https://doi.org/10.1016/j.bcab.2020.101611.

Kemenkes (Kementerian Kesehatan). 2023. Penyakit lyme. [Diakses 8 Mei 2024]. https://yankes.kemkes.go.id/view_artikel/2579/penyakit-lyme.

Kristina, A. D. and A. Setiyono. 2020. Infestasi caplak Ixodidae pada sapi lokal di Kelurahan Balai Gadang Kecamatan Koto Tengan Kota Padang. J. Pusat Inovasi Masyarakat 2(2): 145-152.

Lipinski, C. A. 2004. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov. Today Technol. 1(4): 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007.

Mehlhorn, H., K. A. S. Al-Rasheid, F. A. Ghaffar, S. Klimpel, and H. Pohle. 2010. Life cycle and attacks of ectoparasites on ruminants during the year in Central Europe: Recommendations for treatment with insecticides (e.g. Butox®). Parasitol. Res. 107(2): 425-431. http://dx.doi.org/10.1007/s00436-010-1957-0.

Purwono, R. M., M. Syafitri, R. Widyastuti, B. Prasetyo, D. Andrianto, and D. Maulidia. 2024. In silico study of avocado leaves (Persea americana Mill.) as inhibitor of calcium oxalate urolithiasis. IOP Conf. Ser. Earth Environ. Sci. 1359(1):012130. doi: 10.1088/1755-1315/1359/1/012130.

Reyes, J., C. Ayala-Chavec, A. Sharma, and M. Gulia-Nuss. 2020. Blood digestion by trypsin-like serine protease in the replete Lyme disease vector tick, Ixodes scapularis. Insects 11(3):1–14. https://doi.org/10.3390/insects11030201.

Sakinah, E. N., N. Athoillah, K. Y. W. Putri, and Y. Armiyanti. 2023. In silico determination of fenthion, permethrin, and carbaryl as FFAR2 inhibitors: Type 2 diabetes mellitus

pathomechanism study. JKKI 14(3):236–247. doi: 10.20885/JKKI.Vol14.Iss3.art3.

Shen, C., J. Ding, Z. Wang, D. Cao, X. Ding, and T. Hou. 2020. From machine learning to deep learning: advances in scoring functions for protein–ligand docking. WIREs Comput. Mol. Sci. 10:e1429. https://doi.org/10.1002/wcms.1429.

Spickler, A. R. 2022. Rhipicephalus (Boophilus) microplus and R. australis cattle tick, southern cattle tick, southern cattle fever tick, Asian blue tick. www.cfsph.iastate.edu.

Sysak, S., B. Czarczynska-Goslinska, P. Szyk, T. Koczorowski, D. T. Mlynarczyk, W. Szczolko, R. Lesyk, and T. Goslinski. 2023. Metal nanoparticle-flavonoid connections: synthesis, physicochemical and biological properties, as well as potential applications in medicine. Nanomaterials (Basel) 13 (9):1531. https://doi.org/10.3390/nano13091531.

Syahputra, G., L. Ambarsari, and T. Sumaryada. 2014. Simulasi docking kurkumin enol, bisdemetoksikurkumin dan analognya sebagai inhibitor enzim 12-lipoksigenase. J. Biofisika 10(1):55–67.Tu, Y., L. Gu, D. Chen, W. Wu, H. Liu, H. Hu, Y. Wan, and W. Sun. 2017. Rhein inhibits autophagy in rat renal tubular cells by regulation of AMPK/mTOR signaling. Sci. Rep. 7: 43790. https://doi.org/10.1038/srep43790.

Varlı, M., E. Kim, S. Oh, S. Pulat, R. Zhou, C. D. B. Gamage, B. Gokalsin, N. C. Sesal, K. K. Kim, M. Jeong Paik, and H. Kim. 2024. Chrysophanol inhibits colorectal cancer cell motility and energy metabolism by targeting the KITENIN/ErbB4 oncogenic complex. Cancer Cell Int. 24:253. https://doi.org/10.1186/s12935-024-03434-x.

Wang, Q., W. Li, H. Hu, X. Lu, and S. Qin. 2023. Monomeric compounds from traditional Chinese medicine: new hopes for drug discovery in pulmonary fibrosis. Biomed. Pharmacother. 159:114226. doi: 10.1016/j.biopha.2023.114226.