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Anti-methanogenic effect of pyrogallol in *Spirulina platensis* – molecular docking and dynamics simulation on methyl-coenzyme M reductase

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ABSTRACT: **Methane, along with carbon dioxide and nitrogen oxides, is a key greenhouse gas contributing significantly to the global concern over climate change. This study investigated the anti-methanogenic properties of pyrogallol in *Spirulina platensis* using molecular docking and dynamics simulation on methyl-coenzyme M reductase (MCR). The Swiss ADME web server was used to identify pyrogallol's absorption, distribution, metabolism, and excretion (ADME) properties. Molecular docking studies were conducted using UCSF Chimera with the Vina script as the executor. The docking results were further analyzed through molecular dynamics simulation using Gromacs-2024. ADME analysis indicated that pyrogallol meets Lipinski's Rule of Five. Docking studies revealed that pyrogallol has a binding affinity of 4.6 kJ/mol with 2 hydrogen bonds and 1 hydrophobic interaction. Additionally, the MCR-pyrogallol simulation results showed fluctuating root mean square deviation (RMSD) values that stabilized at t = 26,200 until the end of the simulation with an average value of 2.50 nm. Moreover, the hydrogen bonds formed during the simulation fluctuated, with no bonds observed for more than 75% of the simulation time. The energy released during the simulation reached –300.24 kJ/mol with an average of –5.19 kJ/mol. In conclusion, the pyrogallol compound in *Spirulina plantensis* can potentially inhibit the MCR enzyme, thereby reducing methane production and mitigating the impact of climate change.**

KEYWORDS: **Anti-methanogenic, methyl-coenzyme M reductase, molecular docking, pyrogallol, *Spirulina platensis***

INTRODUCTION

Methane is one of the three major greenhouse gases (GHG), along with carbon dioxide and nitrogen oxides. The global implications of climate change resulting from GHG emissions are a significant concern [1]. The accumulation of methane intensifies climate change, leading to extreme weather events [2], food insecurity [3], and increased human health risks such as respiratory conditions, including asphyxia [4]. In the livestock sector, most methane emissions are generated from the enteric fermentation process of ruminant animals [5]. Annually, ruminants can produce about 86 million metric tons of methane [6, 7]. Besides having a greater contribution to global warming than carbon dioxide (28 times greater) [8], methane emissions constitute a substantial energy loss in ruminant animals [9]. The process of methanogenesis in ruminant animals is carried out by methanogenic archaea [10]. Furthermore, in its conversion, methyl-coenzyme M reductase (MCR) is required to convert carbon dioxide and hydrogen into methane [11]. MCR is the primary complex enzyme that reduces methyl-coenzyme M with coenzyme B in the methane synthesis pathway [12]. Since MCR is the catalytic molecule for the final step of methanogenesis, it is important to target this complex enzyme to reduce methane emissions [13, 14].

Several additive-based methods have been looked into to lower methane emissions [15]. One natural feed additive that can potentially reduce methane emissions in ruminants is microalgae [16]. This could help enhance the sustainability of agricultural practices by incorporating microalgae into environmentally friendly animal diets [17]. *Spirulina platensis* belongs to blue-green microalgae, which has a wide range of nutritional and medical properties [18–20]. In addition to having high-quality protein, *Spirulina platensis* contains bioactive compounds that possess immunomodulatory, antimicrobial, and antioxidant properties [21–23]. Consequently, they aid in disease prevention

and strengthen the immune system. Various phenolic compounds were found in *Spirulina platensis*, with the largest proportion being pyrogallol [24].

In recent years, molecular simulation tools in structural bioinformatics and chemoinformatics have yielded remarkable outcomes in the drug exploration technique [25]. Molecular docking has proven useful in drug discovery and design as this method could predict interactions between small molecules and proteins at the atomic level [26]. Similarly, the previous molecular docking study reported that phytochemical compounds from rhubarb were natural ligands capable of binding to MCR, demonstrating the potential effect to reduce ruminal methane emissions [11]. Furthermore, Khusro et al. [13] demonstrated the potential of *Moringa oleifera L.* related tetradecanoic acid to develop an ideal anti-metanogenic drug in the future, particularly in mitigating methane emissions from horses. In general, other screening on MCR ligand targets had the potential for methane reduction [27,28]. Therefore, we investigated the potential of pyrogallol from *Spirulina platensis* as the MCR inhibitor with anti-methanogenic properties using molecular docking and simulation dynamics.

MATERIALS AND METHODS

Data set and protein-ligand preparation

This study utilizes the MCR sub-unit gamma enzyme obtained from the Uniprot protein database (https://www.uniprot.org/) with ID = A0A1C7D1E3. Homology modeling of the protein structure was performed using the Swissmodel (www.swissmodel.expasy.org/) to obtain the best 3D model for molecular docking studies [29]. Protein optimization was performed using UCSF Chimera by removing water molecules and ligands. Protein preparation included adding hydrogens, assigning charges with gasteiger, and checking side chain structures using the Dunbrack 2010 rotamer library [30].

The bioactive component, pyrogallol, was used as the candidate ligand based on the highest percentage result from the GC-MS analysis conducted by Gabr et al. [24]. The PubChem database (www.pubchem.ncbi.mlm.nih.gov/) was employed to obtain the 3D structure of the pyrogallol compound. Ligand structure minimization was done using UCSF Chimera, adding hydrogen atoms to the ligand based on the considered H-bond (slower). Charge assignment was done using AMBER ff14SB [31].

Molecular docking studies

Molecular docking studies were conducted using UCSF Chimera software with the help of Autodock Vina scripts to execute the molecular docking process [32]. To obtain the best conformation, we used the blind docking method Pan et al. [33] by positioning the grid coordinates according to the protein size. The grid coordinates used in the docking process were (52.45, 32.30, 44.40), with a grid size of (66.30, 43.58, 46.54). The docking results, in the form of the highest affinity values, were used to determine the best position between MCR-pyrogallol. The docking results were visualized in 3D using UCSF Chimera and the Ligplot^{+} to produce visual representations of protein-ligand interactions [34].

ADME study

We utilized Swiss ADME (www.swissadme.ch/) to conduct the absorption, distribution, metabolism, and excretion (ADME) analysis. Lipinski's Rule of Five was applied to assess the suitability of the pyrogallol compound for potential use in livestock to inhibit the MCR enzyme. Lipinski's criteria include a molecular weight below 500 daltons, a high lipophilicity (log P) less than 5, fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, and a molar refractivity between 40-130 [35].

Molecular dynamics simulation

The molecular dynamics simulation employed the Charmm27 force field along with the TIP3P water model. Energy minimization utilized the steepest descent method with a maximum force of 1,000 kJ/mol. The system underwent normal volume and temperature (NVT) equilibration for 100 ps, followed by normal pressure and temperature (NPT) equilibration for 500 ps. The actual molecular dynamics simulation was conducted for 30,000 ps. Ligand and protein preparation for this simulation was done using UCSF Chimera and the Swisspharm web server (www.old.swissparam.ch/). In this simulation, we used NaCl ions to neutralize the system, setting the temperature to 300K and pressure to 1 bar. The default linear constraint solver algorithm constrained all hydrogen atom-based bonds [36]. The results of the molecular dynamics simulation were analyzed in terms of root-mean-square deviation (RMSD), hydrogen bonds changing during simulation, and total energy in this simulation. Gromacs-2024 was used in this molecular dynamic simulation, and the result was visualized using Grace-5.1.25.

RESULTS AND DISCUSSION

Molecular docking studies

The docking results are depicted in Figure 1, illustrating the interaction between the MCR sub-unit gamma and the pyrogallol compound. Molecular docking was employed to visualize and analyze the interaction between the protein and the ligand [37]. The results indicated that the molecular docking between the MCR enzyme and the pyrogallol compound had a binding affinity of -4.6 kJ/mol. This result was considered low compared to the in silico study conducted by Arokiyaraj et al. [11], where the docking between MCR and chrysophanol compound had a binding affinity of -6.92 kJ/mol. However, the study by Dinakarkumar et al. [27] which investigated the interaction between MCR and compound pinacol, had an affinity similar to our study. The binding affinity value indicates the strength of hydrogen bonds or hydrophobic interactions; higher affinity values correspond to stronger binding strength, and vice versa. This parameter reflects the stability of the protein-ligand complex formation, highlighting the significance of new bonds that influence the biological activity of complex molecules [38].

If we look at the interaction shown in Figure 1a, the pyrogallol compound is present in the enzyme cavity. Furthermore, Figure 1b illustrates a hydrogen bond formed with the amino acid residue Glutamate 131 (GLU131). In contrast, pyrogallol engages in hydrophobic interactions with the amino acid residues Aspartate 33 (ASP33), Lysine 132 (LYS132), Lysine 135 (LYS135), Valine 36 (VAL36), and Valine 37 (VAL37). The considerable number of hydrophobic interactions (6 interactions) indicates the strength of the pyrogallol compound in the MCR cavity. These results illustrate that hydrogen bonds and hydrophobic interaction can help stabilize the MCR enzyme with the pyrogallol compound [39].

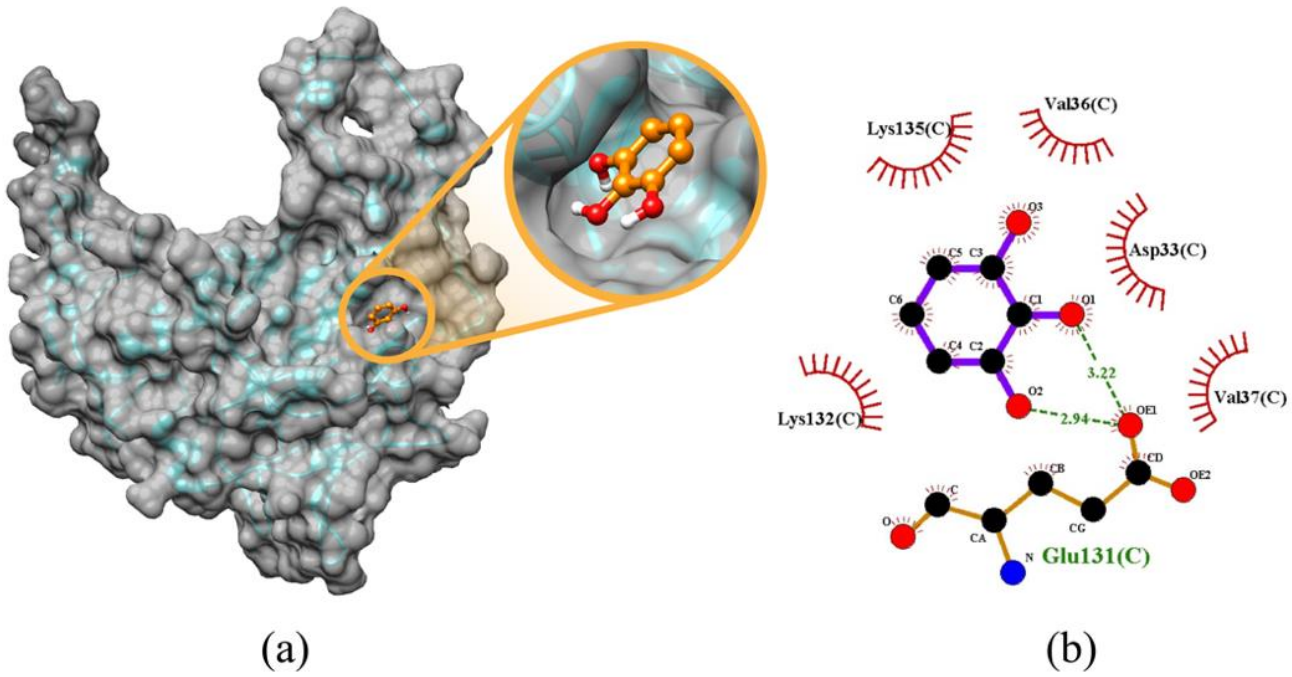

Figure 1. Docking view from MCR sub-unit gamma and pyrogallol; (a) 3D visualization with UCSF Chimera, (b) 2D visualization with LigPlot^{+}

ADME studies

The results of the ADME analysis using the SwissADME web server can be seen in Table 1. The pyrogallol compound with the SMILES Canonical C1=CC(=C(C(=C1)O)O)O has a molecular weight of 126.11 daltons. Molecular weight is one of the important factors because it affects the rate of molecular distribution in the body [40]. The pyrogallol compound can also accept 3 hydrogen atoms and donate 3 hydrogen atoms to the bound compounds. However, pyrogallol has an MLogP value of 0.18, indicating good absorption [41]. Generally, the compound pyrogallol meets four of Lipinski's Rule of Five criteria, except for the molar refractivity value, which is 32.51. This indicates that pyrogallol is typically well absorbed in the intestines and can be effectively distributed once it enters the bloodstream [42]. However, the low molar refractivity value (below 40) may suggest that the compound has insufficient polarity to efficiently traverse cell membranes [35].

Table 1. Compound identify from pyrogallol

Pysicocemichal properties	Value	Lipinski's
Molecular weight (g/mol)	126.11	Yes
Num. H-bonds acceptors	3	Yes
Num. H-bonds donors	3	Yes
Lipophilicity (MLogP)	0.18	Yes
Molar refractivity	32.51	No

Molecular dynamics simulation

Molecular dynamics simulations were performed using the GROMACS-2024 software package. The simulations extended over 30,000 picoseconds (ps) to study the binding dynamics of the pyrogallol compound with the MCR enzyme. The trajectory analysis of the simulation results was used as a reference for analyzing RMSD, hydrogen bond, and total energy. The results from molecular dynamics simulations will elucidate the conformational changes occurring in the MCR-pyrogallol complex throughout the simulation process [43].

The gmx program was employed to analyze the RMSD values throughout the simulation. The highest RMSD value was observed at t = 6,370 ps, reaching 8.03 nm, while the lowest RMSD value was recorded at t = 0 ps, measuring 0.000518 nm. The RMSD values started stabilizing around t = 26,200 ps and remained constant until the conclusion of the simulation at 30,000 ps, averaging 2.50 nm. The RMSD values indicate the stability of the MCR enzyme bound to the pyrogallol compound. In order to evaluate whether the complex system has achieved stability, the average deviation of the complex's conformation from its initial state at a specific time was measured using RMSD [44].

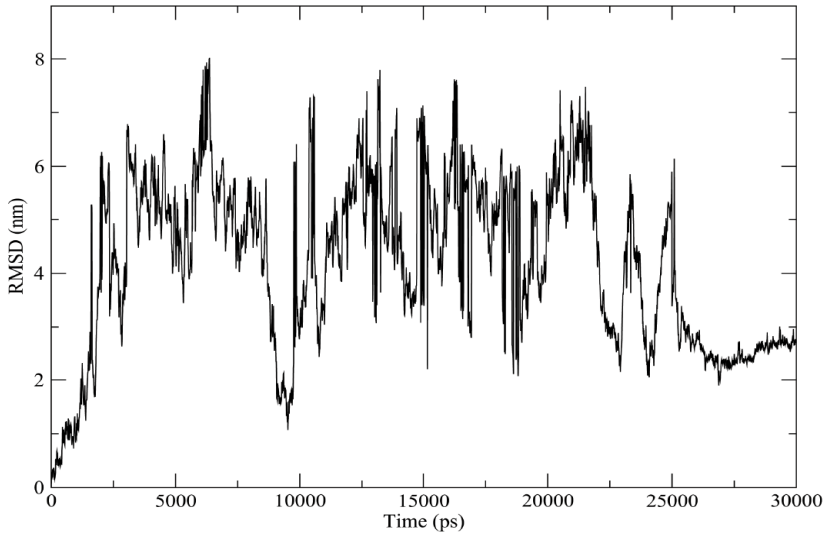

Figure 2. Root mean square deviation (RMSD) of Methyl coenzyme reductase and pyrogallol

The gmx hbond software was utilized to assess hydrogen bond throughout the simulation. Figure 3 illustrates the outcomes of this analysis, demonstrating how hydrogen bonds were formed and dissociated over the course of the simulation. However, it can be observed that hydrogen bonds were often absent during the 30,000 ps simulation, with a total time of 22,800 ps or more than 75% of the simulation time having no hydrogen bonds. The high fluctuations in this simulation study indicate that the system is undergoing conformation changes. However, hydrogen bonds began to form stably at t = 25,330 ps. The highest hydrogen bond formation occurred at t = 27,520 ps, resulting in 4 amino acid residues. If compared, there are differences between the molecular dynamics simulation and the molecular docking study. This indicates that the hydrogen bonds formed are not always stable. The consistency of hydrogen bond stability shows that the protein-ligand complex is in an optimal conformation to function effectively [45], in this case, to inhibit the MCR enzyme.

The energy generated during the simulation procedure was analyzed using the gmx energy program. The results are shown in Figure 4, which indicates that the total energy released during the simulation is -300.24 kJ/mol with an average of -5.19 kJ/mol. Total energy analysis is used to provide an overall view of the thermodynamic stability of the system. Additionally, it can provide insight into the strength of interactions between the MCR

enzyme and pyrogallol compound. Strong interactions are typically characterized by larger negative energies, indicating greater interaction stability [46].

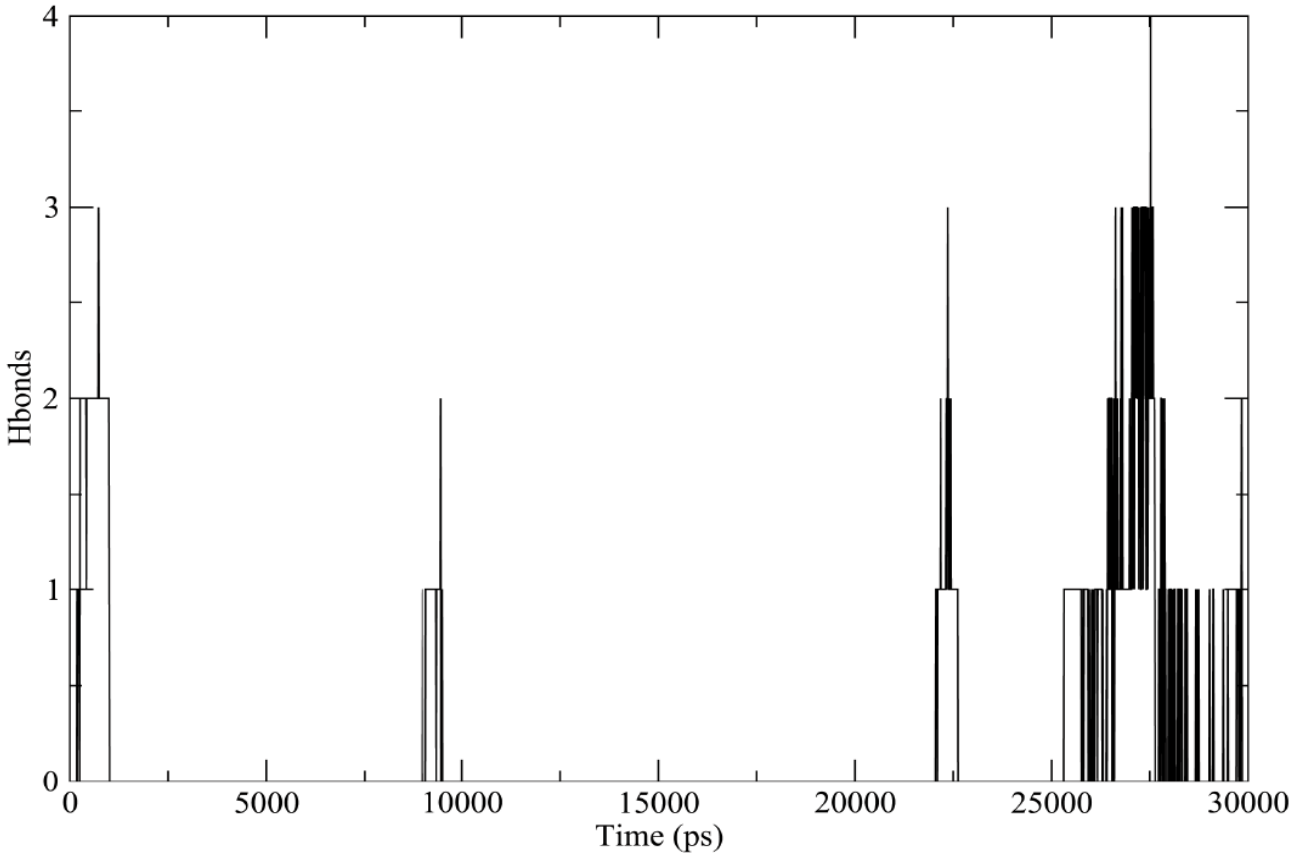

Figure 3. Hydrogen bond numbers of MCR and pyrogallol

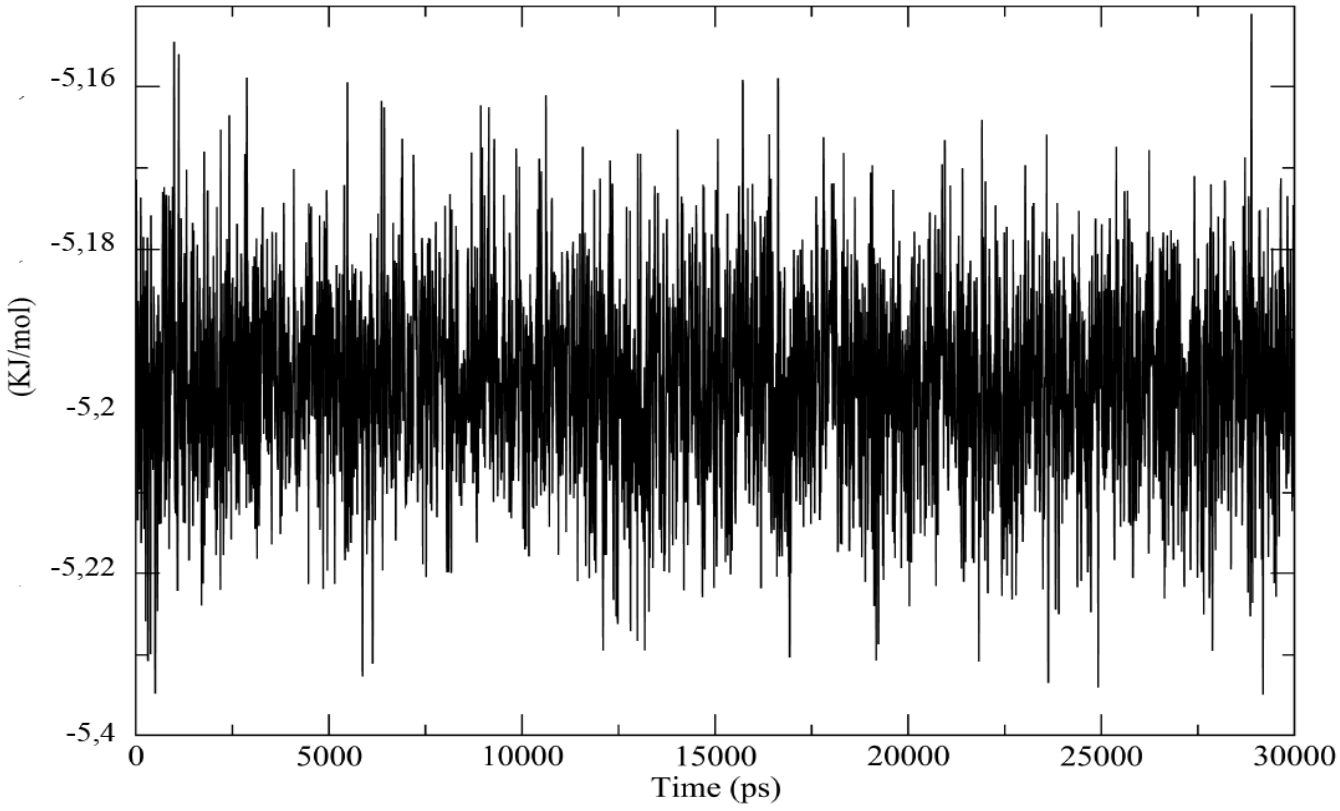

Figure 4. The total energy of MCR and pyrogallol

Implication discussion

The study utilizing MD and simulations has demonstrated that *Spirulina plantensis*, which contains pyrogallol, can interact with the enzyme MCR. This interaction may lead to the inhibition of MCR, potentially reducing methane production from enteric fermentation in ruminant animals. Since MCR is a key enzyme in the methanogenesis process responsible for methane formation [11], mitigating methane production in ruminants could have significant environmental and human health benefits. Methane is a major greenhouse gas that contributes substantially to global warming and climate change [5]. Therefore, reducing methane emissions is crucial not only for ecosystem health but also for human health, as climate change affects clean water availability, agriculture, and air quality—factors directly linked to human well-being [47, 48]. Additionally, methane has a more potent short-term effect in trapping heat in the atmosphere compared to carbon dioxide [8]. Consequently, strategies to reduce methane emissions could lead to more immediate positive impacts in climate change mitigation efforts. In this context, this research highlights the substantial potential of using *Spirulina plantensis* as a feed additive to create more sustainable livestock systems. This approach not only helps to reduce the negative environmental impact of livestock farming but also promotes awareness of more environmentally friendly agricultural practices, ultimately benefiting both humans and animals globally [17, 47]. Thus, the findings of this study are significant not only for enhancing livestock production

CONCLUSION

Pyrogallol has fulfilled Lipinski's Rule of Five in ADME analysis. In molecular docking analysis, the principal binding forces maintaining the stability of the complexes were hydrogen bonding and hydrophobic interaction, as pyrogallol was bound in the interior cavity of methyl-coenzyme M reductase. Furthermore, in molecular dynamics simulation, the binding of methyl-coenzyme M reductase to pyrogallol resulted in a more compact conformation of their structures, showing fluctuation in RMSD values and hydrogen-bonding numbers, although both have the potential to be more stable in the future. Overall, pyrogallol, a bioactive compound in *Spirulina platensis*, has the potential to be used as a methyl-coenzyme M reductase enzyme inhibitor to reduce methane emissions in ruminants, thereby mitigating the impact of climate change.

DECLARATIONS

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Authors' contribution

Muhammad Maulana Sadid: methodology, data collection, simulation, writing, and review. Moh Sofi'ul Anam: conceptualization, writing, data curation, validation, and editing. All authors read and approved the submitted version of the manuscript.

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethics committee approval

This study did not involve the use of live animals; therefore, it did not require approval from an Ethics Committee.

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