Mitigation of Methanogenesis in Ruminants Using Wheatgrass Compounds as Methyl Coenzyme M Reductase Inhibitors: An *In Silico* Study

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Ruminants are significant contributors to methane (CH_4) emissions due to methanogenesis by their gut microbiomes. The enzyme methyl coenzyme M reductase (MCR) is crucial for this process in rumen archaea. Targeting MCR *via* computational tools has emerged as a novel approach to reduce CH_4 emissions in ruminants by inhibiting methanogenesis. This study focused on evaluating wheatgrass (*Thinopyrum intermedium*) compounds as potential MCR inhibitors using *in silico* methods. Initially, 21 wheatgrass compounds were selected, and their drug-likeness traits were assessed using Lipinski's rule of five. Five compounds, namely 2,4,6-trimethyl-1,3-phenylenediamine, Caryophyllene oxide, Caryophyllene, N,N-tetramethylene-.alpha.-(aminomethylene) glutaconic anhydride, and *n*-hexadecanoic acid met all criteria. These compounds were further analysed for absorption, distribution, metabolism, and excretion (ADME) properties using the Swiss ADME tool, confirming their drug-likeness traits with no Lipinski's violation. Molecular docking analysis was performed using the CB-Dock2 tool to assess binding interactions with MCR. The compounds showed binding affinities in the following order: N,N-tetramethylene-.alpha.-(aminomethylene) glutaconic anhydride (-7.3 kcal/mol) > Caryophyllene (-6.8 kcal/mol) > Caryophyllene oxide (-6.7 kcal/mol) > *n*-hexadecanoic acid (-6.3 kcal/mol) > 2,4,6-trimethyl-1,3-phenylenediamine (-6.0 kcal/mol). These findings suggest that the selected wheatgrass compounds have potential as anti-methanogenic agents, positioning them as promising MCR inhibitors for mitigating CH_4 emissions in ruminants.

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INTRODUCTION

There is no doubt that ruminants are crucial for human life as they convert indigestible plant biomass into digestible products like milk and meat (Kamra *et al*. 2012). With rising global demand for these products due to population growth and improved lifestyles (Lan and Yang 2019), the number of domesticated ruminants has significantly

increased. However, this surge raises concerns among ecologists and veterinarians about the associated greenhouse gas emissions, especially methane (CH_4) production. As a matter of fact, the emission of CH_4 from ruminants is directly proportional to the ruminants' population (Islam and Lee 2019). Methane is a potent greenhouse gas, revealing a 25 times higher global warming effect than that of carbon dioxide (CO_2), and ruminants contribute to 16% of global greenhouse gas emissions, accounting for 33% of anthropogenic CH_4 emission (Lan and Yang 2019).

The rumen, an anaerobic fermenter, hosts a diverse microbial community, including bacteria, protozoa, fungi, and archaea (Islam and Lee 2019). Protozoa can constitute up to 50% of the rumen's microbial biomass (Newbold *et al*. 2015), while fungi typically make up around 8%, potentially reaching 20% in sheep (Orpin 1981; Rezaeian *et al*. 2004). Archaea account for a smaller fraction, about 0.3 to 4% (Janssen and Kirs 2008), with bacteria comprising the majority of the microbial biomass (Tapio *et al*. 2017). This microbiome is crucial for fermenting carbohydrates-based feed, producing volatile fatty acids (acetic acid, propionic acid, butyric acids, *etc.*), CO_2, and hydrogen (H_2), which are essential for the ruminant's energy metabolism (Lan and Yang 2019).

Methanogenesis is the process by which CH_4 is produced in the rumen, primarily through the reduction of CO_2 by H_2, facilitated by methanogenic archaea. This process is crucial for clearing H_2 from fermentation (Islam and Lee 2019). Methanogenesis occurs *via* two main pathways: the hydrogenotrophic and methylotrophic pathways (Fig. 1). In the hydrogenotrophic pathway, H_2 and CO_2 are converted into CH_4 by rumen microorganisms, such as bacteria, protozoa, and fungi (Martin *et al*. 2010). Formate, which can be utilized by most ruminal archaea similarly to H_2 and CO_2, is also included in this category (Janssen 2010). The methylotrophic pathway, on the other hand, involves the conversion of methyl groups from substrates like methylamines and methanol into CH_4 (Poulsen *et al*. 2013).

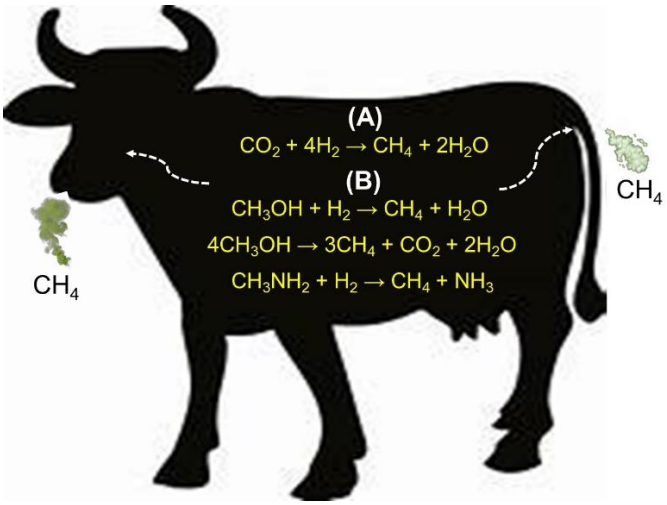

Fig. 1. Methane production from ruminants through (A) hydrogenotrophic pathway and (B) methylotrophic pathway

A variety of methanogens, including species, such as *Methanobacterium* spp., *Methanobrevibacter* spp., and *Methanosarcina* spp., are involved in the final stage of carbohydrate degradation in the rumen, where they convert H_2 and other compounds like formate, CO_2, or methyl donors into CH_4 (Poulsen *et al*. 2013). The final step in biological CH_4 synthesis is catalyzed by the enzyme methyl-coenzyme M reductase (MCR;

EC 2.8.4.1), a membrane-associated enzyme that is unique to methanogens and play a central role in CH_4 biogeochemistry (Khusro *et al*. 2022a). The enzyme is composed of three subunits: α (*mcrA*), β (*mcrB*), and γ (*mcrG*). The α-subunit, encoded by the *mcrA* gene, is highly conserved across all methanogens. This enzyme catalyzes the reduction of methyl coenzyme M (methyl-S-CoM) to CH_4, with coenzyme B serving as the electron donor and coenzyme F_{430} (a nickel-containing tetrahydrocorphin) acting as the prosthetic group (Casañas *et al*. 2015).

In recent years, plethora of *in vitro* studies has been carried out to suppress CH_4 production from ruminants by adding disparate dietary supplements in the feed (Khusro *et al*. 2022b; Elghandour *et al*. 2023, 2024; Santillán *et al*. 2023). However, mitigating CH_4 emission by manipulating the biochemical pathway of methanogenesis process spotlights a new dimension in addressing anti-methanogenic trait in ruminants. Surprisingly, the mitigation of CH_4 emission from ruminants by targeting MCR receptor *via* computational tool is limited. In view of this, the present context was assessed to predict the anti-methanogenic attribute of wheatgrass (*Thinopyrum intermedium*)-associated compounds against MCR receptor in ruminants *via in silico* tools.

EXPERIMENTAL

Compounds of interest

A total of 21 compounds were identified from wheatgrass leaf extract based on previous studies (Durairaj *et al*. 2014; Shakya *et al*. 2014). The chemical structures of these compounds were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF format and were used for the subsequent analyses (Fig. 2).

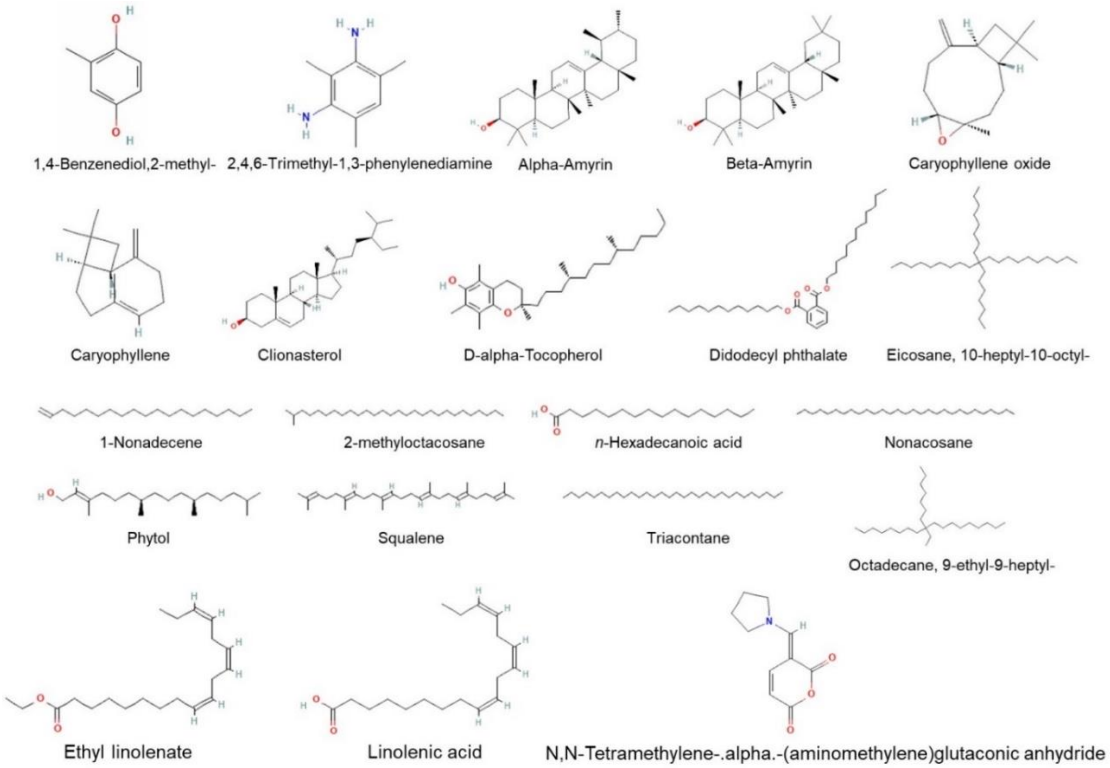

Fig. 2. List of selected wheatgrass compounds

Ligands Selection

Lipinski's rule of five

Lipinski's rule of five was applied to assess the drug-likeness of all the ligands (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). This rule evaluates the drug's viability by considering factors, such as molecular weight, logP, number of hydrogen bond acceptors, hydrogen bond donors, and molar refractivity (Lipinski 2004).

ADME properties analysis

Ligands that satisfied Lipinski's rule of five were further analysed for ADME (absorption, distribution, metabolism, and excretion) properties analysis using the Swiss ADME tool from the Swiss Institute of Bioinformatics (http://www.swissadme.ch/). Canonical SMILES for the ligands were retrieved from PubChem and evaluated for properties such as water solubility (Log mol/L), lipophilicity (Log $P_{o/w}$), gastrointestinal (GI) absorption, blood brain barrier (BBB) permeant, and P-gp substrate status. The Swiss ADME tool, based on a support vector machine algorithm, efficiently analyses datasets of known inhibitor/non-inhibitor and substrate/non-substrate (da Silva *et al*. 2006). These selected compounds were then advanced to molecular docking analysis.

In silico Molecular Docking

Target receptor

The structure of the MCR receptor was obtained from the RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank - IMRO) (http://www.rscb.org/pdb) and saved in PDB format. Prior to docking, non-essential components, such as water molecules and bound inhibitors, were removed from the receptor structure to ensure accurate docking results (Fig. 3).

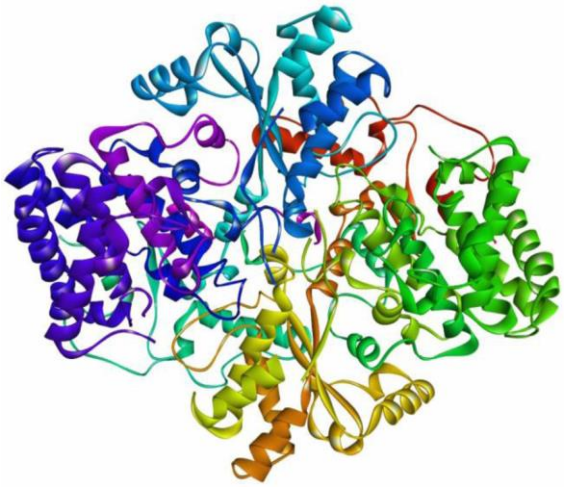

Fig. 3. 3D structure of MCR receptor

Molecular docking and visualization

The interaction between the selected protein and ligands was analyzed using the Cavity detection-guided Blind Docking2 (CB-Dock2) tool (Liu *et al*. 2020). This web-based tool automatically identifies protein cavities, calculates their centers and sizes, and focuses on the top five cavities by default. Blind docking was used to explore potential binding sites and ligand-binding modes by scanning the entire protein surface (Bultum *et*

al. 2022). CB-Dock2 customizes the docking box based on the query ligand and performs molecular docking using AutoDock Vina (Liu *et al*. 2020). The lowest vina score (kcal/mol), typically associated with the largest cavity, was selected as the optimal result for each ligand.

RESULTS AND DISCUSSION

Lipinski's Rule of Five for Compounds

The use of diversified additives in expensive feed formulations, along with the need to assess their CH_4 mitigation potential through *in vitro* or *in vivo* experiments, can be both time-consuming and costly. To address this, researchers are exploring alternative strategies for rapid screening of anti-methanogenic agents. Computational tools have emerged as an effective alternative for veterinarians, helping save time and resources. These short-term *in silico* approaches aim to identify suitable additives without the high cost or duration of traditional testing methods. Through developing cost-effective and efficient screening techniques, it becomes possible to find viable solutions for reducing CH_4 emissions in ruminants while maintaining productivity.

In this investigation, all selected wheatgrass compounds were initially evaluated for drug-likeness properties using Lipinski's rule of five. According to drug-likeness criteria, an ideal ligand should have a molecular mass under 500 Da, fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, lipophilicity (log p) below 5, and molar refractivity between 40 and 130 (Abhishek Biswal *et al*. 2019). Table 1 presents key parameters, such as molecular weight, logP, hydrogen bond acceptors, hydrogen bond donors, and molar refractivity of each compound. Among the tested compounds, 2,4,6-trimethyl-1,3-phenylenediamine, Caryophyllene oxide, Caryophyllene, N,N-tetramethylene-.alpha.-(aminomethylene)glutaconic anhydride, and *n*-hexadecanoic acid met the essential criteria of Lipinski's rule of five, indicating potential for further analyses.

Table 1. Wheatgrass Compounds Analysed by Lipinski's Rule of Five

Phytocomponents	Molecular Formula / Mass (g/mol)	logP	Number of Hydrogen Bond Acceptors	Number of Hydrogen Bond Donors	Molar Refractivity
1,4-benzenediol, 2-methyl-	$C_7H_8O_2$ / 124.14	0.754	2	2	32.35
1-nonadecene	$C_{19}H_{38}$ / 266.5	5.962	0	0	102.25
2,4,6-trimethyl-1,3-phenylenediamine	$C_9H_{14}N_2$ / 150.22	1.794	0	4	48.2
2-methyloctacosane	$C_{29}H_{60}$ / 408.8	9.138	0	0	160.6
Alpha-Amyrin	$C_{30}H_{50}O$ / 426.7	7.826	1	1	153.43
Beta-Amyrin	$C_{30}H_{50}O$ / 426.7	7.837	1	1	153.38
Caryophyllene oxide	$C_{15}H_{24}O$ / 220.35	3.486	1	0	74.46
Caryophyllene	$C_{15}H_{24}$ / 204.35	3.961	0	0	75.11

Clionasterol	$C_{29}H_{50}O$ / 414.7	7.745	1	1	150.19
D-alpha-tocopherol	$C_{29}H_{50}O_2$ / 430.7	7.658	2	1	150.71
Didodecyl phthalate	$C_{32}H_{54}O_4$ / 502.8	8.34	4	0	164.91
Eicosane, 10-heptyl-10-octyl-	$C_{35}H_{72}$ / 492.9	-0.053	6	5	77.14
Ethyl linolenate	$C_{20}H_{34}O_2$ / 306.5	5.297	2	0	103.6
Linolenic acid	$C_{18}H_{30}O_2$ / 278.4	4.325	2	1	92.19
N,N-tetramethylene-.alpha.-(aminomethylene)glutaconic anhydride	$C_{10}H_{11}NO_3$ / 193.2	1.056	3	0	48.07
n-hexadecanoic acid	$C_{16}H_{32}O_2$ / 256.4	4.325	2	1	88.26
Nonacosane	$C_{29}H_{60}$ / 408.8	9.128	0	0	160.65
Octadecane, 9-ethyl-9-heptyl-	$C_{27}H_{56}$ / 380.7	8.536	0	0	149.61
Triacontane	$C_{30}H_{62}$ / 422.8	9.434	0	0	166.12
Phytol	$C_{20}H_{40}O$ / 296.5	5.721	1	1	109.16
Squalene	$C_{30}H_{50}$ / 410.7	8.21	0	0	152.12

ADME Properties Analysis

Table 2 presents the ADME properties of five selected wheatgrass compounds, highlighting their drug-likeness traits. All compounds demonstrated favourable traits, including water solubility, high GI absorption, lipophilicity, BBB permeability, P-gp substrate status, and no Lipinski's rule violations. All the phytocomponents were reported as water-soluble in nature. The lipophilicity values (log $P_{o/w}$) of 2,4,6-trimethyl-1,3-phenylenediamine, Caryophyllene oxide, Caryophyllene, N,N-tetramethylene-.alpha.-(aminomethylene)glutaconic anhydride, and *n*-hexadecanoic acid were 1.64, 3.15, 3.25, 1.65, and 3.85, respectively. All compounds displayed high GI absorption, while 2,4,6-trimethyl-1,3-phenylenediamine, Caryophyllene oxide, Caryophyllene, and *n*-hexadecanoic acid showed BBB permeability. Caryophyllene and N,N-tetramethylene-.alpha.-(aminomethylene)glutaconic anhydride did not permeate the BBB. None of the compounds were P-gp substrates, and there were no violations of Lipinski's rule of five.

Table 2. ADME Properties of Five Selected Compounds

Phytocomponents	Water Solubility (Log mol/L)	Lipophilicity (Log $P_{o/w}$)	GI Absorption	BBB Permeant	P-gp Substrate	Lipinski's Violation	Drug Likeness
2,4,6-trimethyl-1,3-phenylenediamine	-2.21 (Soluble)	1.64	High	Yes	No	0	Yes
Caryophyllene oxide	-3.45 (Soluble)	3.15	High	Yes	No	0	Yes
Caryophyllene	-3.87 (Soluble)	3.25	Low	No	No	0	Yes
N,N-tetramethylene-.alpha.-(aminomethylene)glutaconic anhydride	-1.6 (Very Soluble)	1.65	High	No	No	0	Yes
n-hexadecanoic acid	-5.02 (Moderate soluble)	3.85	High	Yes	No	0	Yes

Molecular Docking and Visualization

Molecular docking of specific ligands with target receptors has proved to be an ideal, cost-efficient screening technique in diverse areas (Khusro *et al*. 2020a; Lavanya *et al*. 2023; Ramasubburayan *et al*. 2023; Mukundh *et al*. 2024). Methanogens rely on MCR for the methanogenesis process, making MCR a prime target for computational approaches aimed at reducing CH_4 emissions in animals. In the present study, the binding affinity energies or docking score, cavity volume, interacting amino acid residues, docking centre, and docking size between selected wheatgrass compounds and receptor are shown in Table 3.

Table 3. Docking Score, Cavity Volume, Interacting Amino Acid Residues, Docking Centre, and Docking Size Between Five Selected Compounds and MCR Receptor

S. No.	Compounds	Docking Score (kcal/mol)	Cavity Volume (Å3)	Interacting Amino Acid Residues	Docking Centre (x, y, z)	Docking Size (x, y, z)
1.	2,4,6-trimethyl-1,3-phenylenediamine	-6.0	6348	**Chain A:** GLN147 MET150 MET233 ILE236 ALA243 GLY244 THR248 **Chain D:** VAL328 GLY329 PHE330 THR331 GLN332 TYR333 ALA334 PHE396 GLY397 GLY398 SER399 PHE443 TYR444 **Chain E:** PHE361 HIS364 SER365 ILE366 TYR367 **Chain F:** LEU117 ARG120	23, 37, -52	35, 25, 35
2.	Caryophyllene oxide	-6.7	6348	**Chain A:** VAL146 GLN147 ARG225 MET233 ILE236 ALA243 GLY244 THR248 ALA252 TYR253 LYS256 ALA258 VAL260 **Chain D:** ARG270 TYR333 PHE443 TYR444	23, 37, -52	35, 25, 35

				Chain E: PHE361 HIS364 SER365 ILE366 TYR367 GLY368 GLY369 HIS379 **Chain F:** LEU117 ARG120 SER154 VAL155		
3.	Caryophyllene	-6.8	6348	**Chain A:** GLN147 MET150 ARG225 ALA243 ALA252 TYR253 **Chain D:** MET324 TYR333 PHE396 GLY397 GLY398 SER399 GLY442 PHE443 TYR444 **Chain E:** PHE362 GLY368 GLY369 VAL381 **Chain F:** LEU117 SER118 GLY119 ARG120 VAL155 HIS156 GLY157 HIS158 SER159	23, 37, -52	35, 25, 35
4.	N,N-tetramethylene-.alpha.-(aminomethylene)glutaconic anhydride	-7.3	6348	**Chain A:** GLN147 MET150 ARG225 TYR253 LYS256 **Chain D:** LEU320 MET324 SER325 GLY329 PHE330 THR331 GLN332 TYR333 PHE396 GLY397 GLY398 SER399 GLY442 PHE443 ASN474 ALA479 MET480 ASN481 VAL482 **Chain E:** PHE361 PHE362 TYR367 GLY368 GLY369 ILE380 VAL381 **Chain F:** LEU117 SER118 GLY119 ARG120 VAL155 HIS156 HIS158	23, 37, -52	35, 25, 35
5.	n-hexadecanoic acid	-6.3	6348	**Chain A:** PRO268 ARG270 TRP319 LEU320 MET324 SER325 PHE330 TYR333 GLY397 GLY398 SER399 LEU441 GLY442 PHE443 TYR444 PRO473 ASN474 ALA479 MET480 ASN481 VAL482 **Chain B:** GLU186 PHE361 PHE362 HIS364 SER365 ILE366 TYR367 GLY368 GLY369 GLY370 ILE374 HIS379 ILE380 VAL381 **Chain C:** LEU117 SER118 GLY119 ARG120 VAL155 **Chain D:** GLN147 ARG225 MET229 MET233 ILE236 ALA243 GLY244 TYR253 LYS256	24, 38, -14	34, 35, 35

The compound 2,4,6-trimethyl-1,3-phenylenediamine, Caryophyllene oxide, Caryophyllene, N,N-tetramethylene-.alpha.-(aminomethylene)glutaconic anhydride, and *n*-hexadecanoic acid predicted binding energy score of -6.0, -6.7, -6.8, -7.3, and -6.3

kcal/mol, respectively. Figure 4 shows the 3D interaction views between compounds and MCR receptor. This is the first *in silico* investigation to predict the CH_4-mitigating potential of wheatgrass-derived bioactive compounds by targeting MCR as the receptor.

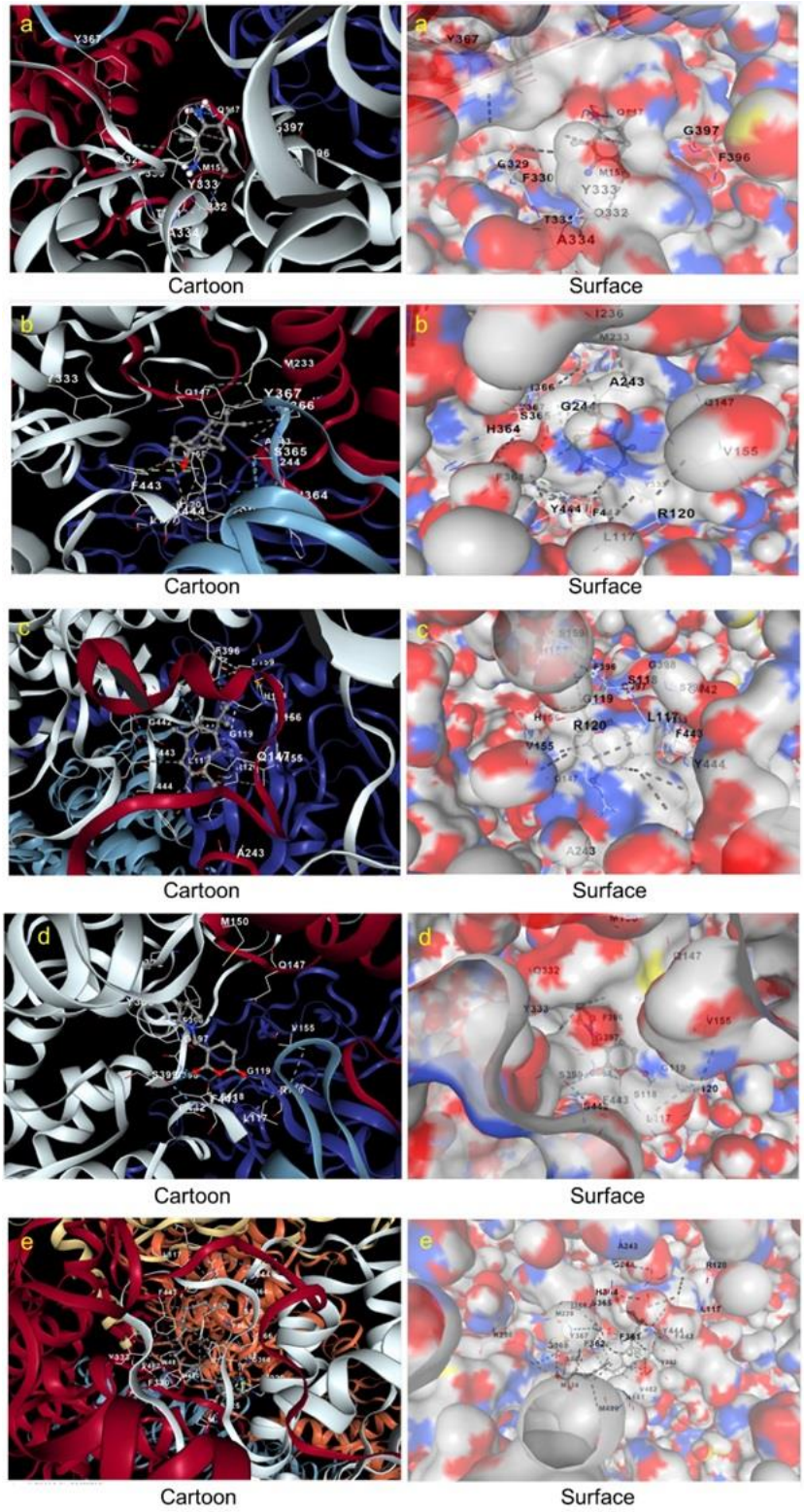

Fig. 4. Molecular docking visualization of: **(a)** 2,4,6-trimethyl-1,3-phenylenediamine, **(b)** Caryophyllene oxide, **(c)** Caryophyllene, **(d)** N,N-tetramethylene-.alpha.-(aminomethylene)

glutaconic anhydride, and **(e)** *n*-hexadecanoic acid with MCR receptor

Previous *in silico* studies highlighted the anti-methanogenic potential of varied plant metabolites by targeting MCR. For instance, Arokiyaraj *et al*. (2019) identified 9,10-anthracenedione, 1,8-dihydroxy-3-methyl, phthalic acid isobutyl octadecyl ester, and diisooctyl phthalate from *Rheum* sp. as promising anti-methanogenic agents in ruminants through molecular modeling. Similarly, Dinakarkumar *et al*. (2021) analyzed 168 compounds from 11 plants, targeting MCR to mitigate CH_4 emissions in ruminants. The study identified rosmarinic acid, biotin, α-cadinol, and 2,4,7,9-tetramethyl-5decyn4,7diol as the most effective MCR inhibitors. Khusro *et al*. (2020b) further demonstrated the pivotal role of compounds like 3,5-bis(1,1-dimethylethyl)-phenol, kaempferol, moringyne, niazimisin, and tetradecanoic acid from *Moringa oleifera* in reducing CH_4 emissions in horses by showing strong binding interactions with MCR using Hex 8.0.0 software. Moreover, bioactive compounds, such as acacetin, matairesinol, methyl tetradecanoate, cis-6-nonenal, syringic acids, limonene, trans-2,4-decadienal, 3-isopropyl-6-methylenecyclohex-1-ene, and 2,5-octanedione, from safflower oil also exhibited strong interaction with MCR, indicating their potential as anti-methanogenic agents in the equine industry (Khusro *et al*. 2022a).

Methanogenesis occurs in natural anaerobic environment and within the digestive tracts of animals, particularly ruminants (Alvarado *et al*. 2014). During this process, methanogens convert varied substrates into CH_4 to obtain energy for their growth and metabolism. Annually, approximately 600 million metric tons of CH_4 are released into the ecosystem through methanogenesis (Khusro *et al*. 2022a). The global warming potential of CH_4 is about 25 times greater than that of CO_2, making CH_4 production a significant environmental threat (Lan and Yang 2019). In agriculture, CH_4 emission from enteric fermentation in ruminants are the largest single source of greenhouse gases and one of the most significant anthropogenic contributors (Palangi and Lackner 2022). Given its detrimental impact, reducing CH_4 emissions from ruminants has become a key focus for researchers worldwide, driven by the urgent need to mitigate the release of this potent greenhouse gas (Khusro *et al*. 2022a). Various strategies, such as altering feed consumption and using dietary additives, are being explored to curb CH_4 production, presenting a promising area of study for environmental conservation and climate change mitigation efforts (Khusro *et al*. 2022b).

In recent years, various *in vitro* strategies have been employed to reduce CH_4 emissions from livestock, with dietary manipulation emerging as one of the most effective approaches. As global demand for meat, milk and other ruminant-derived products continue to rise, incorporating feed additives presents a promising solution to mitigate CH_4 emissions (Khusro *et al*. 2022b). Additives, such as plant extracts, probiotics, plant metabolites, exogenous enzymes, and organic acids, can alter the gut microflora of ruminants, thereby influencing fermentation kinetics and reducing CH_4 emissions (Elghandour *et al*. 2019). Additionally, these supplements enhance feed quality and adjust the dietary proportions, ultimately affecting gut microbial metabolism and further altering fermentation processes (Haque 2018).

Previous research demonstrated that wheatgrass can enhance the growth performance and flesh quality of common carp (Barbacariu *et al*. 2021; Burducea *et al*. 2022), suggesting its potential as a feed additive for other animals, particularly ruminants. The current findings open new avenues for exploring wheatgrass as an additive to reduce CH_4 emissions in livestock.

CONCLUSIONS

1. In summary, among the 21 selected compounds of wheatgrass, five compounds met Lipinski's rule of five criteria.
2. *In silico* analysis revealed strong binding potential of these compounds with the MCR receptor, with N,N-tetramethylene-.alpha.-(aminomethylene)glutaconic anhydride showing the highest docking score of -7.3 kcal/mol using CB-Dock2 tool. The other compounds had lower binding affinities.
3. This study suggested that 2,4,6-trimethyl-1,3-phenylenediamine, Caryophyllene oxide, Caryophyllene, N,N-tetramethylene-.alpha.-(aminomethylene)glutaconic anhydride, and *n*-hexadecanoic acid could be promising anti-methanogenic agents in ruminants.

ABBREVIATIONS

MCR: Methyl coenzyme M reductase
ADME: Absorption, Distribution, Metabolism, and Excretion
PDB: Protein Data Bank
CB-Dock2: Cavity detection-guided Blind Docking2
CH_4: Methane

REFERENCES CITED

Abhishek Biswal, R., Mirunalini, K., Jayshree, P., and Pazhamalai, V. (2019). "Molecular docking analysis of bioactive compounds of *Acacia concinna* against fungal protein," *Journal of Pharmaceutical Sciences and Research* 11(4), 1216–1222.

Alvarado, A., Montañez-Hernández, L. E., Palacio-Molina, S. L., Oropeza-Navarro, R., Luévanos-Escareño, M. P., and Balagurusamy, N. (2014). "Microbial trophic interactions and mcrA gene expression in monitoring of anaerobic digesters," *Frontiers in Microbiology* 5, Article Number 597. DOI: 10.3389/fmicb.2014.00597

Arokiyaraj, S., Stalin, A., and Shin, H. (2019). "Anti-methanogenic effect of rhubarb (*Rheum* spp.) - an *in silico* docking studies on methyl-coenzyme M reductase (MCR)," *Saudi Journal of Biological Science* 26, 1458–1462. DOI: 10.1016/j.sjbs.2019.06.008

Barbacariu, C.-A., Burducea, M., Dîrvariu, L., Oprea, E., Lupu, A.-C., Teliban, G.-C., Agapie, A. L., Stoleru, V., and Lobiuc, A. (2021). "Evaluation of diet supplementation with wheat grass juice on growth performance, body composition and blood biochemical profile of carp (*Cyprinus carpio* L.)," *Animals* 11(9), Article Number 2589. DOI: 10.3390/ani11092589

Bultum, L. E., Tolossa, G. B., Kim, G., Kwon, O., and Lee, D. (2022). "*In silico* activity and ADMET profiling of phytochemicals from Ethiopian indigenous aloes using pharmacophore models," *Scientific Reports* 12, Article ID 22221. DOI: 10.1038/s41598-022-26446-x

Burducea, M., Dincheva, I., Dirvariu, L., Oprea, E., Zheljazkov, V. D., and Barbacariu, C.-A. (2022). "Wheat and barley grass juice addition to a plant-based feed improved

growth and flesh quality of common carp (*Cyprinus carpio*)," *Animals* 12(8), Article Number 1046. DOI: 10.3390/ani12081046

Casañas, M. A., Rangkasenee, N., Krattenmacher, N., Thaller, G., Metges, C. C., and Kuhla, B. (2015). "Methyl-coenzyme M reductase A as an indicator to estimate methane production from dairy cows," *Journal of Dairy Science* 98(6), 4074-4083. DOI: 10.3168/jds.2015-9310

da Silva, C. H., Campo, V. L., Carvalho, I., and Taft, C. A. (2006). "Molecular modeling, docking and ADMET studies applied to the design of a novel hybrid for treatment of Alzheimer's disease," *Journal of Molecular Graphics and Modelling* 25, 169-175. 10.1016/j.jmgm.2005.12.002

Dinakarkumar, Y., Rajabathar, J. R., Arokiyaraj, S., Jeyaraj, I., Anjaneyulu, S. R., Sandeep, S., Karthik, C. S., Appaturi, J. N., and Wilson, L. D. (2021). "Anti-methanogenic effect of phytochemicals on Methyl-Coenzyme M reductase-potential: *In silico* and molecular docking studies for environmental protection," *Micromachines* 12(11), Article number 1425. DOI: 10.3390/mi12111425

Durairaj, V., Hoda, M., Shakya, G., Babu, S. P., and Rajagopalan, R. (2014). "Phytochemical screening and analysis of antioxidant properties of aqueous extract of wheatgrass," *Asian Pacific Journal of Tropical Medicine* 7, S398-404. DOI: 10.1016/S1995-7645(14)60265-0

Elghandour, M., Adegbeye, M. J., Barbabosa-Pilego, A., Perez, N. R., Hernández, S. R., Zaragoza-Bastida, A., and Salem, A. Z. M. (2019). "Equine contribution in methane emission and its mitigation strategies," *Journal of Equine Veterinary Science* 72, 56–63. DOI: 10.1016/j.jevs.2018.10.020

Elghandour, M. M., Maggiolino, A., Alvarado-Ramírez, E. R., Hernández-Meléndez, J., Rivas-Cacerese, R. R., Hernández-Ruiz, P. E., Khusro, A., De Palo, P., and Salem, A. Z. M. (2023). "Marine microalgae as a nutritive tool to mitigate ruminal greenhouse gas production: *In vitro* fermentation characteristics of fresh and ensiled maize (*Zea mays* L.) forage," *Veterinary Sciences* 10(9), Article Number 556. DOI: 10.3390/vetsci10090556

Elghandour, M. M. M. Y., Pacheco, E. B. F., Khusro, A., Tirado-González, D. N., Lackner, M., Ponce-Covarrubias, J. L., De Palo, P., Maggiolino, A., and Salem, A. Z. M. (2024). "Deciphering the role of *Moringa oleifera* seeds and probiotic bacteria on mitigation of biogas production from ruminants," *AMB Express* 14, Article Number 86. DOI: 10.1186/s13568-024-01744-x

Haque, M. (2018). "Dietary manipulation: A sustainable way to mitigate methane emissions from ruminants," *Journal of Animal Science and Technology* 60, Article Number 15. DOI: 10.1186/s40781-018-0175-7

Islam, M., and Lee, S. S. (2019). "Advanced estimation and mitigation strategies: A cumulative approach to enteric methane abatement from ruminants," *Journal of Animal Science and Technology* 61(3), Article number 122. DOI: 10.5187/jast.2019.61.3.122

Janssen, P. H. (2010). "Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics," *Animal Feed Science and Technology* 160, 1-22. DOI: 10.1016/j.anifeedsci.2010.07.002

Janssen, P. H., and Kirs, M. (2008). "Structure of the archaeal community of the rumen," *Applied and Environmental Microbiology* 74, 3619-3625. DOI: 10.1128/AEM.02812-07

Kamra, D. N., Pawar, M., and Singh, B. (2012). "Effect of plant secondary metabolites on rumen methanogens and methane emissions by ruminants," in: *Dietary Phytochemicals and Microbes*, Springer, Amsterdam, Netherlands, pp. 351–370. DOI: 10.1007/978-94-007-3926-0_12

Khusro, A., Aarti, C., and Agastian, P. (2020a). "Computational modelling and docking insight of bacterial peptide as ideal anti-tubercular and anticancer agents," *Biocatalysis and Agricultural Biotechnology* 26, Article ID 101644. DOI: 10.1016/j.bcab.2020.101644

Khusro, A., Aarti, C., Elghandour, M. M., Adegbeye, M. J., Mellado, M., Barbabosa-Pliego, A., Rivas-Caceres, R. R., and Salem, A. Z. M. (2022b). "Dietary manipulation to mitigate greenhouse gas emission from livestock," In: *Handbook of Climate Change Mitigation and Adaptation*, Springer International Publishing, Cham, Switzerland, pp. 2537-2575. DOI: 10.1007/978-1-4614-6431-0_131-1

Khusro, A., Aarti, C., Salem, A. Z. M., Barbabosa-Pliego, A., and Rivas-Caceres, R. R. (2020b). "Methyl-coenzyme M reductase (MCR) receptor as potential drug target for inhibiting methanogenesis in horses using *Moringa oleifera* L.: An *in silico* docking study," *Journal of Equine Veterinary Science* 88, Article ID 102949. DOI: 10.1016/j.jevs.2020.102949

Khusro, A., Sahibzada, M. U. K., Khan, S. U., Rajakrishnan, R., Elghandour, M. M. M. Y., Salem, A. Z. M., Kuppusamy, P., Alcala-Canto, Y., and Tirado-González, D. N. (2022a). "Anti-methanogenic traits of safflower oil compounds against Methyl-coenzyme M reductase receptor in equines: An *in silico* docking analysis," *Journal of Equine Veterinary Science* 113, Article ID 103938. DOI: 10.1016/j.jevs.2022.103938

Lan, W., and Yang, C. (2019). "Ruminal methane production: Associated microorganisms and the potential of applying hydrogen-utilizing bacteria for mitigation," *Science of The Total Environment* 654, 1270–1283. DOI: 10.1016/j.scitotenv.2018.11.180

Lavanya, M., Namasivayam, S. K., Praveena, A., Priyanka, S., Kavisri, M., and Moovendhan, M. (2023). "*In silico* analysis of plants biomass phytochemicals against β2 adrenergic receptor," *Biomass Conversion and Biorefinery* 2023, 1-2. DOI: 10.1007/s13399-023-04241-6

Lipinski, C. A. (2004). "Lead- and drug-like compounds: the rule-of-five revolution," *Drug Discovery Today: Technologies* 1, 337-341. DOI: 10.1016/j.ddtec.2004.11.007

Liu, Y., Grimm, M., Dai, W. T., Hou, M. C., Xiao, Z. X., and Cao, Y. (2020). "CB-Dock: A web server for cavity detection-guided protein–ligand blind docking," *Acta Pharmacologica Sinica* 41, 138–144. DOI: 10.1038/ s41401-019-0228-6

Martin, C., Morgavi, D. P., and Doreau, M. (2010). "Methane mitigation in ruminants: From microbe to the farm scale," *Animal* 4, 351-365. DOI: 10.1017/S1751731109990620

Mukundh, S. T., Veeraraghavan, V. P., Ponnusamy, B., and Jayaraman, S. (2024). "Phytochemical screening and antidiabetic activity of aqueous extract of *Evolvulus alsinoides* leaves: An *in vitro* and *in silico* study," *Journal of Pharmacy and Bioallied Sciences* 16(Suppl 2), S1246-S1248. DOI: 10.4103/jpbs.jpbs_585_23

Newbold, C. J., de la Fuente, G., Belanche, A., Ramos-Morales, E., and McEwan, N. R. (2015). "The role of ciliate protozoa in the rumen," *Frontiers in Microbiology* 6, Article number 1313. DOI: 10.3389/fmicb.2015.01313

Orpin, C. G. (1981). "Fungi in ruminant degradation," In: *Agricultural Science Seminar: Degradation of Plant Cell Wall Material*, Agricultural Research Council, London,

UK, pp. 129–150.

Palangi, V., and Lackner, M. (2022). "Management of enteric methane emissions in ruminants using feed additives: A review," *Animals* 12, Article number 3452. DOI: 10.3390/ani12243452

Poulsen, M., Schwab, C., Jensen, B. B., Engberg, R. M., Spang, A., Canibe, N., Højberg, O., Milinovich, G., Fragner, L., Schleper, C., *et al*. (2013). "Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen," *Nature Communication* 4, Article Number 1428. DOI: 10.1038/ncomms2432

Ramasubburayan, R., Amperayani, K. R., Varadhi, G., Dhanraj, G., Athista, M., Mahapatra, S., and Prakash, S. (2023). "Unraveling bioactive metabolites of mangroves as putative inhibitors of SARS-CoV-2 Mpro and RBD proteins: Molecular dynamics and ADMET analysis," *Journal of Biomolecular Structure and Dynamics* 2023, Article ID 2275185. DOI: 10.1080/07391102.2023.2275185

Rezaeian, M., Beakes, G. W., and Parker, D. S. (2004). "Distribution and estimation of anaerobic zoosporic fungi along the digestive tracts of sheep," *Mycology Research* 108, 1227-1233. DOI: 10.1017/S0953756204000929

Santillán, M. K. G., Khusro, A., Salem, A. Z. M., Pliego, A. B., and Elghandour, M. M. M. Y. (2023). "Potential and valorization of *Salix babylonica* waste leaf extract to mitigate equine fecal production of methane, carbon monoxide, and hydrogen sulphide," *Waste and Biomass Valorization* 14, 1079-1095. DOI: 10.1007/s12649-022-01926-3

Shakya, G., Pajaniradje, S., Hoda, M., Durairaj, V., and Rajagopalan, R. (2014). "GC-MS analysis, *in vitro* antioxidant and cytotoxic studies of wheatgrass extract," *American Journal of Phytomedicine and Clinical Therapeutics* 2, 877-893.

Tapio, I., Snelling, T. J., Strozzi, F., and Wallace, R. J. (2017). "The ruminal microbiome associated with methane emissions from ruminant livestock," *Journal of Animal Science and Biotechnology* 8, Article Number 7. DOI: 10.1186/s40104-017-0141-0

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