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Original Research

Methyl-coenzyme M Reductase (MCR) Receptor as Potential Drug Target for Inhibiting Methanogenesis in Horses Using *Moringa oleifera* L.: An *in Silico* Docking Study

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ABSTRACT

Methane (CH_4) emission from nonruminant livestock, particularly equines, is a colossal burden for veterinarians worldwide. In view of this, the present context was investigated to predict the antimethanogenic attributes of *Moringa oleifera* L. associated phytocomponents by targeting methyl-coenzyme M reductase (MCR) receptor in horses using *in silico* tools. Initially, the pharmacokinetics and ADME (absorption, distribution, metabolism, and excretion) properties of 26 phytocomponents were analyzed using Lipinski's rule of five and Swiss ADME tool, respectively. Among all the tested phytocomponents, 3,5-bis(1,1-dimethylethyl)-phenol, Kaempferol, Moringyne, Niazimisin, and Tetradecanoic acid showed drug-likeness traits with no violation. The molecular docking analysis of selected phytocomponents against MCR receptor was carried out using Hex 8.0.0 docking software. Results estimated the highest binding energy of Tetradecanoic acid against MCR receptor with maximum docking E-value of −142.98 KJ/mol, followed by Niazimisin (−133.98 KJ/mol), Kaempferol (−110.36 KJ/mol), 3,5-bis(1,1-dimethylethyl)-phenol (−93.72 KJ/mol), and Moringyne (−92.62 KJ/mol). In conclusion, Tetradecanoic acid can be utilized as a pronounced antimethanogenic agent in order to develop efficacious CH_4 mitigating drugs by inhibiting the methanogenesis mechanism. Most importantly, this *in silico* outcomes can certainly reduce the cost of *in vivo* studies strategy toward the development of antimethanogenic drugs for horses in the future.

1. Introduction

Livestock are the prime sources of greenhouse gases (GHGs) emission and a colossal concern for global warming. Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are major GHGs produced from the animals [1]. However, CH_4 production from livestock contributes about 9% of total global emission and expected to contribute approximately 18% of total global warming in the next five decades. In addition, CH_4 emission from animals is known to affect not only the ecosystem but also cause reduced growth and productivity [2].

Horses are important members of the Equidae family, which possess an anatomically specialized hindgut that accommodates a microbial ecosystem consisting of diversified microbiota, causing degradation and fermentation of structural polysaccharides of plant cell walls [3]. Methanogens are the most common inhabitants of the hindgut of horses, which convert hydrogen (H_2), and CO_2 into CH_4 using methyl-coenzyme M reductase (MCR) via methanogenesis pathway [4]. Methyl-coenzyme M reductase is the key complex enzyme causing the reduction of methyl−coenzyme M (methyl-CoM) [CH_3-S-CoM, 2-(methylthio)ethanesulfonate] with coenzyme B (CoB) (CoB-S-H, 7-thioheptanoyl-threoninephosphate) to CH_4 and the heterodisulfide of CoM (CoMS-H, 2-thioethane

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sulfonate) and CoB under strictly anaerobic conditions [5]. Methyl-CoM reductase was first characterized by Ellefson and Wolfe [6] as a yellow protein of an apparent molecular mass of 300 kD composed of three different subunits arranged in an $\alpha_2\beta_2\gamma_2$ configuration. The hexameric protein contains two molecules of the tightly but not covalently bound coenzyme F_{430}, which is a Nickel (Ni) porphinoid [7]. Because MCR catalyzes the final step of methanogenesis, it is essential to mitigate CH_4 emission from horses by targeting this complex enzyme.

Over the past few years, the emission of CH_4 from livestock is being mitigated by supplementing various trees leaves into the diet and estimating the production *in vitro*. *Moringa oleifera* L. (Moringaceae), commonly called as "drumstick tree" is a multi-purpose drought tolerant tropical tree that has numerous ethno-pharmacological and agricultural uses due to the presence of potent constituents viz. carotenoids, polyphenols, flavonoids, essential amino acids, and phenolic acids [8]. Surprisingly, the utilization of *M. oleifera* L. as feed supplements in livestock

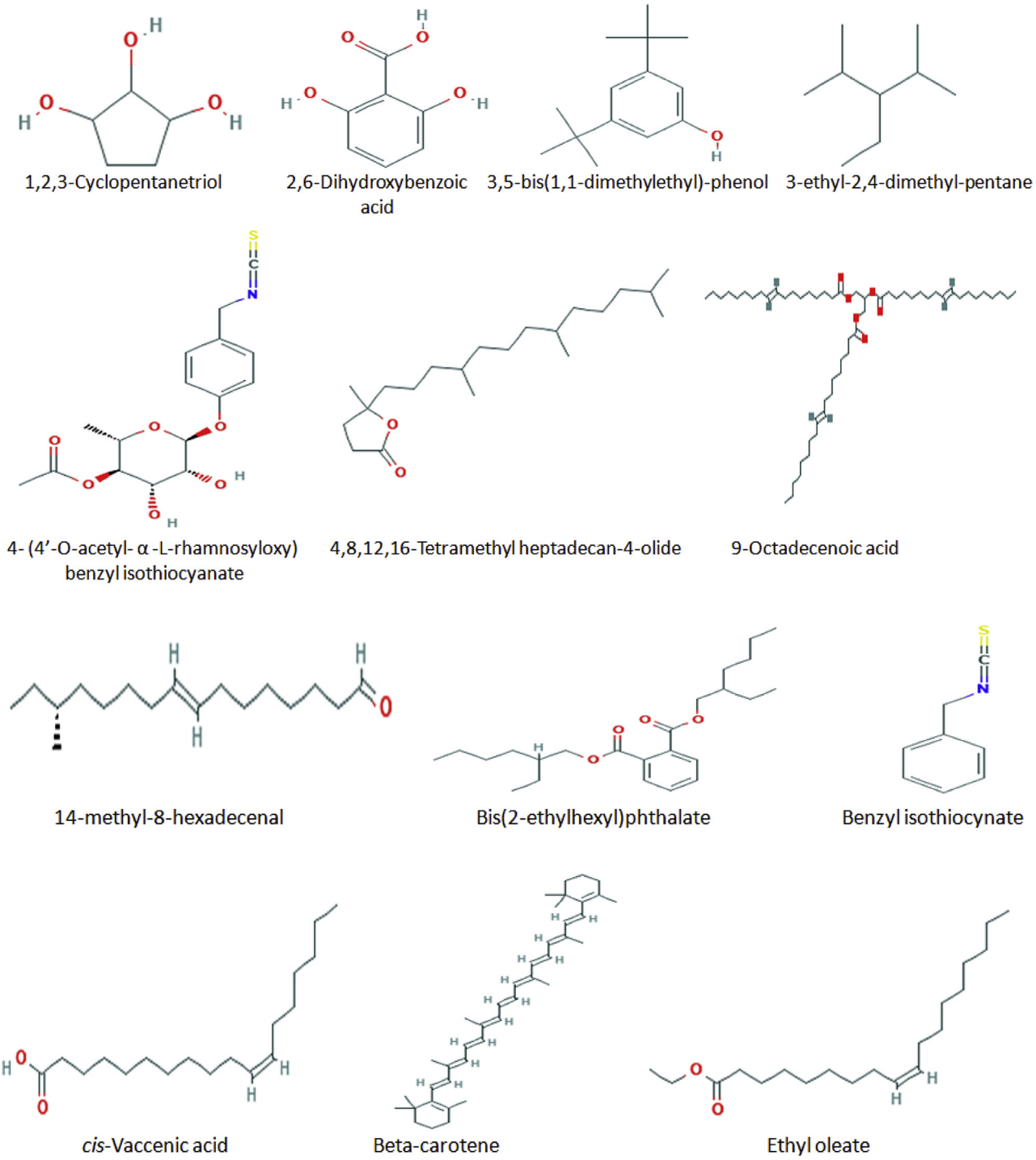

Fig. 1. Structures of phytocomponents of *M. oleifera* L. used.

industries for mitigating the production of CH_4 is scanty, probably undetermined in horses. In view of this, a significant attempt was undertaken in the current context to predict the CH_4 mitigation attributes of potential compounds of *M. oleifera* L. by targeting MCR through *in silico* docking mechanism. In order to reduce the cost of CH_4 mitigation strategies research, initially, we implemented the computational approaches by investigating the interaction between *M. oleifera* L. associated phytocompounds and MCR for its antimethanogenic characteristics using molecular docking tools.

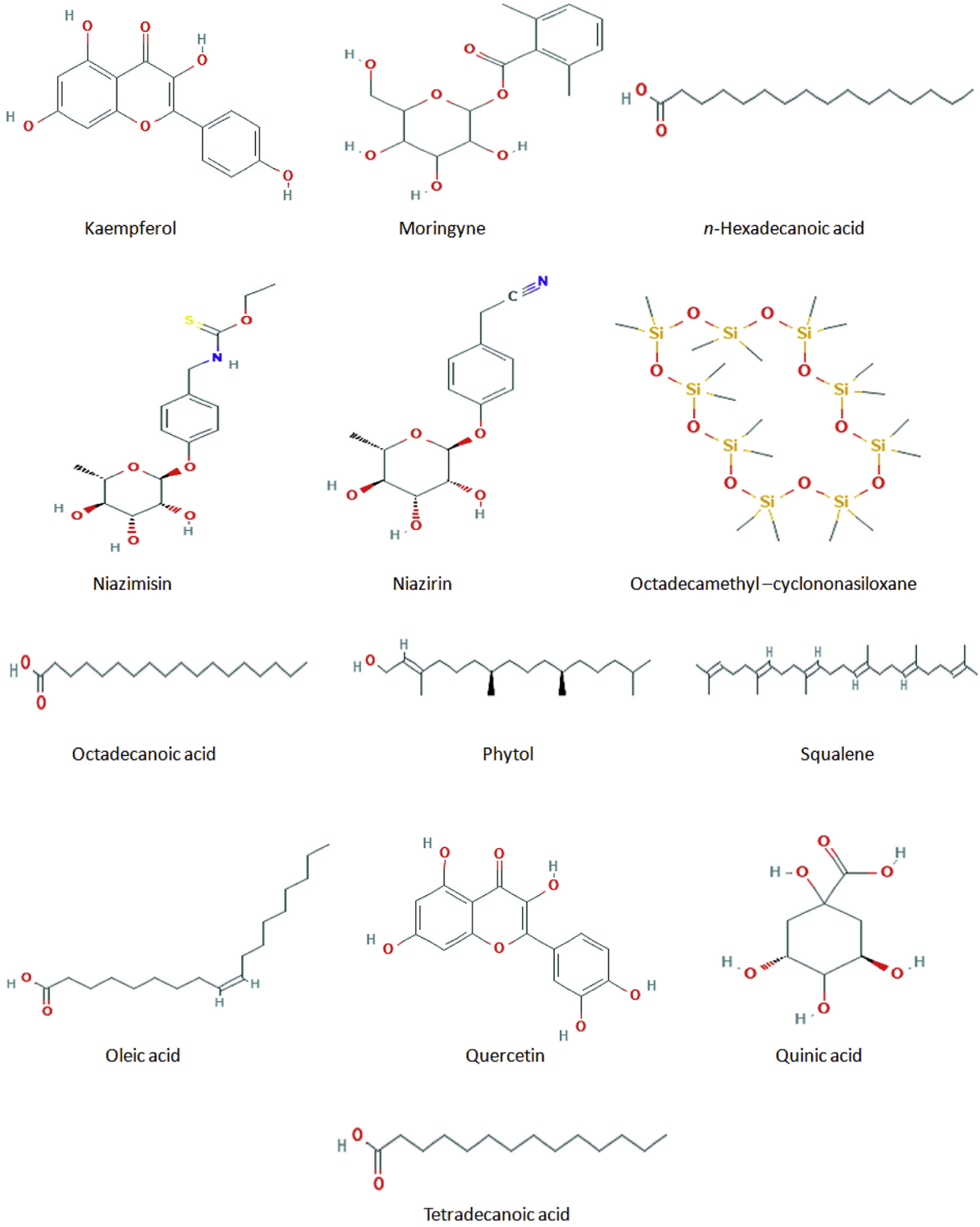

Fig. 1. (*continued*).

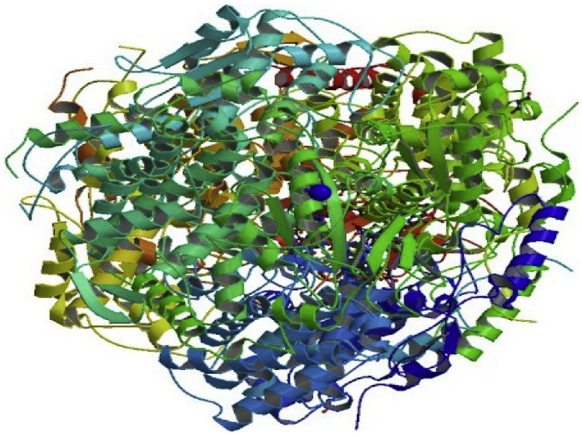

Fig. 2. 3D structure of receptor MCR as retrieved from RCSB PDB (IMRO).

2. Materials and Methods

2.1. Phytoconstituents of Interest

A total of 26 phytocomponents were selected from *M. oleifera* L. according to the prior reports [9,10]. Structures for all the phytocomponents were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF format and continued for further analyses (Fig. 1).

2.2. Ligand selection

2.2.1. Lipinski's Rule of Five

Lipinski's rule of five was implied to determine the drug-likeness of all the ligands (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). This rule not only illustrates the durability but also demonstrates the molecular weight, logP, number of hydrogen bond acceptors, number of hydrogen bond donors, and molar refractivity of the drug candidate [11].

2.2.2. ADME Properties Analysis

Ligands satisfying Lipinski's rule of five were subjected to ADME (absorption, distribution, metabolism, and excretion) properties analysis using the Swiss ADME tool of the Swiss Institute of Bioinformatics (http://www.swissadme.ch/). The canonical SMILES were retrieved from PubChem and evaluated by the Swiss ADME tool. 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 were estimated using this tool. The Swiss ADME tool is based on the principle of vector machine algorithm that can easily analyze data sets of known inhibitor/noninhibitor, as well as substrate/nonsubstrate [12]. These phytocomponents were selected further for molecular docking analysis.

2.3. Target Receptor

The 3D structure of receptor MCR was retrieved from RCSB PDB (Protein Data Bank - IMRO) (http://www.rscb.org/pdb). The complexes bound to the receptor, such as nonessential water molecules and any inhibitors, were removed while docking (Fig. 2).

2.4. Molecular Docking and Visualization

Molecular docking between selected ligands and MCR were analyzed and visualized using Hex 8.0.0 docking software [13]. Hex is an interactive molecular graphics program that reads in molecular coordinate files and displays *in silico* interaction in varied representations and color schemes. The tool identifies the ligand with the best score and calculates the ligand-receptor interaction with the lowest free energy value.

The docking was performed by adjusting the following parameters/features.

Table 1
Phytocomponents analyzed 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
1,2,3-Cyclopentanetriol	$C_5H_{10}O_3$/118.1	0.663	03	03	29.8
2,6-Dihydroxybenzoic acid	$C_7H_6O_4$/154.1	0.148	04	03	31.03
3,5-bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$/206.3	3.201	01	01	69.69
3-ethyl-2,4-dimethyl-pentane	C_9H_{20}/128.2	3.034	00	00	51.15
4- (4′-O-acetyl- α -L-rhamnosyloxy) benzyl isothiocyanate	$C_{16}H_{19}NO_6S$/353.4	2.375	06	02	86.8
4,8,12,16-Tetramethyl heptadecan-4-olide	$C_{21}H_{40}O_2$/324.5	6.021	02	00	113.68
9-Octadecenoic acid	$C_{18}H_{34}O_2$/282.5	−0.053	06	05	77.14
14-methyl-8-hexadecenal	$C_{17}H_{32}O$/252.4	5.004	01	00	90.86
Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$/390.6	5.91	04	00	121.07
Benzyl isothiocynate	C_8H_7NS/149.2	1.553	00	00	41.08
cis-Vaccenic acid	$C_{18}H_{34}O_2$/282.5	4.733	02	01	96.86
Beta-carotene	$C_{40}H_{56}$/536.9	9.913	00	00	188.24
Ethyl oleate	$C_{20}H_{38}O_2$/310.5	5.705	02	00	108.26
Kaempferol	$C_{15}H_{10}O_6$/286.2	0.646	06	04	62.82
Moringyne	$C_{15}H_{20}O_7$/312.3	2.046	07	04	75.3
n-Hexadecanoic acid	$C_{16}H_{32}O_2$/256.4	4.325	02	01	88.26
Niazimicin	$C_{16}H_{23}NO_6S$/357.4	2.157	06	04	90.4
Niazirin	$C_{14}H_{17}NO_5$/279.2	2.012	06	03	69.6
Octadecamethyl−cyclononasiloxane	$C_{18}H_{54}O_9Si_9$/667.3	—	09	00	—
Octadecanoic acid	$C_{18}H_{36}O_2$/284.5	4.937	02	01	99.19
Phytol	$C_{20}H_{40}O$/296.5	5.721	01	01	109.16
Squalene	$C_{30}H_{50}$/410.7	8.21	00	00	152.12
Oleic acid	$C_{18}H_{34}O_2$/282.5	4.733	02	01	96.86
Quercetin	$C_{15}H_{10}O_7$/302.2	0.524	07	05	64.36
Quinic acid	$C_7H_{12}O_6$/192.1	0.353	06	05	40.85
Tetradecanoic acid	$C_{14}H_{28}O_2$/228.3	3.712	02	01	77.32

Table 2
ADME properties of five selected phytocomponents.

Phytocomponents	Water solubility (Log mol/L)	Lipophilicity (Log $P_{o/w}$)	GI absorption	BBB permeant	P-gp substrate	Lipinski's violation	Drug likeness
3,5-bis(1,1-dimethylethyl)-phenol	−4.38 (moderately soluble)	3.89	High	Yes	No	00	Yes
Kaempferol	−3.31 (soluble)	1.58	High	No	No	00	Yes
Moringyne	−1.95 (very soluble)	0.07	High	No	No	00	Yes
Niazimicin	−2.25 (soluble)	0.95	High	No	Yes	00	Yes
Tetradecanoic acid	−4.31 (moderately soluble)	4.45	High	Yes	No	00	Yes

Correlation type Shape + Electrostatics

FFT Mode 3D

Post Processing MM Energies

Grid Dimension 0.6

Receptor range 180

Ligand range 180

Twist range 360

Distance Range 40

The binding energy (KJ/mol) estimated after docking was tabulated.

3. Results

3.1. Lipinski's Rule of Five for Phytocomponents

All the phytocomponents of *M. oleifera* L. were screened for drug-likeness characteristics using Lipinski's rule of five. Table 1 shows the molecular weight, LogP, number of hydrogen bond acceptors, number of hydrogen bond donors, and molar refractivity of all the phytocomponents. Among the tested phytocomponents, 3,5-bis(1,1-dimethylethyl)-phenol, Kaempferol, Moringyne, Niazimicin, and Tetradecanoic acid satisfied all the criteria of Lipinski's rule of five.

3.2. ADME Properties Analysis

Table 2 illustrates the ADME properties of five selected phytocomponents of *M. oleifera* L. The ADME properties predict that all the components are drug-likeness based on water solubility, high GI absorption, an acceptable range of lipophilicity, BBB permeability, P-gp substrate, and lack of Lipinski's violation. All the phytocomponents were water-soluble except 3,5-bis(1,1-dimethylethyl)-phenol and Tetradecanoic acid, which were moderately soluble in water. Moringyne revealed minimum lipophilicity of 0.07 Log $P_{o/w}$. Likewise, 3,5-bis(1,1-dimethylethyl)-phenol, Kaempferol, Niazimicin, and Tetradecanoic acid showed lipophilicity values of 3.89, 1.58, 0.95, and 4.45 Log $P_{o/w}$, respectively.

Table 3
Docking analysis of five selected phytocomponents with MCR.

S. No.	Phytocomponents	Molecular formula/ Mass (g/mol)	Docking E-value (KJ/mol)
1.	3,5-bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$/206.3	−93.72
2.	Kaempferol	$C_{15}H_{10}O_6$/286.2	−110.36
3.	Moringyne	$C_{15}H_{20}O_7$/312.3	−92.62
4.	Niazimicin	$C_{16}H_{23}NO_6S$/357.4	−133.98
5.	Tetradecanoic acid	$C_{14}H_{28}O_2$/228.3	−142.98

3.3. Molecular Docking and Visualization

In view of Lipinski's rule of five and ADME properties of phytocomponents, five potent ligands were selected for molecular docking analysis. Table 3 shows the molecular interaction of 3,5-bis(1,1-dimethylethyl)-phenol, Kaempferol, Moringyne, Niazimicin, and Tetradecanoic acid with the target receptor MCR, estimating docking E-values of −93.72, −110.36, −92.62, −133.98, and −142.98 KJ/mol, respectively. Hydrophobic interaction between ligands and the target protein is shown in Fig. 3. Among the five ligands, Tetradecanoic acid exhibited the highest least energy (minimum binding energy) against MCR.

4. Discussion

Over the past few years, the mitigation of CH_4 emission in herbivorous animals has been recognized as interesting areas for researchers worldwide because of the perceived necessity to curb the emission of this detrimental gas into the ecosystem [14]. Methane emission represents approximately 1.5 ± 0.2% of gross energy and 3.2 ± 0.7% of digestible energy for equids [15].

A plethora of strategies toward the mitigation of CH_4 emission from horses has been implemented by veterinarians. It mainly includes the alteration of animals' diet by supplementing diversified additives viz. fibrolytic enzymes [16], yeast cells [17], *Lactobacillus* sp. [18], and *Staphylococcus* sp [19]. In spite of the inclusion of these nontoxic supplements as a feed additive, only a small percentage of CH_4 mitigation has been achieved. In addition, medicinal plants have also been successfully utilized as ideal feed supplements toward the reduction of CH_4 emission from disparate livestock [2,20,21]. Surprisingly, a study revealing the paramount role of *M. oleifera* L. as CH_4 emission-reducing agent in horses is scarce, probably unavailable. Some chemical inhibitors have been investigated to destroy the pathogenic bacteria, and those inhibitors may be beneficial to the host, which, in turn, affects the microbiota. Thus, it is crucial not only to determine the influence of methanogenic inhibitors on the stability of beneficial microbiota but also discover new targets for CH_4 mitigation.

Methanogens are strictly anaerobic archaea that derive their metabolic energy by converting a few substrates to CH_4 [22]. The MCR is mainly involved in the biological synthesis and anaerobic oxidation of CH_4. The enzyme catalyzes the conversion of methyl-2-mercaptoethanesulfonate (methyl-SCoM) and *N*-7-mercaptoheptanoylthreonine phosphate (CoB_7SH) to CH_4 and the mixed disulfide CoBS-SCoM. The role of Ni in the MCR catalytic cycle is controversial, and two competing catalytic mechanisms for MCR have been proposed. The mechanism I involves the attack of the Ni(I) nucleophile on the methyl group of

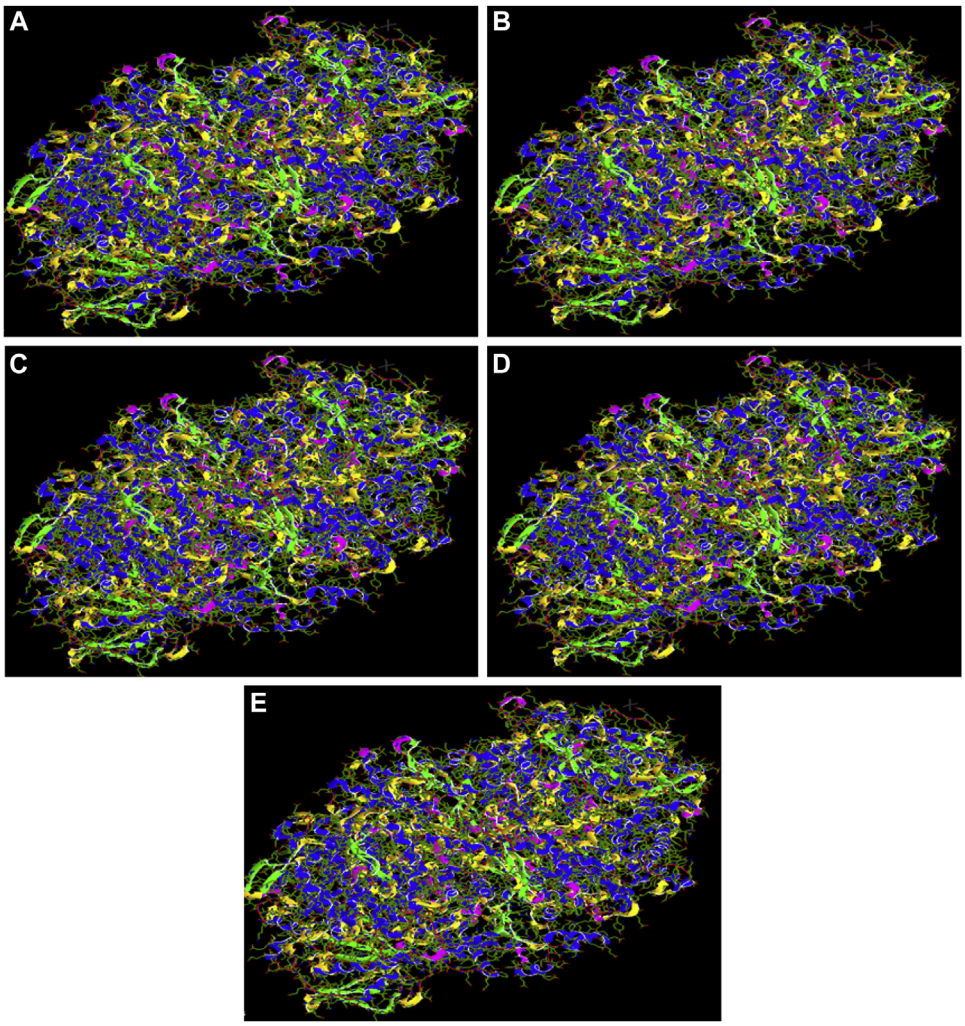

Fig. 3. Molecular docking of (A) 3,5-bis(1,1-dimethylethyl)-phenol, (B) Kaempferol, (C) Moringyne, (D) Niazimicin, and (E) Tetradecanoic acid against MCR. The molecular interaction of 3,5-bis(1,1-dimethylethyl)-phenol, Kaempferol, Moringyne, Niazimicin, and Tetradecanoic acid with the target receptor MCR showed docking E-values of −93.72, −110.36, −92.62, −133.98, and −142.98 KJ/mol, respectively.

methyl-SCoM to generate a methyl-Ni(III) intermediate. This proposed mechanism I is based on mechanistic work with F_{430} model complexes, on the location of substrates in the active site of inactive Ni(II) MCR structures, and on mechanistic and crystallographic studies of the active Ni(I) enzyme with 3-bromopropanesulfonate and methyl halide. Mechanism II begins with Ni(I) attack on the sulfur atom of methyl-SCoM, promoting the homolytic cleavage of the methyl-sulfur bond and generating a methyl radical and a Ni(II) thiolate complex. This mechanism is based on density functional theory calculations and on isotope effects studies of the reaction of MCR with methyl-SCoM and homologous substrate ethyl-SCoM [23].

Computational simulations or approaches are important for selecting bioactive agents because the hypothesis can be tested prior to the time-consuming and resource-demanding process. In the present investigation, we have predicted the pivotal role of phytoconstituents of *M. oleifera* L. as CH_4 mitigating feed supplements in horses by targeting MCR via *in silico* tools. Among tested phytocomponents, 3,5-bis(1,1-dimethylethyl)-phenol, Kaempferol, Moringyne, Niazimicin, and Tetradecanoic acid satisfied all the criteria of Lipinski's rule of five. In general, the standard criteria for drug-likeness of each component were molecular mass should be less than 500 daltons, hydrogen bond donor less than 5, hydrogen bond acceptors less than 10, high lipophilicity less than 5 (log p), and molar refractivity between 40–130 [24]. The ADME traits of each compound predict that the majority of the components are drug-likeness based on the GI absorption in which the percentage of every compound satisfies the maximum absorption rate along with the glycoprotein will not be inhibited [24]. In the present study, 3,5-bis(1,1-dimethylethyl)-phenol, Kaempferol, Moringyne, Niazimicin, and Tetradecanoic acid satisfied the ADME properties. Components revealing values for Lipinski's rule of five and ADME properties in acceptable ranges can be observed for possibilities to ensure the good intestinal absorption or permeation over the gut-blood barrier [25].

The molecular docking of specific ligands onto the selected protein shows potentiality to visualize their docking patterns that indicate their binding affinity and corresponding inhibitory impact [26]. In the present context, target protein MCR was docked with five selected phytocomponents using Hex 8.0.0 software in order to demonstrate their molecular interactions and binding energy. In the present investigation, Tetradecanoic acid was reported as the most potent inhibitor against MCR, thereby showing the highest E-value. This might be due to the fact that Tetradecanoic acid had comparatively higher interaction with MCR. Additionally, this could be because of the fact that the hydrogen bonding played a pivotal role as functional

determinants of protein-ligand interactions, particularly in the inhibition of a complex. In view of the molecular docking results, Tetradecanoic acid revealed good binding interaction with MCR, thereby reducing the methanogenesis activity, which has been directly proportional to increasing horse's performance via distributing metabolic H_2 to fermentation pathways [27]. Previously, Kung et al. [28] demonstrated that the inclusion of 9,10-anthraquinone reduced CH_4 production in ruminants. Similarly, 3-nitrooxypropanol reduced CH_4 emission from ruminants also [29]. Recent *in silico* studies had successfully used Hex 8.0.0 software for evaluating the molecular interaction between ligand of interest and various receptors [30,31]. Our findings provided the further promising role of Hex 8.0.0 tool in understanding ligand-receptor based interaction toward mitigating CH_4 emission from horses by targeting MCR.

5. Conclusions

In a nutshell, the present investigation revealed the promising attributes of bioactive components from *M. oleifera* L. toward mitigation of CH_4 emission in horses by targeting MCR. Among various phytocomponents studied, 3,5-bis(1,1-dimethylethyl)-phenol, Kaempferol, Moringyne, Niazimicin, and Tetradecanoic acid exhibited better drug-likeness characteristics as per Lipinski's rule of five and ADME properties. *In silico* studies demonstrated that Tetradecanoic acid has more specificity toward MCR binding site with maximum docking E-value of −142.98 KJ/mol. The study indicated the potency of *M. oleifera* L. associated Tetradecanoic acid to develop ideal antimethanogenic drugs for equines in the future, particularly in terms of mitigation of CH_4 emission from horses.

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