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Inhibitor Prediction against Methenyl-Tetrahydromethanopterin Enzyme from Methanobrevibacter Ruminantium via Molecular Docking to Mitigate Methane Emissions

Indu Chaturvedi[1](Corresponding Author) and Ashwani Sharma[2,#]

[1]*Department of Animal Nutrition, Central Institute for Research on Goats, Makhdoom, P.O. Farah, Mathura-281 122, UP (India)*
[1,2]*New Era Proteomics Drug Discovery Research C-1/31, Yamuna Vihar New Delhi-110053, India*
E-mail: [1]indu_0012@yahoo.co.in, [2]ashwansharma@gmail.com, [#2]Corresponding author

Abstract: *Methane is the major greenhouse gas produced by the process of methanogenesis carried out by methanogenic bacteria in the rumen of ruminant. The methanogenesis process is regulated by several pathway enzymes present in the methanogenic bacteria, one of them is newly discovered Methenyltetrahydromethanopterin Cyclohydrolase enzyme found in Methanobrevibacter ruminantium. Therefore, there is need to inhibit the activity of this enzyme to regulate the process of methanogenesis which may result in lowering the output the methane gas. Different chemical treatments have been attempted however those are not cost effective and produced several side effects on the ruminant growth. Therefore, currently plant secondary metabolites have been prompted interest to selectively inhibit ruminant methanogenesis. We explored molecular docking method to find selective natural inhibitors of Methenyltetrahydromethanopterin Cyclohydrolase enzyme to regulate methanogenesis. Docking detected higher affinities of bioactive compounds such as δ-Viniferin, Diosmin and Eriocitrin for Cyclohydrolase enzyme as compared to chemical inhibitors Lovastatin and Mevastatin. Therefore, we predicted that ruminant nutrition supplements containing δ-Viniferin, Diosmin and Eriocitrin bioactive compounds can be serve as an effective treatment for reducing methanogenesis process and may lower post methane production in the output.*

1. INTRODUCTION

Methane is a greenhouse gas (GHG), its global warming potential 25 times more than CO2 [1]. Globally, ruminant produces 80 million tonnes of CH4 annually [2]. Enteric CH4 is produced under anaerobic conditions in the rumen by methanogenic Archaea, using CO_2 and H_2 to form CH4, and thus reducing the metabolic H_2 produced during microbial metabolism [3].

Plant extracts with high concentrations of secondary compounds are potential candidates to achieve this problem [4]. Plants contain bioactive secondary plant metabolites which are helpful for methane mitigation [5, 6]. These compounds activate several metabolic reactions in the ruminant cellular system such as the interaction of cholesterol and saponin promotes cell rupture and decreases the growth of methane producing bacteria. The presence of protozoa in the rumen causes protein turnover by predating on bacteria, it increases the nitrogen utilization of the ruminant and may lead to an increase in growth, milk, or wool production [7, 8]. Several flavonoids compounds also proved to decrease methane production [9]. Phenolic acids such as *p*-coumaric acids, ferulic acids, cinnamic acids and phloretic acids and some monomeric phenolics have been found to decrease methane, acetate and propionate production [10]. The rumen is characterized by its high microbial population and it is comprised of different prokaryotes, methanogenic archaea (Methanobrevibacter ruminantium, Methanobacterium formicicum, Methanosarcina Barkeri, Methanomicrobium mobile eukaryotes, protozoa, anaerobic fungi and bacteriophages. Therefore, interest in the rumen methanogens has resulted from the fact that ruminants typically lose 2–15% of their ingested energy solely as methane [11]. As a consequence, new targets or alternative strategy will be implemented to mitigate methane production.

Methenyl-tetrahydromethanopterin (methenyl-H4MPT) cyclohydrolase is found in methanogenic archaea, sulphate-reducing archaea and methylotrophic bacteria [12, 13, 14]. It catalyzes the reversible formation of *N5,N10*-methenyltetrahydromethanopterin (methenyl-H4MPT+) from *N5*-formyltetrahydromethanopterin (formyl-H4MPT) [15,16]. *N5*-formyl-H4MPT+H+ *N5,N10*-methenyl-H_4MPT++H_2O DG°¢ = –5 kJ/mol [12]. This reaction in the forward direction is involved in the reduction of CO_2 to methane and in the autotrophic CO_2 fixation. In the reverse direction it is involved in C_1 unit oxidation to CO_2. The enzyme has been purified from *Methanobrevibacter ruminantium, Methanobacterium thermoautotrophicum* [17, 18], *Methanosarcina barkeri* [19],

Methanopyrus kandleri [20, 21], *Archaeoglobus fulgidus* [13] and *Methylobacterium extorquens* AM1 [22]. There is interest in feed additives with the potential to reduce ruminal methanogenesis. There are a number of experimental studies on plant extracts to reduce the methanogenesis process. In this study, our focus is on finding the potential bioactive compounds which may inhibit the function of Methenyltetrahydromethanopterin Cyclohydrolase enzyme in order to reduce methanogenesis process. We used molecular docking method for computational virtual screening of different plant origin phytochemicals or bioactive compounds to find a potential natural inhibitor for the Methenyltetrahydromethanopterin Cyclohydrolase enzyme.

2. MATERIALS AND METHODS

2.1 Input Receptor file

Crystalline structure of Methenyltetrahydromethanopterin Cyclohydrolase enzyme (PDB Code: 4FIO) of *Methanobrevibacter ruminantium* was obtained from a RCSB protein data bank in the pdb format. Its chain A was selected for further molecular docking study.

2.2. Compounds Database

Information about different phytochemicals and bioactive compounds was obtained from the available literatures that reported the beneficial effect of plant origin secondary metabolites and bioactive compounds in the ruminant growth system. Different classes of compounds were selected as described in the phyto-chemical database (http://www.phytochemicals.info/phytochemicals.php) and available in the literatures. Total 159 phytochemicals were downloaded from PUBCHEM database and their SMILES strings were converted into 3D structure via CORINA server. All files were saved in the pdb file format (http://www.molecular-networks.com/online_demos/corina_demo). Two known chemical compounds known to act as potential inhibitors for methane production Lovastatin and Mevastatin were also selected for our docking study.

2.3 Binding site prediction

Structure of Methenyltetrahydromethanopterin Cyclohydrolase enzyme (PDB Code: 4FIO) was submitted to functional site prediction servers such as PROFUNC and PINTS for putative binding site residues prediction. These residues were further used as a docking target during the protein-ligand docking simulation.

2.4 Molecular Docking

The pdb structure of Methenyltetrahydromethanopterin Cyclohydrolase enzyme (PDB Code: 4FIO, chain A) was submitted to molecular docking with the compounds database by iGemdockv2.1 software. Note: the binding site residues predicted by the servers used as docking target. The *Drug Screening* platform of iGemdock was selected for both the docking studies with parameters such as Population size: 200, Number of generations: 70 and Number of solutions: 3. Top five hits (with lower interaction energies) of bioactive compounds obtained after the iGemdock docking were further submitted to the second step of the docking process by Patchdock docking software. Compounds were ranked based on their interaction energies and fitness values produced by the docking via iGemdock software. On the other hand, Patchdock ranked the compounds based on the Patch-Patch interaction score. The most stable conformations of the bioactive compounds were selected based on the lowest fitness values and higher patchdock interaction scores. Subsequently, post docking analyzes were performed for the binding of bioactive compounds on the enzyme to extract the binding site residues at radius of 8Å taken compounds as a center.

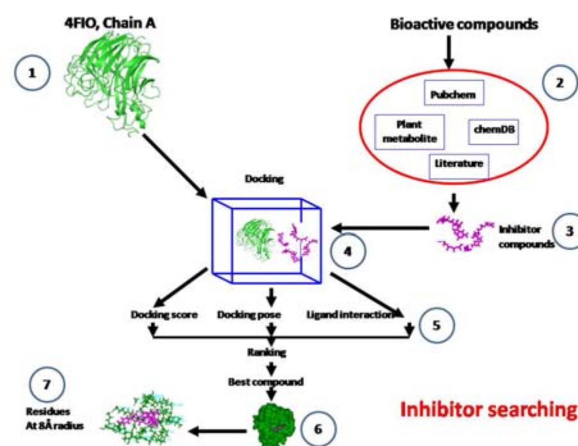


Fig. 1: Schematic for the overall methodology. (1): PDB structure of Methenyltetrahydromethanopterin Cyclohydrolase enzyme (PDB Code: 4FIO, chain A) was obtained from protein data bank. (2): Download the compounds from ligand databases. (3): Obtained inhibitors compounds. (4): Molecular docking against the structure of 4FIO. (5): Post docking analysis. (6): Compounds binding on the enzyme structure. (7): Collection of residues around the bounded ligand at 8Å of radius taking ligand as center.

3. RESULTS

Total 159 bioactive compounds were obtained from the ligand databases. These compounds were docked with the Methenyltetrahydromethanopterin Cyclohydrolase enzyme (PDB Code: 4FIO, chain A). Bioactive compounds δ-Viniferin showed largest affinity for the Cyclohydrolase enzyme with iGemdock interaction energy (fitness value) of -102.06 kcal/mole. Two more compounds, Diosmin and Eriocitrin were obtained similar affinities with the Cyclohydrolase enzyme with iGemdock interaction energies (fitness values) of -101.75 kcal/mole and -101.28 kcal/mole (Table 1). The

affinities produced by δ-Viniferin, Diosmin and Eriocitrin are also unique and not able to detect by other bioactive compounds (Fig. 2: Histogram). Two chemical inhibitors Lovastatin and Mevastatin produced lower affinities with the Methenyltetrahydromethanopterin Cyclohydrolase enzyme as compared to bioactive compounds i.e. δ-Viniferin, Diosmin and Eriocitrin. Their interaction energies are also higher than these bioactive compounds i.e. -81.21 kcal/mole (for Lovastatin) and -73.82 kcal/mole (for Mevastatin) (Table 1).

Docking by Patchdock also detected higher affinities for three bioactive compounds δ-Viniferin, Diosmin and Eriocitrin as compared to chemical inhibitors Lovastatin and Mevastatin. Compounds δ-Viniferin also found to have a higher affinity for the Methenyltetrahydromethanopterin Cyclohydrolase enzyme and produced the best patchdock score of 6232. Second rank obtained by Diosmin with a patchdock score of 6086 followed by Eriocitrin with the score 5822. Similar to iGemdock docking, mevastatin and lovastatin produced lower affinities for the Cyclohydrolase enzyme with patchdock scores of 5106 (lovastatin) and 4664 (mevastatin) (Table 1).

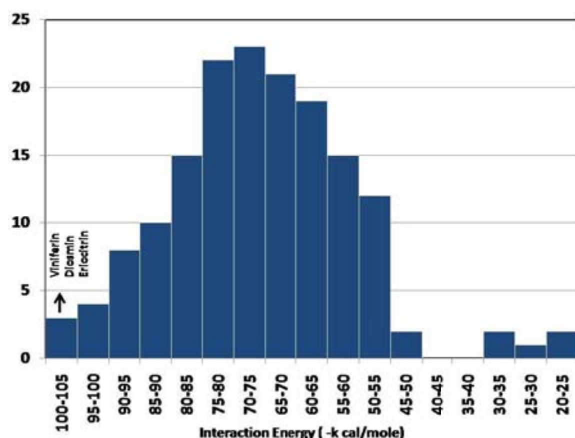


Fig. 2: Distribution of the interaction energies produced after molecular docking of the bioactive compounds with the 4FIO (chain A) by iGemdock software.

Table 1: Docking of bioactive compounds with the 4FIO (chain A).

	iGemdock	**Patchdock**
	Interaction Energy	Score
Compounds	kcal/mole	
δ-Viniferin	-102.06	6232
Diosmin	-101.75	6086
Eriocitrin	-101.28	5822
Chemical Inhibitors		
Lovastatin	-81.21	5106
Mevastatin	-73.82	4664

The selected bioactive compound δ-viniferin is a dehydrodimer resveratrol which is an isomer of ε-viniferin. It is produced in vitro by the oxidative dimerization of resveratrol by plant peroxidases or fungal laccases. It can be isolated from stressed grapevine (*Vitis vinifera*). It was also recently identified in wines and in grape cell cultures. Diosmin is a flavone compound, belong to the flavonoid family (polyphenol compound) found in *Teucrium gnaphalodes*. Eriocitrin is a flavanone-7-*O*-glycoside between the flavanone eriodictyol and the disaccharide rutinose. It is found in high concentration in lemon plant (*Citrus limon*).

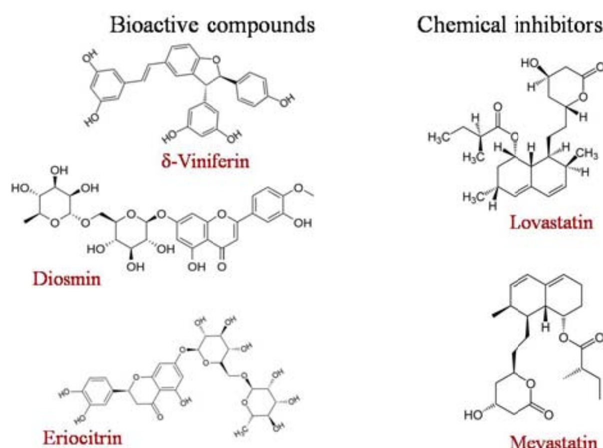
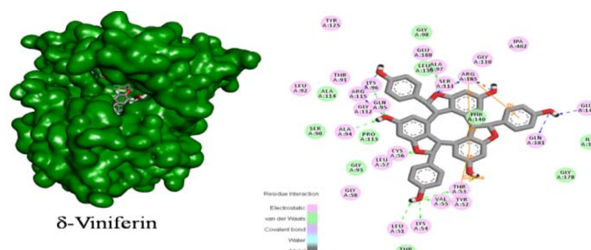


Fig. 3: Different bioactive compounds selected after the docking process.

3.1. Ligand Binding site

Amino acid residues were collected at 8Å of the radius from the docked bioactive compounds δ-Viniferin, Diosmin and Eriocitrin as a center. The phytochemical δ-Viniferin is surrounded by many amino acid residues where LEU51, TYR52, THR53, LYS54, VAL55, CYS56, LEU57, GLY58, THR91, LEU92, ALA94, GLN95, LYS96, GLY110, SER111, GLY112, ARG115, GLU142, ARG185 and GLU188 make an electrostatic cloud around the ligand δ-Viniferin. Among these residues LEU51, TYR52, THR53, LYS54, CYS56, LEU57, ALA94, LYS96, ARG115, GLU142 and ARG185 make hydrogen bond interactions with the δ-Viniferin. In addition, SER90, GLY93, GLY98, PRO113, ALA114, LEU139, THR140, GLY178 and ILE182 make van der waals interaction cloud around the docked ligand δ-Viniferin (Fig. 4).



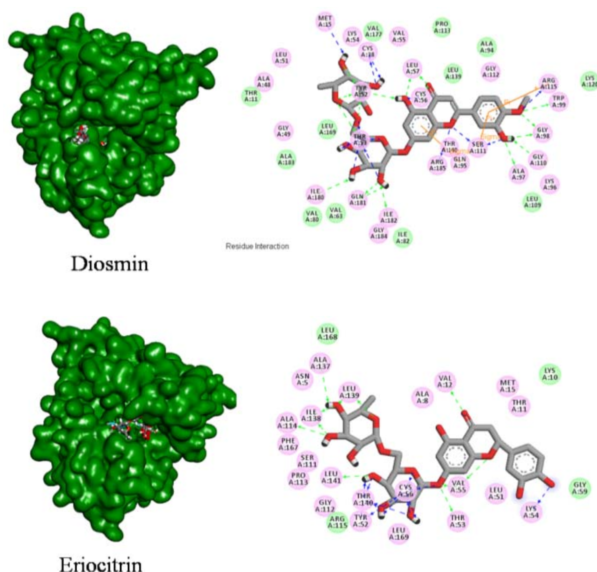


Fig. 4: Binding of bioactive compounds at the cavity of the 4FIO and surrounding amino acid residues at 8Å of radius with ligand as center.

The amino acid residues THR53 and ARG185 involve in making π-sigma and π-cation interactions with δ-Viniferin. In addition to these residues, MET15, CYS38 and SER111 make hydrogen bond interactions with the Diosmin. Two residues SER111 and GLN95 make π-sigma and ARG115 make π-cation interactions with the Diosmin. Similar amino acid residues are surrounding the Eriocitrin ligand at the active site of the protein.

4. DISCUSSION

Methane is the major greenhouse gas, which is responsible for global warming. The large amount of methane is produced by the enteric fermentation in the ruminant gut. This fermentation process is carried out by the action of methanogenic bacteria such as *Methanobrevibacter ruminantium*. Several metabolic pathway enzymes participate in the methane production and one of them is a Methenyltetrahydromethanopterin Cyclohydrolase enzyme that catalyzes the important step of reduction of CO_2 to methane and in the autotrophic CO_2 fixation. Therefore we targeted Methenyltetrahydromethanopterin Cyclohydrolase enzyme for designing inhibitors from natural resources. The Computational molecular docking method was explored for finding the natural bioactive compounds which may inhibit the activity of Cyclohydrolase enzyme. Docking method is less expensive and faster to derive affinity of the ligand for the protein. Our docking method detected that δ-Viniferin, Diosmin and Eriocitrin produced lowest docking energies upon binding with a methenyltetrahydromethanopterin Cyclohydrolase enzyme which predicted their higher affinities for this enzyme. Docking method is also able to detect the binding site residues surrounding δ-Viniferin, Diosmin and Eriocitrin at the active site of Cyclohydrolase enzyme. In addition, two chemical inhibitors, mevastatin and lovastatin produced lower affinities for the Cyclohydrolase enzyme which reveals that these natural compounds can replace the chemical supplements for deriving nutrition for ruminants. This will reduce the cost of deriving food supplements for the ruminant. These natural supplements are easily available and less expensive than the chemical supplements. Several reports are available which reveal the use of natural compounds for inhibiting the production of methane in the ruminant output such as The F420-dependent NADP oxidoreductase enzyme from *Methanobrevibacter smithii* catalyzes the important electron transfer step during methanogenesis it is successfully inhibited by Lovastatin (-22.07 Kcal/mol) and Compactin (Mevastatin) (-21.91 Kcal/mol) [23]. Ethanol and methanol extract of fennel, cloves and garlic had an inhibitory effect on methane production [24]. Therefore, our predicted bioactive compounds may inhibit the activity of methenyltetrahydromethanopterin Cyclohydrolase enzyme which can regulate the methanogenesis process.

5. CONCLUSION

Our first report about molecular docking study of Methenyltetrahydromethanopterin Cyclohydrolase enzyme against the bioactive compound database reveals that δ-Viniferin, Diosmin and Eriocitrin can be act as effective natural supplements in the ruminant nutrition diet in order to reduce methanogenesis and to mitigate the methane output. Our approach will help the experimental biologist to combine computational application in their research. It will also help in specifically designing of ruminant nutrition supplements which may reduce the cost and effectively regulate the methane emission through the ruminant enteric fermentation process.

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REFERENCES

[1] Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., and DeHaan, C., Livestock's Long Shadow: Environment al Issues and Options. FAO, Rome, Italy, 2006.

[2] Beauchemin, K. A., Kreuzer, M., O'Mara, F., and McAllister, T. A., Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture,* 48, January 2008, pp. 21–27.

[3] McAllister, T. A., and Newbold, C. J., Redirecting rumen fermentation to reduce methanogenesis. *Australian Journal of Experimental Agriculture,* 48, January 2008, 7–13.

[4] Teferedegne, B., New perspectives on the use of tropical plants to improve ruminant nutrition. *Proceedings of Nutrition Society,* 59, May 2000, pp. 209–214.

[5] Hu, W., Liu, J., Wu, Y., Guo, Y., and Ye, J., Effects of tea saponins on in vitro ruminal fermentation and growth performance in growing Boer goat. Arch. *Animal Nutrition,* 60, February 2006, pp. 89–97.

[6] Kamra, D. N., Singh, R., Agarwal, N., and Pathak, N. N., Soapnut (Reetha) as natural defaunating agent – its effect on rumen fermentation and in Sacco degradability of jowar hay in buffaloes. *Buffalo Journal,* 16, 2000, pp. 99–104.

[7] Elizabeth Wina, Stefan Muetzel and Klaus Becker., The Impact of Saponins or Saponin-Containing Plant Materials on Ruminant Production — A Review *Journal of Agriculture Food Chemistry*, October 2005, *53* (21), pp. 8093–8105.

[8] Fransis, G., Kerem, Z., Makkar, H. P. S., and Becker, K., The biological action of saponins in animal systems. *A Review British Journal of Nutrition,* 88, December 2002, pp. 587-605.

[9] Broudiscou, L. P., Papon, Y., and Broudiscou, A. F., Effect of dry plant extracts on fermentation and methanogenesis in continuous culture of rumen microbes. *Animal Feed Science Technology,* 87, October 2000, pp. 263–277.

[10] Asiegbu, F. O., Paterson, A., Morrison, I. M., and Smith, J. E., Effect of cell wall phenolics and fungal metabolites on methane and acetate production under *in vitro* conditions. *Journal of General Applide Microbiology,* 41, 1995, pp. 475–485.

[11] Moss, A. R, Jouany, J. P, and Newbold, J., Methane production by ruminants: its contribution to global warming. *Ann Zootechnology,* 49, 2000, pp. 231–253.

[12] Thauer, R. K., Biochemistry of methanogenesis: a tribute to Marjory Stephenson. *Microbiology,* 144, September 1998, pp. 2377-2406.

[13] Klein, A. R., Breitung, J., Linder, D., Stetter, K. O., and Thauer, R. K., *N5,N10*-Methenyltetrahydromethanopterin cyclohydrolase from the extremely thermophilic sulfate reducing *Archaeoglobus fulgidus*: comparison of its properties with those of the cyclohydrolase from the extremely thermophilic *Methanopyrus kandleri. Archaea Microbiology,* 159, 1993, pp. 213-219.

[14] Chistoserdova, L., Vorholt, J. A., Thauer, R. K. and Lidstrom, M. E., C1 transfer enzymes and coenzymes linking methylotrophic bacteria and methanogenic archaea. *Science,* 281, July 1998, pp. 99-102.

[15] Donnelly, M. I., Escalante-Semerana, J. C., Rinehart, K. L. Jr. and Wolfe, R. S., Methenyl-tetrahydromethanopterin cyclohydrolase in cell extracts of *Methanobacterium. Archaea Biochemistry Biophysics,* 242, November 1985, pp. 430-439.

[16] Breitung, J. and Thauer, R. K., Formylmethanofuran: tetrahydromethanopterin formyl-transferase from *Methanosarcina barkeri*: identification of *N5*-formyltetrahydromethanopterin as the product. *FEBS Letters,* 275, November 1990, pp. 226-230.

[17] DiMarco, A. A., Donnelly, M. I. and Wolfe, R. S., Purification and properties of the 5,10-methenyltetrahydromethanopterin cyclohydrolase from *Methanobacterium thermoautotrophicum. Journal of Bacteriology,* 168, December 1986, pp. 1372-1377.

[18] Vaupel, M., Dietz, H., Linder, D. and Thauer, R. K., Primary structure of cyclohydrolase (Mch) from *Methanobacterium thermoautotrophicum* (strain Marburg) and functional expression of the *mch* gene in *Escherichia coli. European Journal of Biochemistry,* 236, February 1996, pp. 294-300.

[19] Te Brömmelstroet, B. W., and Vogels, G. D., Purification and properties of 5,10-methenyltetrahydromethanopterin cyclohydrolase from *Methanosarcina barkeri. Journal of Bacteriology,* 172, February 1990, pp. 564-571.

[20] Vaupel, M., Vorholt, J. A., and Thauer, R. K., Overproduction and one-step purification of the *N5,N10*-methenyltetrahydromethanopterin cyclohydrolase (Mch) from the hyperthermophilic *Methanopyrus kandleri. Extremophiles,* 2, January1998, pp. 15-22.

[21] Breitung, J., Schmitz, R. A., Stetter, K. O., and Thauer, R. K., *N5,N10*-Methenyl-tetrahydromethanopterin cyclohydrolase from the extreme thermophile *Methanopyrus kandleri*: increase of catalytic efficiency (*k*cat/*K*M) and thermostability in the presence of salts. *Archaea Microbiology,* 156, 1991, pp. 517-524.

[22] Pomper, B. K., Vorholt, J. A., Chistoserdova, L., Lidstrom, M. E., and Thauer, R. K., A methenyl-tetrahydromethanopterin cyclohydrolase (Mch) and a methenyl-tetrahydrofolate cyclohydrolase (Fch) in *Methylobacterium extorquens* AM1. *European Journal of Biochemistry,* 261, 1999, pp. 475-480.

[23] Sharma, Ashwani., Chaudhary, P. P., Sirohi, S. K., Saxena, J., Structure modeling and inhibitor prediction of NADP oxidoreductase enzyme from Methanobrevibacter smithii, March 2011 Volume 6(1) www.bioinformation.net

[24] Patra, A. K., Kamra, D. N., and Agarwal, N., Effect of extract of spices on Rumen Methanogenesis, enzyme activities and fermentation of feeds *in vitro*. *Journal of Food science and Agriculture,* 90, February 2009, pp. 511-520.