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Role of Bioinformatics in Climate Change Studies

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ABSTRACT

Climate change is a global concern issue, and climate change study incorporates study on the issue from various perspectives from socio-economic to scientific perspective. All concerned areas of science and technology including bioinformatics plays a major role in the control of climate change to some extent. Deeper study on this issue can contribute substantially towards the solution to this global problem. In bioinformatics study is being conducted with respect to sequencing microbial genome of those microbes which can reduce levels of carbon dioxide, other greenhouse gases and eventually may play a bigger role stabilizing the global climate change.

INTRODUCTION

In 2014 UN Intergovernmental Panel on Climate Change's (IPCC) Synthesis Report states that India is at risk as climate change will have adverse impacts on food production in India hampering the sustainable development. The Indian economy is hugely dependent on climate sensitive sectors such as agriculture, fisheries, forestry and even electricity generation. But it is the poorest people who suffer most from climate change. For example, 58 percent of our people solely rely on agriculture so these new changes in rain or temperature can affect the whole country's food security and economy [1]. IPCC Syn Report 2014 also states about future risks for Asia which are increased flood damage to infrastructure, livelihoods and settlements; heat related human mortality, increased drought related water and food shortage.

The Earth is surrounded by a thick layer of gases which keeps the planet warm and allows plants, animals and microbes to live. These gases work like a blanket. Without this blanket the Earth would be 20–30°C colder and much less suitable for life. But the blanket of gases that surrounds the Earth is getting much thicker [2]. These gases are trapping more heat in

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the atmosphere causing the planet to warm up. This is causing the Earth to heat up, which is called global warming and climate change is happening because there has been an increase in temperature across the world. These gases are called greenhouse gases.

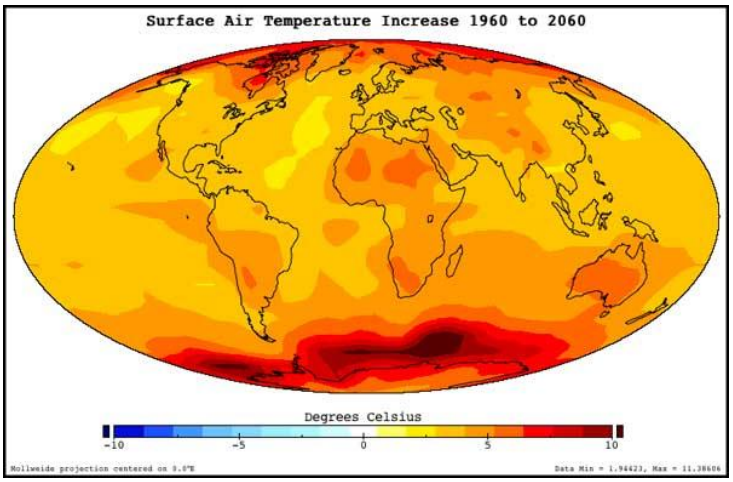

Fig. 1 Annual average global warming by the year 2060 simulated and plotted using EdGCM. Courtesy: NASA

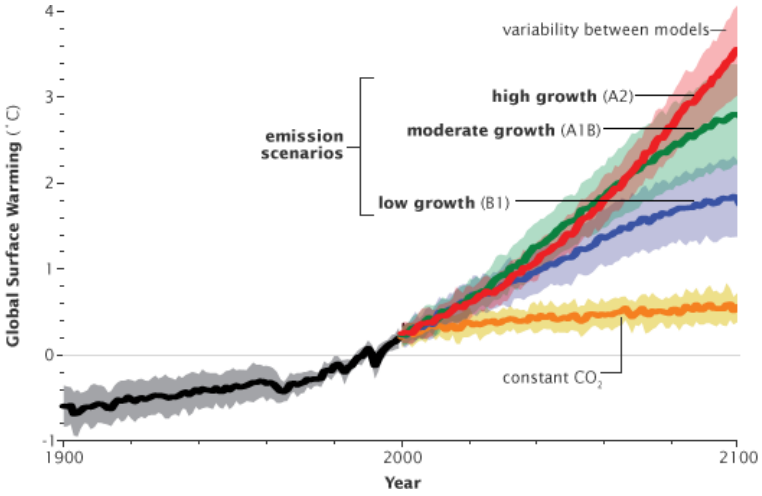

Fig. 2 Model simulations by the UN Intergovernmental Panel on Climate Change estimate that Earth will warm between two and six degrees Celsius over the next century, Courtesy : 2007 IPCC WG1 AR-4

GREENHOUSE GASES AND MICROBES

The three most important greenhouse gases are carbon dioxide, methane and nitrous oxide [3] and these have increased dramatically in recent years due to human activity. The Earth is known as a 'closed system' which means that it produces everything it needs to ensure the survival and growth of its residents. In nature there are chemical cycles such as the carbon cycle to control and balance these gases that surround the Earth. The carbon cycle is a complex series of processes through which all of the carbon atoms in existence rotate. This means that the carbon atoms in your body today have been used in many other molecules since time began e.g. as the carbon found in carbon dioxide in the air. Microbes play an important role as either generators or users of these gases in the environment as they are able to recycle and transform the essential elements such as carbon and nitrogen that make up cells. Bacteria and archaea are involved in the 'cycles' of all the essential elements [4-6].

In the carbon cycle methanogens convert carbon dioxide to methane in a process called methanogenesis [7]. In the nitrogen cycle nitrogen-fixing bacteria such as Rhizobium fix nitrogen, which means they convert nitrogen in the atmosphere into biological nitrogen that can be used by plants to build plant proteins [8]. Other microbes are also involved in these cycles like Photosynthetic algae and cyanobacteria play a key role in the carbon cycle as they carry out photosynthesis and form the basis of food chains in the oceans [9]. Fungi and soil bacteria play a major role in the carbon cycle as they break down organic matter and release carbon dioxide back into the atmosphere.

ROLE OF MICROBES TO REDUCE GLOBAL CLIMATE CHANGE

According to some estimates, microbes constitute about 60% of the earth's biomass and play an important role in natural biogeochemical cycles. Scientists have now started realizing their potential and role in global climate processes. Several applications of microbes have been conceived, such as in cleaning up toxic waste-sites worldwide, energy generation and development of renewable energy sources, management of environmental carbon dioxide related to climate change, detection of disease-causing organisms and monitoring of the safety of food and water supplies, use of genetically altered bacteria as living sensors (biosensors) to detect

harmful chemicals in soil, air or water and understanding of specialized systems used by microbial cells to live in natural environments with other cells.

Prochlorococcus and Synechococcus are single celled cyanobacteria [10] and are the smallest yet most abundant photosynthetic microbes in the ocean. There are around 100 million Prochlorococcus cells per litre of sea water [11]. Researchers estimate that Prochlorococcus and

Synechococcus remove about 10 billion tons of carbon from the air each year; this is about two-thirds of the total carbon fixation that occurs in the oceans and more efficiently than other phytoplankton [12]. Scientists have deciphered the genomes of these two microbes.

What is a genome?
It is a map of the complete genetic make-up of an organism. The basic units of genetic information are called genes. The genome, which is made up of genes, contains all of the biological information needed to build and maintain a living example of that organism. The number of genes an organism has depends to some extent on the complexity of the organism.

With this knowledge scientists hope to understand why these two microbes carry out photosynthesis so successfully. Ultimately being able to harness such microbial power could slow down increases in levels of carbon dioxide and other greenhouse gases and eventually reduce global climate change.

BIOINFORMATICS STUDIES ON MICROBES GENOME

Bioinformatics is also helping in climate change studies. In the last decade, various genome sequencing and metagenomic projects have generated large amounts of genetic data for cyanobacteria. This wealth of data provides researchers with a new basis for the study of molecular adaptation, ecology and evolution of cyanobacteria, as well as for developing biotechnological applications. It also facilitates the use of multiplex techniques, i.e., expression profiling by high-throughput technologies such as microarrays, RNA-seq, and proteomics. However, exploration and analysis of these data is challenging, and often requires advanced computational methods. Also, they need to be integrated into existing framework of knowledge

to use them to draw reliable biological conclusions. Here, systems biology provides important tools. Especially, the construction and analysis of molecular networks has emerged as a powerful systems-level framework, with which to integrate such data, and to better understand biological relevant processes in these organisms. Multiplex data from genomic, transcriptomic, proteomic, and metabolomics studies on cyanobacteria must be studied with informatics approach to draw trustworthy biological inference to be used in the environment remediation. Also studies are made to apply comparative genomics to analyze and characterize cyanobacteria's gene content variations. This study created a computational pipeline to compare gene content of 100 cyanobacteria genomes of diverse species and identify core genes, phylogenetically related species specific genes and genome specific genes.

In 1994, through advances gained by the Human Genome Project, the DOE formulated the Microbial Genome Initiative to sequence the genomes of bacteria useful in the areas of energy production, environmental remediation, toxic waste reduction, and industrial processing. Resulting from that project, six microbes that live under extreme temperature and pressure conditions have been sequenced. By learning the unique protein structure of these microbes, researchers may be able to use the organisms and their enzymes for such practical purposes as waste control and environmental cleanup.

One of such bacteria is Prochlorococcus, which is the smallest unicellular cyanobacterium with a genome size of approximately 2 Mb and profusely found photosynthetic microbe [13] in the ocean that dominates the temperate and tropical oceans. It lacks phycobilisomes that are characteristic of cyanobacteria, and contains chlorophyll b as its major accessory pigment is responsible for a significant fraction of photosynthesis in the world's oceans. In the subtropical Pacific, for example, it often represents 50% of the total chlorophyll This enables it to absorb blue light efficiently at the low-light intensities and blue wavelengths characteristic of the deep euphotic zone. It contributes 30-80% of the total photosynthesis in the oligotrophic oceans, and thus plays a significant role in the global carbon cycle and the Earth's climate.

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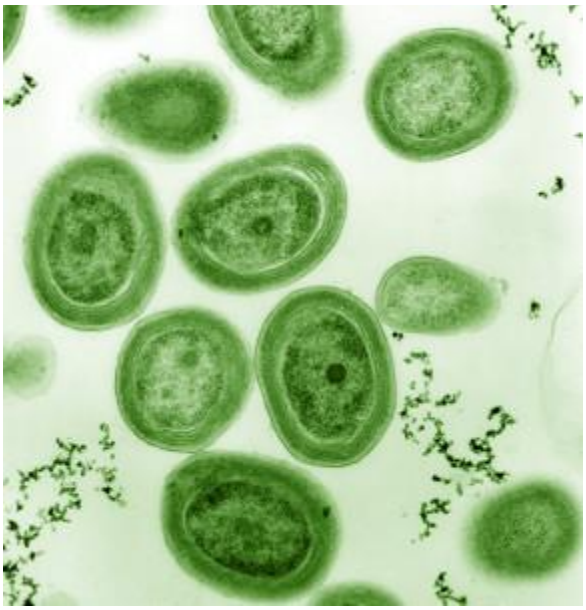

Fig 3(a) Electron micrograph of a cultured strain of *Prochlorococcus*, MED4. (Courtesy : N. Watson and L. Thompson, MIT)

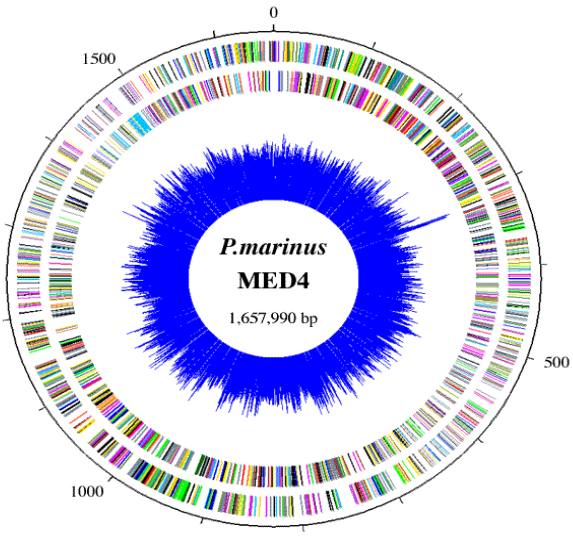

Fig3(b) Circle map of Prochlorococcus marinus MED4 (Source: http://genome.microbedb.jp/cyanobase /MED4)

By contrast, the highly related Synechococcus contains chlorophyll a and phycobilins that are more typical of cyanobacteria. To date, Prochlorococcus is the only photosynthetic organism known to contain this particular combination of pigments. As such, it has recently been suggested to be an extant model for the ancestral photosynthetic bacterium that gave rise to cyanobacteria as well as chloroplasts [14]. Sequence analysis of the Prochlorococcus genome [15] may shed more light on this hypothesis and on cyanobacteria radiation in general.

Description of the complete genome of Prochlorococcus marinus will greatly advance our understanding of the regulation of these globally important processes. Prochlorococcus is an ideal candidate for complete genome sequencing because it is the smallest known phototroph with a relatively small genome (1.8 Mb), it is widespread and abundant and is easily identified and enumerated in situ using flow cytometry and its unique photosynthetic pigment (divinyl chlorophyll) makes its contribution to total photosynthetic biomass in the oceans easily accessed and at least two ecotypes of Prochlorococcus co-exist in the oceans that are distinguished by their photophysiology and molecular phylogeny. One is capable of growth at irradiances where

the other is not. Ultimately, a comparison of the complete genomes of these two ecotypes would provide valuable insights into the regulation of this type of microdiversity in marine microbial systems. In addition, the use of microarray technology for the analysis of gene expression patterns will give us unprecedented insights into how these microbes cope with the dilute environment of the oligotrophic oceans [16]. The role of Molecular Modeling application was also studied for reducing the methane production [17].

Another microbe Deinococcus radiodurans is known for radiation resistance and being used for cleaning up the waste sites that contain toxic chemicals. There are many organisms which use carbon dioxide as their sole carbon source and increasing levels of carbon dioxide emission is one of the major causes of the global climate change. The study of genomes of these microbial organisms, which is possible through bioinformatics, helps in proposing ways to decrease the carbon dioxide content.

The MGP launched by DoE has brought a revolution in the field of microbiology and more work needs to be conducted with help of Bioinformatics in this domain towards waste control and environmental cleanup.

CONCLUSION

In conclusion it can be said that bioinformatics can play a major role in the climate change studies and it can be integral part of climate change studies. Not much work has been done in this area in bioinformatics domain, and more region specific work must be conducted considering microbes of that region and their capability in CO2 reduction.

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Effect of different herbal feed additives on *in vitro* rumen fermentation

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ABSTRACT

In order to observe the effect of different herbal plants/roots on in vitro rumen fermentation pattern, three herbal feed additives i.e Leaves of Clendendrum phlomidis (Arni, T1), roots of Curcuma longa (Haldi, T2), and leaves of Ocimum sanctum (Tulsi, T3) were mixed @ 0.5% in the concentrate mixture or 0.5% in the mixed substrate and assessed individually using goat rumen liquor. After 48 h of incubation total VFA concentration was significantly (P<0.01) higher in T3 than other treatments. However, acetate (A), propionate (P), butyrate and A/P ratio remained unaffected by such treatments. Nitrogen fractions (total-N, NH3-N, TCA-precipitable-N, NPN) in the incubation medium were also similar among three groups. It may be concluded that inclusion of either leaves of Arni or roots of Haldi or leaves of Tulsi @ 0.2% in the substrate mixture (containing concentrate mixture: roughage = 40:60) did not improve IVDMD and rumen fermentation pattern in goats in vitro.

INTRODUCTION

The demand of feed and fodder for livestock is much higher than availability. Various hormones, antibiotics and feed additive have been used to increase the animal productivity. Although the use of antibiotics and hormones has improved the animal performance [1-2] their prolonged use has been discouraged because of their residual effects and development of drug resistant microbes [3,4].

Herbal mixture is eco-friendly and non hazards to both human handlers and animals. They have no side effects and have minimum problem of drug resistance and no residual effects are observed with the use of herbal drugs. The need of the hour is to get safe, affordable and natural organic supplements to improve efficiency of utilization of available feed resource. Natural products with high concentration of secondary metabolite appear to be good for achieving these heath objectives [5-9]. Herbal plants are used in animal feeds as the growth

promoters. They act as antibacterial, antioxidant, anthelmintic, anticoccidial and growth promoters. Majority of medicinal plants do not have the residual effects. Phytochemicals and plant secondary metabolites could increase protein flow to the duodenum [10]. The plants containing saponins have been found to suppress or eliminate protozoa from the rumen and reduce methane and ammonia production [11]. Therefore, the study was conducted to observe the effect of different herbal components on *in vitro* rumen fermentation using goat rumen liquor.

MATERIALS AND METHODS

Three herbal feed additives i.e. Leaves of *Clendendrum phlomidis* (Arni, T_1), roots of *Curcuma longa* (Haldi, T_2), leaves of *Ocimum sanctum* (Tulsi, T_3) were mixed @ 0.5% in the Substrate feed and assessed individually under *in vitro* rumen fermentation system using goat rumen liquor. The substrate was prepared using herbal ingredient containing concentrate mixture (40%), gram straw (40%) and cowpea hay (20%). Rumen fermentation pattern were observed in the in vitro bttles, which were incubated at 39±0.5 ^{0}C for 48h. Total nitrogen, TCA-precipitable-N, NH_3-N and NPN were analyzed as per micro Kjeldahl method AOAC [12]. Total VFAs in rumen fluid were analyzed according to Barnett and Reid (1956)[13]. Fractionation of VFAs rumen liquor was done by Gas Liquid Chromatography according to Erwin *et al.* (1961)[14]. Substrates were analyzed for Proximate AOAC (1984) [12] and cell wall components Goering and VanSoest (1970)[15].

RESULTS

IVDMD and *in vitro* total gas production after 48 h of incubation were not influenced by the incorporation of individual herbal additive in the substrates (Table 1). Whereas, total VFA concentration was significantly ($P<0.01$) higher in Tulsi supplemented *in vitro* bottles than other treatments. However, acetate (A), propionate (P), butyrate and A/P ratio remained unaffected by such treatments. Nitrogen fractions (total-N, NH_3-N, TCA-precipitable-N, NPN) in the incubation medium were also similar among three *in vitro* treatment bottles. Therefore, these

three herbal plants had little or no positive effect on fermentation pattern in goats (under *in vitro* system) when used individual component of such herbal plants.

DISCUSSION

Several reports [16-18] show the adverse effect of essential oils (EO) on ammonia nitrogen by attributed to lower ammonia levels which in turn was due to reduced deamination of amino acids. Whereas, Sardar *et al.* (1997) [19] was reported no change in NH_3-N in the rumen liquor of cattle at various levels of supplementation of EO.

Garlic oil (300 and 3000 mg/l) and benzyl salicylate (300 and 3000 mg/l) also reduced acetate and increased propionate and butyrate proportions [20].

Table 1: Total gas production and fermentation pattern as affected by different herbal constituent under *in vitro* system.

Treatments	T_1 (Arni)	T_2 (Haldi)	T_3 (Tulsi)	S.E.M.	Significance
pH	6.69	6.68	6.59	2.48	NS
TVFA (mmol/dl incubation medium)	9.40^a	8.86^a	11.78^b	0.40	P<0.01
Total-N (mg/dl incubation medium)	86.24	86.24	81.72	1.97	NS
NH_3.N (mg/dl incubation medium)	24.36	22.40	23.28	0.78	NS
NPN (mg/dl incubation medium)	33.60	31.36	34.72	0.86	NS
TCA-precipitable-N (mg/dl)	52.64	54.88	47.00	1.99	NS
Acetate%	68.03	65.16	65.58	0.89	NS

Propionate%	21.41	20.73	21.73	0.31	NS
Butyrate%	10.56	14.11	12.69	0.77	NS
A/P ratio	3.18	3.16	3.04	7.82	NS

Means with different superscripts (a, b) in the row differ significantly.

CONCLUSION

It may be concluded that either *Clendendrum phlomidis leaves* (Arni) *or* roots of *Curcuma longa* (Haldi) or leaves of *Ocimum sanctum* (Tulsi) @ 0.5% in concentrate mixture or 0.5% in the substrate mixture (containing concentrate mixture: roughage = 40:60) did not improve rumen fermentation pattern in goats *in vitro.*

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Dietary Protein Scoring (DPS) Algorithm for Grading of Food in Vital for Major Function of Human Body - A Computational Biology Approach

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ABSTRACT

Cell Synthesizes new protein using both essential and non essential amino acids. Essential amino acids are consumed through food. The proportion of essential amino acid greatly varies in our dietary supplements, so the study of essential amino acid composition will give light on better understanding of the synthesis of some vital human proteins. In this paper, an attempt has been made to rank food supplement based on the a score obtained from a scoring method which is based on the percentage of essential amino acid composition of major constituent proteins of our dietary supplement and the human proteins responsible for vital body functioning The scoring method recommends a particular food for the better synthesis of a human protein. The algorithm constitutes the calculation of percentage of essential amino acids and a scoring method for proteins which human consume in the form of food , as well as the vital proteins which need to be synthesized by human cell for some selected vital body functioning.

INTRODUCTION

The dietary supplement consumed by human is digested and broken into monomers i.e individual amino acid, which in return is carried to the cells and these amino acid are used in synthesis of new protein, vital for smooth functioning of metabolic activities in Human.So the importance of each dietary supplement in the synthesis of new proteins is a major issue in the field of nutrition and dietics. So in this paper we have attempted to prepare a scoring method which is based on the percentage of individual amino acid present in both supplied protein and synthesized protein. So, after making an analysis of the proteins vital for major function of human body following proteins are selected for study Alpha Crystalline, a protein for eyesight[7] , Cadherin, it is a protein responsible for hearing [1], Myosin contains proteins for involuntary heart beat[2],Immunoglobulin protein are responsible for the protection of body against pathogen[3], Trypsin is responsible for digestion[4], Somatotropin stimulates for growth[5],

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Neurotransmitter for nerve conduction[6.], Cotransporter for amino acid absorption in kidneys [8] Haemoglobin, a metalloprotein responsible for blood formation [9].

The sequences of these proteins are collected from various protein databases. A program has been written in java which calculates the percentage of essential amino acid in the proteins. On the basis of this analysis ,a chart will be prepared showing varying degrees of percentage of amino acid of these proteins.

Now regular food commodities are explained in details, for eg- major constitute protein of chicken consumed by Human is myosin protein, so intake of chicken would increase the proportion of myosin protein in our diet. Similarly Fish, Egg, Milk, Cocoa, Soyabean, Dal, Wheat, Bread, Butter are chosen from which the major constituent protein is taken into account. This will also have a considerable variation and again a chart is prepared. Based on both the charts and comparing amino acid composition of one vital human protein against multiple food proteins. Analysis will be made to identify optimum food protein which can be proved to be best suited for the synthesis of that particular human protein. Such Scoring method will help in ranking various food supplements. The algorithm constitutes the calculation of percentage of essential amino acids and a scoring method for proteins which is human consumed in the form of food , as well as the vital proteins which needs to be synthesized by human cell for some selected vital body functioning.

MATERIALS AND METHODS

MATERIALS

JDK 1.6

The JDK includes the JRE plus command-line development tools such as compilers and debuggers that are necessary or useful for developing applets and applications.

Protein Sequence Databases

UNIPORT- UniProt is a comprehensive, high-quality and freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing projects (http://www.uniprot.org/help/uniprotkb). RCSB-It is a protein database (URL:

http://www.rcsb.org). PIR: A resource to assist researchers in the identification and interpretation of protein sequence information (URL : http://pir.georgetown.edu/).

NMR Protein Sequence

Nuclear magnetic resonance spectroscopy of proteins (usually abbreviated protein NMR) is a field of structural biology in which NMR spectroscopy is used to obtain information about the structure and dynamics of proteins. From the above mentioned databases only the NMR Sequences were retrieved for the food proteins (as given in Table 2.1) and vital human proteins (as given in table 2.2). The data is given here in tabular form.

Table 2.1 Major Constituent Protein of Dietary Supplement

ID	Source	Protein Name
Q39837(l=119,Mw=13,046)	Soyabean Seed	Soyabean Albumin-1
Q6X1C0 (L=460,Mw=50,722)	Dal	Crocetin glucosyl transferase
Q9EEU3(328..37,364)	Fish	Myosin Light chain
P13538(1939..223145)	Chicken	Myosin Heavy chain
P32765(221..24,039)	Choclate	Seed protein
P02702(241..27,992)	Milk	Folate Receptor protein
Q517K9	Wheat	Ribosomal protein
P02752(238..27,211)	Egg	Yolk albumin
Q84JT3	Cauliflower	Cauliflower protein

Table 2. 2 Vital Human Protein

ID	Protein Name	Functionality
Q5T2W1	Na(+)/H(+) exchange regulatory cofactor NHE- RF3	Transmission of signals
Q9NZD4	Alpha-hemoglobin-stabilizing protein	Maintenance of RBC in blood
Q14896	Myosin-binding protein C	Responsible for involuntary cardiac responses
Q9UBF9	Human Myotilin	Provides immunity
P01236	Somatotropin Prolactin	Responsible for growth
P07477	Trypsin -1	Helps in digestion
2V37:A	Cadherin	Aids in hearing
1IIY:A	Alphacrystalline	Improves vision
P17787	Neuronal acetylcholine	Works as NeuroTransmitter

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METHOD

Sequence Retrieval

Protein sequence were retrived from the mentioned database based on the protein name.Only the non redundant NMR sequences were retrieved.Sequences were saved as fasta files.

Sequence Analysis

To calculate the percentage of essential amino acids of the given sequences a Java program was written using JDK 1.6 which reads the fasta file and calculate the percentage of essential amino acid.

Table 2.2(a). Amino Acid Composition of Major Constituent Proteins of Diet

Name	%H	%I	%L	%K	%M	%F	%T	%Y	%V
Chicken	2.22	4.85	10.11	10.83	2.78	2.94	4.74	2.17	4.54
Milk	3.73	2.9	5.81	4.98	2.07	3.32	4.56	3.73	3.73
Fish	0	6.71	7.38	7.38	3.36	7.38	4.03	1.34	6.04
Soyabean	1.68	5.04	5.88	5.04	4.2	6.72	5.04	2.52	4.2
Wheat	1.08	1.61	3.23	4.30	1.08	1.61	3.23	4.30	1.08
Dal	2.17	4.13	9.57	4.78	1.96	3.48	4.35	2.17	8.26
Choclate	0.9	4.07	7.24	5.88	0.9	4.52	7.24	2.26	9.5
Rice	3.27	3.27	6.12	2.04	1.22	2.04	5.71	1.63	5.71
Cauliflower	3.13	3.91	10.55	7.03	4.69	2.34	1.56	3.91	3.91

Table 2.2(b) Amino Acid Composition of Vital Human Protein

Name	%H	%I	%L	%K	%M	%F	%T	%Y	%V
Myosin	1.81	4.32	7.54	6.67	1.73	3.38	5.65	2.43	8.79
Haemoglobin	1.96	1.96	13.73	5.88	2.94	4.9	2.94	3.92	6.86
Cotransporter	2.7	3.66	8.48	8.48	2.12	2.5	4.43	2.7	10.02
Somatotropin	3.96	6.17	14.1	5.29	2.64	2.2	3.52	3.08	5.29
ImmunoGlobulin	2.01	4.22	7.03	6.22	1.81	3.82	5.42	2.61	5.22
Cadherin	0	7.62	5.71	4.76	0	3.81	6.67	0.95	14.29
AlphaCrystalline	0.99	6.93	7.92	4.95	0.00	2.97	9.90	2.97	3.96

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Trypsin	1.62	6.48	7.29	5.67	1.21	2.43	4.05	4.05	7.69
Neurotransmitter	2.39	4.98	11.16	3.59	2.79	5.58	6.18	2.99	9.16

SCORING METHOD

SEQUENCE LENGTH DIFFERRENCE	SCORING METHOD	SCORE
Length of FP = Length of HP		
FP(%K) = HP(%K)	YES as , FP(%K) - HP(%K) =0	0
FP(%K) < HP(%K)	YES as , FP(%K) -HP(%K) <0	-ve
FP(%K) > HP(%K)	YES as ,FP(%K) - HP(%K) >0	+ve
Length of FP < Length of HP		
FP(%K) = HP(%K)	YES as , FP(%K) -HP(%K) <0	-ve
FP(%K) < HP(%K)	YES as, FP(%K) -HP(%K) <0	-ve
FP(%K) > HP(%K)	If $\frac{FP(\%K)\times Len(FP)}{100} - \frac{HP(\%K)\times Len(HP)}{100} < 0$	-ve
	If $\frac{FP(\%K)\times Len(FP)}{100} - \frac{HP(\%K)\times Len(HP)}{100} > 0$	+ve
	If $\frac{FP(\%K)\times Len(FP)}{100} - \frac{HP(\%K)\times Len(HP)}{100} = 0$	0
Length of FP > Length of HP		
FP(%K) = HP(%K)	FP(%K) -HP(%K) >0	+ve
FP(%K) < HP(%K)	If $\frac{FP(\%K)\times Len(FP)}{100} - \frac{HP(\%K)\times Len(HP)}{100} < 0$	-ve
	If $\frac{FP(\%K)\times Len(FP)}{100} - \frac{HP(\%K)\times Len(HP)}{100} > 0$	+ve
	If $\frac{FP(\%K)\times Len(FP)}{100} - \frac{HP(\%K)\times Len(HP)}{100} = 0$	0
FP(%K) > HP(%K)	FP(%K) -HP(%K) >0	

HUMAN PROTEIN

Y gm of HP_I weights = X_I Dalton, Total HPi strands,$N_i(HP_I) = X_i(HP_i)/Mw\ (HP_i)$

$N_Q(HP_I) = n_q(HP_i)^* N_i(HP_I)$

Where

$N_Q(HP_I)$ is the total no. of Q residues in (HP_i), $X_i(HP_i)$ is the protein required in dalton,

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Mw (HP_i) is the molecular weight of human protein, $N_i(HP_I)$ is the total strand required for synthesis.

Food Protein

Y gm of FP_I weights = X_I Dalton. Total FPi strands,$N_i(FP_I) = X_i(FP_i)/Mw\ (FP_i)$.

$N_Q(FP_I) = n_q(FP_i)* N_i(FP_I)$

Where $N_Q(FP_I)$ is the total no. of Q residues in (FP_i), $X_i(FP_i)$ is the protein required in dalton,

Mw (FP_i) is the molecular weight of human protein, $N_i(FP_I)$ is the total strand required for synthesis.

FORMULA

$$\mathbf{S_{IL}=(FP_i)\ * X_i(FP_i)/Mw\ (FP_i)\ -(HP_i)* X_i(HP_i)/Mw\ (HP_i)\ )}$$

Where

S_{IL}=Total score of Ith food protein against Lth Human Protein.

FP is the list of Food Protein. 0<I<10.

HP is the list of Human Protein . 0<L<9

Algorithm

```
ProteinScore(FP,hp,A,x,y)
        m=length(FP)
        n= length(A)
        for j ← 1 to m
            for i ← 1 to n
                sumx=sumx+x_i
            for i ←1 to n
```

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$sum_y = sum_y + y_i$

$S_J = sum_x - sum_y$

Next j

max=0

For j ← 1 to m

If S_j > max then

Max= S_j

for j ← 1 to m

if max= S_j then

return P(j)

break

An algorithm to calculate highest score value is calculated. The above algorithm recommends the best food protein for the synthesis of a vital human protein

3 RESULTS

The food items with positive score signify that the proportion of amino acid is surplus against synthesized protein requirements. While on the other hand, the negative score signifies the deficit of supplied protein obtained through diet.

Table 3.3 Food Scores against Vital Activity in Human

	Heart	Blood Oxygenation	Kidney	Growth	Immunity	Hearing	Eye Sight	Digestion	Neurotransmission
Chicken	+2.86	+0.09	+0.09	-1.07	+6.82	+1.37	+4.59	+4.69	-3.64
Milk	-7.49	-10.26	-10.26	-11.42	-3.53	-8.98	-8.98	-5.66	-13.99
Fish	+1.3	-1.47	-1.47	-2.63	+5.26	-0.19	-0.19	+3.13	-5.2

Soyabean	-2	-4.77	-4.77	-5.93	+1.96	-3.49	-3.49	-0.17	-8.5
Wheat	-20.8	-23.50	-23.51	-24.73	-16.82	-22.29	-19.07	-18.97	-27.3
Dal	-1.45	-4.22	-4.22	-5.38	+2.51	-2.94	-2.94	+0.38	-7.95
Choclate	+0.19	-2.58	-2.58	-3.74	+4.15	-1.3	-1.3	+2.02	-6.31
Rice	-11.31	-14.08	-14.08	-15.24	-7.35	-12.8	-12.8	-9.48	-17.81
Cauliflower	-1.29	-4.06	-4.06	-5.22	+2.67	-2.78	-2.78	+0.54	-7.79

4 CONCLUSIONS

The scoring method recommends a particular food for the better synthesis of human protein. The method has been devised to rank various food items as it provides an easier approach for the nutritionists and dieticians to classify food items. There are extremely wide future scope of this work in which new parameters will be incorporated for the improvement and accuracy of the scoring method.

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Effect of various herbal supplements on in vitro rumen fermentation using goat rumen liquor

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ABSTRACT

To see the effect of three herbal feed additives i.e, fruits of Emblica officinalis (Amla), leaves of Azadirachta indica (Neem) and leaves of Tephrosia purpuria (Mokh) were mixed @ 0.5% in the mixed substrate and assessed individually under in vitro rumen fermentation system using goat rumen liquor. Total gas production and rumen fermentation pattern were observed in the in vitro bottles, which were incubated at 39 0C for 48h. TVFA (P<0.001) were significantly higher in T1 than other two treatments. pH reduced in this treatment. Whereas, ammonia-N (P<0.05), NPN (P<0.01) were significantly higher in Mokh treated group than other groups. However, acetate (A), propionate (P), butyrate and A/P ratio remained unaffected by such treatments. Therefore, it may be concluded that supplementation of Amla fruits @ 0.5% in the mixed substrate improved in vitro rumen fermentation pattern than other herbal plants.

INTRODUCTION

Manipulation of rumen microbial ecosystem for enhancing fibrous feed digestibility, reducing methane emission and nitrogen excretion by ruminants to improve their performance are some of the most important goals for animal nutritionists. Herbal feed additive could either influence feeding pattern or influence the growth of favourable microorganism in the rumen. Plant extracts are found to improve the activity of certain digestive organs (gall bladder, pancreas), thereby increasing the secretion of bile salts and pancreatic fluid [1,2]. Herbs can stimulate the secretion of various digestive enzyme which in turn may improve the efficient utilization of nutrient or stimulate the milk secreting tissues in mammary glands, resulting in improved production and reproductive performance of dairy animals [3-8]. Therefore, the study

was conducted to compare different herbal additives on *in vitro* rumen fermentation and gas production.

MATERIALS AND METHODS

Three different herbal feed additives i.e. fruits of *Emblica officinalis* (Amla) T_1, leaves of *Azadirachta indica* (Neem)T_2 and leaves of *Tephrosia purpuria* (Mokh)T_3 were mixed @ 0.5% in Substrate assessed individually under *in vitro* rumen fermentation system using goat rumen liquor. The substrate was prepared using herbal ingredient containing concentrate mixture (40%), gram straw (40%) and cowepea (20%). Rumen fermentation pattern were observed in the *in vitro* bottles, which were incubated at 39 ^{0}C for 48h. Total nitrogen, TCA-precipitable-N, NH_3-N and NPN were analyzed as per micro Kjeldahl method AOAC (1984)[9]. Total VFAs in rumen liquor were analyzed according to Barnett and Reid (1956)[10]. Fractionation of VFAs was done by Gas Chromatograph according to Erwin *et al*. (1961)[11]. Substrates were analyzed for Proximate AOAC (1984)[9] and cell wall components[12].

RESULTS

TVFA were significantly higher in T_1 than other two treatments. At the same time pH reduced in this treatment. (Table 1). Whereas, ammonia-N, NPN were significantly higher in T_3 than other groups. However, acetate (A), propionate (P), butyrate and A/P ratio remained unaffected by such treatments. Therefore, it may be concluded that supplementation of Amla fruits @ 0.5% in the concentrate mixture or 0.5% in the mixed substrate improved *in vitro* rumen fermentation pattern than other herbal plants.

DISCUSSION

Different herbal additives indicated variable responses on *in-vitro* N-metabolism. Ammonia nitrogen in the fermentation medium under *in-vitro* system was not affected by inclusion of eucalyptus oil [13] as also reported earlier with inclusion of peppermint oil [14]. Sachan Jyoti (2011)[15] and Kumar (2007)[16] did not find pH change upon addition of peppermint oil and *curcuma longa*. All the values of pH were within normal range (6.5-7.0)

showing no adverse effect of *curcuma longa* addition on rumen environment. Variable responses of other herbal additives on VFA production pattern were reported by different workers. Extract of garlic caused decreased in acetate: propionate ratio [17]. Patra *et al.* (2006)[18] showed that adding water extracts of Neem seeds decreased total ruminal volatile fatty acid (VFA) concentrations, ratio of acetate to propionate and ruminal feed digestibility. In the present study also Neem leaves reduced VFA concentration.

Table 1: Total gas production and fermentation pattern as affected by different herbal constituents under *in vitro* system.

Treatments	T_1 (Amla)	T_2 (Neem)	T_3 (Mokh)	S.E.M.	Significance
pH	6.57^a	6.77^c	6.70^b	2.24	P<0.001
TVFA (mmol/dl incubation medium)	10.12^b	6.52^a	6.58^a	0.47	P<0.001
Total-N (mg/dl incubation medium)	89.60	87.36	99.68	2.31	NS
NH_3-N (mg/dl incubation medium)	25.48^a	24.92^a	27.32^b	0.40	P<0.05
NPN (mg/dl incubation medium)	30.24^a	30.24^a	39.20^b	1.39	P<0.01
TCA-precipitable-N (mg/dl)	59.36	57.16	60.48	1.80	NS
Acetate%	68.88	63.82	66.90	1.33	NS
Propionate%	21.20	22.62	21.60	0.34	NS
Butyrate%	9.92	13.56	11.50	1.07	NS
A/P ratio	3.26	2.84	3.11	0.10	NS

Means with different superscripts (a, b) in the row differ significantly.

CONCLUSION

It may be concluded that supplementation fruits of *Emblica officinalis* (Amla) @ 0.5% in the mixed substrate improved *in vitro* rumen fermentation pattern than *Azadirachta indica* (Neem) and *Tephrosia purpuria* (Mokh).

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Molecular Docking and Dynamics Studies for discovery of potent drug candidate among compounds of *Lepidium sativum* for *Clostridium perfringens* Enterotoxin

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ABSTRACT

BACKGROUND: *Clostridium perfringens enterotoxin (CPE) is a pore forming toxin which damages target cells by disrupting the selective permeability of the plasma membrane and causes food poisoning and diarrhea. Domain-I (C-CPE, residues 184-319) of CPE is responsible for binding to the specific receptor such as claudin (CLDN) and it is reported that the second extracellular loop of claudins (specially, CLDN3 and CLDN4) located on the C-terminal acts as receptor of CPE. C. perfringens has acquired resistance against most of the antibiotics available in the market. Thus there is a need to identify new potent inhibitors for treating CPE induced infections.*

METHODOLOGY: *: In order to obtain the most potent inhibitors of CPE, different computer-aided drug designing methodologies, like virtual screening, molecular docking and molecular dynamics simulations are utilized.*

RESULTS: *In order to identify the various CPE inhibitors, plant extracts (metabolites) of Lepidium sativum (Halim sak) is chosen and 20 compounds are virtually screened. The screened molecules are then filtered and molecular docking study is carried out. Finally, 3 molecules showed satisfactory interactions with claudin binding residues of claudin binding domain of CPE and taken for further analysis. Ligand binding stability of these 3 protein–ligand complexes has been assessed as well as analyzed with the help of molecular dynamics simulations.*

CONCLUSION: *The interactions between CPE and ligands are studied by various computational approaches and the results of the molecular docking and molecular dynamics simulations showed that Semilepidinoside A and B, chemical compounds found in the extract of Lepidium sativum, has the ability to be stabilized at the claudin binding pocket of CPE thereby inhibiting the toxin to bind with the tight junction proteins of the host and thus reducing the chance of causing infection by CPE.*

INTRODUCTION

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Clostridium perfringens enterotoxin(CPE) is a causative agent of food poisoning which damages intestinal epithelia cells of the host by disrupting the selective permeability of the plasma membrane.CPE consist of a single chain polypeptide of 319 amino acids[1]. *Clostridium perfringens* has acquired resistance against most of the antibiotics(Specially, tetracycline and lincomycin) available in the market. Several investigations have been conducted to study the antimicrobial resistance pattern of *Clostridium perfringens* and it has been shown that the organism is resistant to mostly used antibiotics, Many antibiotics have been developed for treating *Clostridium perfringens* infections but CPE acquired resistance to most of them .That is why there is a need to find new potential leads for treating *Clostridium perfringens* infections.

It has been observed that, certain members of the claudin family, which are the components of tight junction and act as receptor of the target cells binds to the C-terminal domain of CPE(C-CPE, residues 184-319) by recognizing it [2-4] and the death of target cells occur due to forming of physiological pores to disrupt the selective permeability of the plasma membrane by N-terminal region of CPE[5-7].These physiological pores are composed of large complexes formed by CPE and members of claudin family.[2,8]The binding between C-CPE and claudin is well established and it is found that residues, specially, TYR^{306}, TYR^{310}, TYR^{312} and LEU^{315} are important for the interaction[9-13].According to crystal structure of C-CPE, these residues organizes a cleft space that is considered to interact directly with claudins [2]. Claudins are the tight junction, tetra-transmembane proteins. The second extracellular loop of the claudin is responsible for binding to CPE and recently designated as CPE-SR for CPE sensitivity region[15]. Thus, this claudin binding pocket of CPE represents a major target for drug rediscovery against cattle-borne CPE infection.In our study, we used Lepidium sativum, a wild edible plant which has anti-diarrheic and entero-protective activity[16]. A Combined Ligand-Based Virtual Screening of *Lepidium sativum* metabolites were performed for CPE inhibitory activity and its Docking studies were also conducted and taken for further analysis by molecular dynamics studies[24-31]. The stable ligand thus generated may provide guidance to discover novel inhibitors of CPE infection by highlighting the important binding features of *Lepidium sativum* extracts.

2. MATERIALS AND METHODS

MODELLING AND PRELIMINARY EXPERIMENTS ON CPE AND CLAUDIN

In this experiment, the binding affinities of that of the Clostridium perfringens enterotoxin,CPE (PDB ID: 3AM2) to that of claudin-4,CLDN4 (UniProt ID: 014493) has been observed. At the very beginning, as a crystal structure of CLDN4 is not available till date, a three-dimensional structure of CLDN4 is predicted by homology modeling with the help of MODELLER 9.10 and refined by UCSF Chimera[17,18]. Protein-protein docking has been performed taking the "CPE" as the Receptor and "Claudin-4" as the Ligand, with the help of the HADDOCK Web Server in order to check the interaction between them[19]. It has been seen that after getting the protein-protein docking results, the first best energy favorable conformation of the cluster I shows the significant result which is very much similar to the information provided in the earlier literatures on CPE- CLDN4 complex and it also satisfy our predicted model of CLDN4.

VIRTUAL SCREENING OF THE PLANT METABOLITES

Virtual screening is a technique which is performed to retrieve new molecules from databases for further biological testing. The main purpose of virtual screening is to identify new novel inhibitors of PBP4 of CPE. .The software, Raccoon offers user-friendly screening analysis tools. Using the Raccoon software virtual screening was performed. In the virtual screening, we used Lipinski's rule of five as query to search potential leads against CPE from metabolites dataset (20-Metabolites) which was downloaded from Knapsac family database. The hit molecules obtained from database were filtered by applying Lipinski's rule of five and finally we got 6 ligands(metabolites) whis shows good score of druglikeness[20,21].

MOLECULAR DOCKING STUDIES

A. Protein and Ligand Preparation

Referring the various literatures we found that probably inhibition of Claudin binding pocket of CPE may effectively reduce the CPE induced infection. The Clostridium perfringens enterotoxin was observed and retrieved from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) with PDB id 3AM2.The active sites of protein were identified on the basis of the literatures on CPE-Claudin complexes. The 3D structure of components of Lepidium sativum and its physiochemical properties were retrived from Knapsac Family Database for identifying its drug likeliness [20]. The components were filtered on the basis of Lipsinki's rule of five using OSIRIS ((http://www.organicchemistry.org/prog/peo/) and Molinspiration [22] that satisfies Structure Activity Relationship (SAR) properties.

B. Optimisation of Protein and Ligand

The protein CPE downloaded from PDB (PDB id 3am2) was prepared for docking by deleting all hetero atoms, ligands and water molecules and optimized by minimization of energy by using UCSF Chimera.Ligands- Ligands designed was obtained from Knapsac family database as mol format and was optimized by using UCSF Chimera **.**

C.Molecular Descriptors

The molecular descriptors of screened ten components of Lepidium sativum were predicted by loading them into an online server, OSIRIS property explorer [22]. This prediction process depends on comparison between precomputed set of structural moieties whose properties are already known and the structural moieties of loaded molecules. Molecular descriptors like clogP, solubility, drug score and side effects such as mutagenicity, carcinogenicity and teratogenicity were determined. To calculate the overall drug score, OSIRIS combined logP, logS, molar mass, drug-likeness and toxicity risks into a single number to predict the molecule's over all drug potential. Finally, we got six ligands, which do not show any toxicity risk on human metabolism.

D. Docking Studies

All the 6 ligands , were docked with CPE protein in the software AutoDock 4.2 (MGL Tools for Ligand Docking) using a genetic algorithm and simulated annealing approach to explore wide range of ligand conformational flexibility and rotational flexibility of selected receptor

hydrogens . In the aspect of producing binding energy estimates, the AutoDock computes minimum binding energy with Claudin receptor pocket for all the ligands. Standard customized parameter settings were used to evaluate the protein-ligand interactions [23].

E. Visualization of Docked Complex

The docked complex was visualized in UCSF Chimera showing how the ligand interacts with the protein CPE.

Molecular Dynamics Simulations

After selecting the three best models, generated from docking programs, four rounds of Molecular Dynamics simulations has been performed, in order to check the dynamics of the interacting complexes. Except protein-protein docked model all the simulations are done for a time scale of 20ns. For protein-protein docked model simulation are done for a timescale of 30ns which is used as a control to analyzing the final results [24].

The 4-simulations performed are represented as follows:

I. Optimized CPE with Lepidine C(ligand id 28460).

II. Optimized CPE with Semilepidinoside A C(ligand id 28987).

III. Optimized CPE with Semilepidinoside B C(ligand id 28988).

IV. Optimized CPE with (homology modeled) full-length CLDN4.

All the three rounds of Molecular Dynamics simulations are performed using GROMACS force-field GROMOS96 43A1, on the linux operating system, for a time period of 20ns. Target CPE (PDB ID 3AM2) has been downloaded from Protein Data Bank (PDB) Fig.4. It is a pore forming toxin consisting of A chains having molecular weight 36658.99 Da belonging to class alpha beta and toxin protein family (Pfam Accession no: PF03505).

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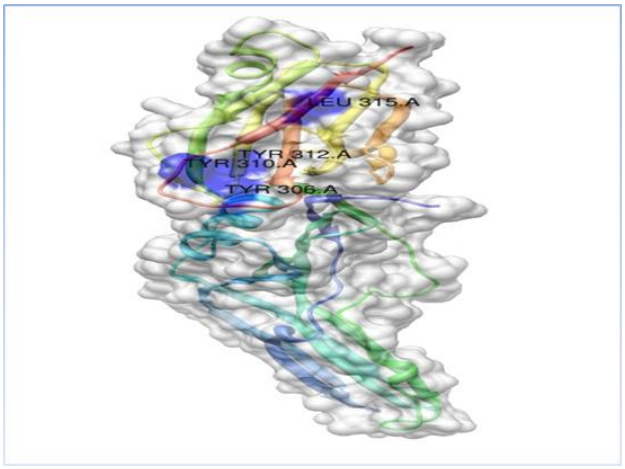

Fig.1. *Clostridium perfringens* enterotoxin (PDB ID:3AM). Putative interactin residues Tyr^{306}, Tyr^{310}, Tyr^{312} and Leu^{315} are labelled.

Protein downloaded from RCSB PDB was loaded as the receptor and modelled CLDN4 was loaded as the ligand on the HADDOCK web server for protein–protein docking. Other standard default parameters were set for protein–protein docking. HADDOCK clustered 176 structures in 5 clusters which represents 80% of the water refined models HADDOCK generated. The statistics of the top 5 clusters are shown below:

Table 1. Molecular Docking Results: HADDOCK web server.

CLUSTER NO.	CLUSTER SIZE	HADDOCK SCORE	RMSD*	VDW ENERGY	ELECT. ENERGY	DESOLV. ENERGY	BURRIED SURFACE AREA	Z-SCORE
1	59	-123.2±6.6	17.9 ± 0.1	-84.7 ± 5.0	-161.4 ± 20.1	-12.5 ± 3.7	2320.4 ± 79.5	-1.2
2	55	-85.2 ± 5.8	21.3 ± 0.5	-55.8 ± 3.9	-83.8 ± 18.2	-15.0 ± 5.6	1361.4 ± 86.3	0.7
3	33	-97.1 ± 7.6	14.9 ± 0.4	-55.8 ± 10.4	-169.9 ± 16.7	-15.2 ± 6.0	1707.3 ± 205.6	0.1
4	25	-118.5±4.5	17.6 ± 0.2	-91.7 ± 9.8	-139.3 ± 4.9	-6.9 ± 12.2	2689.1 ± 175.9	-1.0

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5	4	-69.6 ± 4.5	-8.9 ± 0.1	-46.5 ± 3.4	-155.5 ± 41.0	-3.4 ± 6.7	1559.5 ± 60.7	1.4

Z-score indicates how many standard deviations from the average this cluster is located in terms of score (the more negative the better). As it is observed that in cluster 1 the HADDOCK score and Z-score are more negative than that of the other clusters. Therefore, we have chosen the 1st conformation to be the most appropriate CPE–CLDN4 complex (Fig.2).

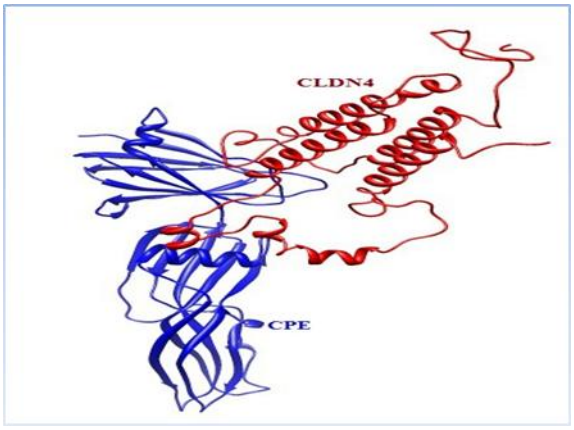

Fig.2. The best energy favourable conformation of the cluster 1 obtained from Haddock web server.

PDB ID 3AM2 has been prepared for ligand docking by UCSF Chimera. Initial coordinates were energy minimised by GROMACS and energy minimised structure was used as the receptor for the selected ligands.

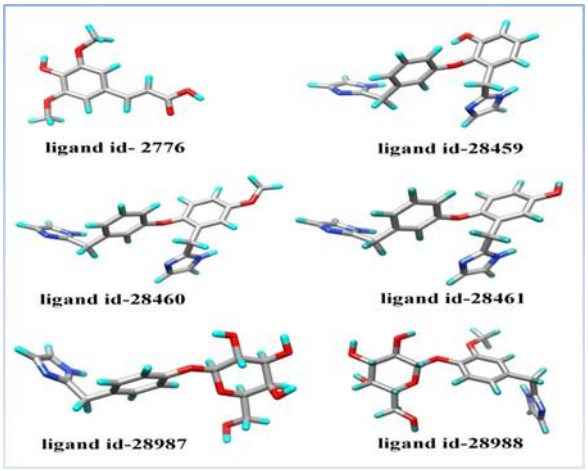

Fig.3. The least possible energy conformation of the ligands after geometry optimisation.

All the ligands were geometry optimised to its least possible energy conformation (Fig.6). All the compounds of *L. sativum* were screened for its toxicity properties. Among them the best compounds showing maximum drug likeliness are listed in Table 3 where we find that the

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compounds of *L. sativum* have the properties of non-mutagenic, non-tumorogenic, non-irritant and no-reproductive effect.

Table 2. Toxicity Properties of Ligands: OSIRIS Property Explorer.

LIGAND ID	28460	28459	2776	28461	28987	28988
Mutagenic	NO	NO	NO	NO	NO	NO
Tumorogenic	NO	NO	NO	NO	NO	NO
Irritant	NO	NO	NO	NO	NO	NO
Reproductive	NO	NO	NO	NO	NO	NO
ClogP	2.72	2.44	1.12	2.44	-0.81	-0.81
Solubility	-3.55	-3.24	-1.29	-3.24	-1.02	-1.02
Molecular weight	360	346	295	346	336	336
Druglikeness	1.26	0.38	4.52	0.86	-5.06	-5.06
Drug score	0.71	0.66	0.93	0.71	0.47	0.47

The chosen best ligands were then docked with the claudin binding pocket of the CPE by AutoDock 4.2 in a customised grid box and grid parameter (X-dimension=80, Y-dimension=70, Z-dimension=66 and X-centre=32.77.and Y-centre=28.607, Z-centre=22.699 at a grid spacing of 0.375 Å). The results are summarised in Table 3.

Table 3. Molecular Docking Results: AutoDock 4.2

LIGAND ID	28460	28459	28461	28987	28988	2776
Binding Energy	-6.91	-5.9	-4.94	-4.78	-4.52	-4.13
Ligand Efficiency	-0.26	-0.23	-0.19	-0.2	-0.17	-0.26
Inhibitory Constant (in µM)	8.64	47.62	240.48	315.59	486.35	939.13
Inter Molecular Energy	-9	-7.98	-7.03	-7.46	-7.5	-54.92
Total Internal Energy	-0.86	-1.28	-0.75	-2.06	-2.99	-0.78

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No. Hydrogen Bonds	2	3	2	4	4	2
Hydrogen bond forming residues	311, 313	311,313, 313	256, 306	306, 310, 311,311	307,307, 310,311	227,313

The ligands showing maximum binding affinity and formed hydrogen bonds with the residues of the claudin binding pocket (Tyr^{306}, Tyr^{310}, Tyr^{312} and Leu315) were taken for further analysis by MD simulations and listed in Table 4.

Table 4. Ligands which showed the lowest binding energy and formed maximum hydrogen bonds with C-CPE were subjected to molecular dynamics simulations.

LIGAND ID	28460	28987	28988
Binding Energy	-6.91	-4.78	-4.52
Ligand Efficiency	-0.26	-0.2	-0.17
Inhibitory Constant (in µM)	8.64	315.59	486.35
Inter Molecular Energy	-9	-7.46	-7.5
Total Internal Energy	-0.86	-2.06	-2.99
No. Hydrogen Bonds	2	4	4
Hydrogen bond forming residues	311,313	306,310,311,311	307,307,310,311

It is observed from the Table 4 that the ligand 28460 has the highly negative binding energy (Fig.7.) with binding efficiency of –0.26. Out of 20 conformation generated by AutoDock 4, this conformation shows a stable binding with Pro^{311} and Ser^{313}.

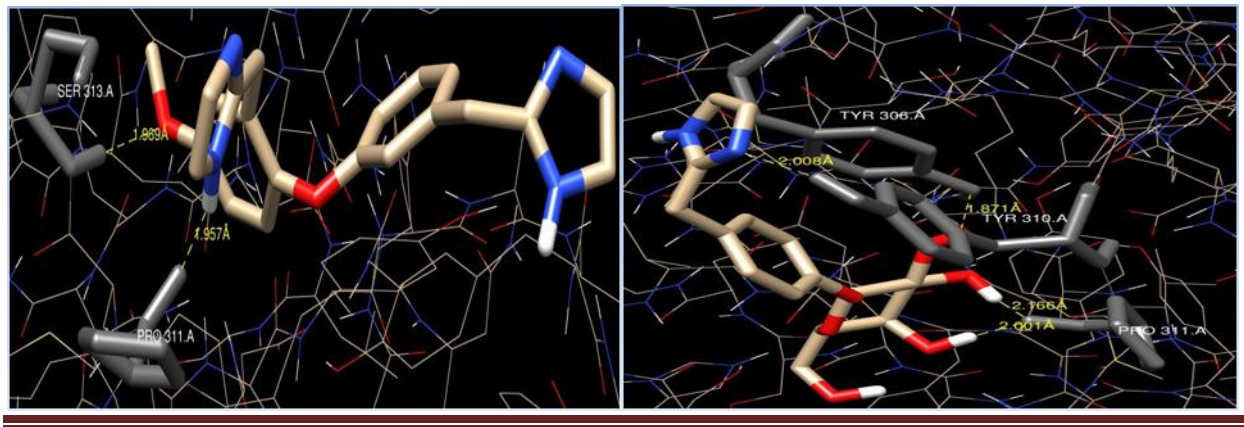

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Fig.4. Ligand 28460 formed hydrogen bonds with the residues PRO^{311} and SER^{313} obtained from AutoDock 4.2.

Fig.5. Ligand 28987 formed hydrogen bonds with the residues TYR^{306}, TYR^{310} PRO^{311} obtained from AutoDock 4.2.

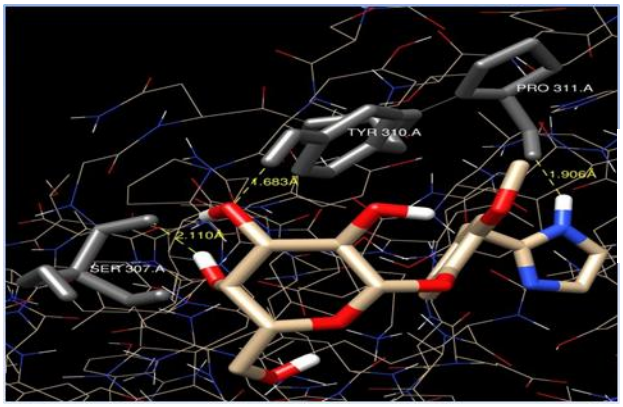
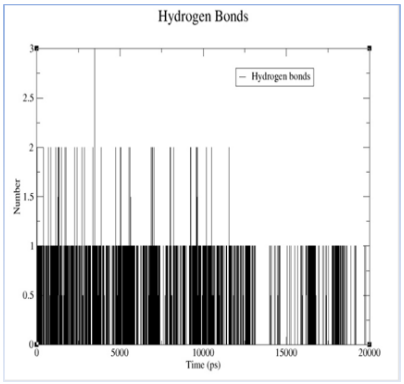


Fig.6. Ligand 28988 formed hydrogen bonds with the residues TYR^{307}, TYR^{310} PRO^{311} obtained from AutoDock 4.2.

Based on binding energy and hydrogen bond formed, docking results were analysed. The results were compared to find out the best ligand which can block the claudin binding pocket of CPE. Based on these observations, 28460, 28987 and 28988 are the most potent ligand among the other since they have well-occupied at the described pocket of the toxin. Thus the *in silico* method adopted in the present study helped in identifying the ligands for the treatment of CPE induced infection. This method reduces the time and cost in designing a drug as well as in analysing the drug likeliness before it enters in to actual drug discovery process and the clinical trials.

HYDROGEN BONDS



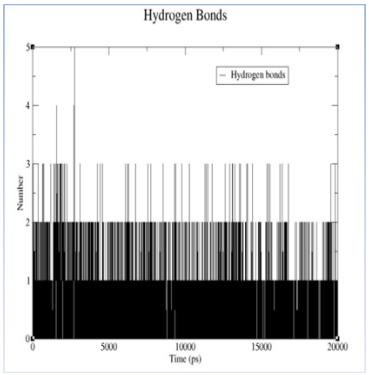


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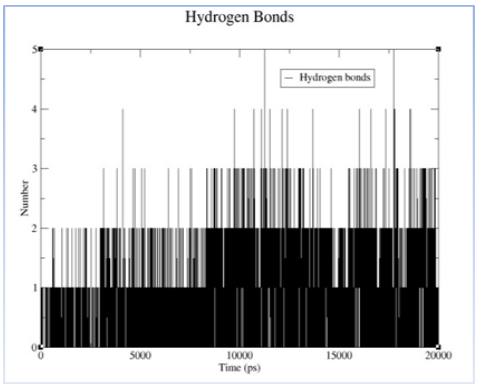
Fig.7. Hydrogen bonds formation pattern of CPE-28460

Fig.8. Hydrogen bonds formation pattern of CPE-28987 complex.



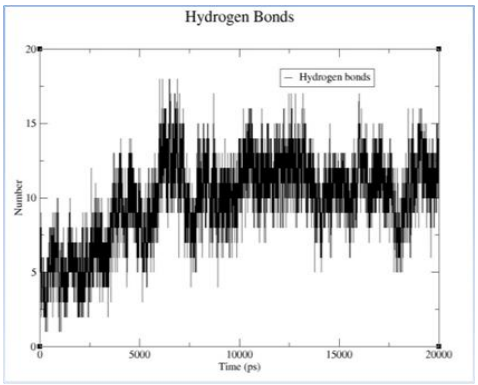


Fig.9. Hydrogen bonds formation pattern of CPE-28988

Fig.10. Hydrogen bonds formation pattern of CPE-CLDN complex.

The *g_hnum* program of GROMACS calculates the number of hydrogen bond formation. Here, the number of hydrogen bond formation by the CPE–28460 complex seems to be decremented after 10 ns when compared with CPE–28987 and CPE–28988 complexes. Similarly, the number of hydrogen bond formation is more in CPE–28987 complex in comparison to CPE–28460 complex. The number of hydrogen bond formation in CPE–28988 complex seems to be incremented from 5 ns to till the end of the simulation. However, the maximum limit of hydrogen bond formation increases up to 4 over the time in CPE–28988 complex which we cannot find in the previous complexes. The hydrogen bonds formation pattern of CPE–CLDN complex cannot be compared with the other CPE–ligand complexes as they do not poses any similarity

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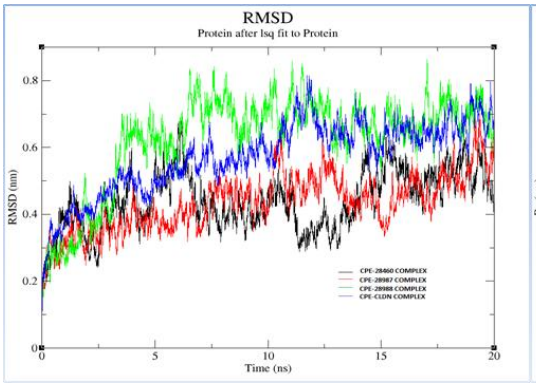

Fig.11. RMSD comparison graph of CPE-ligand complexes.

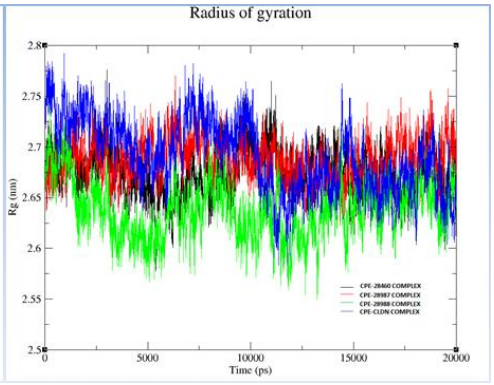

Fig.11. Radius of gyration comparison graph of CPE-ligand complexes.

Careful observation suggests a close resemblance of RMSDs of three protein–ligand complexes, namely, CPE–CLDN4, CPE–28988 and CPE–28987. Although all the plots overlapped at the end point of the simulation, there is a significant instability observed in case of CPE–28460 complex throughout the simulation. From this analysis it is suggested that the compounds 28987 and 28988 have the affinity to bind with CPE similar to CLDN4.we can infer that radius of gyration seems to be declined from 2.7 to 2.6 nm till 5 ns, but maintains a stability throughout the simulations then onwards. This suggests that there is no additional folds occur during the simulation upon ligand binding.

CONCLUSION AND FUTURE WORK

In our study, all the ligands, which were virtually screened from *L. sativum* were docked with CPE. The ligands which showed the lowest binding energy and formed hydrogen bonds with the claudin binding residues of C-CPE were subjected to molecular dynamics simulation to check the binding stability. Based on binding energy and hydrogen bond formed, docking results were analysed. The results were compared to find out the best ligand which can block the claudin binding pocket of C-CPE. Based on these observations 28460, 28987 and 28988 can be considered as the most potent ligands among the other ligands. The stability of CPE–ligand complexes were studied by using various tools of GROMACS. Based on root mean square deviations, radius of gyration, average distance between ligands and claudin binding residues of C-CPE and number of hydrogen bond deviations MD simulation results were analysed.

Molecular dynamics simulation results clearly indicate that all the three ligands are mostly stable during the simulation except for the ligand 28460. The ligand 28460 shows somewhat unstable behaviour during the simulation and it can be ignored. From virtual screening, molecular docking studies and MD simulation it is observed that ligand 28987 and ligand 28988 are highly potent inhibitors of CPE. Hence, we concluded that 28987 (Lepidine A) and ligand 28988 (Lepidine B) which are the compounds of wild edible plant *L. Sativum* can be used for treating *C. perfringens* enterotoxin induced infections. Future directions include experimental validation of the ligands and developing inhibitors for the CPE with better understanding in terms of molecular dynamics.

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