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In silco Comparative Modeling of Acetyl Glutamate Kinase from *Kappaphycus Alvarezii*

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Abstract
Seaweed or algae belongs to a group of plant like organisms that grow in the sea some algae are one celled organisms such as micro algae, they generate energy through photosynthesis the groups (or phyta) of seaweeds that are commonly consumed included : Green algae, brown algae, Red algae and Blue green algae. The red seaweed, *Kappaphycus alvarezii*, was evaluated for its potential to prevent signs of metabolic syndrome through use as a whole food supplement. Major biochemical components of dried *Kappaphycus* are carrageenan (soluble fiber ~34.6%) and salt (predominantly potassium (K) 20%) with low overall energy content for whole seaweed. K. alvarezii is a source of kappa carrageenan which is used as an additive in food, pharmaceutical and cosmetic products. The protein of acetyl glutamate kinase is an enzyme that catalyses the chemical reaction it is antimicrobial protein. Hence the protein acetyl glutamate kinase has been taken for analysis. The Primary and Secondary analysis were carried out using bioinformatic tool like protparam, Gym motif server, net surfp and signal p. Homology modeling of acetyl glutamate kinase protein was done through using Geno3D.

Keywords
acetyl glutamate kinase*, Kappaphycus alvarezii,* protparam, Gym motif server, net surfp and signal p.

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INTRODUCTION
Algae are a large and diverse group of simple plant like organisms ranging from unicellular to multicellular forms. The largest and most complex marine algae are called seaweeds (Hardy and Guiry, 2006). Seaweeds are a group of macroscopic marine algae that form the biomass in the intertidal zone and the term seaweeds and sea vegetables are used interchangeably (Wong and Cheung, 2002). Seaweeds are multicellular and macrothallic. Seaweeds are salt water tolerant, land dependent plants growing almost exclusively at narrow interface where land and sea meet. They are photosynthetic and must be firmly attached to a stratum to stay in the photic zone where they can receive sufficient sunlight (Smith, 2004; Yang, 2002). Seaweeds are also called the benthic marine algae which just mean attached algae that live in the sea (Rindi, 2004). Seaweeds are macrophytic algae, a primitive type of plants lacking true roots, stems and leaves. Most seaweeds belong to one of the three divisions namely Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). There are about 900 species of green seaweed, 4000 red species and 1500 brown species found in

nature. A large variety of red and green seaweeds are found in subtropical and tropical waters, while brown seaweeds are more common in cooler and temperate waters. Seaweeds grow abundantly along the Tamil Nadu and Gujarat coasts and around Lakshadweep and Andaman and Nicobar Islands. There are also rich seaweed beds around Mumbai, Ratnagiri, Goa, Karwar, Varkala, Vizhinjam and Pulicat in Tamil Nadu and Chilka in Orissa. Out of approximately 700 species of marine algae found in both inter-tidal and deep-water regions of the Indian coast, nearly 60 species are commercially important. Agar yielding red seaweeds such as Gelidiella acerosa and Gracilaria species are collected throughout the year.

ACETYL GLUTAMATE KINASE:

In enzymology, an acetyl glutamate kinase (EC 2.7.2.8) is an enzyme that catalyzes the chemical reaction: ATP + N acetyl-L-glutamate ⇌ {\display style \right left harpoons} ADP + N-acetyl-L-glutamyl 5-phosphate. Thus, the two substrates of this enzyme are ATP and N-acetyl-L-glutamate, whereas its two products are ADP and N-acetyl-L-glutamyl 5-phosphate. This enzyme belongs to the family of transferases, specifically those transferring phosphorus-containing groups (phosphotransferases) with a carboxy group as acceptor. This enzyme participates in urea cycle and metabolism of amino group.

METHODOLOGY:

Target protein of acetyl glutamate kinase in l retrieved from NCBI database. The retrieved sequence is submitted to gym motif sever to know the motif analysis of acetyl glutamate kinase protein. The retrieved sequence is submitted to protparam server to know the sequence analysis of acetyl glutamate kinase protein. The retrieved sequence is submitted to netsurfp and signal p server for functional analysis of acetyl glutamate kinase protein. The retrieved sequence is submitted to genome3d server, to get the three-dimensional structural analysis of acetyl glutamate kinase protein.

RESULT AND DISCUSSION:

1. NCBI:

Protein Sequence:

>YP_009449628.1 acetylglutamate kinase (chloroplast) [Kappaphycus alvarezii]

MFNSFQYIKYLINTLPFIKEYVGKTVVIKYGGSVMLNSELKKKVINDILFLFAFGIKPVLIHGGGPIINH
WLNRLKIEPKFHNGIRLTDEFTMEVVQMVLSGKINKDLVSLLNRNSSYAIGLSGVDGNLITVSPLFKDSN
NLVGKVDNINADLLIMLLDSGHIPVISSIGVNINGQFYNINADTVAGDIAIALRAEKLILLTDTPGIMYN
INDSNTLIKHINSEQVIELKEKNIISGGMIPKVECCIHALRSNVKEAHIIDGRLEHSLLFELFTINRVGS
MLTL

Nucleotide Sequence:

>NC_036637.1:56789-57643 Kappaphycus alvarezii chloroplast, complete genomeATGTTTAATTCTTTTCAGTATATAAAGTATTTGATTAATACTCTTCCTTTTATAAAAGAGTATGTAGGTA
AAACAGTTGTTATTAAATATGGTGGTTCTGTTATGTTAAATAGTGAATTAAAAAAAAAAGTTATTAATGA
TATATTATTTTTATTTGCTTTTGGTATTAAGCCCGTTTTAATTCATGGGGGTGGGCCTATTATTAATCAC
TGGTTGAATAGGTTAAAAATAGAACCTAAGTTTCATAATGGTATTCGTTTAACTGATGAATTTACAATGG
AAGTTGTTCAAATGGTCCTTTCTGGTAAAATAAATAAAGATTTAGTTTCTTTATTAAATAGAAATAGTAG
TTATGCTATCGGTTTATCAGGTGTAGATGGCAATTTAATTACTGTTTCTCCTTTATTTAAAGATTCTAAT
AATTTAGTTGGAAAGGTTGATAATATAAATGCAGATTTATTAATAATGTTACTTGATTCAGGTCATATTC
CTGTAATTTCTAGTATTGGTGTAAATATTAATGGTCAATTTTATAACATTAATGCTGATACTGTAGCTGG
AGATATAGCAATAGCGTTGAGAGCAGAGAAATTAATTTTATTAACAGATACACCGGGAATCATGTATAAT
ATAAATGATTCAAATACACTTATTAAGCATATTAATAGTGAACAGGTAATCGAGCTAAAAGAGAAGAATA
TTATTTCTGGTGGTATGATTCCTAAAGTAGAGTGTTGTATACATGCATTAAGGTCCAATGTTAAGGAGGC
ACATATTATAGATGGAAGATTAGAACATTCTTTGTTATTTGAGTTATTCACAATTAATAGAGTTGGTTCT
ATGTTAACTCTTTAA

The above results show the fasta format sequence of acetyl glutamate kinase protein.

PRIMARY ANALYSIS:

1. **PROTPARAM:**

10	20	30	40	50	60
MFNSFQYIKY	LINTLPFIKE	YVGKTVVIKY	GGSVMLNSEL	KKKVINDILF	LFAFGIKPVL
70	80	90	100	110	120
IHGGGPIINH	WLNRLKIEPK	FHNGIRLTDE	FTMEVVQMVL	SGKINKDLVS	LLNRNSSYAI
130	140	150	160	170	180
GLSGVDGNLI	TVSPLFKDSN	NLVGKVDNIN	ADLLIMLLDS	GHIPVISSIG	VNINGQFYNI
190	200	210	220	230	240
NADTVAGDIA	IALRAEKLIL	LTDTPGIMYN	INDSNTLIKH	INSEQVIELK	EKNIISGGMI
250		260	270		280
PKVECCIHAL		RSNVKEAHII	DGRLEHSLLF		ELFTINRVGS MLTL

Number of amino acids: 284

Molecular weight: 31514.96

Theoretical pI: 7.78

Amino acid composition:

Ala (A) 10	3.5%
Arg (R) 7	2.5%
Asn (N) 26	9.2%
Asp (D) 13	4.6%
Cys (C) 2	0.7%
Gln (Q) 4	1.4%
Glu (E) 13	4.6%
Gly (G) 22	7.7%
His (H) 8	2.8%
Ile (I) 37	13.0%
Leu (L) 35	12.3%
Lys (K) 20	7.0%
Met (M) 8	2.8%
Phe (F) 12	4.2%
Pro (P) 8	2.8%
Ser (S) 19	6.7%
Thr (T) 11	3.9%
Trp (W) 1	0.4%
Tyr (Y) 7	2.5%
Val (V) 21	7.4%
Pyl (O) 0	0.0%
Sec (U) 0	0.0%
(B) 0	0.0%
(Z) 0	0.0%
(X) 0	0.0%

Total number of negatively charged residues (Asp + Glu): 26
Total number of positively charged residues (Arg + Lys): 27

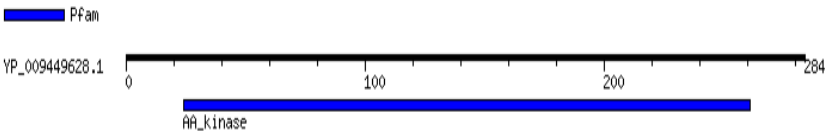
Atomic composition:

Carbon	C	1431
Hydrogen	H	2314
Nitrogen	N	372
Oxygen	O	404
Sulfur	S	10

Formula: $C_{1431}H_{2314}N_{372}O_{404}S_{10}$
Total number of atoms: 4531
Extinction coefficients:
Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.
Ext. coefficient 16055
Abs 0.1% (=1 g/l) 0.509, assuming all pairs of Cys residues form cystines
Ext. coefficient 15930
Abs 0.1% (=1 g/l) 0.505, assuming all Cys residues are reduced
Estimated half-life:
The N-terminal of the sequence considered is M (Met).
The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).
Instability index:
The instability index (II) is computed to be 34.80
This classifies the protein as stable.
Aliphatic index: 123.84
Grand average of hydropathicity (GRAVY): 0.260
The above results show the primary analysis of acetyl glutamate kinase
GYM MOTIF SERVER: Motif Analysis

Result of MotifFinder

Number of found motif: 1



Pfam (1 motif)

Pfam	Position(Independent E-value)		Description
AA_kinase	24..261(3.9e-46)	Detail	PF00696, Amino acid kinase family

Database:Pfam
Entry:AA_kinase
LinkDB: AA_kinase
Original site: AA_kinase
ALL LINKS:
Chemical reaction (4)
KEGG ENZYME (4)
Gene (29884)
KEGG GENES (29884)
Protein sequence (137791)
UniProt (136013)
SWISS-PROT (1778)
3D Structure (92)
PDB (91)
SCOP (1)
Protein domain (5)
InterPro (1)
Pfam (1)
PROSITE(DOC) (2)
NCBI-CDD (1)
Literature (1)
PubMed (1)
All databases (167777)

The above results show the motif analysis of acetyl glutamate kinase protein.

SECONDARY ANALYSIS:

NETSURP:

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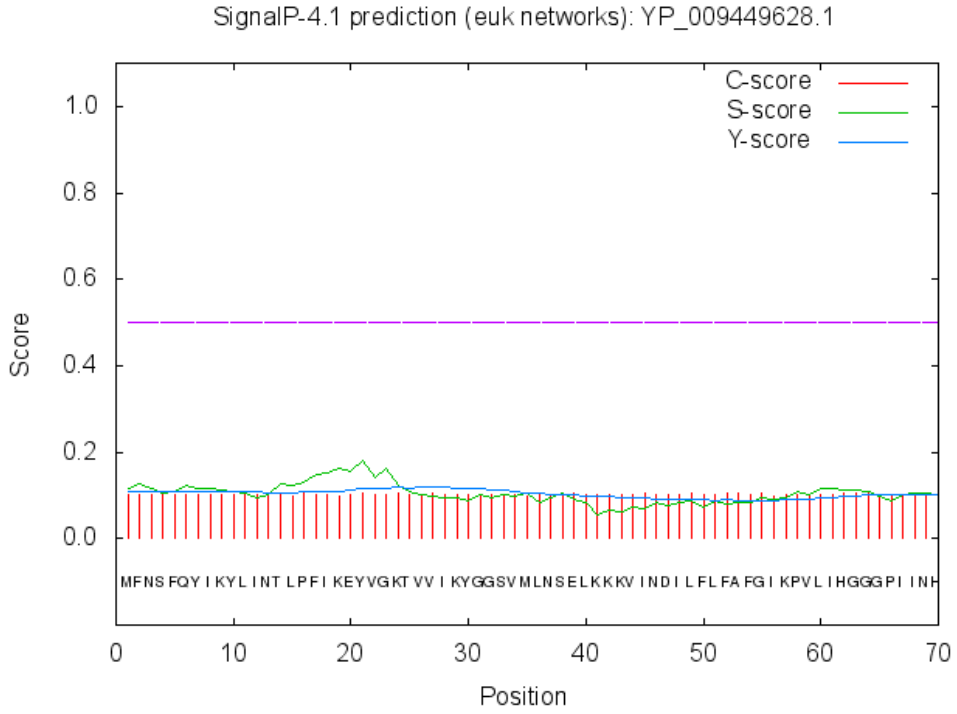
# For publication of results, please cite:
# A generic method for assignment of reliability scores applied to solvent accessibility predictions.
# Bent Petersen, Thomas Nordahl Petersen, Pernille Andersen, Morten Nielsen and Claus Lundegaard
# BMC Structural Biology 2009, 9:51 doi:10.1186/1472-6807-9-51
#
# Column 1: Class assignment - B for buried or E for Exposed - Threshold: 25% exposure, but not based on RSA
# Column 2: Amino acid
# Column 3: Sequence name
# Column 4: Amino acid number
# Column 5: Relative Surface Accessibility - RSA
# Column 6: Absolute Surface Accessibility
# Column 7: Z-fit score for RSA prediction
# Column 8: Probability for Alpha-Helix
# Column 9: Probability for Beta-strand
# Column 10: Probability for Coil
E M  YP_009449628.1       1   0.787 157.519  -1.700   0.003   0.003   0.994
E F  YP_009449628.1       2   0.560 112.312  -0.433   0.053   0.005   0.942
E N  YP_009449628.1       3   0.621  90.958   1.050   0.109   0.005   0.886
E S  YP_009449628.1       4   0.361  42.356  -0.477   0.858   0.002   0.139
E F  YP_009449628.1       5   0.492  98.704   0.169   0.923   0.002   0.076
```

E Q	YP_009449628.1	6	0.591	105.517	1.163	0.923	0.002	0.076
B Y	YP_009449628.1	7	0.238	50.839	1.325	0.970	0.001	0.030
B I	YP_009449628.1	8	0.038	7.030	0.249	0.970	0.001	0.030
E K	YP_009449628.1	9	0.467	96.062	1.747	0.970	0.001	0.030
B Y	YP_009449628.1	10	0.104	22.203	1.024	0.970	0.001	0.030
B L	YP_009449628.1	11	0.030	5.456	0.798	0.988	0.000	0.012
B I	YP_009449628.1	12	0.175	32.357	0.031	0.970	0.001	0.030
B N	YP_009449628.1	13	0.264	38.664	-0.235	0.970	0.001	0.030

The above results show the secondary structure analysis of acetyl glutamate kinase

SIGNAL P:



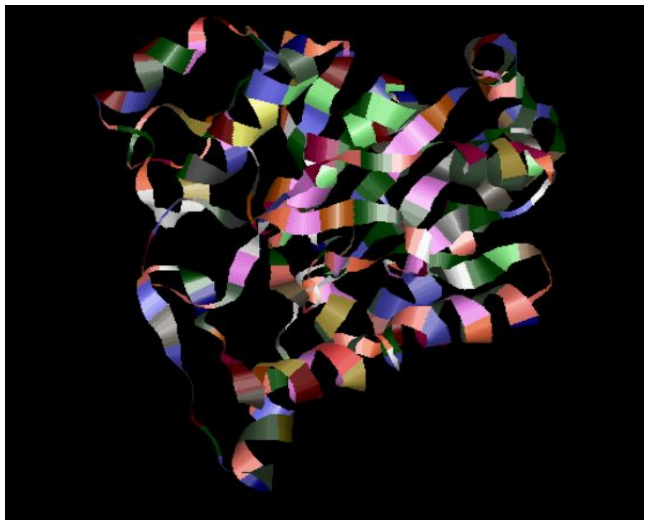
# Measure	Position	Value	Cutoff	signal peptide?
max. C	64	0.109		
max. Y	24	0.119		
max. S	21	0.180		
mean S	1-23	0.128		
D	1-23	0.123	0.500	NO

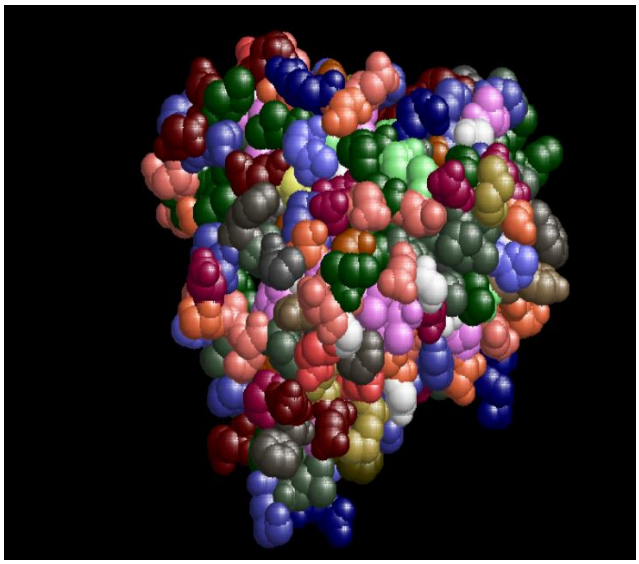
Name=YP_009449628.1 SP='NO' D=0.123 D-cutoff=0.500 Networks=SignalP-TM

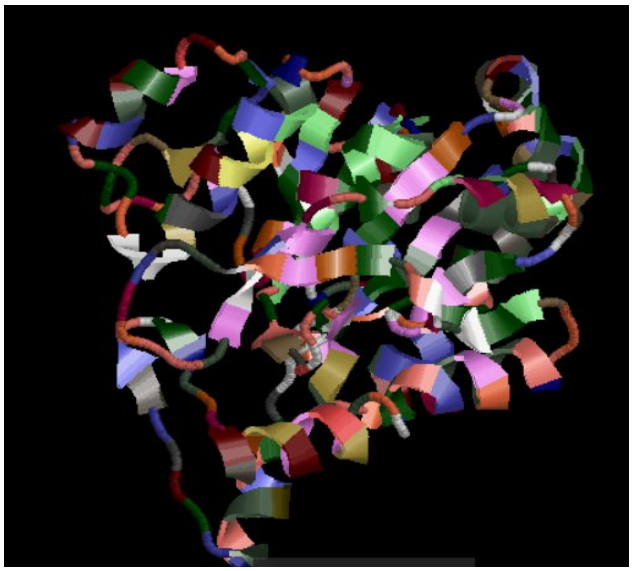
The above results show the signal peptides sequence of acetyl glutamate kinase protein.

HOMOLOGY MODELING:

GENOME3D:







The above results show the tertiary structure of acetyl glutamate kinase.

CONCLUSION:

Marine organisms are a rich source of structurally novel and biologically active metabolites. Of all the species the algae of the present study is Kappaphycus alvarezii, a red algae. The sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India, in the form of dry and living sample. Antioxidant potential of the red algae (Kappaphycus alvarezii) was determined by estimation of vitamin C, vitamin E and heavy metals such as Selenium and Magnesium in previous studies. From estimation of biochemical composition, it is observed that the protein is as high as 18.78 gm compared to all other substances like, phenols, lipids, carbohydrates, fat, sterols. The primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Hence the species can serve as functional food with vital nutritional and biological value. The acetyl glutamate kinase is the terminal complex of the electron transfer chain, an antimicrobial protein. Results of this study suggest the utility of acetyl glutamate kinase for various nutritional products for use as health or nutraceutical supplement. The protein sequence of acetyl glutamate kinase was retrieved from NCBI database. Structural and sequence analysis of acetyl glutamate were done using bioinformatics online tools. A comprehensive study of the protein may be further used in research.

REFERENCES:

Abdullah, M.I., Fredriksen, S., 2004. Production, respiration and exudation of dissolved organic matter by the kelp Laminaria hyperborea along the west coast of Norway. Journal of the Marine Biological Association of the UK 84: 887.

Baich A, Vogel HJ (1962). "N-Acetyl-gamma-Ilutamokinase and N-acetylglutamic gamma-semialdehyde dehydrogenase: repressible enzymes of arginine synthesis in Escherichia coli". Biochem. Biophys. Res. Commun. 7: 491–6. doi:10.1016/0006-291X(62)90342-X. PMID 13863980.

Bolton, John J. (23 July 2010). "The biogeography of kelps (Laminariales, Phaeophyceae): a global analysis with new insights from recent advances in molecular phylogenetics". Helgoland Marine Research. 64 (4): 263–279. doi:10.1007/s10152-010-0211-6 – via SpringerLink.

Chellamuthu VR, Ermilova E, Lapina T, Lüddecke J, Minaeva E, Herrmann C, Hartmann MD, Forchhammer K. A widespread glutamine-sensing mechanism in the plant kingdom. Cell. 2014 Nov 20;159(5):1188-1199.
doi: 10.1016/j.cell.2014.10.015.

Chen, Huihui; Zhou, Dong; Luo, Gang; Zhang, Shicheng; Chen, Jianmin (2015). "Macroalgae for biofuels production: Progress and perspectives". Renewable and Sustainable Energy Reviews. 47: 427–437. doi:10.1016/j.rser.2015.03.086.

Christie, H., Jørgensen, N.M., Norderhaug, K.M., Waage-Nielsen, E., 2003. Species distribution and habitat exploitation of fauna associated with kelp (Laminaria hyperborea) along the Norwegian coast. Journal of the Marine Biological Association of the UK 83, 687-699.

Eberhardt D, Jensen JV, Wendisch VF. L-citrulline production by metabolically engineered Corynebacterium glutamicum from glucose and alternative carbon sources. AMB Express. 2014 Dec;4(1):85. doi: 10.1186/s13568-014-0085-0. Epub 2014 Dec 10.

Hebsibah Elsie B, Subashini.G, Nithya.G and Shoba.K. (2018); Purification and Identification of Antioxidant Peptides from the Skin Protein Hydrolysate of Marine Fish (Aurigequula Fasciata). European journal of Pharmaceutical and Medical Research.5 (10). 371 – 378.Issn No 2394-3211.

Kalpana K., Manjuvani S., Shoba K. In Silico Comparative Modeling of Maturase K Protein in Cymbopogon martinii Plant. Research & Reviews: A Journal of Bioinformatics. 2018; 5(3): 30–36p.

Revathi G, Shoba K, Hebsibah Elsie B, "Gene Expression and Structural analysis of NADH Dehydrogenase subunit 2 protein in Sargassum Muticum", IJRAR - International Journal of Research and Analytical Reviews (IJRAR), Volume.6, Issue 1, Page No pp.720-733, February-2019, E-ISSN 2348-1269, P- ISSN 2349-5138.

Shoba K, Sowmiya S and Dr. Mazher sultana, World Journal of Pharmaceutical and Life Sciences, ISSN 2454-2229, Vol. 3, Issue 1, 427-436.

Shoba K., Manjuladevi M, Mazher sultana, Biochemical analysis and gene expression profiling on collagenase protein in fiddler crab, World journal of pharmacy and pharmaceutical sciences, issn 2278 – 4357, volume 6, issue 3, 747-756

Shoba K., Hebsibah Elsie B. And Bavya sri S. In silico Peptide Modeling Studies and Structural Analysis on Ribulose -1, 5 Bisphosphate Carboxylase In GracilariaEdulis, World Journal Of Pharmacy And Pharmaceutical Sciences, 2018, Volume 7, Issue 3, 1086-1095, Issn 2278 – 4357.

Shoba K., Kalpana K., Protein Modeling and Drug Docking Studies on Potential Protein Target (E. coli–dosP)

and Compound Aldehyde (Sumatriptan) using Bioinformatics Tools. Research & Reviews: A Journal of Bioinformatics. 2018; 5(3): 9–18p.

Shoba. K, Hebsibah Elsie. B and Jayakumari. S. Sathya. R. (2018); Insilico Structural Analysis and Drug Docking Stuidies On Ribulose -1, 5 Bisphosphate Carboxylase In Gracilaria Edulis. International journal of advanced research. 6 (9). 159-165] (ISSN 2320-5407).

Shoba. K, Nithy.G and Deepa.L. (2018); Biochemical Analysis and Peptide Modeling of Lysozyme in Indian Fenneropenaeus indicus shrimp species. International journal of advanced research. 6 (9). 159-165] (ISSN 2320-5407).

Shoba.k and Dr. Mazher sultana, Three - dimensional structure and motif prediction studies on collagenase protein in fiddler crab, International journal of novel trends in pharmaceutical sciences, Issn: 2277 – 2782, volume 6, issue 4, pages 79 – 83.

Shoba.K, Hebsibahelsie.B, insilico homology modeling ofribulose-1, 5-bisphosphate carboxylase protein in gracilariaedulis, world journal of pharmacy and pharmaceutical sciences, 2017, volume 6, issue 8, 396-406, issn 2278 – 4357.

Shoba.K, Lavanya.G, Identification Of De Novo Peptide and Motif Prediction On Porphyria Protein (Hmbs) Using Insilico Tools, Universal Journal Of Pharmacy ,2018, Volume 8, Issue 1, Issn2320-303x.

Shoba.K, Lavanya.G, Tertiary Structural Prediction And Drug Binding Studies On Mutated Gene (Hmbs) In Human Porphyria,International Journal Of Novel Trends In Pharmaceutical Science,2018,Issn 2277 -2782,Volume 8,Issue 1

Usha.K and Shoba.K, Functional analysis & pepptide structure modeling of sphyastatin protein in scylla paramamosain, International Journal of Current Science and Technology, Volume 7, Issue, 01(A), pp. 666-669, January 2019, ISSN: 2320-8090.