ISSN PRINT 2319 1775 Online 2320 7876

*Research paper*  UGC CARE Listed ( Group -I) Journal Volume 11, S Iss 1,2022

# *Piper Longum* Carboxylase Oxygenase Inhibitor Protein Three Dimensional Structure Modelling and Ramachandran Plot Analysis

**Dr Manisha**[1]
Associate Professor and Head of Botany Department , Chinmaya Degree College, BHEL, Haridwar, Uttarakhand, manisha26solanki@gmail.com

**Saumya Surekha**[2]
Research scholar, Department of Biochemistry, Panjab University, Chandigarh
surekha.saumya@gmail.com

**Dr. Beema Jainab S.I**[3]
Assistant Professor Justice Basheer Ahmed Sayeed College for Women (Autonomous)
Chennai - 600 018 beemajainab.s@jbascollege.edu.in

**M.Krishnaveni**[4]
Assistant Professor in Biochemistry, Nadar Saraswathi College of Arts & Science, Theni.
venibio87@gmail.com

## ABSTRACT
Long pepper (*Piper longum*), also known as Indian long pepper or thippali, is produced for its fruit, which is dried and used as a spice and condiment. Long pepper tastes similar to *Piper nigrum*, which produces black, green, and white pepper, but it is spicier. This study seeks NCBI FASTA sequences for several proteins. BLAST is then used to find similar sequences. Swiss model modelled homology. The template model was created. This program's comparative modelling predicts protein sequence identification with 94.95% accuracy. The template protein's e value is 0.0, and chain A may score 990 for 100% similarity. Ramachandran plots verified the protein structure. Ramachandran plot analysis found 97% of residues in the desired locations. Other methods described and examined the projected 3D model.

**Keywords:** Medicinal plants, Homology modellling, Structure prediction, Sequence similarity

## INTRODUCTION
Pepper comes from Sanskrit meaning long pepper (pippali). Long pepper (*Piper longum*), often known as Javanese, Indian, or Indonesian long pepper, is a Piperaceae flowering vine grown for its dried, spice-like fruit. P. nigrum, which produces black, green, and white pepper and has a similar but frequently hotter flavour, is a close relative of long pepper. Fruits are

ISSN PRINT 2319 1775 Online 2320 7876

*Research paper* **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed ( Group -I) Journal Volume 11, S Iss 1,2022**

pungent due to piperine, an alkaloid. Java is home to P. retrofractum, another long pepper. Topical application lowers inflammation and muscular pain. Ayurveda says it rejuvenates. *P. longum* causes intestinal gas and appetite. *P. longum* root infusion stimulates placental expulsion after birth [1]. Historical use of the full plant and components like the fruit has not been documented. Hepatotoxicity, depression, diabetes, cancer, inflammation, and other disorders may be treated with this inexpensive, readily available herb [2].

D-ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), the main enzyme of plant photosynthesis, needs carbamylation of Lys201 of the large subunit and Mg(2+) coordination to become catalytically competent. 6-phosphogluconate (6PG) and reduced NADPH enhance activation in biochemical studies. The structural process remains unknown. Here are the crystal structures of activated rice Rubisco with NADPH, 6PG, or 2-carboxy-D-arabinitol 1,5-bisphosphate (2CABP). Loop 6 at the catalytic site and a few other loops are open-state in the NADPH and 6PG complexes. 2CABP complex structures are closed [3].

A significant source for bioinformatics tools and services is the NCBI (National Center for Biotechnology Information). Every day, new information is retrieved from and added to this database of DNA, RNA, and protein sequences. BLAST compares amino acid and nucleotide sequences [4]. Heuristic sequence alignment. NCBI hosts this tool. BLAST may be used to locate homologous species or identify species. These findings are presented graphically, with a table displaying the corresponding BLAST score for each hit identified [5].

Homology modelling is a structural biology technique that significantly contributes to both experimentally and systematically determined structure and known protein structure [6]. Homology modelling includes template modelling, side chain modelling, model optimization, and validation [7]. By integrating a protein's sequence (Target) into a known structure, this process creates a previously unidentified protein structure (Template). Given a minimum 30% sequence homology between the target and the template [8]. The Swiss PDB viewer's Swiss Model is a fully automated protein homology modelling service [9]. This server's goal is to open up protein modelling to all life science research across the globe [10].

Ramachandran plots confirm protein structure and conformations in RAMPAGE server [11].It is used to visualize the energetically permissible regions for the amino acid residues and backbone dihedral angles in protein structure [12]. Three distinct regions that were allowed and outliers were indicated on this graph. , and the protein's structural validity was confirmed [13].

The current study focuses on the modelling and analysis of a *Piper longum*-related carboxylase oxygenase inhibitor in three dimensions using data from the Swissmodell server. Ramachandran plot analysis was used to validate the structure.

ISSN PRINT 2319 1775 Online 2320 7876

*Research paper*  UGC CARE Listed ( Group -I) Journal Volume 11, S Iss 1,2022

## Material and methods

### Sequence retrieval

FASTA-formatted *Piper longum* carboxylase oxygenase inhibitor protein sequences are taken from the NCBI database.

### Simple Local Alignment Search Instrument (BLAST)

After a search, the BLASTn suit website provided *Piper longum* related carboxylase oxygenase inhibitor protein query sequences. Mega blast protein selection for very similar sequences followed. BLAST.

### Swiss model homology modelling

Template Search BLAST and HH Blits searched SWISS-MODEL template library. SMTL's main amino acid sequence BLAST searched the target sequence. 173 templates were found. BLAST searches the goal collection using the SMTL main amino acid sequence. 173 templates were found. The first generation of HH blits against NR20 was used to create a preliminary profile. Then, the obtained profile was compared to all SMTL profiles. Templates totaled 2341.

### Template Choice

The target-template alignment's characteristics have been used to forecast each detected template's quality. Then, for model creation, the greatest quality templates have been chosen.

### Building model

ProMod3 models target template alignment. The template replicates the coordinates that are the same between the target and the template to the model. A fragment library is used to change additions and deletions. Rebuild side chains. Finally, a force field is utilized to regularize the geometry of the created model. PROMOD-II is used to model loops if ProMod3 fails.

### QA Model

The QMEAN scoring function assessed residue model quality. SWISS-MODEL has trained QMEAN word weights to improve performance.

### Simulation

Homology transfers template ligands to the model under the following circumstances. The target and template preserve the residues in contact with the ligand, the model contacts the ligand, and the protein does not conflict with it. The template library marks biologically relevant ligands. If any of these four prerequisites aren't satisfied, the model won't contain the ligand. The model summary explains why some ligands were excluded.

### Oligomeric conservation

Template quaternary structure annotation models target sequence oligomeric form. The supervised machine learning approach Support Vector Machines (SVM) uses interface conservation, structural grouping, and other template attributes to determine quaternary structure quality (QSQE). A model developed using a given alignment and template has a QSQE score of 0 to 1 that predicts interchain links. Higher values imply reliability. The GMQE score, which measures the model's tertiary structure accuracy, increases.

ISSN PRINT 2319 1775 Online 2320 7876

*Research paper* **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed ( Group -I) Journal Volume 11, S Iss 1,2022**

### Ramachandran plot study of RAMPAGE structure validation

The structure was modelled, and after that, the RAMPAGE server received the PDB coordinate file. The most liked parts supported the server plot, which is a graphical depiction of a protein's three-dimensional structure. Different colours are used to indicate the areas, and the protein model was confirmed.

## Result

### Sequence retrieval from NCBI

```
>ABR26368.1 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit
(chloroplast) [Piper longum]
MSPQTETKAYVGFKAGVKDYKLTYYTPEYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVW
TDGLTSLDRYKGRCYHIEPVAGEENQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRI
PPAYSKTFQGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECLRGGLDFTKDDENVNSQP
FMRWRDRFVFCAEALYKAQAETGEIKGHYLNATAGTCEEMIKRAVFARELGVPIVMHDYLTGGFTANTSL
AHYCRDNGLLLHIHRAMHAVIDRQKNHGMHFRVLAKALRMSGGDHVHAGTVVGKLEGEREITLGFVDLLR
DDFVEKDRSRGIYFTQDWVSMPGVLPVASGGIHVWHMPALTEIFGDDSVLQFGGGTLGHPWGNAPGAVAN
RVSLEACVQARNEGRDLASEGNEIIREAAKWSPELAAACEVWKEIKFEFEAMDTL

>ABR26356.1 maturase, partial (chloroplast) [Piper longum]
MEKLKGYLETFRSEQKHFLYPLLFQEYIYALGHDPGLNRPIPYESIENLGYGDKSSSLIVKRLIIRMHKQ
NHFLISCNENYFQQNQLLGRKNNLHSKIISEAFSIIVEIPFSLQLVSCLEKKREIEKSHNLRSIHSIFSF
FEDNIFYLYHISDVLIPYPIHPEILVQTLRYWIQDVPSLHLLRIFLYEYCHSGSLISKKKFFSFSKKENE
RLSLFIYNSHVYEWESVFLFIRKQSYHLRSISWEALLERVHFYGKIEHLEVVLCNDFQKALRLFKDSFMH
YVRYRGKSLLISKGTDLLMKKWKYHFIYLWQCNFHLWSQLHRIHINQLDNRSFHFLGYVSSVRRNLSVVK
SQMLENSFLMETSVKKFETIVPIISLIDSLSKEKFCNLSGHPTSKAIWADLSDSDIMERFGRVCRNLSHY
YSGCSKKQILYRIKYILRLSCARTLARKHKSTVRTFLKKLGSGFWKEFLAEEEQVLSYFFPRSYPTSYRS
NKDKERIWYLDITHTNDLTNHE

>UVI62139.1 ribulose-1,5-bisphosphate carboxylase/oxygenase large
subunit, partial (chloroplast) [Piper longum]
ETKAYVGFKAGVKDYKLTYYTPEYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLT
SLDRYKGRCYHIEPVAGEENQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIPPAYS
KTFQGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYG

>UVI62127.1 maturase K, partial (chloroplast) [Piper longum]
LRYWIQDVPSLHLLRIFLYEYCHSGSLISKKKFFSFSKKENERLSLFIYNSHVYEWESVFLFIRKQSYHL
RSISWEALLERVHFYGKIEHLEVVLCNDFQKALRLFKDSFMHYVRYRGKSLLISKGTDLLMKKWKYHFIY
LWQCNFHLWSQLHRIHINQLDNRSFHFLGYVSSVRRNLSVVKSQMLENSFLMETSVKKFETIVPIISLID
```

**Fig 1: Carboxylase oxygenase inhibitor protein sequences linked to *Piper longum* in FASTA formats collected from NCBI**

### Sequence similarity search

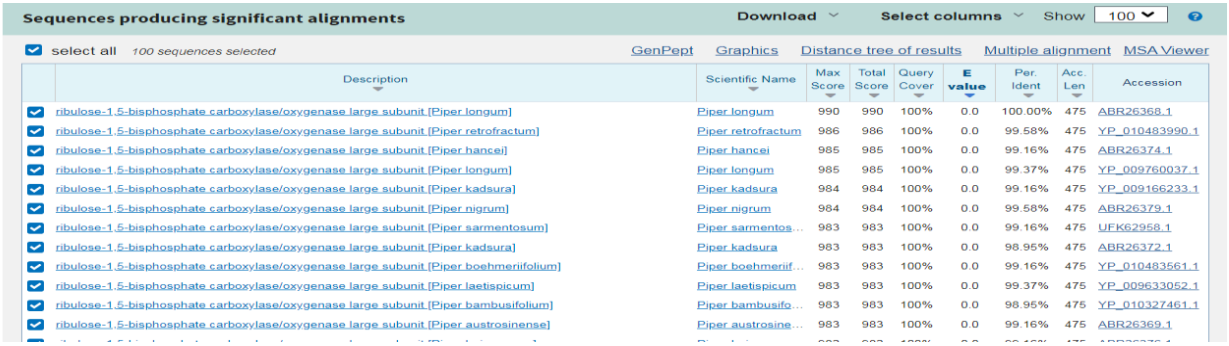


**Fig 2: Basic Local Alignment Search Tool similarity search *Piper longum* carboxylase oxygenase inhibitor sequences**

ISSN PRINT 2319 1775 Online 2320 7876

*Research paper*  UGC CARE Listed ( Group -I) Journal Volume 11, S Iss 1,2022

## Protein structure prediction

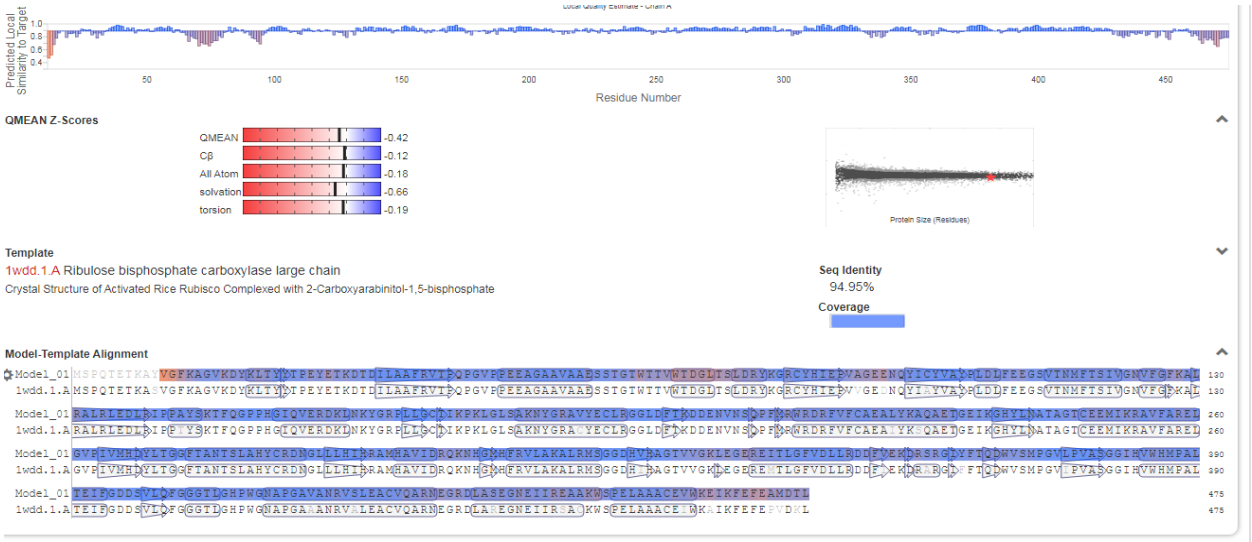


**Fig 3: Swiss model sequence identity predicted the protein structure at 94.95%.**


**Fig 4: Templates for carboxylase oxygenase inhibitors linked to *piper longum***

ISSN PRINT 2319 1775 Online 2320 7876

*Research paper*  UGC CARE Listed ( Group -I) Journal Volume 11, S Iss 1,2022

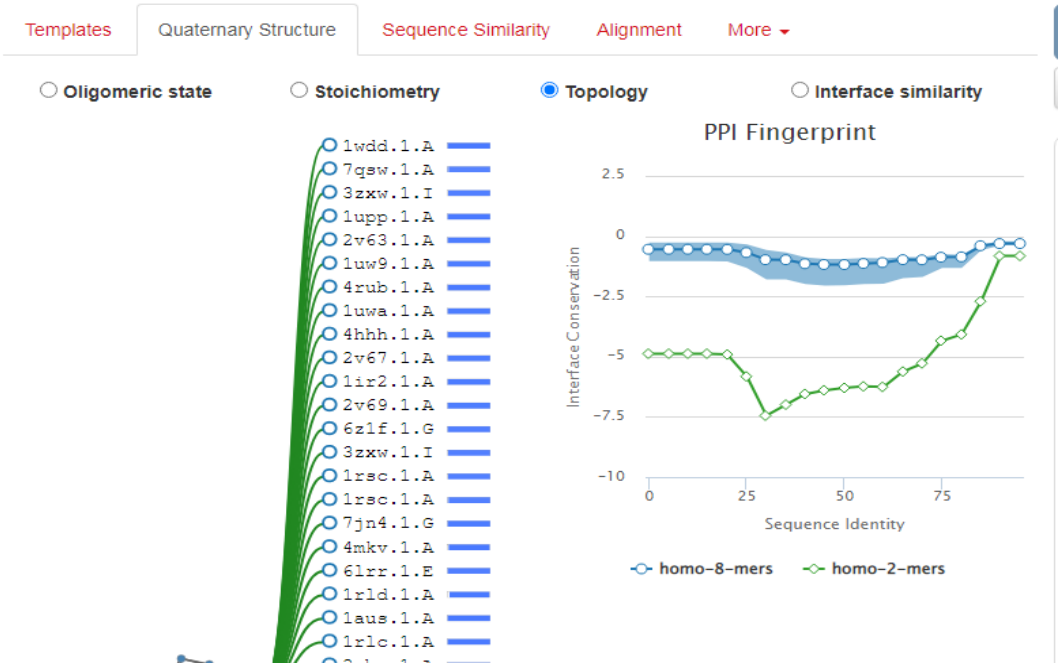


**Fig 5: Related carboxylase oxygenase inhibitor Topology to *Piper longum***

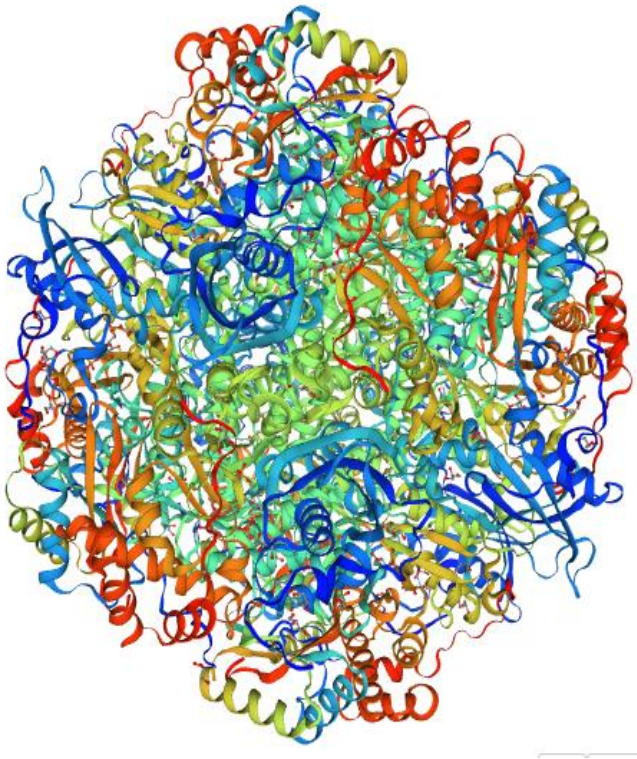

**Fig 6: The carboxylase oxygenase inhibitor linked to *Piper longum* as shown in Pymol**

ISSN PRINT 2319 1775 Online 2320 7876

*Research paper*  UGC CARE Listed ( Group -I) Journal Volume 11, S Iss 1,2022

**Structure validation using Ramachandran plots**

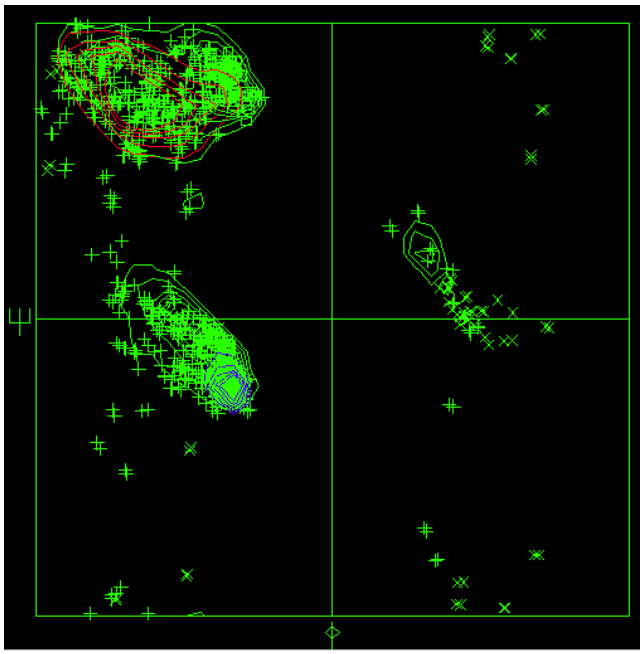

**Fig 7: Ramachandran plots predict protein structural quality. High-quality Ramachandran plots cover 97% of torsional angles.**

## CONCLUSION

FASTA formats for carboxylase oxygenase inhibitor sequences linked to *Piper longum* taken from NCBI. The sequence utilized by the BLAST server is 100% identical. The template protein's e value is 0.0, and chain A may score 990 for 100% similarity. The top fifty templates and Swiss model for protein structure prediction are mentioned. The filtered list was supplemented with 50 templates that were unsuitable for modelling. (1wdd,5iuo,2v63,1uwa,1upp) In order to improve our knowledge of the protein universe and its characteristics, computational structural modelling techniques have proven themselves as a viable supplement to experimental structural biology efforts. In this project, comparative modelling approaches have developed into fully automated processes that make it simple to get trustworthy 3D models and expand the range of protein model users and applications. The Ramachandran plot is most important for predicting protein structure quality based on experimental data (X-ray crystallography, NMR and Cryo-EM).Protein structure is represented as a number of torsional angles falling in the banned zone. A high quality structure encompasses the whole set of torsional angles in the permissible area. In addition to experimental approaches, the Ramachandran plot is often used to verify protein structures derived from homology modelling or ab-initio procedures.

ISSN PRINT 2319 1775 Online 2320 7876

*Research paper*  UGC CARE Listed ( Group -I) Journal Volume 11, S Iss 1,2022

## REFERENCE


1. KR Kirtikar, BD Basu.Indian Medicinal Plants, Orients Longman, Mumbai, India (1980), pp. 21-28
2. Rastogi RP, Malhotra BN. *Compendium of Indian Medicinal Plants*. CDRI, Luckhnow and New Delhi, India: Nisc 1993: 504-857.
3. Matsumura, H., Mizohata, E., Ishida, H., Kogami, A., Ueno, T., Makino, A., ... & Kai, Y. (2012). Crystal structure of rice Rubisco and implications for activation induced by positive effectors NADPH and 6-phosphogluconate. *Journal of molecular biology*, *422*(1), 75-86.
4. Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L. BLAST+: architecture and applications. BMC Bioinformatics 10, 421-430 (2009)
5. Remmert, M., Biegert, A., Hauser, A., Söding, J. HHblits: lightning-fast iterative protein sequence searching by HMM-HMM alignment. Nat Methods 9, 173-175 (2012)
6. Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 46(W1), W296-W303 (2018).
7. Guex, N., Peitsch, M.C., Schwede, T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. Electrophoresis 30, S162-S173 (2009).
8. Bienert, S., Waterhouse, A., de Beer, T.A.P., Tauriello, G., Studer, G., Bordoli, L., Schwede, T. The SWISS-MODEL Repository - new features and functionality. Nucleic Acids Res. 45, D313-D319 (2017).
9. Benkert, P., Biasini, M., Schwede, T. Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics 27, 343-350 (2011).
10. Bertoni, M., Kiefer, F., Biasini, M., Bordoli, L., Schwede, T. Modeling protein quaternary structure of homo- and hetero-oligomers beyond binary interactions by homology. Scientific Reports 7 (2017).
11. Hollingsworth SA and Karplus PA (2010), A fresh look at the Ramachandran plot and the occurrence of standard structures in proteins, Biomol Concepts, 1(3-4): 271–283.
12. Ramachandran GN, Ramakrishnan C and Sasisekharan V (1963), Stereochemistry of polypeptide chain configurations, Journal of Molecular Biology, 7: 95–9.
13. Ramachandran GN and Sasisekharan V (1968), Conformation of polypeptides and proteins, Advances in Protein Chemistry,Advances in Protein Chemistry, 23: 283–437.