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# Structural and Functional Characterization of *Hericium erinaceum*, Manganese Peroxidase as an Antioxidant against Iron Induced Parkinson's Disease

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**Abstract:** ***Hericium erinaceum, (lion's mane) is an edible and medicinal mushroom in the tooth fungus group. This has great therapeutic benefits in treating Parkinson's disease mainly because of the stimulation of the Nerve Growth Factor. Most research was focused on the therapeutic effects of the medicinal mushrooms, but little information is available about their antioxidant properties. In this paper, a bioinformatics and molecular modeling approach was adopted to explore properties and structure of Hericium erinaceum antioxidant proteins. The antioxidant proteins studied for the neuroprotective effect by free radical scavenging are manganese peroxidase 1(MnP1) and manganese peroxidase 2(MnP2). Physico-chemical characterization interprets properties such as p[I], EC, AI, GRAVY and instability index and provides data about these proteins and their properties. Prediction of motifs, patterns, disulfide bridges and secondary structure were performed for functional characterization. Three dimensional structures of these proteins were not available as yet in PDB. Therefore, homology models for these antioxidant proteins were developed. The modeling of the three dimensional structure of these proteins shows that models generated by Swiss model were more acceptable in comparison to that generated by (PS) [2]-V[2], Phyre[2]. The quality and reliability of the models were checked using Ramachandran Plot calculation and by using ERRAT. These structures will provide a good foundation for functional analysis of experimentally derived crystal structures.***

**Keywords:** Parkinson's disease, Hericium erinaceum, Computational tools, antioxidants, neuroprotection, Homology model.

## 1. Introduction

Free radicals play important roles in many physiological and pathological conditions. In general, excess of free radicals caused by the imbalance between free radical generation and scavenging may contribute to disease development. Free radicals can damage membranes, proteins, enzymes and DNA, increasing the risk of diseases such as cancer, Alzheimer's, Parkinson's, angiocardiopathy, arthritis, asthma, diabetes, and degenerative eye disease [1].

In recent years mushrooms have been shown to possess valuable antioxidants of great nutritional and therapeutic value [2]. Many studies have found that some species of mushrooms are having many therapeutic properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immune stimulatory effects. They accumulate a variety of secondary metabolites, including phenolic compound, polyketides, terpenes and steroids. A detailed analysis of protein sequences which are responsible for these properties, their probable structures and mode of action has yet to be accomplished [3].

*Hericium erinaceum*, (lion's mane) an edible medicinal mushroom in the tooth fungus group is considered for the present study. Native to North America, Europe and Asia. it is long used in traditional Chinese and traditional Japanese medicine [4]. It contains a number of health promoting substances including antioxidants and beta glucan. Dietary supplementation with this stimulates the synthesis of Nerve Growth factor (NGF) and promotes the process of myelination. There by help to slow the progression of degenerative neurological disorder such as Alzheimer's and Parkinson's [5] Mushrooms have become attractive as functional food and as a source of physiologically beneficial compounds including antioxidants [5]. Considering the importance of complementary and alternative medicine in prevention of Parkinson's, this study is undertaken to evaluate the antioxidant properties of *Hericium erinaceum*. So that it can be supplemented in the diet of Parkinson's disease patient.

Parkinson's disease (PD), the second most common neurodegenerative disorder which is characterized by the damage of an area of brain called Substantial nigra (SN) [6], [7]. This area influences the involuntary movements. This disorder is idiopathic and consequently no cure exists. Mechanisms leading to the degeneration of melanized neurons in the brain stem and particularly in the substantial nigra (SN) in patients with Parkinson's disease (PD) are still unknown[8]. An excessive hydrogen peroxide in post mortem of frontal cortex of a patient with PD gives the observation that it may be a direct indicator of oxidative stress [9].Although antioxidant defense and repair systems are available in humans and other organisms to protect them against oxidative damage, these systems are insufficient totally prevent the damage[9]. There are many epidemiological studies suggest that the consumption of polyphenol–rich foods and beverages is associated with a reduced risk of many diseases, in which polyphenol is linked to the antioxidant properties. The consumption of dietary antioxidants will help to prevent free radical damage [10].Antioxidants are substances which when present at low concentration compared to those of an oxidizable substrate, significantly delay or prevent the oxidation of that substrate

[11].They are capable or preventing or attenuating damages such as lipid peroxidation, oxidative damage to membranes, glycation of proteins and inactivation of enzymes caused by free radicals [12]. There are several evidences that show that oxidative stress resulting from reactive oxygen species including free radicals such as hydroxl(OH), superoxide($O_2$), nitric oxide(NO), nitrogen dioxide($NO_2$), peroxyl (ROO) and non free radical like hydrogen peroxide and singlet oxygen play an important role in the development of several pathological conditions as lipid peroxidation, protein oxidation, DNA damage and cellular degeneration [2].

Manganese peroxidase or MnP, These enzymes belongs to the family oxidoreductases to be specific class II peroxidases, whose main function is lignin degradation in white rot fungi, However the specific roles of manganese is uncertain [13]. In P.D an elevation of iron with staging of disease has been observed in SN, especially in the Zona Compacta (ZN).The iron in SNZC is thought to induce oxidative stress, there by iron can full fill the role of a neurotoxin [14]. In contrast to this pro-oxidant effect of iron, manganese causes neither lipid peroxidation nor nigral injury nor dopamine depletion [15]. Manganese dose dependently, particularly at lower doses, protect nigral neurons from iron induced oxidative injury and dopamine depletion. Manganese also suppressed acute increase in the dopamine turnover and contralateral turning behavior induced by iron. Surprisingly manganese catalyzed the Fenton reaction, the conversion of hydrogen peroxide to hydroxyl radicals. This shows that iron and manganese are two transition metals mediating opposite effects in the nigrostratal system as pro-oxidant and antioxidant respectively. Thus atypical antioxidative properties of manganese protect SN compacta from iron induced oxidative stress, there by act as a potent therapeutic agent and successfully studied in both animal models and clinical studies [16]. Since high doses of manganese revealed a reversible oxidative injury to nigrostratial dopaminergic neurons, care should be taken in to consideration in dosage determination [17].

During the past few decades, the uses of natural antioxidants have received increased interest due to the concerns about the possible ill effects generated by the use of synthetic antioxidants [18].Natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer and cheaper than synthetic ones; thus they are more readily acceptable to the modern consumers [19]. The WHO estimated that approximately 80% of the world population uses natural products as a source of drugs [20]. Most of the research was focused on the therapeutic effects of the medicinal mushrooms, but little information is available about their antioxidant properties [21].

The knowledge of the mechanisms of action of most medicinal mushrooms is scant and exploration of their use as therapeutic agents is limited. Therefore there is a need to implement newer techniques to determine their potential uses. Most research was focused on the therapeutic effects of the medicinal mushrooms, but little information is available about their antioxidant properties. High antioxidant activities in mushrooms can suppress active oxygen species, which are related to ageing and diseases. They also might be developed into functional foods or drugs in future [22].With the advent of proteomics and genomics this problem can be partially alleviated with these efficient methods for rapid identification and analysis of these antioxidants.

Computational tools provide researchers to understand physicochemical and structural properties of proteins. A large number of computational tools are available from different sources for making predictions regarding the identification and structure prediction of proteins. The major drawbacks of experimental methods that have been used to characterize the proteins of various organisms are the time frame involved, high cost and the fact that these methods are not amenable to high throughput techniques. *In silico* approaches provide a viable solution of these problems .The amino acid sequence provides most of the information required for determining and characterizing the molecule's function, physical and chemical properties. Computationally based characterization of the features of the proteins found or predicted in completely sequenced proteomes is an important task in the search for knowledge of protein function.

In this study the antioxidant proteins of *Hericium erinaceum* have been selected for which three dimensional structures were not available at the protein data bank (PDB).These proteins are manganese peroxidase 1(MnP1) and manganese peroxidase 2(MnP2), which can be used as a natural source of antioxidants against iron induced oxidative damage in Parkinson's. Hence to describe its structural features and to understand molecular function, the model structures for these proteins were constructed.

## 2. Materials and Methods

Sequences of antioxidant proteins of *Hericium erinaceum* were retrieved from Uniport, a public domain protein database [23]. Table 1, shows the protein sequences considered in this study. The antioxidant proteins sequences were retrieved in FASTA format and used for further analysis.

**[Table-1]**

**Table 1:** Protein sequences considered for the study

| *No* | *Antioxidant Protiens* | *Accession No.* | *Length* | *Description* |
|---|---|---|---|---|
| 1 | MnP1 | E2FB79 | 359 | Manganese peroxidase 1 |
| 2 | MnP2 | H9C7S6 | 361 | Mangasense peroxidase 2 |

### 2.1 Physico-chemical characterization

For physico-chemical characterization, theoretical isoeletric point ($p^I$), molecular weight, total number of positive and negative residues, extention coefficient [24] ,Instability index [25], aliphatic index [26] and grand average hydropathy (GRAVY) [27] were computed using the Expasy's ProtParam server [28].The results were shown in Table 2.

**[Table-2]**

**Table 2:** ParameterscomputedusingExpasy'sProtParamtool

| *Antioxidant proteins* | *Accession No.* | *Sequence Length* | *M.wt* | *$p^I$* | *-R* | *+R* | *EC* | *II* | *AI* | *GRAVY* |
|---|---|---|---|---|---|---|---|---|---|---|
| MnP1 | E2FB79 | 359 | 38177.7 | 4.35 | 48 | 20 | 11500 | 52.97 | 78.38 | -0.042 |
| MnP2 | H9C7S6 | 361 | 38102.6 | 4.52 | 45 | 21 | 6000 | 55.59 | 76.84 | 0.006 |

### 2.2 Subcellular Localization

Subcellular localization of any protein is important in understanding the protein function. Prediction of subcellular localization of the protein was carried out by CELLO v.2.5 [29]. The results are shown in table 3.

### 2.3 Functional Characterization /Functional Annotation of the Protein

The PRED - TMR server performed the identified of transmembrane regions [30]. Table 4 represents the transmembrane region identified for these antioxidant proteins. Disulfide bonds important in determining the functional linkages Table 5 shows prediction of "SS" bonds using the primary structure (Protein sequence data) by the tool CYS_REC identifies the position of cysteins, total number of cysteins present and pattern, if present, of pairs in the protein sequence. To hypothetically annotate the function of the proteins Profunc was used. It was discovered that protein is involved in four biological processes metabolic process, response to oxidative stress, to stress and to stimulus. The biochemical function of the protein is binding, catalytic activity, heme and ion binding. To further investigate the function of protein by finding its family it was searched in the Prosite is a database of protein families and domains [31].Table 6 represents the output of Prosite [32]. That was recorded in terms of the length of amino residues of protein with specific profiles and patterns.

### 2.4 Secondary Structure Prediction

SOPMA was employed for calculating the secondary structural features of the antioxidant protein sequences considered for this study [33]. The results were presented in Table 7.

**[Table-3]**

**Table 3:** CELLO prediction of subcellular localization

| Antioxidant proteins | Accession No. | Localization | Reliability |
|---|---|---|---|
| MnP1 | E2FB79 | Extracellular | 1.746 |
| MnP2 | H9C7S6 | Extracellular | 2.126 |

**[Table-4]**

**Table 4:** Transmembrane regions identified using PRED-TMR server

| *Antioxidant Proteins* | *Accession No.* | *Transmembrane region* | *Length* | *Type of Protein* |
|---|---|---|---|---|
| MnP1 | E2FB79 | 0 | 0 | Soluable |
| MnP2 | H9C7S6 | 0 | 0 | Solubale |

**[Table-5]**

**Table 5:** Disulfide (SS) bond pattern of pairs predicted, by CYS_REC.

| **Antioxidant proteins** | **Accession No.** | **CYS_REC** |
|---|---|---|
| MnP1 | E2FB79 | Cys26-Cys39, Cys38-Cys306, Cys58-Cys142, Cys270-Cys335 |
| MnP2 | H9C7S6 | Cys26-Cys39, Cys38-Cys308, Cys58-Cys144, Cys272-Cys337 |

**[Table-6]**

**Table: 6.** Functional characterization of proteins of *Hericium erinaceum* by Prosite

| **Antioxidant Proteins** | **Accession No.** | **Motif Found** | **Profile** | **Position in the protein** | **Description** |
|---|---|---|---|---|---|
| MnP1 | E2FB79 | PEROXIDASE_2<br>PEROXIDASE_1 | PEROXIDASE_4 | 62-73<br>189-199 | Heme peroxiades are Heme contacting peroxidase carries out a number of oxidative reactions using hydrogen peroxide as the electron acceptor. |
| MnP2 | H9C7S6 | PEROXIDASE_2<br>PEROXIDASE_1 | PEROXIDASE_4 | 62-73<br>191-201 | Heme peroxiades are Heme contacting peroxidase carries out a number of oxidative reactions using hydrogen peroxide as the electron acceptor. |

**[Table-7].**

| **Antioxidant Proteins** | **MnP1** | **MnP2** |
|---|---|---|
| Secondary Structure | E2FB79 | H9C7S6 |
| Alpha helix | 37.60% | 35.18% |
| 310 helix | 0.00% | 0.00% |
| Pi helix | 0.00% | 0.00% |
| Beta bridge | 0.00% | 0.00% |
| Extended strand | 7.52% | 8.86% |
| Beta turn | 1.95% | 2.49% |
| Bend region | 0.00% | 0.00% |
| Random coil | 52.92% | 53.46% |
| Ambiguous states | 0.00% | 0.00% |
| Other states | 0.00% | 0.00% |

### 2.5 3D Structure prediction using homology approach

The modeling of the three dimensional structure of the protein was performed by three homology modeling programs swiss model, $(PS)^2$-$V^2$, Phyre$^2$ to built the final three dimensional structure . The template selection and

template target alignment are the critical steps for this template based modeling methods.

### 2.6 Quality and reliability assessment

The overall sterochemical property and quality of the modeled protein was assessed by Ramchandran plot analysis [34] .Reliability of the model was further checked by ERRAT that analyzes the statistics of non-bonded interactions between different atom types and plots the value of the error function versus position of a 9- residue sliding window, calculated by comparison with statistics from highly refined structures. Furthermore, superimposition of query and template structure, and visualization of generated models was performed using SuperPose Version 1.0 [35].

## 3. Results and Discussion

Table 1 shows the antioxidant protein sequences of *Hericium erinaceum* considered in this study. These antioxidant protein sequences were retrieved from the Uniport, a public domain protein data base. These protein sequences were retrieved in FASTA format and used for further analysis. Parameters computed using Expasy's ProtParam tool was represented in Table 2. The calculated isoelectric point ($p^I$) will be useful because at $p^I$, solubility is least and mobility in an electro focusing system is zero. Isoelectic point is the PH at which the surface of protein is covered with charge but net charge of protein is zero. At $p^I$ proteins are stable and compact. The computed $p^I$ value of MnP1 and MnP2 is less than 7 ($p^I < 7$) indicates that these antioxidant proteins were considered as acidic. The computed $p^I$ will be useful for developing the buffer system for purification by isoelectirc focusing method. Although Expasy's ProtParam computes the extinction coefficient for 276,278,279,280 and 282 nm wavelengths, 280 nm is favored because proteins absorb light strongly there while other substances commonly in protein solutions do not. Extinction coefficient of MnP1 at 280 nm is ranging from 11000 to 11500 $M_{-1}$, $cm_{-1}$ with respect to the concentration of cysteine. Extinction coefficient of MnP2 at 280 nm is ranging from 5500 to 6000 $M_{-1}$, $cm_{-1}$.The computed extinction coefficients help in the quantitative study of protein-protein and protein-ligand interactions in solution. The N terminal of both the protein is M (met-methionine) so the estimated half life is 30 hours (mammalian reticulates, invitro), 20 hours (yeast, in vivo), and 10 hours (*Escherichia coli*, in vivo).In MnP1 Ala and Thr are found to be rich in the protein 1 with 12% and 8.6% respectively. In MnP2 Ala, Gly, Fe and Thr are found to be rich in the protein 2 with 12% of Ala and 7.5 % of others respectively.The instability index provides an estimate of the stability of protein in a test tube. There are certain dipeptides, the occurrence of which is significantly different in the unstable proteins compared with those in the stable ones. The methods assigns a weight value of instability .Using these weight values it is possible to compute an instability index (II).A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable (Guruprasad et al., 1990).The instability index value for both MnP1 and MnP2 antioxidant proteins were found to be 52.97 and 55.59 respectively. The results classified both the antioxidant proteins as unstable. The aliphatic index (AI) which is defined as the relative volume of a protein occupied by aliphatic side chains (A, V, I and L) is regarded as a positive factor for the increase of thermal stability of globular proteins. Aliphatic index for the antioxidant protein sequences ranged from 67.26-84.98.The very high aliphatic index of the antioxidant protein sequences indicates that these proteins may be stable for a wide temperature range. The lower thermal stability was indicative of a more flexible structure when compared to other antioxidant protein. MnP1 shows the aliphatic index of 78.38 and MnP2 shows 76.84 aliphatic index. This high level obtained and within the range of aliphatic index of antioxidant proteins, shows that both the proteins under study are highly stable for wide range of temperature. The Grand Average Hydropathy (GRAVY) value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence. GRAVY indices of MnP1 and MnP2 are -0.042 and 0.006. This low range of value indicates the possibility of better interaction with water.

Functional analyses of these proteins include prediction of transmembrane region, disulfide bond and identification of important motifs. PRED -TMR distinguishes between membrane and soluble proteins from amino acid sequences, predicts the transmembrane helices for the former. This server classifies both MnP1 and MnP2 as soluble proteins. As disulfide bridges play an important role in determining the thermostability of these proteins, CYS_REC was used to determine the Cysteine residues and disulphide bonds. Possible pairing and pattern with probability were presented in Table 5. Results shows that both the antioxidant proteins contain disulphide linkages.

The functions of antioxidant proteins of *Hericium erinaceum* were analyzed by submitting the amino acid sequence to Prosite server. Sequence of a particular cluster of residue types, which is variously known as pattern, motif, signature or fingerprint. These motifs, typically around 10 to 20 amino acids in length, arise because specific residues and regions thought or proved to be important to the biological function of a group of proteins are conserved in both the structure and sequence during evolution. Prosite analysis suggested the functionality of these proteins with profiles and patterns identified for characteristic functionality were represented in Table 6.

The secondary structure of *Hericium erinaceum* antioxidant proteins were predicted by SOPMA, which correctly predicts of amino acids for a state description of the secondary structure prediction. The secondary structure indicates whether a given amino acid lies in a helix, strand or coil. Secondary structure features as predicted using SOPMA were represented in table 7.The results revealed that random coils dominated among secondary structure elements followed by alpha helix, extended strand and beta turns for all sequences.

Three dimensional structures are predicted for the proteins where such data is unavailable. There is lack of experimental structures for these proteins considered for this study. The

modeling of the three dimensional structure of the protein was performed by $(PS)^2$ -$V^2$ homology modeling server. The Φ and Ψ distribution of the Ramachandran Map generated by of non glycine, non proline residues were summarized in Table 8.

**[Table 8]**

**Table 8:** Ramchandran Plot calculation with Rampage and comparative analysis of models from the three modeling tools

| Server | Residues in different regions | Antioxidant protiens | |
|---|---|---|---|
| | | MnP1 | MnP2 |
| Swiss Model | Residues in the most favored region | 96.4 | 96.9 |
| | Residues in the allowed region | 2..4 | 2.8 |
| | Residues in the outlier region | 1.2 | 0.3 |
| $(PS)^2 – V^2$ | Residues in the most favored region | 96.6 | 95.8 |
| | Residues in the allowed region | 2.5 | 2.8 |
| | Residues in the outlier region | 0.8 | 1.4 |
| $Phyre^2$ | Residues in the most favored region | 95.2 | 95.5 |
| | Residues in the allowed region | 3.0 | 3.6 |
| | Residues in the outlier region | 1.8 | 0.9 |

A comparison of results obtained from the three different software tools in table 8 shows that the models generated by Swiss model **[Figure- 1]** and [**Figure- 2]** was more acceptable in comparison to $Phyre^2$ and $(PS)^2 – V^2$.The final modeled structures were visualized by Swiss PDB viewer that was shown in Figure 1.The stereo chemical quality of the predicted models and accuracy of the protein model was evaluated after the refinement process using Ramachandran Map calculations computed with RAMPAGE. The assessment of the predicted models generated by swiss model was shown in Figure 2.The main chain parameters plotted are Ramachandran plot quality, peptide bond planarity, Bad non bonded interaction, main chain hydrogen bond energy, C-alpha residues were classified according to its regions in the quadrangle. The red regions in the graph indicate the most allowed regions where as the yellow regions represent allowed regions. Glycine is represented by triangles and other residues are represented by squares. The result revealed that the modeled structure for MnP1 has 96.4% residue in the favorable region and MnP2 has 96.9% residues in the favorable region. The distribution of the main chain bond angles were found to be within the limits for these proteins. Such figures assigned by Ramachandran plot represent a good quality and reliability of the predicted models.

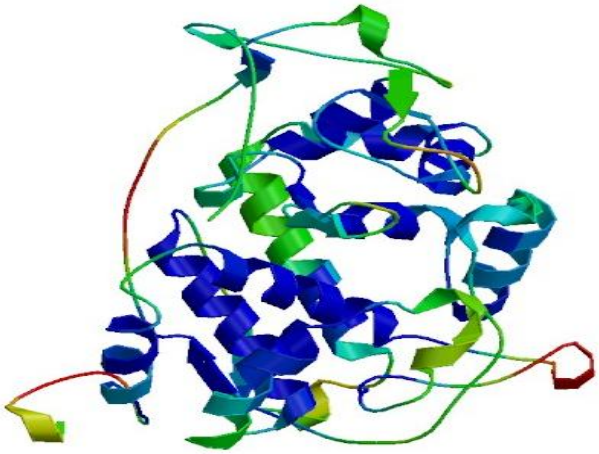

**Figure 1:** Predicted 3D structure of MnP1 antioxidant Protein

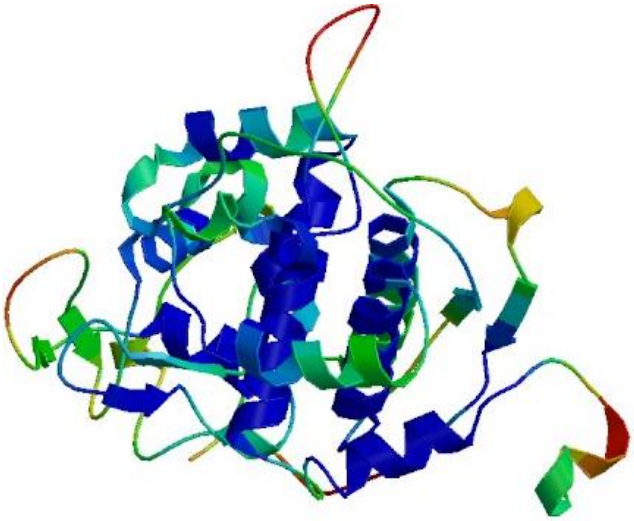

**Figure 2:** Predicted 3D structure of MnP2 antioxidant Protein

Reliability of the model was further checked by ERRAT that analyzes the statistics of non – bonded interactions between atom types and plots the value of the error function versus position of a 9- residue sliding window, calculated by comparison with statistics from highly refined structures. Results from ERRAT were shown in Table 9, represents that the models are of good quality, and the results of the selected models built using swiss model are shown in [**Figure- 3]** and [ **Figure- 4]** for MnP1 and MnP2 respectively.

**[Table 9]**

**Table 9:** Calculated ERRAT values for prediction of model

| Antioxidant Proteins | Servers Used | | |
|---|---|---|---|
| | Swiss Model | $(PS)^2$-$V^2$ | $Phyre^2$ |
| MnP1 | 80.495 | 77.410 | 81.23 |
| MnP2 | 86.943 | 81.138 | 82.51 |

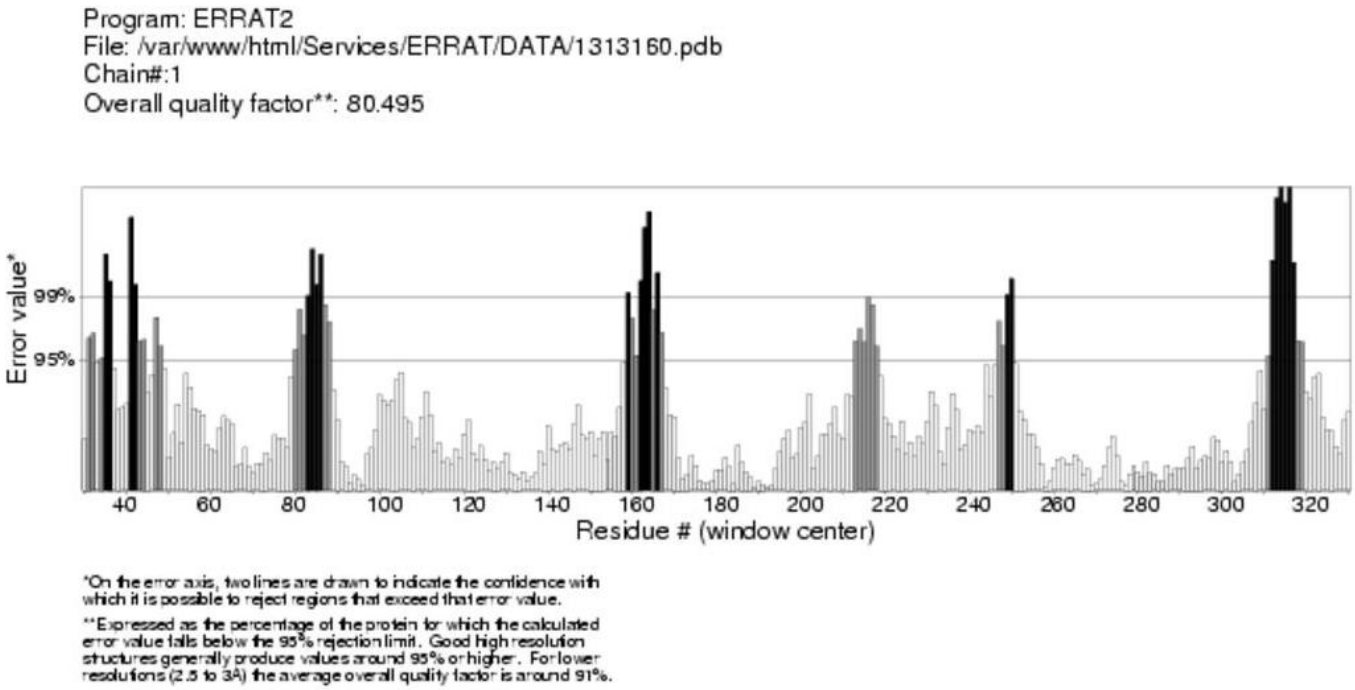


**Figure 3:** Overall quality factor checked by ERRAT for MnP1

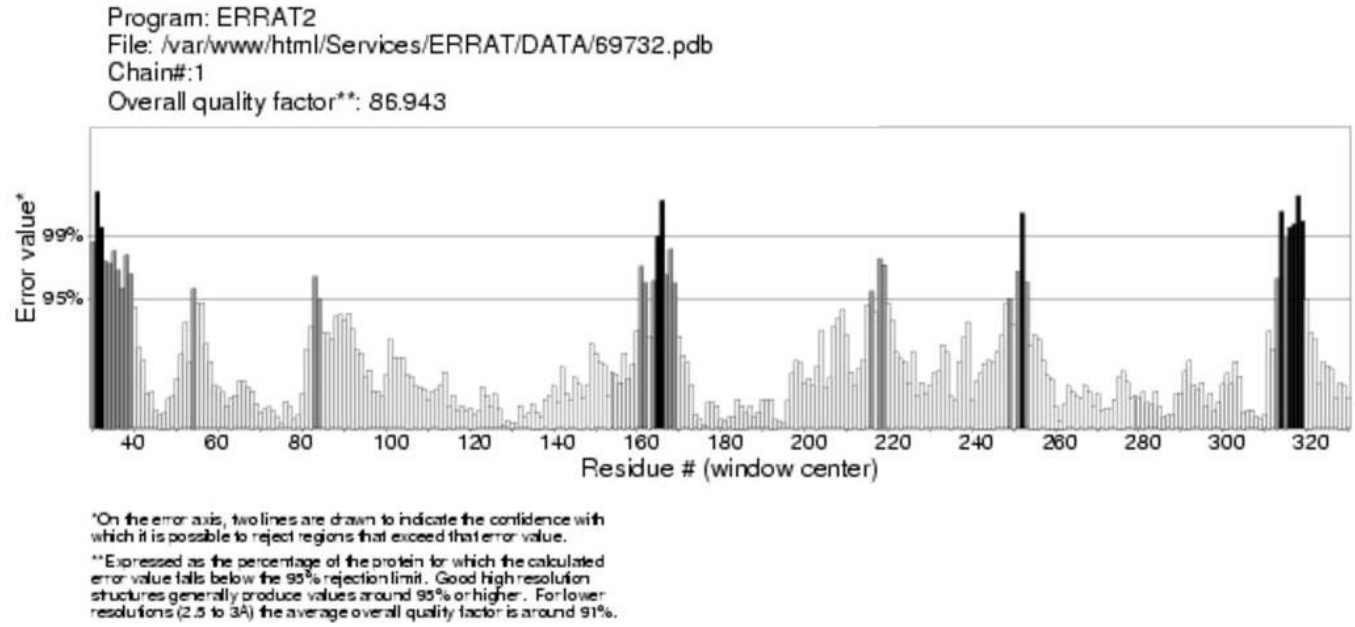


**Figure 4:** Overall quality factor checked by ERRAT for MnP2

Furthermore, superimposition of query and template structure, and visualization of generated models was performed using SuperPose Version 1.0 .It has been found that protein structures with RMSD (Root Mean Square Deviation) Values less than 2 Å are found to be structurally similar. The results of superimposition were shown in Table 10.

**[Table 10]**

**Table 10:** Calculated RMSD values

| Antioxidant Proteins | Accession Number | RMSD Values |
|---|---|---|
| MnP1 | E2FB79 | 0.27 |
| MnP2 | H9C7S6 | 0.74 |

## 4. Conclusion

Our main objective of this study was to perform sequence analysis, structure analysis and homology modeling on Hericuim antioxidant proteins MnP1 and MnP2, since no reports regarding this available. We used various sequence and structure analysis tools that helped in understanding of the sequence and its structure. Physico chemical characterization were performed by computing isoelectric point, molecular weight, total number of positive and negative residues, extention coeffient, instability index,aliphatic index ,grand average hydropathy (GRAVY). Functional analysis of these proteins was performed by PRED-TMR. For these proteins disulfide linkages, motifs and profiles were predicted. Furthermore, protein was functionally annotated by using Prosite and by searching

conserved domain of the protein. As a part of present study, we used homology modeling approach to propose the first 3D structure of MnP1 and MnP2 antioxidant proteins. The overall stereochemical property and quality and reliability of the modeled proteins were assessed by Ramchandran plot analysis and ERRAT. Furthermore, superimposition of query and template structure, and visualization of generated models was performed using SuperPose Version 1.0.The predicted 3D structure will provide more insight in understanding the structure and function of the proteins.

These structures will provide a good foundation for functional analysis of experimentally derived crystal structures. More over these structures can be used for drug designing, understanding the iron induced Parkinson's Disease conditions and also can use as manganese containing antioxidants supplements specially for iron induced - Parkinson's disease treatment.

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