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In silico Characterization, Homology Modeling and Virtual Screening of Selected Natural Compounds as Modulators of *Salmo salar* and *Teratodon nigrovirdis* Tyrosinase Protein

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A B S T R A C T

Colour patterns are the most distinct phenotypic traits in the majority of the living organism including fishes and considered as one of the main influencing factors in consumers' buying decisions. Color traits are regulated by mainly four types of pigments stored in chromatophores. Melanin, is one of the most important pigment of cells, responsible for the coloration. Tyrosinase is a characteristics enzyme in melanin biosynthesis. Modulation of tyrosinase is of significance in the ornamental fish industry, food fishes as well as in humans. By modulating the tyrosinase activity, it is possible to alter the body pigmentation and will help to develop different colour varities of fish and also treatment of hyper pigmentation disorder. The green spotted puffer fish (*Teratodon nigrovirdis*), an ornamental fish and Atlantic salmon (*Salmo salar*), an important aquaculture species were selected because of their commercial importance. The present study was aimed to characterize the tyrosinase enzyme of green spotted puffer fish (*Teratodon nigrovirdis*) and Atlantic salmon (*Salmo salar*) and to identify the natural compounds as putative modulators of tyrosinase. 3D models of tyrosinase protein were predicted by performing a comparative homology modeling program with the help of Chimera 1.1.3, Modeller and Swiss-model, and predicted structure was validated by the energy minimization method using SAVES Server including Procheck, Verify 3D and Errat tools. The physicochemical properties revealed that the fish tyrosinase protein is hydrophobic in nature, acidic, having a high extinction coefficient, unstable, thermolabile for a range of temperatures. The functional properties identified that *T.nigrovirdis* and *S. salar* tyrosinase protein sequence had transmembrane-segment and random coils dominated the secondary structure followed by an alpha helix, extended strands and beta turns. The results obtained from the Saves server showed that the models predicted by Swiss-model were more reliable compared to models developed by other tools. A structure-based virtual screening method helped to identify tyrosinase modulators. Three compounds out of 13000 from the ZINC database were identified as putative modulators of fish tyrosinase having binding energy -8.7 Kcal/mol to -8.2 Kcal/mol. Among which, 3-Methyl-2-phenylquinoline-4-carboxylic acid, 2-(4-chlorobenzyl)-5-(3-nitrophenyl)-2H-1,2,3,4-tetraazole and Kaempferol were considered as potential putative modulators as they had low binding energy and had acceptable properties for standardized drugs. Further, these compounds can be tested in-vivo for their efficacy and usefulness in altering the color pattern of the fishes.

Introduction

Colour and its patterns are the most distinct phenotypic traits in the majority of the living organism including fishes and are the result of diverse pigments synthesized by chromatophores. Melanins are unique pigmented biopolymers synthesized by melanocytes and deposited within melanosomes. The chemical nature of melanin along with its size, number, shape and distribution governs skin pigmentation. Tyrosinase is one of the key enzyme of melanogenesis pathway where it oxidizes L-tyrosine to melanin. In melanogenesis, tyrosinase oxidizes tyrosine to dopaquinone; a reaction which is the rate-determining step in the synthesis of melanin since the remainder of the reaction sequence can spontaneously proceed at the physiological pH values (Halaban *et al.,* 2002). Tyrosinase gene family is categorized into three groups viz., tyrosinase (Tyr, TYR), tyrosinase related protein-1(Tyrp-1, TYRP-1) and tyrosinase related protein-2 (trp-2, TRP-2) and widely available in plants, animals, microorganism, etc. ((Wang *et al.,* 2007; Bagherzadeh *et al.,* 2015). Tyrosinase plays a major role in photoprotection, and thermoregulation, however, the accumulation of the abnormal amounts of melanin in different parts of the skin results in various kinds of physiological conditions (Hasegawa, 2010; Rao *et al*., 2013). Alteration in melanin pigment synthesis also affects the appearance, taste, nutrition values of agricultural and horticultural goods and leads to economic loss (Artés *et al*., 1998). Color and its patterns are also the main influencing factors in consumers' buying decisions for food fishes as well as ornamental fishes. Therefore, introducing more efficient modulators is of great importance in medical products and industry including agricultural, aquaculture and cosmetics. Salmonids fish of the genera *Salmo* attract the attention of both researchers and the general public, on account of their biological characteristics and wide use in aquaculture, sport and recreational fishing, and research (Garcia de Leaniz *et al*., 2007). It has been widely reported that the melanin-based coloration in *Salmo salar* reduces their acceptability in the commercial market and it is of importance to understand the tyrosinase structure and functions along with its putative modulators which will help the salmon industry. The green spotted *Teratodon nigrovirdis*, an ornamental fish is a vertebrate model organism and the knowledge of tyrosinase of this species will help to extend it to other species also. Homology modeling and molecular docking have recently developed as a powerful method complementing traditional high through put screenings. Computational chemistry and chemo informatics play an important role in preliminary drug research. Keeping these points in mind the present study was taken up with objectives of In silico characterization, Homology modeling and virtual screening of selected natural compounds as modulators of *Salmo salar* and *Teratodon nigrovirdis* tyrosinase protein.

Materials and Methods

Sequence retrieval

The tyrosinase family like tyrosinase (tyr), tyrosinase related protein-1a (tyrp-1a, tyrp-1) tyrosinase related protein-1b (tyrp-1b) and Dct protein sequences of *Teratodon nigrovirdis* and *Salmo salar* retrieved in FASTA format from UniprotKB, a public domain protein database. Six unique proteins were selected and considered for this study (Table 1).

Primary structure analysis

The physicochemical parameters were computed using the Expasy's ProtParam tool **(**http://web.expasy.org/protparam/).

Transmembrane region analysis

The identification of transmembrane regions of a protein was determined by the SOSUI server **(***harrier.nagahama-i-bio.ac.jp/sosui/).* Hydrophobicity score and plot were obtained using the Kyte and Doolittle method by keeping a window size of 7 (Figure 1).

Secondary structure analysis

SOPMA server (https://npsa prabi.ibcp.fr/NPSA/npsa_sopma.html) was used to predict the secondary structure of the protein in the form of α-helical, β-strand and coiled regions in percentages.

Tertiary structure prediction

The modeling of the 3D structure of the tyrosinase amino acid sequence of *Teratodon nigrovirdis* (H3CX59) and *Salmo salar* (Q19VI0) was predicted by SWISS-MODEL, and Chimera 1.1.2. The predicted model was validated by SAVES including PROCHECK, verify 3D, and ERRAT server. Python molecular viewer (PyMol) was used to visualize the tertiary structure of the protein (Sanner, 1999).

Naturals compounds library preparation

A total of 13000 natural compounds were downloaded from ZINC12 Database (www.zinc.org) in SDF format. The compounds were prepared for docking study into PDB format followed by PDBQT with the help of Openbabel software.

Virtual screening and molecular docking

Grid box, for docking, was made with the help of autodock tool 4.2. The docking was based on complete structure docking and at different active sites positions.

Ligand prediction

The criterion for selecting the ligands was based on their lowest binding efficiency, threshold value being -7 kcal/mol.

Toxicity analysis

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties was tested by ADMETsar server and lipinski rule of five (*www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp*).

Results and Discussion

Primary structure analysis

The primary structure analysis was done and different parameters computed using the Protparam tool were tabulated in table 2. The analyses of the amino acid sequences revealed that the primary structure of the majority of the fish tyrosinase sequence is hydrophobic in nature due to the presence of high non-polar residue. Moreover, the primary structure analysis revealed that the *Teratodon nigrovirdis and Salmo salar* fish tyrosinase have high residues of acidic and basic amino acid, this might be involved in salt bridge formation.

Physicochemical characteristics

The Molecular weight of the protein was calculated by adding the average isotopic masses of amino acids in the target protein and the average isotopic mass of one water molecule. The value of molecular weight ranged from 47576 to 60985.88 Dalton (Table 2). Isoelectric point (pI) is the value at which the surface of the protein is covered with the charge but the negative and positive charges are equal hence, the net charge of the protein is zero. In the present study, the value of the

Isoelectric point ranged from 5.44 to 6.15 (< 7) reveals that all of them are acidic in nature except Q19VI1 (Table 2). The calculated isoelectric point (pI) will be useful because at pI, solubility is least and mobility in an electro focusing system is zero (Arora *et al*., 2009). The extinction coefficient of tyrosinase at 280 nm is ranging from 75205 to 117185 M^{-1} cm^{-1} with respect to the concentration of Cys, Trp, and Tyr. The high extinction coefficient indicates the presence of a high concentration of Cys, Trp and Tyr. The Instability Indices (II) for the proteins was above 40 with the value ranging from 38.81 to 54.98 (Table 2), which indicated that they are less stable within a solution except for Q19VI1. All the proteins had negative Grand Average Hydropathy (GRAVY) scores with the value ranging from -0.230 to -0.413, which meant that they are hydrophilic in nature and have better interaction with water. The Aliphatic Index (Ai) evaluates the relative volume of the protein occupied by the aliphatic side chains. Based on the results attained, it indicated that AI values were average with the value ranging from 68.75 to 78.88, which indicated that the proteins would vary over an array of temperatures.

Transmembrane analysis

The SOSUI server performs the identification of transmembrane helices with their corresponding length and differentiates membrane proteins from soluble proteins (Hirokawa *et al*., 1998). The present study revealed that all selected proteins were membrane proteins having one transmembrane helix except Q19VI0 (Table 3). The transmembrane region predicted was found to be rich in hydrophobic amino acids and it is also validated by Kyte and Doolittle mean hydrophobicity plot (Figure 1) in which many points lie above the 0.0 line and a clear peak was observed in a plot that indicates about transmembrane helix.

Secondary structure analysis

SOPMA server was used to derive quantitative values for the number of alpha-helices, beta sheets and coils present within the amino acid stretch of the protein. The predicted secondary structure of tyrosinase in the present study revealed that random coils dominated the secondary structure followed by the alpha helix, extended strands and beta turns for all sequences. While other features of secondary structure such as 310 helix, Pi helix, Ambiguous states, Bend region, and Beta Bridge were not found (Table 4). Random coils refer to the disordered and rapidly fluctuating set of conformation assumed by denatured protein and other proteins in solution (Rungwala *et al*., 2011).

Table.1 Tyrosinase gene family retrieved from UNIPROTKB

S.NO	ACCESSION NUMBER	SEQUENCE DESCRIPTION	ORGANISM
01	Q19VI0	Tyrosinase	*Salmo salar*
02	Q19VI1	Dopachrome tautomerase	
03	Q19VH9	Tyrosinase-related protein 1	
04	H3CX59	Tyrosinase	*Teratodon nigrovirdis*
05	H3D173	Tyrosinase-related protein 1	
06	H3BZP5	Dopachrome tautomerase	

Table.2 Amino acid composition of considered Tyrosinase (in percentage) computed using the ExPasy tool

S.NO	Accession Number	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
01	Q19VI0	5.9	6.6	4.6	4.6	2.8	4.1	5.3	9.4	2.8	3.3	8.7	1.3	3.5	5.2	5.7	6.8	7.0	2.2	4.4	5.9
02	Q19VI1	5.4	5.6	6.4	6.8	3.1	3.1	3.9	7.7	3.1	3.1	9.8	2.9	1.4	6.0	5.6	7.7	6.4	1.9	3.5	6.8
03	Q19VH9	7.3	6.2	6.4	5.9	1.7	4.3	4.3	5.7	2.8	5.0	7.1	1.2	1.9	5.5	7.3	6.4	7.8	2.1	4.0	7.1
04	H3CX59	6.9	7.4	5.0	6.7	2.8	3.3	4.6	7.2	3.1	1.7	10.4	2.0	2.6	4.4	6.7	8.3	4.3	3.0	3.5	6.1
05	H3D173	7.9	7.4	5.3	5.9	3.2	4.7	4.0	7.8	3.0	3.8	6.0	1.1	1.7	5.7	7.2	6.8	5.5	1.9	3.4	7.8
06	H3BZP5	5.4	6.4	6.7	5.6	3.1	3.1	5.0	7.5	2.5	4.2	10.0	2.1	1.3	6.7	6.4	6.9	5.8	1.9	3.1	6.2

Table.3 The physicochemical parameter of considered Tyrosinase is computed using the ExPasy ProtParam tool

S. NO.	Accession Number	No of amino acid	M. wt.	PI	(-) R	(+) R	EC	II	AI	GRAVY
01	Q19VI0	543	60985.88	5.77	54	43	102635	47.60	69.67	-0.286
02	Q19VI1	518	57980.18	5.80	55	44	82820	38.81	75.44	-0.291
03	Q19VH9	422	47576.38	5.44	43	31	75205	47.04	75.09	-0.307
04	H3CX59	540	60982.65	5.95	61	51	117185	54.98	71.52	-0.413
05	H3D173	529	58929.13	6.15	52	45	82820	43.07	68.75	-0.328
06	H3BZP5	519	58430.01	5.66	55	44	79840	41.25	78.88	-0.230

Table.4 Transmembrane region identified by SOSUI Server

S.No	Accession number	N terminal	Transmembrane region	C terminal	Type	Length
01	Q19VI0	1	MVLLVVLGSLLQMLFLRSCVGQF	23	PRIMARY	23
		481	QWLLGAGLIGAILAGIVMTTGAL	503	PRIMARY	23
02	Q19VI1	468	VFVLGSTLGGVFLGLLVLLLVLV	490	PRIMARY	23
03	Q19VH9	366	TEIITIAMVAALVIVAVIFAATT	388	PRIMARY	23
04	H3CX59	1	QPALMWLVIFTGILLNVTPSHQQ	23	PRIMARY	23
05	H3D173	4	RVWIMLARCVFLLLSAAVVRAQF	26	PRIMARY	23
06	H3BZP5	474	TEIITMGVVIALVVVAVIFAATT	496	PRIMARY	23

Table.5 Calculated secondary structure elements by SOPMA Server

S.NO	Accession Number	Alpha helix	Extended	Beta turn	Random coil
01	Q19VI0	30.94	11.23	1.47	56.35
02	Q19VI1	27.99	13.51	2.90	55.60
03	Q19VH9	32.94	10.90	0.71	55.45
04	H3CX59	30.74	11.85	2.04	55.37
05	H3D173	29.11	10.78	2.27	57.84
06	H3BZP5	25.63	13.49	2.41	58.57

Table.6 Validation of modeled protein structure using different tools

Servers	Property	Q19VI0	H3CX59
SWISS MODEL			
ERRAT	Overall Quality factor	91.4425	89.3365
Verify3D	(3D-1D score > 0.2)	92.24	92.91
PROCHECK	Residues in most favored regions	86.1%	84.7%
	Residues in additional allowed regions	12.5%	14.2%
	Residues in generously allowed regions	1.4%	1.1%
	Residues in disallowed regions	0.0%	0.0%
CHIMERA 1.1.2			
ERRAT	Overall Quality factor	58.8621	54.9784
Verify3D	(3D-1D score > 0.2)	77.53%	77.96%
PROCHECK	Residues in most favored regions	89.5%	87.3%
	Residues in additional allowed regions	8.9%	11.0%
	Residues in generously allowed regions	1.1%	1.5%
	Residues in disallowed regions	0.4%	0.2%

Figure.1 Kyte and Doolittle hydropathy plot for Tyrosinase of *Salmo salar*

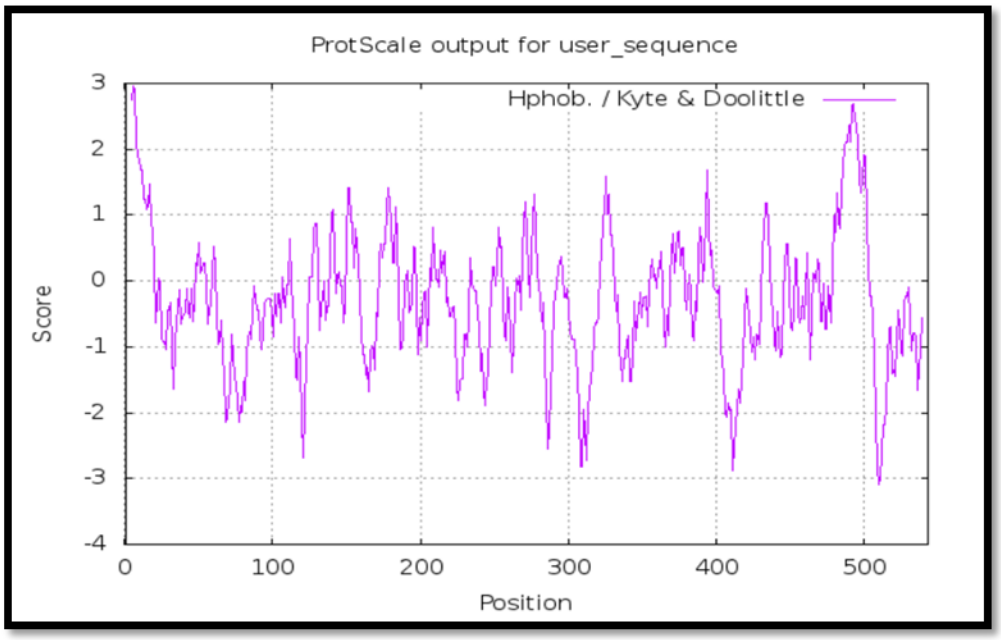


Fig.2 Ramachandran plot of the predicted Tertiary structure of Tyrosinase protein of *Salmo salar* (Q19VI0) by Procheck (Saves Server)

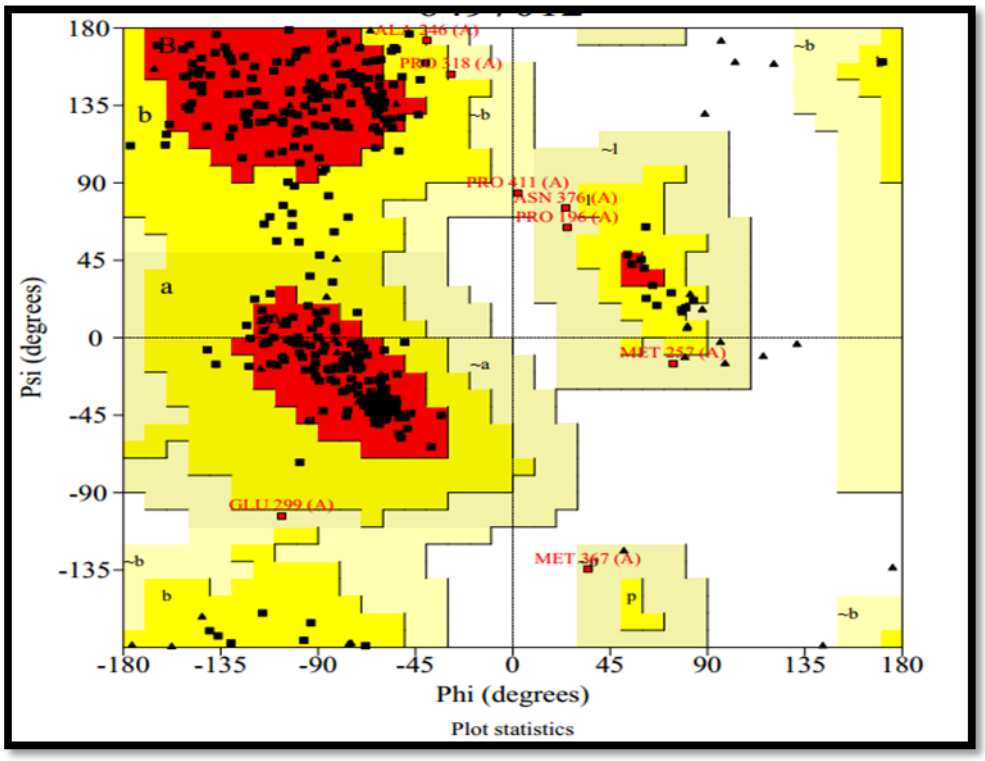


Fig.3 Ribbon representation of predicted tertiary structure of tyrosinase protein of *Salmo salar* using Swiss- model (Helix - cyan, Sheet- orange red, Loop – Magenta)

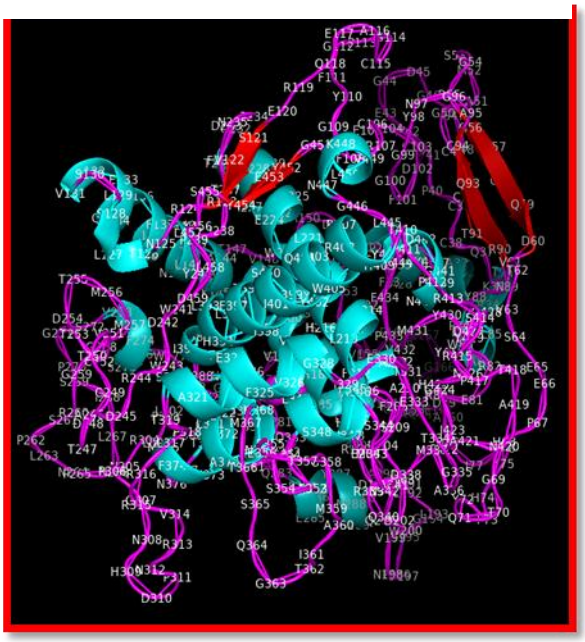


Fig.4 Surface representation of predicted tertiary structure of tyrosinase protein of *Teratodon nigrovirdis* using Swiss- model (Helix - cyan, Sheet- orange red, Loop – Magenta)

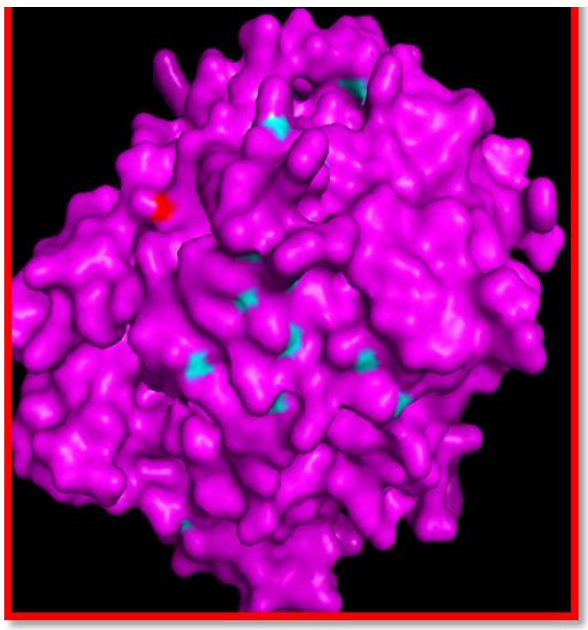
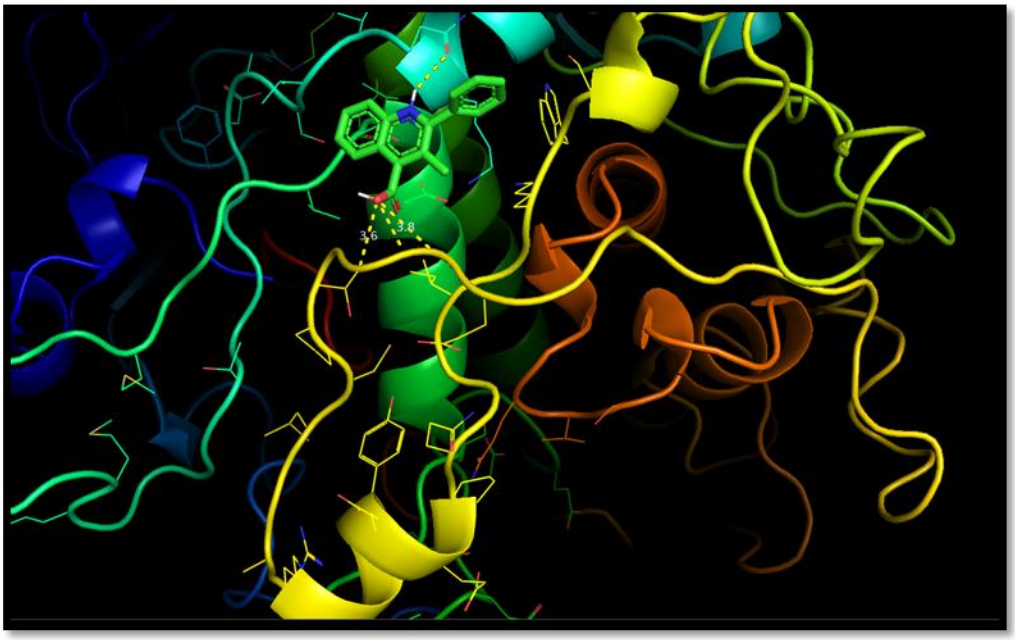


Table.7 Top 3 compound after virtual screening and toxic properties analysis

S.NO	ZINC ID	BINDING EFFICIENCY (kcal/mol)
01	ZINC35860	-8.7 kcal/mol
02	ZINC00090084	-8.5 kcal/mol
03	ZINC00119983	8.2 kcal/mol

Fig.5 Predicted Docked structure of Tyrosinase protein of *Salmo salar* using Autodock vina, Viewed in PyMol



Tertiary structure prediction

SWISSMODEL was used to predict the 3D structures of proteins. 5m8l.1 (5, 6-dihydroxyindole-2-carboxylic acid oxidase) were selected as templates from the PDB database for Q19VI0 and H3CX59 respectively based on sequence identity (45.14% and 45.71%). The final modeled structures are shown in Figure 2**.** The amino acid residues 86.1% and 84.7% lie in the most favored regions of Ramachandran Plot as revealed by PROCHECK analysis for the structure modeled for Q19VI0 and H3CX59 respectively. The predicted structures conformed well to the stereochemistry indicating reasonably good quality and were used for further analysis.

Grid box dimension

Tyrosinase protein sequence of *Salmo salar* and *Teratodon nigrovirdis* was selected for virtual screening against 13000 compounds downloaded from the Zinc database. The grid box parameter values were adjusted to confirm the maximum binding affinity and best conformational pose of ligand – protein complexes. The grid parameter values for *Salmo salar* and *Teratodon nigrovirdis* tyrosinase were adjusted into specific coordinates having center_x (37.2354832692), center_y (29.5828041517), center_z (-17.6576), size_x (62.0255238487), size_y = (63.5933591266), size_z = (25.0000) in angstrom.

Virtual screening

A virtual screening experiment was employed on all ligands against the predicted structure of *Salmo salar* and *Teratodon nigrovirdis* tyrosinase by Autodock vina. The protein – ligand docked complexes were further evaluated on the lowest binding energy (kcal/mol) values. The best docked energy complexes with the lowest binding values were visualized in PyMol. The estimated ADME properties proved that selected compounds were suitable for usage in fishes. Three compounds out of 13000 from the ZINC database were identified as putative modulators of fish tyrosinase having binding energy -8.7Kcal/mol to -8.2Kcal/mol and rmsd 0.000 from lower bound as well as upper bound. Among which, 3-Methyl-2-phenylquinoline-4-carboxylic acid, 2-(4-chlorobenzyl)-5-(3-nitrophenyl)-2H-1,2,3,4-tetraazole and Kaempferol were considered as potential putative modulators as they had low binding energy and had acceptable properties for standardized drugs.

In conclusion, the knowledge acquired by the results of the predicted tertiary structure of *Salmo salar* and *Teratodon nigrovirdis* Tyrosinase protein provides an insight into tyrosinase protein structure and function. The *Salmo salar* and *Teratodon nigrovirdis* tyrosinase protein is hydrophobic in nature due to the presence of non-polar residue.

The identified modulators may be significance to determine its modulating activity in the treatment of some dermatological diseases associated with melanin hyper pigmentation in human as well as food fishes, preventing undesirable browning of fruits and vegetables that change their color, taste, and nutritive values occur as a result of over activity of the enzyme.

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