Original Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INSILICO CHARACTERISATION OF PROTEINS OF *SALVINIA MOLESTA* D.S.MITCHELL AN AQUATIC WEED AND ASSESSMENT OF NANOPARTICLE SYNTHESISING ABILITY OF CLOSELY RELATED PLANT SPECIES

***[1]T. G. NITHYA,[1]SWETHA K ,[2] JAYANTHI J AND [3]RAGUNATHAN MG**

[1]Department of Biotechnology, Faculty of Science and Humanities, SRM University Kattankulathur, India.
[2]G.S.Gill Research Institute, Guru Nanak College,Chennai-India
[3]Department of Advanced Zoology and Biotechnology,Guru Nanak College,Chennai-India.

ABSTRACT

The main aim of the study is to analyse the proteins of *Salvinia molesta* using various bioinformatics tools.The proteins were structurally as well as functionally characterized using tools like protparam,SOPMA and SOSUI. NAD(P)H quinone oxidoreductase protein in *Salvinia molesta* was selected for evaluation and protein BLAST was performed.Primary structure prediction were performed to evaluate physical and chemical characteristics of proteins *viz*.,Molecular weight, pI etc.The secondary structures like alpha helix, beta turns etc.,were studied.Transmembrane protein was identified and analysed.NAD(P)Hquinone oxidoreductase possess the abilty to reduce metals and hence *Salvinia molesta* was predicted for its ability to synthesis nanoparticles.Finally, phylogenetic analysis was carried out using NJ plot.Phylogenetic tree revealed the closeness of *Salvinia molesta* with plants like *Azolla caroliniana, Lygodium japonicum* etc.,revealing the efficacy of these plants also in synthesising nanoparticles.

KEYWORDS: ***S.molesta*,BLAST,Expasy,NAD(P)H,quinine Oxidoreductase, Nanoparticles.**

T. G. NITHYA
Department of Biotechnology, Faculty of Science and Humanities, SRM University Kattankulathur, India.

INTRODUCTION

Back from ancient time people were searching for cure or medicament for various diseases and looked for drugs in nature.[1] Pants play a chief role in our life.They are often considered as treasure house which has raw materials for drug production.[2] Medicinal plants were widely used by man to treat illness caused by pathogenic microorganisms.The presence of bioactive compounds in medicinal plants play a significant role in curing diseases.The use of medicinal plants in drug production is a safe process as there is mostly no side effects seen after their consumption.*Salvinia molesta* is a floating fern which is commonly known as Kariba weed or Giant Salvinia. *Salvinia molesta* is native to Brazil .They belong to the *Salviniaceae* family.They are Pteridophytes as they reproduce and disperse through spores.*Salvinia molesta* when submerged in water has an extraordinary property of long-term air retention on the surface of the leaves due to their unusual microstructure.[3] *Salvinia molesta* grow in ponds , ditches, rivers, marshes, slow-flowing streams , rice fields and lakes.[4] Giant *Salvinia* grows the best in high light intensities and warm temperatures..When the nutrients like nitrogen and phosphorus are present in large amounts in the water it leads to faster growth of *Salvinia molesta*.If the buds of *Salvinia molesta* are exposed to -3°C , it may lead to the death of this fern but their leaves have the ability to survive in very cold conditions if they are under water or submerged.[5] The rapid and aggressive growth of the fern *Salvinia molesta* has lead to the overall coverage of water bodies inhibiting the growth of the native vegetation, as it removes almost all the available nutrients.Death of *Salvinia molesta* causes the aquatic life to perish because the mats of the dead *Salvinia molesta* sinks to the bottom to decay and also simultaneously take up the dissolved oxygen needed by the fishes.[6] Thus *S.molesta* is considered as largest noxious weed in the world and menace to aquatic environment. Still,controversially *S.molesta* exhibited potent antioxidant and antibacterial activity against selected pathogens and also exhibited significant cytotoxic activity against cancer cell lines.[7] Insilico approach are widely used by researchers to analyse and characterise various proteins present in different plant species.Different bioinformatics tools were used for their analysis.There are various drawbacks in experimental work to study and characterise proteins of an organism using wet lab methodologies and hence insilico analysis gives a valuable data in this regard.There are exorbitant cost and additional time involved in experimental methods. So accurate results can be obtained by Insilico approach whereas the possibility of obtaining results by experimental work cannot be guaranteed.[8] Nanotechnology is a branch of science which deals with the ability to control and manipulate matter ranging from a scale less than a nanometre up to 100 nm.[9] Nanoparticles are nano scaled particles which are less than 100 nm in diameter.Nanoparticles were conventionally produced by physical and chemical process but these methods were costly and needed many trained professional working on it. Nanoparticles synthesis by this methods was also found to be hazardous to the environment around us.Thus an alternative method using plant extracts are used to synthesise nanoparticles. This method using plant extract is inexpensive and not hazardous to the environment .[10] Lately nanoparticles are used widely in modern medicine. They are used for gene and drug delivery to treat serious illness.[11] Thus this study is to analyse the ability of *S.molesta* and its related species to synthesise nanoparticles.Using bioinformatics tools, the proteins of *S.molesta* were indentified and their importance was studied thoroughly by examining their structure and functional characteristics.The results obtained were used to form hypothesis so that successful experimental work can be carried out in predicted plants.Eleven different proteins were identified in *S.molesta* out of which NAD(P)H quinone oxidoreductase were subjected for further analysis as it plays a crucial role in nanoparticles synthesis in plants.

MATERIALS AND METHODS

Sequence Retrieval

The FASTA sequence of the proteins present in the plant *Salvinia molesta* was obtained from Genbank database from NCBI(http://www.ncbi.nlm.nih.gov).[12] (Table 1)

Primary Structure prediction

The primary structure prediction for the proteins present in *Salvinia molesta* was obtained using Expasy's protparam tool. This tool computes different physio - chemical characteristics like molecular weight, theoretical pI, amino acid composition, atomic composition, estimated half-life, extinction coefficient,aliphatic index ,instability index, and GRAVY (grand average of hydropathicity of the proteins) (http:// www.us. expasy. org/ tools /protparam .html).[13] Table2)

Secondary Structure Prediction

The secondary structure prediction for the proteins was done using SOPMA (Self Optimized Prediction Method with Alignment).The results are obtained by analysing the FASTA sequence of the proteins. (Table 3)

Functional characterization

Functional characterization was done by SOSUI server. This server provided the information about presence or absence of transmembrane and their solubility. (Table 4).

Table 1
Proteins of Salvinia molesta

Accession Number	Name of the Protein
S5CJ29	Photosystem II D2 protein
AOA23J5H1	NAD(P)H quinone oxidoreductase subunit
B5SUJ6	ATP Synthase beta subunit
B5SUJ5	ATP Synthase beta subunit
Q95CC2	ATP Synthase beta subunit
J7FDL7	Photosystem II D1 protein
B5SUK2	Rubisco large subuni
B5SUK1	Rubisco large subunit
Q95C99	Small Ribosomal protein 4
AOAO23J5H2	Ribosomal proteinL2
AOAO23J5H4	Maturase

Table 2
Parameters computed using Expasy's Protparam tool

Protein	Accession No.	Length	Mol. Weight	Pi	-R	+R	EC	II	AI	GRAVY
Photosystem II D2 protein	S5CJ29	353	46854.0	5.99	53	45	57090	37.99	81.93	-0.260
NAD(P)H quinone oxidoreductase subunit	AOA23J5H1	495	34023.7	7.55	28	29	57675	39.70	121.39	0.724
ATP Synthase beta subunit	B5SUJ6	364	39427.7	5.03	46	35	21890	40.60	89.48	-0.155
ATP Synthase beta subunit	B5SUJ5	382	41296.8	4.94	49	36	21890	40.24	89.84	-0.152
ATP Synthase beta subunit	Q95CC2	394	42594.3	4.96	50	37	21890	39.89	90.81	-0.134
Photosystem II D1 protein	J7FDL7	353	38821.4	5.20	27	15	73005	35.32	96.77	0.373
Rubisco large subunit	B5SUK2	424	46854.0	5.99	53	45	57090	37.99	81.93	-0.260
Rubisco large subunit	B5SUK1	436	48160.5	6.19	54	48	58580	36.29	81.24	-0.267
Small Ribosomal protein 4	Q95C99	201	23010.5	10.78	18	37	9065	40.10	95.52	-0.575
Ribosomal protein L2	AOAO23J5H2	116	13403.3	10.8	9	25	11920	41.65	75.52	-0.748
Maturase	AOAO23J5H4	92	10970.7	10.0	7	16	13075	80.60	94.35	-0.375

Mol.wt- molecular weight, pI - isoelectric point , -R - number of negative residues, +R - number of positive residues, EC - Extinction coefficient , II - instability index , AI - aliphatic index, GRAVY - Grand average Hydropathicity

Table 3
Secondary structure prediction of proteins of S.molesta

Secondary Structure	S5CJ29	AOA23 J5H1	B5SUJ6	B5SUJ5	Q95CC2	J7FDL7	B5SUK2	B5SUK1	Q95C99	AOAO2 3J5H2	AOAO2 3J5H4
Alpha helix	38.81%	41.62%	35.99%	35.08%	35.79%	34.28%	34.43%	33.49%	28.86%	11.21%	30.43%
Secondary helix	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Pi helix	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Beta bridge	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Extended strand	23.51%	23.84%	21.70%	21.99%	21.83%	24.36%	18.16%	18.81%	22.39%	43.97%	27.17%
Beta turn	10.48%	7.27%	8.79%	9.16%	9.14%	11.05%	15.33%	15.37%	9.45%	14.66%	7.61%
Bend region	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Ambiguous states	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Other states	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Random coil	27.20%	27.27%	33.52%	33.77%	33.25%	30.31%	32.08%	32.34%	39.30%	30.17%	34.78%

Table 4
Transmembrane Region Predicted By SOSUI Tool For NAD(P)H quinone oxidoreductase subunit (AOA23J5H1), amino acid sequence of membrane protein and possess 14 transmembrane helices.

S.No.	N terminal	Transmembrane region	C terminal	Type	length
1	**9**	SFGDFPILPESILILGLLTIIAI	31	SECONDARY	23
2	**44**	YRISLVSLLISMALLLYQWNIMS	66	PRIMARY	23
3	**81**	NISRLFLLICSLLSISLSVDYVR	103	PRIMARY	23
4	**106**	KTAMAEFSLFILTAGSGGMLLCR	128	SECONDARY	23
5	**132**	SITVYVALECLGLSSYLLSGYA	153	SECONDARY	22
6	**167**	LSMGGVSSSLLMYGFSLLYGLSG	189	SECONDARY	23
7	**212**	LQTSLALVAAGMAFKLSLVPFHQ	234	SECONDARY	23
8	**247**	VVAFFSVTSKVAALALSTRLFSI	269	SECONDARY	23
9	**280**	IALGILAILSMISGNPIAVTQTS	302	SECONDARY	23
10	**306**	MPAYSSISQIGYIMIGIIAA	325	SECONDARY	20
11	**335**	ITHTFIYIFMNLGTFACIILFSL	357	SECONDARY	23
12	**379**	LSSVLCSSSLGGIPPLSGFFGKL	401	SECONDARY	23
13	**415**	PVSVALITSVISIYYYLKVIKLM	437	PRIMARY	23
14	**465**	VEIAMIFCASASILSGILIDPII	487	PRIMARY	23

SEQUENCE SIMILARITY

BLAST (Basic Local Alignment Search Tool)was performed to evaluate the sequence similarity. Out of the 11 proteins present in *S.molesta* the FASTA sequence NAD(P)H quinone oxidoreductase subunit (AOA23J5H1) was inputted in protein BLAST search.Protein BLAST thus anlysed proteins of different plant species which has regions of sequence similarity with the sequence.(Table 5).

PHYLOGENETIC ANALYSIS

The Phylogenetic analysis for the protein NAD(P)H quinone oxidoreductase subunit (AOA23J5H1) present in *S.Molesta* was performed to analyse the functional and structural similarity among the related species obtained through BLAST.The FASTA sequence of all the proteins was loaded in Clustal X and complete alignment was performed.The alignment files are saved in .dnd format.The NJ plot was downloaded and the sequence in dnd format was given to obtain a phylogenetic tree (Figure 1).

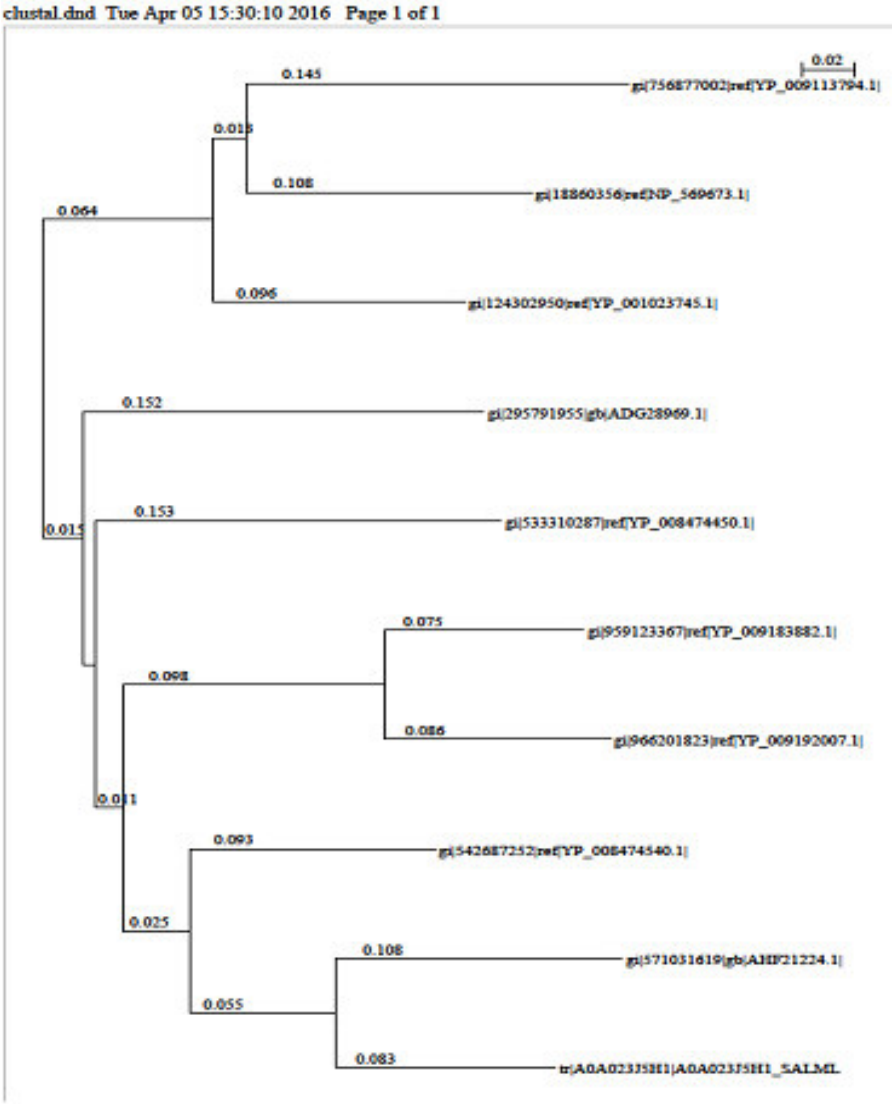
Table 5

List of plants species with higher identity with sequence of NAD(P)H Quinone Oxidoreductase protein in S.molesta

SNO	PLANT NAME	IDENTITY PERCENTAGE
1	*Salvinia molesta*	100 %
2	*Azolla carolinania*	82%
3	*Marsilea crenta*	77%
4	*Angiopetris evecta*	67%
5	*Lygodium japanoicum*	67%
6	*Woodwardia unigemmata*	67%
7	*Gleichenia japonica*	67%
8	*Cyrtomium falcatum*	66%
9	*Psilotum nudum*	65%
10	*Stangeria eriopus*	61%

Figure 1

Phylogenetic Analysis of related plants similar to NAD(P)H quinone oxidoreductase of S.molesta



RESULTS

The primary structure prediction performed using protparam tool from Expasy revealed information about the physical and chemical characteristics of proteins present in *Salvinia molesta.*The result computed using protparam tool indicates that protein Rubisco (B5SUK1) has the highest molecular weight and Maturase (A0A023J5H4) has the lowest molecular weight.The protein NAD(P)H quinoue oxidoreductase has the highest number of amino acids. pI or isoelectric point of 7 proteins of *S.molesta* were less than 7 which indicates that they are acidic. pI of 3 proteins were more than 7 which indicates they are basic.Protein NAD(P) H quinone oxidoreduclase was found to be neutral.Instability index helps in estimating the stability of the protein.Proteins whose instability index lesser than 40 are stable in nature.The computed instability index values by protparam revealed that 6 proteins of *Salvinia molesta* are stable in nature.The Aliphatic index of the proteins ranged from 81.24 - 121.39. GRAVY value refers to the degree of hydrophobicity and hydrophilicity of the amino acids present in the proteins.The lesser the GRAVY value,better the interaction with water.Ribosomal protein L2 of *S.molesta* has the least GRAVY values.The Secondary structure prediction of the proteins in *S.molesta* indicated that alpha helix, extended strand, beta turns and random coils are predominantly found in them.SOSUI server predicted that the protein NAD(P)H Quinone oxidoreductase is soluble in nature.Protein BLAST was carried out using the FASTA sequence of the protein NAD(P)H quinone oxidoreductase.It revealed that many proteins of different plant species have regions in their sequence similar to the protein NAD(P)H quinone oxidoreductase of *S.molesta*.

DISCUSSION

In the present study *Salvinia molesta* an abundantly available aquatic weed species were chosen and subjected for *insilico* methods to compare the closeness in sequence of NAD(P) H quinone oxidoreduclase ,a protein responsible for nanoparticle synthesis.The phylogenetic tree obtained ,revealed the distant relationship of *Salvinia Molesta* with *Stangeria eriopus,Psilotum nudum and Angiopterius evecta and Gleichenia japonica* (below 68%)whereas they are closely related related to *Azolla caroliniana, Lygodium japonicum* and *Crytomium falcatum.*Similar kind of *insilico* analysis were not performed using aquatic weeds till date.A large number of medicinal plants are being exploited and evaluated from nature for the profitable production of drugs[14] .Green nanotechnology refers to the process of synthesising nanoparticles from plants and microbes.Protein assays revealed that NADH dependant oxidoreductase is the main reason for nanoparticles synthesis.The NADH is oxidised to NAD + by reductases and so it gains ability to reduce metal ions.[15] The protein NAD(P)H quinone oxidoreductase of *Salvinia molesta* are concentrated in this study because it is responsible for synthesis of nanoparticles.On performing sequence similarity and Phylogenetic analysis of several plant species which are particularly weeds were found to be similar to *S.molesta* .Therefore, these weeds including *Salvinia molesta* can be used to synthesise nanoparticles and therapeutic agents as they are abundant and available free of cost.

CONCLUSION

The present study is an cost effective method to find relevant plant species possessing nanoparticle synthesing ability when provided with suitable precursors which can be further carried out for laboratory experimental methods to obtain significant nanoparticles by green synthesis from plants.

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