



## STUDY ON INSILICO ANALYSIS OF PROTEINS OF *RHINACANTHUS NASUTUS-ACANTHACAE*

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### ABSTRACT

Medicinal plants are staging a comeback and 'renaissance' is happening all over the globe. The Medicinal plants products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. So the medicinal plants have a promising role in to prevent and as well as to cure the diseases. Andrographis is an important genus of the family Acanthaceae known for its ethanomedicinal claims and for a variety of medicinal properties. *Rhinacanthus nasutus* has been used in various folk medicinal preparations and its chemical composition and pharmacological activities have been elaborated recently. Functional characterization of a protein sequence is one of the most frequent problems in biology. This task is usually facilitated by accurate three-dimensional (3-D) structure of the protein. The number of protein sequences that can be modeled, as well as the accuracy of the prediction, is increasing steadily because of the growth and number of known protein sequences and structures as well as improvements in the modeling software. It is currently possible to model, with useful accuracy. Significant parts of approximately one half of all known protein sequences. This research report render a futuristic summary of bioinformatics study of *Rhinacanthus nasutus* with emphasis on the current development and future directions, which shall provide tools and resources necessary to understand and uphold advances in this important field. The aim of the present study, 2 proteins of *Rhinacanthus nasutus* were analysed using bioinformatics tools. Structural prediction and functional characterization of proteins of *Rhinacanthus nasutus* were done using Expasy Protparm server, 3D structure was done using SWISS MODEL. Plants of different family showing identity 80% and above were selected and its sequences retrieved, aligned using Clustal Omega. phylogenetic tree was constructed for the aligned sequence. Structure prediction showed that  $\alpha$  – helix, random coil,  $\beta$  – turn and extended strand predominates. Phylogenetic analysis of pbsA of *Rhinacanthus nasutus* reveals that the Plants of *Martynia annua*, *Proboscidea Fragrans*, Veronica glandulosa, *Salvia castanea*, *Duranta erecta*, *Paeonia*

lactiflora, *Lithospermum lactitolium*, *Dioscorea subcalva* and *Mandenovia komarovii* family are closely related. Hence the present article includes the detailed exploration of Computational tools of protein, and bioinformatic aspects of *R.nasutus* in an attempt to provide a direction for further research.

**Key words:** *Rhinacanthus nasutus*, Pharmaceutical uses, Photosystem II Protein ( psbA), Bioinformatics.

## INTRODUCTION

Herbal drugs are the potential source of therapeutic aid for the treatment and prevention of number of ailments. Plants and plant based medications have been man's prime therapeutic tools since the dawn of civilization and are still among the frontline treatments for a variety of ailments. Medicinal plants are an important resource to traditional society's health care systems. They have always been counting emphases on the herbal medicines as a potential pipeline for novel bioactive molecules. The rich biodiversity of plants make them a treasure house for obtaining new and novel compounds either themselves as drugs or lead molecules for drugs with different mechanism of action. Medicinal plants contain several different phytochemicals or secondary metabolites that may act individually, additively or in synergy to improve health [1].

The different plant extracts have different modes of action for curing diseases. They can also be applied as food preservatives [2]. Deforestation, forest degradation and fragmentation, biological invasion and unsustainable extraction of medicinal parts seems to have adversely affected the populations of native species and brought changes in distribution pattern of various plant species, including *Andrographis*. Genus *Andrographis* (Acanthaceae) is medicinally important taxa of 26 species native to India, with extreme species diversity occurring in southern Peninsular India. Southern part of the Eastern Ghats is comparatively possessing important endemic medicinal plant species and requires attention of conservation.

The drugs are derived from the whole plant or from different parts like leaves, stem, bark, root, flower, seed, etc. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing country such as India; the contribution is as much as 80% [3]. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world.

The genus *Rhinacanthus* comprises of about 25 species confined to the Old World tropics and subtropics. Within the *Acanthaceae* family, it placed in the *Justiciinae* subtype [4]. *R.nasutus* is widely distributed in some parts of sub-continent, in the region of Southeast Asia and China [5]. It is typically found wild in the road bushes and is shade loving perennial shrub commonly known as rangchita.

The *R.nasutus* (Nagamalle) is cultivated particularly as a medicinal plant has been used in treatments and preventions of diverse diseases as folklore medicines. Different parts of *R.nasutus* have used in traditional medicine for the treatment in diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension and several skin diseases [6]. Different parts of *R.nasutus* have used in traditional medicine for the treatment in diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension and several skin diseases. In the some experiments, it has potential effects for treatment of some diseases like to treat cancer, liver disorders, skin diseases, peptic ulcers, helminthiasis, scurvy, inflammation and obesity [7].

In this study two protein sequences of *R.nasutus* were selected and analyzed with the help of computational tools. One such important protein is psbA is a light-driven water: plastoquinone oxidoreductase that uses light energy to abstract electrons from H<sub>2</sub>O, generating O<sub>2</sub> and a proton gradient subsequently used for ATP formation. It consists of a core antenna complex that captures photons, and an electron transfer chain that converts photonic excitation into a charge separation. The D1/D2 (PsbA/PsbA) reaction center heterodimer binds P680, the primary electron donor of PSII as well as several subsequent electron acceptors [8]. In silico approach provide useful information by identifying the primary, secondary and tertiary structure predictions which can be used for further analysis. The king of bitter medicinal plant *R.nasutus* is known to possess medicinal uses, but the biological and pharmacological properties are unexplored. This is the first report of the in silico protein analysis of *R.nasutus*.

## **MATERIALS AND METHODS**

*Rhinacanthus nasutus* used in the study was identified and the reference material has been kept under reference GRD/SC/05/16-17. Fresh whole plant was collected randomly from the region of in around Yercaud *Servarayan* Hills, Salem, Tamilnadu. Fresh plant material was air dried and then homogenized to fine powder and stored in air tight bottle. The extracts were then, dried in vacuum and stored in a refrigerator.



**Fig 1:** *Rhinacanthus nasutus* (Linn.)

### Sequence Retrieval

The FASTA sequence of the proteins [TABLE: 1] were retrieved from Genbank database hosted by the NCBI (<http://www.ncbi.nlm.nih.gov>) [9].

**Primary Structure Prediction:-** For Physio-chemical characterization, theoretical Isoelectric Point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average of hydropathy (GRAVY) were computed using the Expasy Protparam server[10] (<http://us.expasy.org/tools/protparam.html/>).

### Secondary structure prediction

SOPMA (Self Optimized Prediction Method with Alignment) was used for the secondary structure prediction.

### Functional characterization

SOSUI and TMHMM v.2.0 tools were used to characterize whether the protein is soluble or trans membrane in nature. Inter Pro is an integrated resource for protein families, domains and functional sites. Inter Pro incorporates the major protein signature databases into a single resource. These include: PROSITE, which uses regular expressions and profiles, PRINTS, which uses Position Specific Scoring Matrix-based (PSSM-based) fingerprints, ProDom, which uses automatic sequence clustering, and Pfam, SMART, TIGRFAMs, PIRSF, SUPERFAMILY, Gene3D and PANTHER, all of which use hidden Markov models (HMMs). Superfamily and molecular function were predicted by Inter pro protein sequencing and classification[11].

(<http://www.ebi.ac.uk/interpro/>).

### Sequence Alignment

Sequence alignment of psbA was performed using pair wise sequence alignment tool (NCBI- BLAST) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and multiple sequence alignment was done using the EBI-CLUSTAL OMEGA (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) tool. Clustal Omega also has powerful features for adding sequences to and exploiting information in existing alignments, making use of the vast amount of precomputed information in public databases like Pfam [12]. The emphasis of this work was to find the regions of sequence similarity, which in other words allows us to yield functional and evolutionary relationships among the proteins considered in this study.

### Phylogenetic Analysis

The phylogenetic analysis of psbA was performed to determine the number of proteins that share common structural and functional features. As an input to Clustal Omega all sequences in Fasta formats were supplied with default options. The output was analyzed for sequences that are aligned for the complete length, scores, alignment, conserved residues, substitutes and semi conserved substituted residue patterns. The phylogenetic tree was constructed based on the bootstrap Neighbour Joining (NJ) method [13]. The stability of the internal nodes was assessed by bootstrap analysis with 1000 replicates.

### RESULTS AND DISCUSSION:

*Rhinacanthus nasutus* Kurz. (family Acanthaceae) is a valuable plant which is widely distributed and cultivated in South China, Taiwan, India and also in Thailand. *Rhinacanthus nasutus* contains enormous amount of bioactive constituents. In addition, it possesses wide range of pharmacological activities. Hence the plant can be used to treat many diseases, and can be used in various pharmaceutical formulations and drug development studies [14].

**Table 1: PRIMARY STRUCTURE OF PROTEINS OF RHINACANTHUS NASUTUS**

S.NO	ACCESSION NUMBER	PROTEIN	LENGTH
1	ADD48243.1	Ribulose biphosphate carboxylase (rbcL)	234
2	ACF15349.1	Photosystem II protein (psbA)	24

**Table 2: PARAMETERS COMPUTED USING EXPASY'S PROTPARAM TOOL.**

S.NO	PROTEIN	ACCESSION NUMBER	LENGTH	MOL.WT	PI	-R	+R	EC	II	AI	GRAVY
1	RbcL	ADD48243.1	234	26249.79	6.24	29	28	39225	30.1 1	74.23	-0.381
2	PsbA	ACF15349.1	24	2590.85	5.27	3	1	-	50.2 1	73.33	-0.379

Mol. Wt – molecular weight(Daltons), pI – Isoelectric point, -R - Number of negative residues, +R – Number of Positive residues, EC – Extinction Coefficient at 280 nm, II – Instability Index, AI – Aliphatic Index, GRAVY – Grand Average Hydropathicity.

The primary structure prediction was done with the help of protparam tool (Table 2). The parameters were computed using Expasy's protparam tool which revealed that the molecular weights for two different proteins as 26249.79 (Ribulose biphosphate carboxylase), 2590.85 (PsbA). The pI of two protein was less than 7 which indicated that they are acidic in character. The proteins are found to be compact and stable at their pI [15]. Among the two proteins one is showed instability index lesser than 40, indicating that the protein are stable.

Aliphatic index of the proteins ranged between 73.33-74.23. The computed extinction coefficients help in the quantitative study of protein-protein and protein-ligand interactions in solution. The range of GRAVY (Grand Average of Hydropathicity) of *Rhinacanthus nasutus* proteins was found to be -0.379 to -0.381. The lowest value of GRAVY indicates the possibility of better interaction with water [16].

The secondary structure prediction of *Rhinacanthus nasutus* proteins (Table-3) was analyzed by SOPMA which revealed that alpha helix, extended strand, beta turn and random coil, were more predominant. In all the two proteins alpha helix dominates which is followed by random coil, extended strand and beta turn. The secondary structure were predicted by using default parameters (Window width: 17, similarity threshold: 8 and number of states: 4). TMHMM v.2.0 and SOSUI predicted that 2 proteins were soluble protein.

**TABLE 3: SECONDARY STRUCTURE OF PROTEINS OF *RHINACANTHUS NASUTUS***

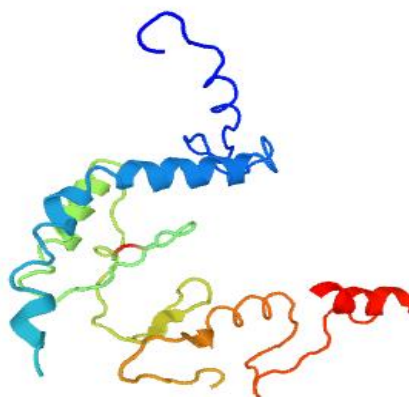
S.no	Secondary sutures	Protein	
		RbcL	psbA
1	Alpha helix	35.05	0.00
2	3 <sub>10</sub> helix	0.00	0.00
3	Pi helix	0.00	0.00
4	Beta bridge	0.00	0.00
5	Extended strand	21.46	29.17
6	Bend region	10.73	0.00
7	Beta region	0.00	0.00
8	Random coil	34.76	70.83
9	Ambiguous states	0.00	0.00
10	Others	0.00	0.00

Secondary structure prediction of proteins by SOPMA revealed that  $\alpha$  – helix, random coil,  $\beta$  – turn and extended strand were more prevalent. In rbcL , PsbA,  $\alpha$  – helix predominates, whereas rbcL, PsbA random coil region was frequent (Table: 3). In rbcl, extended strand dominates followed by random coil and  $\alpha$  – helix. Domains are evolutionary units, often identified as recurring sequence or 3D structure [16]. Inter pro tool analysis of proteins of *Rhinacanthus nasutus* revealed its super family, molecular function (Table: 4).



**TERTIARY STRUCTURE OF *RHINACANTHUS NASUTUS***

Fig-2: 3D STRUCTURE OF RIBULOSE BIS PHOSPHATE CARBOXYLASE



**TABLE 4: INTERPRO RESULTS OF PROTEINS OF *RHINACANTHUS NASUTUS***

S.NO	ACCESSION NUMBER	SUPER FAMILY	MOLECULAR FUNCTION
1	ADD48243.1	Ribulose biphosphate carboxylase	Mg ion binding Ribulose bis po4 carboxylase activity
2	ACF15349.1	PsbA	photosynthetic reaction

Photosystem II (PSII) is a multiprotein complex that catalyzes the light-driven water-splitting reactions of oxygenic photosynthesis. The job of determination of phylogenetic relationship of various organisms is a difficult one as the living world exhibits unimaginable diversity with respect to its species content. This diversity is not only reflected in phenotypic characters but also in ultra-structural, biochemical and molecular features. Phenotypically

similar organisms may have contrasting biochemical and molecular features. Evolutionary relationship was done with *rbcl* is a plant plastidial gene. [17].

Recently, several investigators have used *psbA* sequences for barcoding or species identification as well as for phylogenetic analysis. Universal barcode markers need to be evaluated for a broader spectrum due to morphological/geographical variation and reticulate evolution in plant species [18].

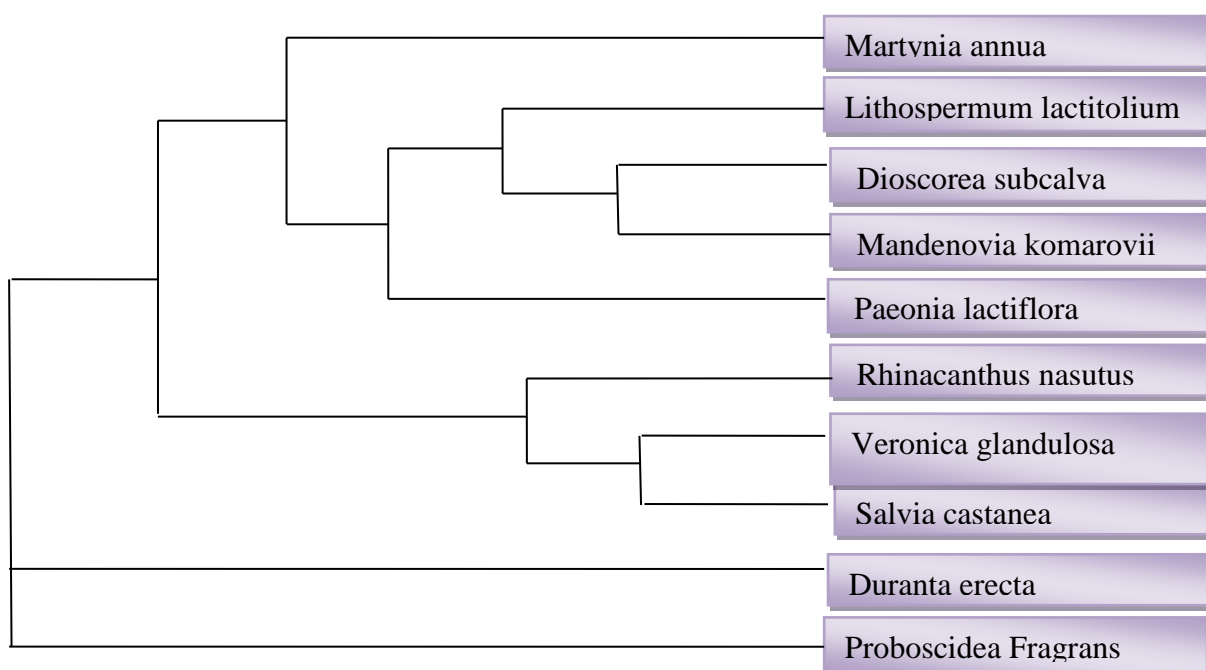
The *psbA* of *Rhinacanthus nasutus* was subjected to BLASTp analysis to find the other plant species having the same query protein. The results obtained showed that more than 45 plant species belonging to 10 different family have 80 % and above similarity. From the above hits one plant species from each family was randomly selected for evolutionary analysis in this study. The list of these plant species, accession number, identity score and are given in [Table: 5].

**TABLE 5: LISTS OF PLANT SPECIES SHOWING SIMILARITY OF 90% AND ABOVE WITH THE PHOTOSYSTEM II PROTEIN ( *psbA* )**

S.NO	Plant Species containing <i>psbA</i> protein	Family	Accession Number	Identity
1	<i>Rhinacanthus nasutus</i>	Acanthaceae	ACF15349.1	100%
2	<i>Martynia annua</i>	Martyniaceae	AFP7379.1	95.5%
3	<i>Proboscidea Frangans</i>	Martyniaceae	AFP73787.1	95.7%
4	<i>Veronica glandulosa</i>	Veroniceae	ACS88004.1	95.7%
5	<i>Salvia castanea</i>	Lamiaceae	AGJ81166.1	95.7%
6	<i>Duranta erecta</i>	Verbenaceae	AGC23809.1	95.7%
7	<i>Paeonia lactiflora</i>	Paeoniaceae	APY28383.1	91.7%
8	<i>Lithospermum lactitolium</i>	Boraginaceae	AEG79680.1	91.7%
9	<i>Dioscorea subcalva</i>	Dioscoreaceae	ADR10337.1	91.7%
10	<i>Mandenovia komarovii</i>	Apiaceae	ABN13429.1	91.7%

The phylogeny concluded from a single marker gene or protein sequence only reflects evolution of that particular gene. A multiple sequence alignment was done for 10 plant species using Clustal Omega. The tool was run with default parameters and the phylogenetic tree was drawn [Fig 3]. The results revealed that the psbA protein of *Rhinacanthus nasutus* of Acanthaceae family was closely related to whereas *Martynia annua*, *Proboscidea Fragrans*, *Veronica glandulosa*, *Salvia castanea*, *Duranta erecta*, *Paeonia lactiflora*, *Lithospermum lactitolium*, *Dioscorea subcalva* and *Mandenovia komarovii*. Therefore the plants of these families can be assayed for producing pharmacologically effective substances.

**Fig-3: PHYLOGENETIC TREE OF PHOTOSYSTEM II PROTEIN ( psbA ) CONTAINING PLANTS**



## CONCLUSION

Now a day's focus on plant research has been increased throughout the world and data have collected to show the immense potential of plants used in various traditional systems. To best of our knowledge, ours is the first study to show these functional aspects of proteins of *Rhinacanthus nasutus*. Further investigations are needed to determine how protein

functions in these plant processes and to provide a more comprehensive understanding of the evolution of this unusual gene.

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