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Research Article

STRUCTURAL INVESTIGATION AND INSILICO CHARACTERISATION OF PROTEINS OF ANDROGRAPHIS ECHIOIDES- AN INDIGENOUS MEDICINAL PLANT

Nirubama, K[1]., Shabanabegum, M*[2]., Rubalakshmi, G[3] and Prabhakaran.S[3]

[1,3]GRD Bio Clinical Research, Rasipuram - 637408, Namakkal District, Tamil Nadu, India
[2]Department of Biochemistry, Muthayammal College of Arts & Science, Rasipuram-637408. Namakkal District, Tamil Nadu, India

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ABSTRACT

Plant-derived phytochemicals have been traditionally used as a natural remedy in the treatment of diverse ailments. They are the backbone of traditional medicine. In this content, there is an interest in developing novel lead molecules from plant sources because of their higher biological activity, safety, and lower cost as compared to the synthetic drugs. So the medicinal plants have a promising role in to prevent and as well as to cure the diseases. *Andrographis* (Acanthaceae) is a genus of about 40 species, various members of which have a reputation in indigenous medicine. *Andrographis echoidies* has been used in various folk medicinal preparations and its chemical composition and pharmacological activities have been elaborated recently. However there remains still a huge scope for use of modern scientific methods - genomics, proteomics and bioinformatics in this plant. Bioinformatics shall facilitate analysis and integration of information from these related fields to enable the identification of genes and gene products and elucidate the functional relationships between genotype and observed phenotype. This research report provides a state-of-the-art overview of bioinformatics study of *Andrographis echoidies* with emphasis on the current progress and future directions, which shall provide tools and resources necessary to understand and promote advances in this important field. The aim of the present study, 2 proteins of *Andrographis echoidies* were analysed using bioinformatics tools. Structural prediction and functional characterization of proteins of *Andrographis echoidies* 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 α – helix, random coil, β – turn and extended strand predominates. Phylogenetic analysis of Maturase K of *Andrographis echoidies* reveals that the plants of Verbenaceae, Bignonaceae, and Fabaceae family are closely related. *Andrographis echoidies* an endangered medicinal plant has to be analysed further for identifying its various medicinal properties.

INTRODUCTION

Everywhere in the world research has been carried out to explore the hidden drugs and to utilize the healing property of herbs. In India, the ayurvedic system has described a large number of such medicines based on plants or plant product and the determination of their morphological and pharmacological or pharmacognostical characters can provide a better understanding of their active principles and mode of action [1]. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care [2]. Still there is a requisite for the development of scientific technology for the enhancement of medicinal plants and their products. For the isolation of lead compounds, assorted classes of plant species have been analysed with the help of upgraded Ayurvedic traditional methods and other advanced scientific exploration. Innumerable therapeutic agents can be obtained from medicinal plants after screening of secondary metabolite compounds [3].

Andrographis echiodes(L.)Nees belongs to the family of acanthaceae. It is widely distributed in the tropical India, Srilanka. Andrographis echiodes(L.) Nees is traditionally used as anti inflammatory, febrifuge, cooling, alternative for cuts and wounds. The extract of the whole plant is used to cure fever [4]. Andrographis echioides is identified as one of the most important medicinal plants in kanjamalai hills of Salem, which can be used for many ailments like cuts, wounds and

*Corresponding author: **Shabanabegum, M***
Department of Biochemistry, Muthayammal College of Arts & Science, Rasipuram-637408. Namakkal District, Tamil Nadu, India

fever [5]. Androechin a new chalcone glucoside from Andrographis echioides was suspected to possess all the medicinal properties as that of Andrographis paniculata [6].This plant is used to relieve griping, irregular stools and loss of appetite in case infants and in debility and certain farms of dyspepsia. However, information on the chemical composition and bioactivity of this species is very rare. Andrographis echioides is often substituted for kalmegh in tribal medicine. A dried plant of Andrographis echioides is used by the tribal's to keep up the moisture content of economically important crops during the hot summer. Medicinal properties of this plant are more or less similar to those of Andrographis paniculata. Nano synthesized Andrographis echoides exhibited appreciable alpha glucosidase inhibitory effects when compared with standard drug acarbose [7]. In this study two protein sequences of Andrographis echiodes were selected and analyzed with the help of computational tools. One such important protein is **Maturase K** (matK) is a plant plastidial gene. Universal matK primers can be used for DNA bar coding of angiosperms [8]. In silico approach provide useful information by identifying the primary, secondary and tertiary structure predictions which can be used for further analysis.

MATERIALS AND METHODS

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 Protparm 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 Maturase K (ALN49191.1.) 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 phylogentic analysis of Maturase K 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

Andrographis is an important genus of the family Acanthacae known for its ethanomedicinal claims and for a variety of medicinal properties [14]. *Andrographis echioides* 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 [15].

Table 1 Primary Structure of Proteins of Andrographis Echioides

S.NO	Accession Number	Protein	Length
1	AOAOS2CVR5	Ribulose bisphosphate carboxylase	184
2	AOAOS2CWS4	Maturase K	243

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 20519.44 (Ribulose bisphosphate carboxylase), 29140.63 (Maturase K). The pI of one protein was less than 7 which indicated that they are acidic and one protein was greater than 7 which showed that it is basic in character. The proteins are found to be compact and stable at their pI [16]. 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 79.5-85.8. 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 Andrographis echioides proteins was found to be -0.241 to -0.912. The lowest value of GRAVY indicates the possibility of better interaction with water [17].

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	Ribulose bisphosphate carboxylase	AOAOS2CVR5	184	20519.44	6.57	21	21	30620	28.11	79.51	-0.241
2	Maturase K	AOAOS2CWS4	243	29140.63	10.11	14	33	60640	47.26	85.80	-0.912

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 secondary structure prediction of Andrographis echioides 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 Andrographis Echoidies

S.NO	Secondary structure	AOAOS2CVR5	AOAOS2CWS4
1	Alpha helix	35.33%	31.28%
2	3_{10} helix	0.00%	0.00%
3	Pi helix	0.00%	0.00%
4	Beta bridge	0.00%	0.00%
5	Extended strand	19.57%	27.57%
6	Bend region	11.41%	10.29%
7	Beta region	0.00%	0.00%
8	Random coil	33.70%	30.86%
9	Ambiguous states	0.00%	0.00%
10	Others	0.00%	0.00%

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

Tertiary Structure of Andrographis Echioides

Table 4 Interpro Results of Proteins of Andrographis Echiodes

S.NO	Accession number	Super family	Molecular function
1	AOAOS2CVR5	Ribulose bisphosphate carboxylase	Mg ion binding Mono oxygenase activity
2	AOAOS2CWS4	Maturase K	RNA splicing

Evolutionary relationship was done with Maturase K. Maturase K (matK) is a plant plastidial gene. The protein it encodes is an intron maturase, a protein that splices introns. It is used to promote RNA splicing. Universal matK primers can be used for DNA barcoding of angiosperms.

DNA barcoding relies on the information encoded in the nucleotide sequences of a standard region of the genome as a tool for species identification. The Consortium for the Barcode of Life (CBOL) plant working group recommended the 2-locus combination of ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit (*rbcL*) and maturase K (*matK*) as the standard plant barcode based on assessments of recoverability, sequence quality and levels of species discrimination (CBOL Plant Working Group, 2009). These two regions of chloroplast DNA were chosen based on two main criteria: efficient recovery of good-quality sequences and high levels of species discrimination [18].

Recently, several investigators have used *rbcL* and *matK* 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 [19].

The Maturase K *Andrographis echoides* 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.

Fig-1 3D Structure of Ribulose Bis Phosphate Carboxylase

Fig-2 MATURASE - K

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 Spieces Showing Similarity of 80% And Above With The Maturase K Protein

S.NO	Plant Species containing Maturase K protein	Family	Accession Number	Identity
1	Petrea Volubilis	Verbenaceae	AEI17878.1	83%
2	Halleria lucida	Snap dragon family	AEK35012.1	82%
3	Freylinia lanceolata	Scrophylariaceae	AFU49804.1	83%
4	Stachytarpheta frantzii	Verbenaceae	AFI17901.1	83%
5	Harpagophytum procumbens	Pedaliaceae	AEX68624.1	82%
6	Martynia annua	Martyniaceae	AFI16750.1	83%
7	Eccremocarpus scaber	Bignoniaceae	AEH26568.1	83%
8	Teedia pubescens	Fabacaeae	AKJ77115.1	83%
9	Buddleja officinalis	Buddhejaceae	AMS24268.1	82%
10	Mimulus alatus	Phrymaceae	AKM99766.1	83%

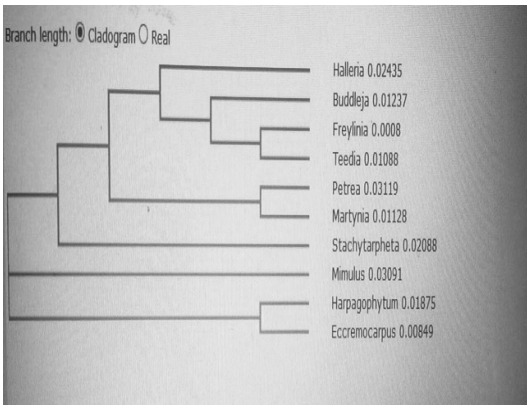

Fig-3 Phylogenitic Tree of Maturase –K Protein Containing Plants

Maturase K protein

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 Maturase K protein of *Andrographis echoides* of Acanthaceae family was closely related to whereas *Petrea Volubilis, Haleria lucida, Martynia annua*. Therefore the plants of these families can be assayed for producing pharmacologically effective substances.

CONCLUSION

Since time immemorial people have tried to find medications to alleviate pain and cure different illnesses. In every period, every successive century from the development of humankind and advanced civilizations, the healing properties of certain medicinal plants were identified, noted, and conveyed to the successive generations. In this study, proteins of *Andrographis echoides* were selected. Expasy's ProtParam tool predicted the physio-chemical characters of the proteins. Phylogenetic study revealed the close and distant relationship of Maturase K protein of *Andrographis echoides* of Acanthaceae family with the plants of other family. Further analysis are required for drug target identification.

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