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Antiviral Proteins from Family Amaranthaceae: An Overview

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ABSTRACT: Amaranthus, the fifth largest genus of the family Amaranthacea which covers around 60 species throughout world caught the attention of biological and biomedical researchers because of the presence of antiviral proteins specifically ribosome-inactivating proteins. These antiviral proteins exhibited N-glycosidase, DNAse, RNAse, immunomodulatory activities. Current article discuss the role of antiviral proteins present in family amaranthaceae.

KEYWORDS: Amaranthus; Amaranthaceae; Antiviral Activity; Medicinal Value; RIP.

INTRODUCTION

The amaranthus represent an horticulturally important group of annual herbaceous plants represented by total of 60 species in the world flora under Amaranthus L., which is the fifth largest genus in the Family: Amaranthacea out of which about 20–25 species occurring in India. Amaranthus species are grown in several parts of the world including Mexico, Central and South America, India and Africa where they are mostly consumed as leafy vegetables (‘green amaranthus’) or grain crops (‘grain amaranthus’). Green amaranthus serve as one of the most delicious leafy vegetables and are a rich source of protein (up to 5.6% on fresh weight basis), requisite vitamins (A, B, C, folic acid), minerals (Ca, Mg, K, P, Na, N, Fe, Mn, Zn) and fibres (5.25%)**[1,2].** Besides, they contain other biologically pro-health compounds such as the antioxidant squalene and carotenoids**[3]** and thus, are recommended as a nutritious food with medicinal properties for young children, lactating mothers and for patients with fever, hemorrhage, anemia or kidney complaints. Of several species cultivated in tropical and sub-tropical areas of India Amaranthus tricolor is probably the highest yielding leafy vegetable crop species with its excellent nutraceutical value. On the other hand, the attraction of the grain amaranthus (A. hypochondriacus, A. cruentus and to some extent A. caudatus) to both earlier civilizations and modern consumers is due to the thousands of tiny but highly nutritious pinkish white or golden seeds that are unusually high in protein (14–16%) with a well-balanced amino acid composition and particularly an elevated lysine content. Thus, amaranthus qualify as an ideal food source for people of low income-food deficit countries (LIFDC). Besides, some Amaranthus species (e.g. A. caudatus, ‘love-lies-bleeding’; A. hypochondriacus, ‘Prince’s Feather’; A. tricolor, ‘Joseph’s coat’) are valued as ornamentals being gifted either with floral splendor due to their radiantly coloured inflorescence or variegated foliage. Owing to their high C4-type photosynthetic efficiency and high yielding ability coupled with minimum of cultivation constraints and moderate tolerance to drought, salinity and heat, amaranthus have evolved as a nutritionally rich food crop to serve as an essential component of the sustainable horticulturesystem in tropics **[4]**.Here, we are concern only antiviral protein which isolated from Amaranthus.

Antiviral protein

Many plants contain proteins that are capable of inactivating ribosomes and accordingly are called ribosome-inactivating proteins or **RIPs**. These typical plant proteins receive a lot of attention in biological and biomedical research because of their unique biological activities

toward animal and human cells. In addition, evidence is accumulating that some RIPs play a role in plant defense and hence can be exploited in plant protection against different

viruses, fungi and bacterial pathogens and are also known as antiviral proteins (AVPs) **[5-11]**. Most of the newly discovered enzymic activities of RIPs are

- DNase activity on supercoiled DNA **[12]**
- RNase activity **[13]**
- Depurination of capped mRNAs **[14]**
- Superoxide dismutase (SOD) activity **[15] [16]**
- Phospholipase activity **[17]**
- Antioxidant activity **[18]**
- Inducing cell death by apoptosis**[19]**

But widely accepted mechanism suggests that RIPs possess characteristics N-glycosidase activity that inactivates the ribosomes, inhibiting protein synthesis irreversibly **[20]**. A number of phylogentically diverse plants has been reported to contain proteins which acts as powerful inhibitors of eukaryotic ribosomes. Ribosome-inactivating proteins (RIPs) are classified as type 1 and 2. Type 1 RIP has a unique enzymic polypeptide chain with N-glycosidase activity on the ribosomal RNA that irreversibly impair protein synthesis by enzymatically modifying the EF-2-dependent GTPase activity of the subunit. Type 2 RIP is consisting in two polypeptide chains linked by a disulfide bonding, A-chain being the enzymic chain able to attack the 60S ribosomal subunit and B-chain being a lectin able to recognize membrane sugars, mostly galactose residues. Type 1 RIPs are relatively abundant and to date nearly 30 have been isolated, the best known of which are saporin and pokeweed antiviral protein. The pokeweed antiviral protein, the first of the non-toxic proteins to be purified, was shown to inhibit protein synthesis in the host cells. Subsequently it was found that both the toxins and the `A-chain-like` proteins, like pokeweed antiviral protein (PAP), the wheat-germ inhibitor, the Momordica charantia inhibitor and gelonin, strongly inhibit an eukaryotic ribosome. An extract from Bryonia dioica (bryony) seeds, which inhibits protein synthesis, also had antiviral activity, and an extract from Dianthus caryophyllus leaves, whose antiviral activity was known, had a strong inhibitory effect on protein synthesis. Type 2 RIPs, which can enter cells through the interaction of their lectin moiety with the cell membrane, are among the most potent natural toxins because of their capacity to bind most intact cells, the best known of which is ricin. The use of antiviral proteins (AVPs) from plants is one of the promising approaches to control viral diseases. Some well-characterized AVPs are from **[21,22,23,24,25,26]**:

- *Phytolacca americana*,
- *Mirabilis jalapa*,
- *Dianthus caryophyllus*,
- *Clerodendrum aculeatum*,
- *Amaranthus viridis*, and
- *Trichosanthes kirilowii* .

Antiviral Protein from *Amaranthus Tricolor* Leaves

An antiviral protein (AVP), imparting high level of resistance against sunnhemp rosette virus (SRV) was purified from the dried leaves of Amaranthus tricolor. The purified protein (**AAP-27**) exhibited 98% inhibition of local lesion formation at a concentration range of_30 µg/ ml. Since extracts from both fresh and dry A. tricolor leaves inhibited local lesion formation by sunnhemp rosette virus in its hypersensitive host, *C. tetragonoloba* (Guar) to the same extent, the dried leaves were used to extract the antiviral activity. The antiviral protein (AVP) was purified from the leaves of *A. tricolor* var. Lal Chulai. Sunnhemp rosette virus (SRV) was used as test virus and C. tetragonoloba as test plant for bioassay of the antiviral protein. The antiviral activity was expressed in terms of percent inhibition of lesion formation on the test plant leaves by the virus. **[27]**.

Protein fraction	Total protein (mg) [From 60 g of dried leaves]	Percent inhibition [Concentration range of 30µg /ml]
Crude extract	2207.0	95.5
Ammonium sulphate fraction 60–80%	320.0	94.1
DEAE-cellusose chromatography		
Unabsorbed fraction	96.4	93.4
Absorbed fraction	156.2	12.0
CM-sepharosechromatography 0.1M NaCl		
Fraction I	14.4	88.9
Fraction II	8.2	10.0
CM-sepharosechromatography 0.2M NaCl		
Fraction III	15.4	98.5
Superose-12 chromatography	1.8	98.3

Table 1: Purification of antiviral protein (AAP-27) from the leaf extracts of *Amaranthus tricolor*

Molecular weight of AAP-27: Molecular weight (Mr) of the purified AAP-27 as determined by both calibrated gel permeation chromatography and SDS–PAGE was observed to be _27 kDa (fig 1) .

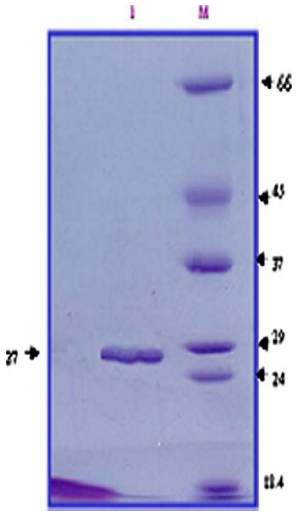

Fig. 1. SDS–PAGE of purified AAP. Purified AAP. (lane 1). Molecular weight markers (M).

Isoelectric focusing and amino acid composition: The isoelectric focusing pattern showed the protein to migrate towards the cathodic end of the focused gel and the pI was calculated to be 9.8 (results not given). This confirmed that the protein is basic in nature. The basic nature of protein was further confirmed by amino acid analysis, which shows that lysine (38.4%) and arginine (24.8%) formed the major constituents of the protein.

N-glycosidase activity: Aniline treatment of rRNA extracted from AAP-27 treated *C. tetragonoloba* ribosomes resulted in generation of specific RNA fragments (lane 2) due to their N-glycosidase activity(fig 2).

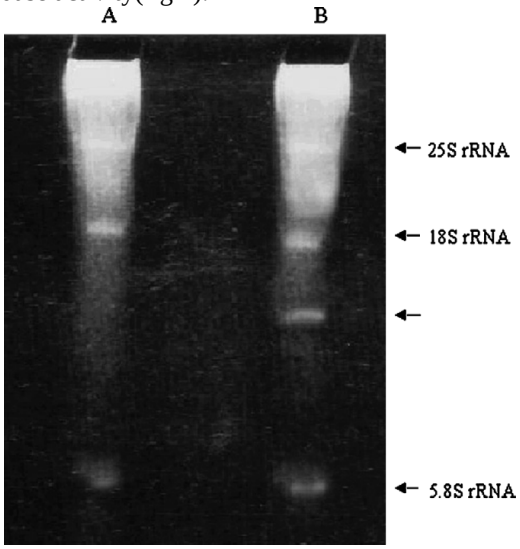

Fig. 2. N-glycosidase activity of purified AAP on *Cyamopsis tetragonoloba* rRNA. Control rRNA (lane 1) and AAP treated rRNA (lane 2).

An Antiviral Protein from *Celosia cristata* Leaves

Celosia cristata, an ornamental plant of *Amaranthaceae* family is a potential candidate for the presence of strong antiviral proteins. Two potent growth stage dependent glycoproteins, CCP-25 and CCP-27 have been reported to be present in the leaves of this plant at flowering stage. An antiviral protein named **CCP-27** was purified from the leaves of *Celosia cristata* at the post-flowering stage by anion-exchange, cation-exchange, and gel-filtration chromatography. It exhibited resistance against sunnhemp rosette virus in its test host *Cyamopsis tetragonoloba*. It also exhibited deoxyribonuclease activity against supercoiled pBlueScript SK+ plasmid DNA. It was found to nick supercoiled DNA into nicked circular form at lower protein concentration followed by nicked to linear form conversion at higher protein concentration. CCP-27 also possesses strong ribonuclease activity against *Torula* yeast rRNA **[28]**.

DNase activity: CCP-27 exhibited strong DNase activity. When pBlueScript SK+ DNA having all the three forms, i.e. supercoiled, nicked, and linear (Fig. 3a), was incubated with various amounts of CCP-27, it was found that the extent of supercoiling was obviously altered. The supercoiled form of the pBlueScript SK+ DNA when incubated with 2 and 4 µg of CCP-27, first nicked to give a nicked circular form, which moved significantly slower than the supercoiled DNA through the agarose gel. When the CCP-27 concentration was increased to 6 µg, the linear form of DNA emerged which migrated faster than the nicked circular form but slower than the supercoiled DNA. From this finding, it can be suggested that AVP/RIP-associated DNase activity was conformation specific, i.e. RIPs only cleave supercoiled DNA in a twostep process, first nicking a single strand before linearizing the DNA by second strand cleavage. The results are in line with the earlier findings [8-13]. In order to make sure that band shift was not due to the binding of CCP-27 onto pBlueScript SK+ DNA, attempts were made to separate the two species before electrophoresis. This was achieved by digestion with proteinase K, after incubation of DNA with CCP-27. The DNA was then extracted with phenol:chloroform (1 : 1) and precipitated with ethanol.

RNase activity: For RNase activity test, as the gel was stained with Toluidine blue, which interacts with RNA to give blue color, the unstained portion indicated the position of RNase activity. Figure 4 (lane *1*) shows a single unstained band that was found to co-migrate with purified CCP-27 (2 µg), indicating that CCP-27 does possess a strong RNase activity. The presence of only one band with RNase activity corresponding to the position of CCP-27 suggests that there was no other contaminating nuclease with the purified CCP-27 preparation. Lane *2* (Fig. 4) shows the position of positive control (2 mg RNase A). The ribonucleolytic activity on naked rRNA was also reported in case of a- and b-momorcharin [14].

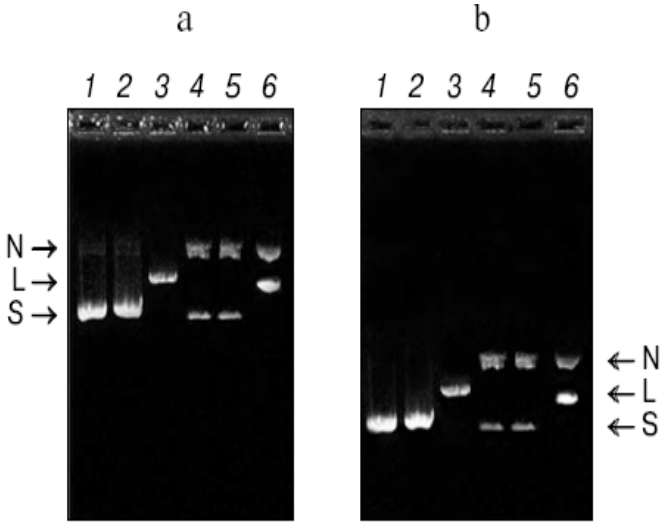

Fig. 3. a) DNase activity test. When incubated with 2 or 4 mg of CCP-27, pBlueScript SK+ DNA supercoiled form (S) first gives rise to nicked form (N) (lanes *4* and *5*) and with 6 mg of CCP-27 gives rise to linear form (L) (lane *6*). Lanes: *1*) plasmid; *2*) control (pBlueScript SK+ DNA was incubated without CCP-27); *3*) plasmid restricted with *EcoR*I. b) DNase activity test after proteinase K treatment. Same changes in banding patterns indicate that band shifting was not due to binding of the protein with plasmid DNA.

Masayuki et al. [24] observed 50% inhibition of local lesion formation in cowpea leaves when treated with a 23 kD RNase like glycoprotein (figaren) from *Cucumis figarei*, 24 h before to 1 h after incubation with cucumber mosaic virus. Figaren also digested double stranded RNase extracted from cucumber mosaic virus infected tobacco tissues. Thus, there may be a positive correlation between antiviral activity and RNase activity.

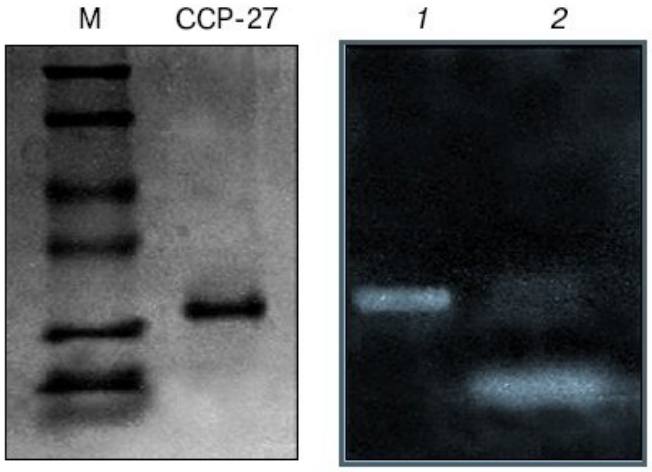

Fig. 4. RNase activity test. CCP-27 was separated using 12% SDS-PAGE with *Torula* yeast RNA. Following electrophoresis, the gel was stained with Toluidine blue O to show the bands with RNase activity. The unstained portions indicate the presence of RNase activity. Lanes containing the protein marker M and CCP-27 were cut from the gel and stained with Coomassie blue to indicate location on the protein. Lanes: *1*) 4 mg CCP-27; *2*) 2 mg RNase A (positive control).

Bioactive Peptides in *Amaranthus hypochondriacus* Seed

Amaranthus hypochondriacus seeds are rich in protein with a high nutritional value, but little is known about their bioactive compounds that could benefit health. The objectives of this research were to investigate the presence, characterization, and the anticarcinogenic properties of the peptide lunasin in amaranth seeds. Furthermore, to predict and identify other peptides in amaranth seed with potential biological activities. ELISA showed an average concentration of 11.1 µg lunasin equivalent/g total extracted protein in four genotypes of mature amaranth seeds. Glutelin fraction had the highest lunasin concentration (3.0 µg/g). Lunasin was also identified in albumin, prolamin and globulin amaranth protein fractions and even in popped amaranth seeds. Western blot analysis revealed a band at 18.5 kDa, and MALDI-TOF analysis showed that this peptide matched more than 60% of the soybean lunasin peptide sequence. Glutelin extracts digested with trypsin, showed the induction of apoptosis against HeLa cells. Prediction of other bioactive peptides in amaranth globulins and glutelins were mainly antihypertensive. This is the first study that reports the presence of a lunasin-like peptide and other potentially bioactive peptides in amaranth protein fractions**[29]**.

Antiviral proteins amarandin 1 and 2 from *Amaranthus viridis* leaves

Inventors have isolated and purified amarandin 1 and 2 ribosome-inactivating proteins having antiviral activity from Amaranthus viridis leaves, an edible crop in Korea. The first 10 amino-terminal amino acid sequences of amarandin 1 and 2 are determined as**[30]**,

Ala-Asp-Leu-Thr-Phe-Thr-Val-Thr-Lys-Asp-Gly 11 -(amarandin 1) (SEQ ID NO:1), and

Val-Asn-Pro-Thr-Phe-Val-Val-Thr-Met-Ser 10 -(amarandin 2) (SEQ ID NO:2).

Amarandin 1 and 2 can be extracted from leaf tissue of the plant Amaranthus viridis wherein they are present in two forms having different molecular weight, respectively 28,000 and 30,000; said molecules seem to be two different proteins although no definitive conclusion has been drawn. The biochemical characteristics of these two proteins are similar: alkaline isoelectric point is remarkably basic for both (pI.about.9.5); Their action are enzymatic, as the IC 50 (50% inhibitory concentration) value calculated on rabbit reticulocyte lysate are identically of the order of 10 -11 M. As little as 0.3 ng/ml of amarandin 1 and 2 is inhibitory, amarandin 1 and 2 are very effective inhibitory of ribosome function in vitro, apparently by interfering with EF-2 (elongation factor 2) mediated translocation of the nascent peptide chain along the ribosome.

Also, amarandin 1 and 2 of Amaranthus viridis were shown to reduce the infectivity of tobacco mosaic virus (TMV) and

other plant viruses, although interestingly the antiviral property was not demonstrable in the host plant itself. The antiviral spectrum of amarandins may include TMV, watermelon mosaic virus, zucchini mosaic virus, cauliflower mosaic virus, potato virus X and Y, potato leafroll virus, odontoglossum ringspot virus, and cucumber mosaic viruses etc., all of which are readily quantitated by mixing with the above proteins and rubbing onto susceptible leaves. There was actually a 95% decrease in the production of extracellular infectious virus, suggesting that a late stage of the virus replication cycle was inhibited.

Amino Acid Sequencing Analysis of Amarandin 1 and 2

Amarandins were subjected to SDS-PAGE in the presence of 2-mercaptoethanol. Protein electroblotted onto PVDF membranes were detected by staining with 0.2% Coomassie Blue R-250 (w/v) in methanol:water:acetic acid (50:40:10) for 3 min. The membrane was washed in methanol:water:acetic acid (48:47:5), and the protein band was cut out of the PVDF and applied to Beckman 890C amino acid Sequencer equipped with a Sequemat P-6 autoconverter. An Altex 345C HPLC and a Hewlett-Packard 3390A integrator were used to analyze the products, according to the methods of Tarr.

Immunomodulatory effects of Alternanthera tenella Colla aqueous extracts

Alternanthera tenella Colla extracts are used in Brazilian traditional folk medicine to treat a variety of infectious diseases as well as inflammation and fever. In this work, the immunomodulatory, anti-inflammatory and potential toxic effects of cold (CAE) and hot (HAE) aqueous extracts of A. tenella were investigated in vivo. In addition, analyze the phytochemical properties of both extracts. BALB/c mice were immunized in vivo with sheep red blood cells and concomitantly inoculated intraperitoneally (i.p.) with each extract (50, 100 or 200 mg/kg). Specific antibody producing cells were enumerated using plaque-forming cell assays (PFC) and anti-SRBC IgG and IgM serum levels were measured via enzyme-linked immunosorbent assay. Body and lymphoid organ weights were determined after treatments in order to evaluate toxic effects. Carrageenan-induced paw edema was employed to investigate anti-inflammatory activity in mice inoculated i.p. with CAE or HAE (200 or 400 mg/kg). Phytochemical screening was performed using spectrometric and chromatographic approaches and revealed that CAE possessed higher tannin and flavonoid levels than HAE. PFC numbers were increased after treatment with CAE (100 mg/kg) four days after immunization, as were the serum antibody titers after four and seven days, suggesting immunostimulatory activity through modulation of B lymphocyte functions. Body and organ weights did not show major changes, suggesting thatextracts administered to mice did not induce significant toxicity. Both extracts had significant anti-inflammatory activity in the paw edema assay. These results suggested that aqueous extracts from A. tenella contained several chemical compounds that possess positive and/or negative modulator effects on the immune system, which appeared to correlate with tannin and flavonoid levels in those extracts. In summary, these studies provide important insight into the biological activities of A. tenella**[31]**.

Antiviral proteins from *Achyranthes aspera*

In an in vitro assay the methanolic extract of *A. aspera* leaves (100 µg) revealed significant
inhibitory effects on the Epstein-Barr virus early antigen induced by the tumour promoter 12-
O-tetradecanoylphorbol-13-acetate in Raji cells. The fraction containing mainly non-polar
compounds showed the most significant inhibitory activity (96.9 % and 60 % viability) **[32]**.

Bioactive peptides from *Celocia argentea*

Cyclic peptides comprise a class of naturally occurring molecules, which exhibit a range of biological activities. Their cyclic nature often provides more lipophilicity and membrane permeability, because of reduced zwitterionic character. Furthermore, restricted bond rotation results in rigid backbone conformation with more affinity and selectivity for binding proteins. During search for bioactive peptides from medicinal plants,found that aseries of unique bicyclic peptides, celogentins isolated from *Celocia argentea* (Amaranthaceae) remarkably inhibit the tubulin polymerization**[33]**.

Protein fraction	Total protein (mg) [From 40 g of dried leaves]	Test sample concentration,µg/ml	Percent inhibition
Crude extract	28.70	28	81.5
Ammonium sulphate fraction 60–80%	21.52	130	93.0
DEAE-cellusose chromatography			
Unabsorbed fraction	12.30	195	92.8
CM-sepharsechromatography 0.05M NaCl			
Peak I	3.68	145	98.1
CM-sepharsechromatography 0.1M NaCl			
Peak II	3.25	140	8.5
Peak III	4.10	182	2.3

Table 2: Purification of antiviral protein from leaf extract of *Celosia cristata*

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