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Mirabilis antiviral protein studies and its potential applications

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Abstract

Plants are an important source of different natural products, which have been utilised as medicine. One such natural product is protein, which has different applications like plant protection, therapeutic agent for different diseases. Ribosome inactivating proteins (RIPs) are one of such proteins which has N-glycosidase activity, due to which they can modify large mRNA and inhibit further translation. Different types of RIPs have been isolated from plants, crop plants and microorganisms. They are classified into three groups; Type I, Type II and Type III based on their physical properties. It has also been observed that more than one RIP can be isolated from one source, which indicates the presence of different isoforms in that source. One such frequently used RIP for medical purpose is Mirabilis Antiviral Protein (MAP), isolated from roots, leaves and seeds of *Mirabilis jalapa* L. MAP comes under the category of Type I RIP. MAP exhibits wide range of biological activities, such as antiviral, anticancer and antibacterial. The protein is lysin rich with pI 9.1 and molecular weight close to 24 kDa as determined by SDS-PAGE. It is highly rigid and thermostable protein, and also reported to express and maintain its antiviral activity at high temperature.

Key words: Ribosome inactivating proteins, mirabilis antiviral protein

1. Introduction

Since ancient times, different parts of plants are used as source of medicine for treatment of many diseases. They are invaluable source of phytochemicals and proteins, which have been utilized as traditional medicine in many countries. Different types of proteins have been investigated with selective toxicity and used for different purposes such as for transgenic plant protection (Logemann *et al*., 1992; Lodge *et al*., 1993), treatment of different types of diseases like cancer, treatment of HIV, Hepatitis B, *etc*. (Olsnes and Pihl, 1982; Pastan and Fitzgerald, 1991; Au *et al*., 2000; Fan *et al*., 2009). Ribosome inactivating proteins (RIPs), are one such class of proteins which are isolated not only from plants but also from certain fungi and bacteria. These proteins have N-glycosidase activity, due to which they can modify large mRNA and inhibit further translation (Stirpe *et al*., 1992).

Most of RIP literature elucidates its isolation, characterization and different applications, since the proteins show selective toxicity (Gasperi-Campani *et al*., 1985; Barbieri *et al*., 1993). Also many other studies focused on enzymology, uptake of RIPs into target cells and subsequent transport to its target (Cavallaro *et al*., 1995; Sandvig and Van Deurs, 1999). All these investigations provide a knowledge regarding biochemical and medicinal properties of RIPs. In recent years with the help of recombinant DNA technology, investigation of RIPs activities has been completely revealed. These studies not only lead to an improved understanding of RIPs expression and its N-glycosidase activity but also discovered new enzymatic activities which suggest that RIPs biology is quite complex.

1.1 Historical view

In 1925, first RIP was extracted from pokeweed (*Phytolacca americana*), which showed the inhibition of viral infection (Irvin, 1983). Therefore, it has been linked to plant defence mechanisms. Stirpe *et al.* (1992) and Barbieri *et al.* (1993) extracted the protein from 50 plants and checked its activity. They found that most of the extracts showed *in vitro* translation inhibitory activity. Purification of these proteins led to their identification as RIPs. Further investigation showed that they are not only present in plants but also in crop plants such as wheat, maize and barley (Coleman and Roberts, 1982).

Endo *et al*. (1987) found an enzymatic activity of RIP. They discovered that RIP has a N-glycosidase activity which specifically remove adenine which is corresponding to A 4324 residue in rat 28S rRNA (Endo and Tsurugi, 1987; Endo *et al.*, 1987). This adenine present in R-sarcine loop, which is a 14 nucleotide region, conserved in large rRNAs from bacteria to humans (Bailey-Serres *et al.*, 1998).

The first “A” of a GAGA sequence is the RIPs substrate, which forms the core of putative tetraloop enclosed by a short base paired stem (Correll *et al.*, 1998; Gutell *et al.*, 1993; Orita *et al.*,1993). The translation is blocked by an irreversible modification of target A residue, by blocking EF-1 and EF-2 dependent GTPase activity. This inhibit the binding of ribosome to EF-2. Due to this translation inhibitory activity of RIPs, these proteins are toxic to cells (Nilsson *et al.*, 1986). Subsequently it has been observed that most of the RIPs remove more than one adenine from ribosome. Apart from ribosome, they also release adenine from poly-A tail and form DNA. Due to these enzymatic activities, they are also termed as polynucleotide: adenosine glycosidase (Barbieri *et al.*, 1997; Bolognesi *et al.*, 2002).

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2. Types of RIPs and their characteristics

Based on their physical properties, RIPs are classified into three groups as follows (Mundy *et al.,* 1994):

Type I: It is composed of single polypeptide chain of approximate molecular weight of 30 kDa (Barbieri *et al.,* 1993; Irvin, 1975). These are basic proteins which have not only many highly conserved active cleft residues and secondary structure within the active site region (Mlsna *et al.,* 1993; Monzingo *et al.,* 1992), but also distinctly different with respect to the overall sequence and post-translational modification (Hartley and Lord, 1993). The receptor alpha-2 macroglobulin is responsible for the binding and endocytosis of Type I RIPs. This mechanism was explained with the help of RIPs Saporin and Trichosanthin (Chan *et al.,* 2000). Study by Carvalloet *et al.* (1995) showed that there is an interaction between Type I RIPs and cell membrane mediated with the help of alpha-2-macroglobulin receptor and carried out endocytosis of Type I RIPs. Once inside cell, they disturb translation of cell.
Example: Pokeweed antiviral protein (PAP) and saporin.

Type II: These are heterodimer proteins consisting of A and B subunits, each with approximate molecular weight of 30 kDa (Stirpe *et al.,* 1978). The A chain has RIP activity and is linked to B-chain, which is endowed with galactose binding lectin through a disulphide bond (Stirpe *et al.,* 1978). B-chain binds to glycoproteins and/or glycolipids present on cell membrane of eukaryotic cells and mediate transportation of A-chain into cytoplasm (Lehar *et al.,* 1994; Olsnes and Sandvig, 1988; Sandvig *et al.,* 1976; Steeves *et al.,* 1999) then they access to rRNA and disturb the translation mechanism (Olsnes and Pihl, 1973).
Example: Ricin and Abrin

Type III: These are synthesized as inactive precursors, *i.e.* proRIPs. To form an active RIPs, proRIPs require proteolytic processing event (Peumans *et al.,* 2001). These RIPs are less common than Type I and II RIPs and are not used for therapeutic purpose. These have been characterized only from maize and barley (Walsh *et al.,* 1991; Bass *et al.,* 1992; Chaudhry *et al.,* 1994).

Example: Maize and Barley

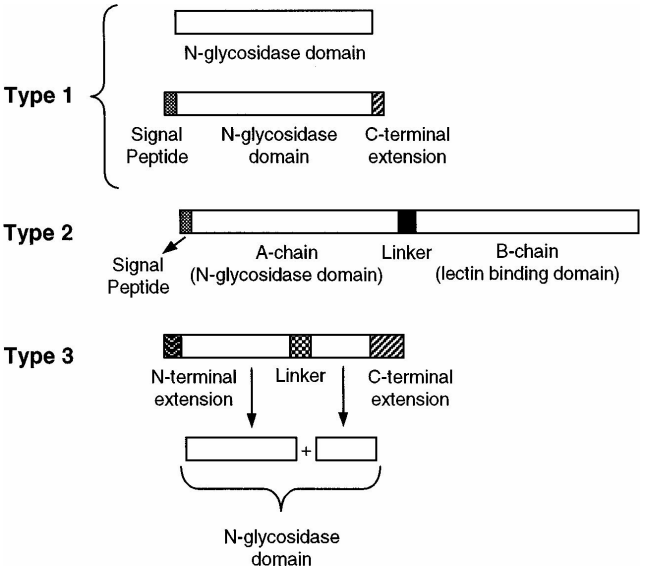

Figure 1: Classification of RIPs

3. Dispersion of RIPs

Due to their unique enzymatic activity towards eukaryotic cells, these proteins had received attention in research for their use as a therapeutic agents. To understand their mode of action and to optimize their therapeutic applications, different RIPs have been isolated from about 50 plants species covering 17 families. Some of them include many RIPs-producing species such as Cucurbitaceae, Euphorbitaceae, Nyctagaceae (Stirpe, 1992). Many plants which are from taxonomically unrelated families showed presence of RIPs which are not only structurally similar but also apparently identical in mode of action. Most of the RIPs are present in roots, leaves and seeds of the plants. It has also observed that more than one RIP can be isolated from one source, which showed similar amino acid sequence and most likely comprise a multigene family, for example, RIPs isolated from seeds of *Saponaria officinalis*, *Momordica charantia* and *Mirabilis jalapa* and leaves of *Dianthus carophyllus* and *Phytolacca americana* leaves (Stirpe *et al.,* 1992). One of such frequently used protein for medical purpose is Mirabilis Antiviral Protein (MAP).

Many studies have led to an improved understanding of MAP gene expression and activity against pathogens and cancerous cell also have uncovered new enzymatic activities that are suggestive of MAP biology being quite complex. This article summarizes work related to MAP activities and its different applications.

4. Mirabilis antiviral protein (MAP)

Mirabilis jalapa L., a native Peruvian plant, which is found to contain proteins in leaves and roots (MacBride, 1951). These proteins showed inhibition of mechanical transmission of certain plant viruses (Verma and Kumar,1979; Kubo *et al.,*1990; Takanami *et al.,* 1990; Vivanco, 1999). This protein is further named as Mirabilis Antiviral Protein (MAP), (Habuka *et al.,* 1992). This protein was purified with the help of cation exchange chromatography, by use of CM-sepharose CL6B column. The protein was revealed to be lysin rich with pI 9.1 and molecular weight close to 24 kDa as determined by SDS-PAGE (Habuka *et al.,* 1991; Takanami *et al.,* 1990). Several isoforms of MAP have been identified in the seeds of *M.jalapa*, MAP (27.788 kD), MAP-2 (30.412 kD), MAP-3 (29.771 kD), and MAP-4 (29.339 kD). Main isoform from seed, *i.e* MAP was present in roots while the second abundant isoform from seed, *i.e*. MAP-4 was also isolated from leaf tissue (Bolognesi *et al*., 2002).

Habuka *et al.* (1989) showed that MAP is made up of single polypeptide with 250 amino acid. They also revealed a complete amino acid sequence and it was observed that amino acid sequence has 24% homology with ricin A chain and also contain two homologus regions with ricin A and shiga like toxi. Region from Ile 163 to Ile 175, IQMVSEAARFKYI contain glutamic acid residue (Glu 168) which was corresponding to the Glu 177 from ricin A chain and Glu 167 of shiha like toxin. The glutamic acid residue of both these proteins has been proved to be an accepted active site for ribososme inactivation (Hovde *et al.,* 1988; Schlossman *et al.,* 1989). The MAP is highly rigid and thermostable protein, this property is due to the presence of intramolecular disulphide bond (Habuka *et al.,* 1989). In *E. coli*, recombinant biological active MAP was expressed at 42°C without inactivation (Habuka *et al.,* 1990), whereas ricin A chain was expressed but inactivated at that temperature, because of absence of intramolecular disulphide bond

(O'Hare *et al.,* 1987; Piatak, 1988). In addition to this MAP also reported to maintain its antiviral activity even after the treatment at 85ºC for 30 min (Takanami *et al.,* 1990).

5. Application of mirabilis antiviral proteins

A: Potential to cleave supercoiled DNA

MAP also has ability to cleave supercoiled double stranded DNA to acquire nick circular and linear forms (Zullies, 2006). This property of nucleic acid cleavage by MAP has been used to identify the presence of this protein in crude extract preparation of plant (Zullies, 2006).

B : Antibacterial activity

RIPs of *M. jalapa* leaves has bacteriostatic activity to *Staphylococcus epidermidis* with MIC value of 10 mg/ml. while it does not inhibit the growth of *Propionibacterium acnes* (Rumiyati, 2014).

C : Antiviral activity

Studies of Kubo *et al.* (1990) showed that MAP extracted from roots showed that it inhibit mechanical transmission of tobacco mosaic, cucumber green mottle mosaic, potato Y, turnip mosaic and cucumber mosaic virus. In other studies, effect of MAP extracted from roots of *Mirabilis jalapa* were studied against potato virus X, potato virus Y, potato leaf roll virus, and potato spindle tuber viroid. The roots extract containing MAP was sprayed on test followed by viral inoculation 24 h later. 100% inhibition was observed as corroborated by infectivity assays and the nucleic acid spot hybridization test. Antiviral activity of MAP extracts was observed against mechanically transmitted viruses but not against aphid-transmitted viruses. Purified MAP showed the same antiviral effect as the crude extracts (Vivanco, 1999). This antiviral activity is nonspecific regarding the characteristics of the virus and hence may have agricultural and clinical applications (Vivanco, 1999).

D: Anticancer activity

MAP was observed to be more toxic to cancerous cells than normal cells. MAP isolated from leaves showed different extent of cytotoxicity against T47D and SiHa cell lines while it was relatively less cytotoxic to mononuclear cell (Zullies, 2006). In addition, many studies had demonstrated that MAP indicated its potent anticancer activity through apoptotic pathway. Its activity was confirmed by DNA fragmentation and cell morphology change methods (Zullies, 2003, Watthanachaiyingcharoen *et al.,* 2007). In our study, we have observed that MAP isolated from roots showed more specific cytotoxic activity against cancer cell line such as MACF-7, A549, HCT 116, than the normal cell line, *i.e.* Vero (Kale and Mukundan, 2015). Recombinant RIPs can be made available on a scale which could meet the high demand of its use as a potential therapeutic agent for treating cancer cells (Salehzadeh and Arasteh, 2012).

6. Conclusion

RIPs commonly has an important enzymatic activities such as RNA N-glycosidase and adenine polynucleotide glycosidase activity. One of such RIP is Mirabilis antiviral protein which has vast applications in medical and therapeutic field. MAP showed inhibitory effect against plant viral infections, therefore has potential to use in agriculture. It also showed antibacterial and anticancer activity, more emphasis should be given for identifying its potential activity which will throw more insight into cytotoxic drug development and treatment of diseases.

Conflict of interest

We declare that we have no conflict of interest.

References

Au, T.K.; Collins, R.A.; Lam, T.L.; Ng, T.B.; Fong, W.P. and Wan, D.C.C. (2000). The plant ribosome inactivating proteins lufûn and saporin are potent inhibitors of HIV-1 integrase. FEBS Lett., **471**:169-72.

Bailey-Serres, J. and Gallie, D.R. (1998). A Look Beyond Transcription: Mechanisms Determining mRNA Stability and Translation in Plants. Rockville, MD: Am. Soc. Plant Physiol., 145-52.

Barbieri, L.; Battelli, M.G. and Stirpe, F. (1993). Ribosome-inactivating proteins from plants. Biochim. Biophys. Acta, **1154**:237-82.

Barbieri, L.; Valbonesi, P.; Bonora, E.; Gorini, P.; Bolognesi, A. and Stirpe, F. (1997). Polynucleotide:adeoine glycosidase activity of ribosome-inactivating proteins: effect on DNA RNA and poly(A). Nucl. Acids Res., **25**:518-22.

Bass, H.W.; Webster, C.; OBrian, G.R.; Roberts, J.K.M. and Boston, R.S. (1992). A maize ribosome-inactivating protein is controlled by the transcriptional activator Opaque-2. Plant Cell, **4**:225-34.

Bolognesi, A.; Polito, L.; Lubelli, C.; Barbieri, L.; Parente, A. and Stirpe, F. (2002). Ribosome-inactivating and adenine polynucleotide glycosylase activity in *Mirabilis jalapa* L. tissues. J. Biol. Chem., **277**(16):13709-16.

Cavallaro, U.; Nykjaer, A.; Nielsen, M. and Soria, M.R. (1995). Alpha 2-macroglobulin receptor mediates binding and cytotoxicity of plant ribosome-inactivating proteins. Eur. J. Biochem., **232**:165-71.

Chan, W.L.; Shaw, P.C.; Tam, S.C.; Jacobsen, C.; Gliemann, J. and Nielsen, M.S. (2000). Trichosanthin interacts with and enters cells *via* LDL receptor family members. BBRC, **270**:453-57.

Chaudhry, B.; Mueller, U.F.; Cameron Mills, V.; Gough, S. and Simpson, D. (1994). The barley 60 kDa jasmonate-induced protein (JIP60) is a novel ribosome-inactivating protein. Plant J., **6**:815-24.

Coleman, W.H. and Roberts W.K. (1982). Inhibitors of animal cell-free protein synthesis from grains. Biochim. Biophys. Acta, **696**:239-44.

Correll, C.C.; Munishkin, A.; Chan, Y.L.; Ren, Z.; Wool, I.G. and Steitz, T.A. (1998). Crystal structure of the ribosomal RNA domain essential for binding elongation factors. Proc. Natl. Acad. Sci. USA, **95**:13436-41.

Endo, Y. and Tsurugi, K. (1987). RNA N-glycosidase activity of ricin A-chain: mechanism of action of the toxic lectin ricin on eukaryotic ribosomes. J. Biol. Chem., **262**:8128-30.

Endo, Y.; Mitsui, K.; Motizuki, M. and Tsurugi K. (1987). The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes: the site and the characteristics of the modification in 28S ribosomal RNA caused by the toxins. J. Biol. Chem., **262**:5908-12.

Fan, J.M.; Zhang, Q.; Xu, J.; Zhu, S.; Ke, T.; Gao, D.F. and Xu Y.B. (2009). Inhibition on Hepatitis B virus *in vitro* of recombinant MAP30 from bitter melon. Mol. Biol. Rep., **36**:381-388.

Gasperi-Campani, A.; Barbieri, L.; Battelli, M.G. and Stirpe, F. (1985). On the distribution of ribosome-inactivating proteins amongst plants. J. Nat. Prod., **48**:446-54.

Gutell, R.R.; Gray, M.W. and Schnare, M.N. (1993). A compilation of large subunit (23S and 23S-like) ribosomal RNA structures. Nucleic Acids Res., **21**:3055-74.

Habuka, N.; Akiyama, K.; Tsuge, H.; Miyano, M.; Matsumoto, T. and Noma, M. (1990). Expression and secretion of Mirabilis antiviral protein in *E. coli* and its inhibition of *in vitro* eukaryotic and prokaryotic protein synthesis. JBC, **265**:10988-92.

Habuka, N.; Miyano, M.; Kataoka, J. and Noma, M. (1991). *Escherichia coli* ribosome is inactivated by Mirabilis antiviral protein which cleaves the N-glycosidic bond at A2660 of 23 S Ribosomal RNA. J. Mol. Biol., **221**:737-43.

Habuka, N.; Miyano, M.; Kataoka, J.; Tsuge, H. and Noma, M. (1992). Specificities of RNA N-glycosidase activity of Mirabilis antiviral protein variants. JCB, **267**:7758-60.

Habuka, N.; Murakami, Y.; Noma, M.; Kudo, T. and Horikoshi, K. (1989) Aminoacid sequence of Mirabilis antiviral protein, total synthesis of its gene and expression in *Escherichia coli*. J. Biol. Chem., **264**:6629-6637.

Hartley, M.R. and Lord, J.M. (1993). Structure, function and applications of ricin and related cytotoxic proteins. ***In***: Biosynthesis and Manipulation of Plant Products, ed. D Griesson, pp:210-39. New York: Chapman & Hall.

Hovde, C.J.; Calderwood, S.B.; Mekalanos, J.J. and Collier, R.J. (1988). Evidence that glutamic acid 167 is an active-site residue of Shiga-like toxin I. Proc. Natl. Acad. Sci., **85**(8):2568-2572.

Irvin, J.D. (1975). Purification and partial characterization of the antiviral protein from *Phytolacca americana* which inhibits eukaryotic protein synthesis. Arch. Biochem. Biophys., **169**:522-28.

Irvin, J.D. (1983). Pokeweed antiviral protein. Pharmacol. Ther., **21**:371-87.

Kale, D.K.C. and Mukundan, U. (2015). Cytotoxicity against tumor cell lines of a purified Mirabilis antiviral protein isolated from root of *Mirabilis jalapa*. World Journal of Pharmaceutical Research, **4**(2):1696-1710.

Kubo, S.; Ikeda, T.; Imaizumi, S.; Takanami, Y. and Mikami Y. (1990) A potent plant virus inhibitor found in *Mirabilis jalapa* L. Ann Phytopathol. Soc. Japan, **56**:481-87.

Lehar, S.M.; Pedersen, J.T.; Kamath, R.S.; Swimmer, C. and Goldmacher, V.S. (1994). Mutational and structural analysis of the lectin activity in binding domain 2 of ricin B chain. Protein Eng., **7**:1261-66.

Lodge, J.K.; Kaniewski, W.K. and Tumer, N.E. (1993). Broad-spectrum virus resistance in transgenic plants expressing pokeweed antiviral protein. Proc. Natl. Acad. Sci., **90**:7089-93.

Logemann, J.; Jach, G.; Tommerup, H.; Mundy, J. and Schell, J. (1992). Expression of a barley ribosome-inactivating protein leads to increased fungal protection in transgenic tobacco plants. Biotechnology, **10**:305-8.

MacBride, J.F. (1951). Euphorbiaceae, spurge family. ***In***: Flora of Peru. Chicago Field Museum of Natural History, Chicago, pp:543-44.

Mlsna, D.; Monzingo, A.F.; Katzin, B.J.; Ernst, S. and Robertus, J.D. (1993). Structure of recombinant ricin A chain at 2.3 A. Protein Sci., **2**:429-35.

Monzingo, A.F. and Robertus, J.D. (1992). X-ray analysis of substrate analogs in the ricin A-chain active site. J. Mol. Biol. **227**:1136-45.

Mundy, J.; Leah, R.; Boston, R.; Endo, Y. and Stirpe F. (1994). Genes encoding ribosome-inactivating proteins. Plant Mol. Biol. Rep., **12**:S60-62.

Nilsson, L.; Asano, K.; Svensson, B.; Poulsen, F.M. and Nygard, O. (1986). Reduced turnover of the elongation factor EF-1 X ribosome complex after treatment with the protein synthesis inhibitor II from barley seeds. Biochim. Biophys. Acta, **868**:62-70.

O'Hare, M.; Roberts, L.M.; Thorpe, P.E.; Watson, G.J.; Prior, B. and Lord, J.M. (1987). Expression of ricin A chain in *Escherichia coli*. FEBS Lett., **25**(1):73-78.

Olsnes, S and Pihl, A. (1982). Different biological properties of the two constituent peptide chains of ricin, a toxic protein inhibiting protein synthesis. Biochemistry, **12**:3121-26.

Olsnes, S. and Sandvig, K. (1988). How protein toxins enter and kill cells. Cancer Treat. Res., **37**:39-73.

Orita, M.; Nishikawa, F.; Shimayama, T.; Taira, K.; Endo, Y. and Nishikawa, S. (1993). High-resolution NMR study of a synthetic oligoribonucleotide with a tetranucleotide GAGAloop that is a substrate for the cytotoxic protein, ricin. Nucleic Acids Res., **21**:5670-78.

Pastan, I. and Fitzgerald, D. (1991). Recombinant toxins for cancer treatment. Science, **254**:1173-76.

Peumans, W.J.; Hao, Q. and Van Damme, E.J. (2001) Ribosome-inactivating proteins from plants: More than N-glycosidases? FASEB, **15**:1493-1506.

Piatak (1988). Expression of soluble and fully functional ricin A chain in *Escherichia coli* is temperature sensitive, using M13-derived vectors: an efficient and general proce J. Biol. Chem., **263**(10):4837-4843.

Rumiyati,; Nugrahani, A.W.; Sismindari,; Lukitaningsih E. and Yuliati, T. (2014). Potential of Ribosome-inactivating proteins (RIPs) of *Mirabilis jalapa* L. as an antiacne: Effect on proliferation of cultured sebocyte cells and its antibacterial activities against *Propioni bacterium* acnes and *Staphylococcus epidermidis*. International Journal of Pharmaceutical Chemistry, **4**(4):130-133.

Salehzadeh A. and Arasteh A. (2012). Expressing of rice ribosome inactivating protein as tool for treatment of cancer cells. Scientific Research and Essays, **7**(1):61-65.

Sandvig, K. and Van Deurs, B. (1999). Endocytosis and intracellular transport of ricin: recent discoveries. FEBS Lett., **452**:67-70.

Sandvig, K.; Olsnes, S. and Pihl, A. (1976). Kinetics of binding of the toxic lectins abrin and ricin to surface receptors of human cells. J. Biol. Chem., **251**:3977-84.

Schlossman, D.; Withers, D.; Welsh, P.; Alexander, A.; Robertus, J. and Frankel A. (1989). Role of glutamic acid 177 of the ricin toxin A chain in enzymatic inactivation of ribosomes. Mol. Cell Biol., **9**(11):5012-5021.

Steeves, R.M.; Denton, M.E.; Barnard, F.C.; Henry, A. and Lambert, J.M. (1999). Identification of three oligosaccharide binding sitesnin ricin. Biochemistry, **38**:11677-85.

Stirpe, F.; Barbieri, L.; Battelli, M.G,; Soria, M. and Lappi, D. A. (1992). Ribosome-inactivating proteins from plants: present status and future prospects. Biotechnology, **10**:405-12.

Stirpe, F.; Gasperi-Campani, A.; Barbieri, L.; Lorenzoni, E. and Montanaro, L. (1978). Inhibition of protein synthesis by modeccin, the toxin of *Modecca digitata*. FEBS Lett., **85**:65-67.

Takanami ,Y.; Kuwata, S.; Ikeda, T. and Kubo, S. (1990). Purification and characterization of the anti-plant viral protein from *Mirabilis jalapa* L. Ann. Phytopathol. Soc. Japan, **56**:488-94.

Verma, H.N. and Kymar, V. (1979). Prevention of potato plants from viruses and insect vectors. J. Indian Potato Assoc., **6**:157-61.

Vivanco, J.M. (1999). Antiviral and Antiviroid Activity of MAP-containing extracts from *Mirabilis jalapa* roots. Plant Disease, **83**(12):1116-21.

Walsh, T.A.; Morgan, A.E. and Hey, T.D. (1991). Characterization and molecular cloning of a proenzyme form of a ribosome-inactivating protein from maize: novel mechanism of proenzyme activation by proteolytic removal of a 2.8-kilodalton internal peptide segment. J. Biol. Chem., **266**:23422-27.

Watthanachaiyingcharoen, R.; Utthasin, P.; Potaros, T. And Suppakpatana, P. (2010). Proteins from *Mirabilis jalapa* possess anticancer activity *via* an apoptotic pathway. J. Health. Res., **24**(4): 161-165.

Zullies, I.; Kawati; Sujadi and Sismindari. (2006). Cytotoxicity against tumor cell lines of a Ribosome - inactivating protein (RIP) like protein isolated from leaves of *Mirabilis jalapa* L. Malays J. Pharm. Sci., **4**(1):31-41.

Zullies, I.; Sudjadi,; Widyaningsih, E.; Dyah, P. and Sismindari (2003). Induction of apoptosis by protein fraction isolated from the leaves of *Mirabilis jalapa* L. on HeLa and Raji cell-line. Oriental Pharmacy and Experimental Medicine, **3**(3):151-156.