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PROGRESS IN RIBOSOMAL INACTIVATING PROTEIN (RIP) STUDIES: RECENT REVIEW OF POTENTIAL APPLICATIONS

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ABSTRACT

It is reported that some plants contain molecules that inactivate the ribosomes by inhibiting the protein synthesis through their N-glycosidase enzymatic activity. The molecules are identified to be group of proteins namely ribosomal inactivating proteins (RIPs). The RIPs have received a lot of attention in recent biomedical research because of their unique biological and enzymatic activities towards animal and human cells. RIPs can be toxic or non toxic based on their cytotoxicity. The first RIP, Ricin was identified from Ricinus communis seeds (castor beans) and this one was important milestone for plant protein identification. This paper reviews elaborately on recent progress in RIP studies - classification, biological and enzymatic activity and also its potential applications in medical research. Information on structural aspects of RIP compiled from the protein data bank and represented in this review paper will provide insight into researchers who aspire to work on cytotoxic drugs.

KEY WORDS

Ribosomal inactivating protein (RIP), Biological activity of Ribosomal inactivating protein (RIP), Structural classification of Ribosomal inactivating protein (RIP), anticancer activity, HIV effect

INTRODUCTION

Ribosomal inactivating proteins (RIPs) are widely distributed among higher plants (Mehta and Boston 1998)[1]. RIPs have been identified and purified from both monocotyledons and dicotyledons and have not yet been purified in Gymnosperms (Barbieri et al. 1993)[2]. Different RIPs have been reported in various plant species covering approximately 17 families. Some families include *Cucurbitaceae, Euphorbiaceae, Poaceae*, and families belonging to the super order Caryophyllales (Sharma et al. 2004)[3]. RIPs with various types of bioactivity are located in a variety of tissues, including leaves, seeds, roots and tubers in the same plant (Shu et al. 2009)[4]. RIPs are mainly acts as toxic and non-toxic activity based on their cytotoxicity. The first non-toxic RIP was identified from *Ricinus communis seeds (castor beans)* namely *Ricinus* agglutinin (RAC) (Svinth et al. 1998)[5].

Many biological effects such as the toxicity of castor bean seeds (*Ricinus communis*), jequirity bean (*Abrus precatorious*) and also the abortifacient activity of some plants like *Trichosanthes kirilowii* and *Momordica charantia* (Peumans et al. 2001)[6], has been proved to be due to the presence of RIPs. These plants inactivate ribosomes which inhibit protein synthesis by their *N*-glycosidase activity (Park et al. 2006)[7]. The RIPs have attracted a lot of attention in biomedical research and development towards animal and human cells. The interest in RIPs has arisen because an understanding of this enzymatic activity can enhance the exploitation of the unique properties and activities of RIPs. RIPs show diverse applications like immunotoxicity (Battelli et al. 1996)[8], abortifacient (Yeung et al. 1988)[9], and anti-human immunodeficiency virus (HIV) agents (Barbieri et al. 1993, Park et al. 2006)[2, 7]. RIPs show some unique

bioactive properties including antiviral, antifungal, antibacterial and antitumor activity (Shu et al. 2009, Stirpe and Battelli 2006)[4, 10]. This paper reviews the structural and functional characteristics of RIPs and their potential applications.

TYPES OF RIBOSOMAL INACTIVATING PROTEIN

RIPs have been classified into three different types depending on the presence or absence of atleast one polypeptide chain with lectin activity.

- **Type 1**

Type 1 RIPs are composed of a single polypeptide chain with a molecular mass of approximately 30 kDa. Some examples are Pokeweed Antiviral Protein (PAP), Mirabilis Antiviral Protein (MAP) and Camphorin. It is the most common and has been identified in more than 30 plants (Barbieri et al. 1993)[2]. In some Type 1 RIP containing plants, a galactose specific lectin is also present as a separate molecule (Eg: *Trichosanthus kirilowii* seeds) (Shu et al. 2009)[4].

- **Type 2**

Type 2 RIPs have two chain proteins. It is composed of two polypeptide chains a molecular weight of approximately 60 - 120 kDa. The A chain has RNA N-glycosidase activity (Mol. Wt approx. 30 kDa) and the B chain binds to galactose specific lectin (Mol. Wt approx. 35 kDa) (Stirpe and Battelli 2006)[10]. The two polypeptide chains are linked by disulphide bonds and other non covalent bonds (Peumans et al. 2001)[6]. The B chain because of its carbohydrate-binding lectin interacts with cell membranes and facilitates the uptake of RIP into the cytosol space (Barbieri et al. 1993, Sharma et al. 2004)[2, 3]. Type 2 RIP is usually divided into two categories, toxic and non toxic Type 2 RIPs (discussed later). Ricin, Abrin, Modeccin, Volkensin and Ebulin 1 are common examples of Type 2 RIPs (Montfort et al. 1987)[11].

- **Type 3**

Type 3 RIPs have an N-terminal domain closely related to the A chain of RIPs and linked to an unrelated C-terminal domain with unknown function. For example, The RIP, JIP-60 (Mol. Wt – 60kDa) obtained from barley (*Hordeum vulgare*) (Sharma et al. 2004, Reinbothe et al. 1994)[3, 12].

DISTRIBUTION IN NATURE

Ribosomal inactivating proteins (RIPs) are commonly found in plants. Type 1 RIPs were found in various plants including some that are eaten raw like spinach (Ishizaki et al. 2002)[13] and tomato (Barbieri et al. 2006)[14]. The occurrence of Type 1 RIPs is higher when compared to Type 2 RIPs. Toxic Type 2 RIP, Aralin has been found in the shoots of *Aralia eleta* (Tomatsu et al. 2003)[15], but can be safely consumed as the RIP denatures on cooking. RIPs called Ricin are present only in the seeds of *Ricinus communis* plant, whereas Saporin is present in all tissues of soap-wort, including leaf, stem and root (*Saponaria officinalis*) (Ferreras et al. 1993)[16]. Sometimes both Type 1 and Type 2 RIPs have been found in the same plant (e.g. *Sambucus nigra* (Ferreras et al. 2000)[17], *Cinnamomum camphora* (Ling et al. 1995)[18] and toxic and non-toxic RIPs may coexist (e.g. Ricin and *Ricinus* agglutinin in castor beans, while Nigrin-B was found to be of both types (Hartley et al. 1996)[19].

Many bacteria and fungi also produce RIPs that are capable of bringing about inhibition of protein synthesis. However their mode of action is different from that of plant RIPs. The Type 2 RIPs from plants show surprising similarities with toxins produced by some bacteria such as *Corynebacterium diphteriae* and *Pseudomonas aeruginosa* which produced the RIPs diphtheria toxin (DT) and Pseudomonas exotoxin (PE) respectively. Here the toxicity of the RIP is due to its association with lectin and Elongation Factor 2 (EF2) ADP ribosylase (Morimoto and Bonavida 1992)[20]. *Escherichia coli* and *Shigella dysenteriae* also produce a RIP, namely Shiga toxin (ST) (Brigotti et al. 2002)[21], which associates with lectin and rRNA N-glycosidase (Barbieri et al. 1993, Narayanana et al. 2005)[2, 22]. However, the ribosome inactivation activity of Shiga toxin is identical to that of plant RIPs (Narayanana et al. 2005)[22]. RIPs are also produced by calluses and cultured cells of the algae *Laminaria japonica* and fungi *Lentinus edodes* and mushrooms. A RIP-like glycosylase activity was also found in mammalian cell and tissue cultures (Peumans et al. 2001)[6]. Cells from RIP producing plants do not always produce RIPs in culture, and commonly differences were observed among cultures from the same species (*Phytolacca americana*). For example, differences

were found in the production of the *Mirabilis Antiviral* Protein (MAP) from *Mirabilis jalaba* cultured cells (Barbieri et al. 1993)[2].

OCCURRENCE

- **Type 1 Ribosomal inactivating proteins**

The first Type 1 RIP, Pokeweed Antiviral Protein (PAP) found in *Phytolacca americana* leaves prevent Tobacco Mosaic Virus (TMV) infection (Barbieri et al. 1993, Duggar and Armstrong 1925)[2, 23]. It has been studied for its inhibitory activity of plant viruses and recognized as an inhibitor of protein synthesis (Dallal and Irvin 1978)[24]. Few other studies on Type 1 RIPs have been discussed in Table 1 (Barbieri et al. 1993, Stirpe and Battelli 2006, Hartley et al. 1996, Stirpe and Barbieri 1986, Fracasso et al. 2010)[2, 10, 19, 25, 26].

- **Type 2 Ribosomal inactivating proteins**

Ricin, the protein was first purified from the seeds of *Ricinus communis* (castor beans) (Stillmark 1888)[27]. The identification of ricin was an important milestone in biochemistry because for the first time a well-defined biological activity was discovered in a plant protein. The property of Ricin is attributed to its toxicity which causes the agglutination of erythrocytes (Barbieri et al. 1993)[2]. Later on, other agglutinating property of RIP was discovered from the plant seeds of *Abrus precatorius*, namely Abrin. This protein played an important role in the early development of immunology (Peumans et al. 2001)[6]. Small amounts of Abrin and Ricin induced immunity in rabbits when fed with the respective plant seeds. This was a fundamental observation in the history of immunology (Ehrlich 1892)[28]. Modeccin the toxin from *Adenia digitata* (Modecca), suspected to be similar to Ricin (Green and Andrews 1923)[29], was independently identified as a toxic Type 2 RIP (Refsnes et al. 1977)[30]. Type 2 RIPs, Modeccin and Volkensin were present in the roots and seeds of the respective plants. Other highly toxic Type 2 RIPs were found in other plants such as *Passifloraceae*, *Adenia goetzii*, *Adenia lanceolata* and *Adenia stenodactyla* (See Table 2). Thus, Type 2 RIPs seem to be particularly frequent among *Adenia* plants, many of which contain galactose-binding lectins (Pelosi et al. 2005)[31].

TOXIC AND NON TOXIC TYPE 2 RIBOSOMAL INACTIVATING PROTEINS

Type 2 RIPs divided into two groups, toxic and non-toxic, based on the considerable differences in their cytotoxicity. Differences exist within the toxic RIPs. Abrin, Volkensin and the toxin from *Adenia stenodactyla* (Pelosi et al. 2005)[31], are more potent than Ricin. The reasons for these differences are not known and they are involved in the binding and entry of the proteins into the cells and the intracellular destination, degradation and exocytosis of the proteins (Stirpe and Battelli 2006)[10]. The first non-toxic Type 2 RIP was identified from *Ricinus communis* seeds (castor beans) namely *Ricinus* agglutinin (RAC) which may be due to its reduced capacity to translocate (Svinth et al. 1998)[5]. Some of toxic and non toxic Type 2 RIPs are shown in Table 2 (Barbieri et al. 1993, Stirpe and Battelli 2006, Hartley et al. 1996, Stirpe and Barbieri 1986, Fracasso et al. 2010) [2, 10, 19, 25, 26].

RIPs do something more other than just inactivating ribosomes as observed from the lesions causing activity reported by Ricin and Volkensin (Battelli 2004)[32], may be due to high doses or due to their immunotoxin (Battelli et al. 1996)[8]. An important factor is the production of cytokines, which may be released by macrophages damaged by RIPs. This leads to apoptosis (Narayanana et al. 2005)[22] and inflammatory reactions (Stirpe and Battelli 2006)[10].

STRUCTURE

The study of both primary and three dimensional RIP structures have been attempted.

i. Primary structure

The Type 1 RIPs are similar with the A chain of Type 2 RIP and this Type 2 RIP has different the B chain. A close examination indicates that the sequence similarity between the amino-terminal is much higher than that of the carboxyl-terminal sequences (Peumans et al. 2001)[6]. Type 1 RIPs have been sequenced from nearly 22 different sources and vary in their resolution and ligands binding ability to the substrate. RIPS are structurally classified into single, double and multi chains. Double chains are also divided into two types, homo and hetero model

chains. The results compiled from a Protein data bank are shown in Table 3.

ii. Three-dimensional structure

The first 3-dimensional structure of RIP was reported in Ricin with the resolution of 2.8 Å. Ricin is a globular, glycosylated heterodimer linked by a single disulfide bond. The A chain consists of 267 amino acids with approximately 50% of the polypeptide arranged in alpha-helices and beta-sheets. Three individual domains are distinguished from the A chain, which forms the active site of RNA N-glycosidase activity. The B-chain is a bilobal structure in which the domains are homologous. Each B chain domain contains galactose binding site in the polypeptide chain by the tripeptide Asp-Val-Arg (Montfort et al. 1987)[11].

BIOLOGICAL ACTIVITIES

The two main biological properties of RIPs are (i) inhibition of the multiplication of plant viruses and (ii) extremely potent cytotoxicity (Bolognesi et al. 2002)[33]. The direct effect of both types of RIPs on cell structure and function is an irreversible damage to ribosomes. Here the larger subunit becomes unable to bind with the elongation factors, resulting in the arrest of protein synthesis. It has similar potency in cell free systems, but has different toxicity to cells and animals (Barbieri et al. 1993)[2].

ENZYMATIC ACTIVITY

RIPs are classified as rRNA N-glycosidases in the enzyme nomenclature (EC 3.2.2.22). It has been suggested that RIPs specifically cleave the N-C glycosidic bond of the single adenine base (A4324 in rat liver rRNA) in the tetra loop sequence (GAGA) at the top of a stem in 28S rRNA called sarcin/ricin (S/R) loop (Park et al. 2006, Stirpe and Battelli 2006)[7, 10]. In addition to its *N*-glycosidase activity, some RIPs have DNase, DNA glycosylase, and apurinic pyrimidinic lyase activities (Li et al. 1991)[34]. It has been suggested that the nuclease activities are due to the contamination by other enzymes, whereas all RIPs tested to remove Adenine from DNA and also from poly (A) (Sharma et al. 2004)[3]. The enzymatic activity of RIP was defined as polynucleotide adenosine glycosidase, which changed to adenine polynucleotide glycosylase (APG) in the EC nomenclature of nucleic acid glycosylases (Bolognesi et al. 2002)[33]. Some RIPs have specific DNA nuclease activity against supercoiled, covalently closed, circular plasmid DNA and single stranded phage DNA (Sharma et al. 2004)[3].

RIPs have been found in nucleic acids, randomly removing adenine residues from single-stranded regions of nucleic acids and guanine residues from wobble base-pairs in hairpin stems. This substrate recognition and enzymatic activity depend on the physical availability of nucleotides and their denaturation increases with their interaction with RIPs (Park et al. 2006)[7]. Apart from adenine and guanine glycosidase activity, RIPs exhibit a number of novel enzymatic activities such as RNase, DNase, Phosphatase activity on lipids (Helmy et al. 1999)[35], Phosphatase activity on nucleotides (Chen et al. 1996)[36], Chitinase activity (Shih et al. 1997)[37] and Superoxide Dismutase (SOD) (Li et al. 1996)[38] activities. These observations suggest that RIPs may possess dual biochemical activities and multiple biological roles (Park et al. 2006)[7].

APPLICATIONS

i) Antiviral activity

RIPs from many sources have shown to possess effective activity against both plant and animal virus infections. All types of RIPs have antiviral activity against plant, fungal and animal viruses except the Type 2 RIPs obtained from *Eranthis hyemalis* (Kumar et al. 1993)[39] and *Sambucus nigra* (Chen et al. 2002)[40]. The antiviral activity of the RIPs in both plants and animals is not caused by a direct effect on virions before cell infection. This observation states that the antiviral RIPs which are often extra cellular, led to the local suicide hypothesis where in, RIPs selectively gain entrance into the cytosol of infected cells and inactivate the ribosomes thereby preventing virus replication (Hartley et al. 1996)[19].

Pokeweed Antiviral Protein (PAP) protects suitable indicator plants from viral infections which include DNA and RNA viruses. They also prevent their replication in human cells of polio virus, cytomegalo virus, influenza virus, herpes simplex virus and HIV

(Chen et al. 1991)[41]. The replication of HIV in cells was inhibited by RIPs and could be used in AIDS therapy (Shaw et al. 2005)[42]. High-level expression of the PAP was harmful to transfected tobacco (Lodge et al. 1993)[43] and bent-grass (Dai et al. 2003) [44] plants. RIP active on tobacco ribosomes inhibited the formation of local lesions caused by tobacco mosaic virus infection in tobacco leaves (Taylor et al. 1994) [45].

Trichosanthin from the plant *Trichosanthus kirilowii*, selectively inhibits HIV RNA and protein accumulation without affecting host-cell's gene expression (Barbieri et al. 1993)[2]. However Trichosanthin gave disappointing results, as sometimes the administered protein aggravated neurological (Garcia et al. 1993)[46] or mental symptoms and caused allergic reactions (Byers et al. 1994)[47]. MAP is effective against the tobacco (*Nicotiana tabacum*) mosaic virus, potato (*Solanum tuberosum*) virus X, potato virus Y, and viroids such as the potato spindle tuber viroid (Kubo et al. 1990)[48]. This antiviral activity is nonspecific regarding the characteristics of the virus and hence may have agricultural and clinical applications (Hartley et al. 1996)[19].

ii) Antifungal and Antibacterial activity

RIPs have some bioactive properties, which include antibacterial, antifungal and insecticidal activities. Thus the induced expression of a RIP from *Phytolacca heterotepala* and Asparagus (*Asparagus officinalis*) enhanced resistance of tobacco plants to the fungi *Alternaria alternate* and *Botrytis cinerea* (Corrado et al. 2005)[49], whereas transfection of *Vitis vinifera* with barley RIP did not improve its resistance against the fungi, *Uncinula necator* and *Plasmopara viticola* (Bornhoff et al. 2005)[50]. Fungal ribosomes have been shown to be more sensitive to barley seed RIP than mammalian ribosomes (Roberts and Selitrennikoff 1986)[51] and this RIP needs the support of two enzymes namely alpha-glucanase and chitinase, to enter the fungal cell. Barley seed RIP along with the enzymes which are known to degrade the cell walls, acted synergistically in the inhibition of mycelial growth of *Trichoderma reesei, Fusarium sporotrichioides* (Leah et al. 1991)[52]. Transgenic tobacco provided significantly enhanced protection against the soil borne fungal pathogen *Rhizoctonia solani*, when compared with the barley transgene level (Jach et al. 1995)[53]. RIPs were highly effective in inhibiting the growth of *Trichoderma reesei* and *Fusarium sporotrichioides* (barley pathogen), *Rhizoctonia solani* (potato pathogen) and *Botrytis cinerea* (Pea pathogen) (Barbieri et al. 1993)[2]. There are possible differences both in the activity of the various RIPs and the sensitivity of fungal species towards them. Also two more proteins, from the mushrooms *Hypsizygus marmoreus* and *Lyophyllum shimeji* reported as RIPs have shown to be active against of fungi (Lam and Ng 2001)[54].

iii) Abortifacient activity

The roots of *Trichosanthus kirilowii* have been used to induce abortion, due to the effect of Trichosanthin, a protein used as abortifacient in official Chinese medicine. Actually, RIPs are not abortifacient, in that they do not induce abortion by causing contractions of the uterus or a hormonal imbalance. However sometimes they cause the death of the fetus by killing Syncytiotrophoblasts. These cells are highly sensitive to RIPs because macrophages have a high capacity of protein uptake, and taking up a large amount of RIP (Battelli et al. 1992)[55]. The abortifacient activity of Trichosanthin was attributed to its effect on ribosomes (Stirpe and Battelli 2006)[10].

iv) Anticancer activity

RIPs were found to be more toxic to tumor cells than to normal cells (Lin et al. 1970)[56]. It is possible that the RIPs are more harmful to malignant cells because they have a high rate of protein synthesis while actively proliferating, and also because they are more sensitive to toxins (Stirpe and Battelli 2006)[10]. Extracts of *Viscum album* (mistletoe) and *Ximenia americana*, both containing the Type 2 RIPs, Viscumin and Riproximin (Table 2), have been used as anticancer agents. RIPs may have some beneficial effect on cancer patients, not only by acting directly on cancer cells, but also by exerting strong stimulation of the immune system and inducing the production of cytokines in the cells under the effect of several RIPs (Yamasaki et al. 2004)[57].

v) Immunotoxins

RIPs are used as therapeutic agents against cancer and HIV infection. RIPs have been chemically linked

or genetically fused with carriers mainly antibodies, growth factors, hormones and lectins in odrer to make them selectively toxic to a target cell. For this, Type 1 RIPs and isolated A chains of Type 2 RIPs have been used. But Type 2 RIPs are not suitable because B chains can bind to any cell (Rosenblum 2004)[58]. Most research on immunotoxins has been focused on the cancer therapy. RIP containing immunotoxins should have some advantages over the conventional chemotherapeutic agents. They can be very potent, as they act on both dividing and non dividing cells without inducing resistance. A possibility is the use of immunotoxins administered intravesically for bladder cancer therapy. A Saporin containing immunotoxin could be safely administered to severely immunodeficient patients (French et al. 1996)[59]. RIP-containing immunotoxins and a fibroblast growth factor-saporin conjugate specific for bladder tumor cells have been prepared and submitted to clinical trials (Battelli et al. 1996)[8]. Muscle-specific immunotoxins are potential immunotherapeutic agents for the treatment of focal muscle spasm, myasthenia gravis and strabismus, by destroying oculomotor muscles (Hott et al. 1998)[60]. Other possible uses of immunotoxins are for ophthalmology, Alzheimer's disease and to prevent corneal cell proliferation (Stirpe and Battelli 2006)[10].

vi) Bioweapons

Toxic Type 2 RIPs are potential biohazards, especially Ricin has been considered as a possible weapon for warfare and terrorist attacks. Sources of other toxic RIPs are not as easily available as castor beans, but their toxins could be obtained by biotechnological techniques. Ricins and related toxins could be used to contaminate water supplies or large amounts of food. Serious concerns that Ricin or similar toxins could be disseminated in the air as dust or aerosol in terrorist attacks have prompted a number of studies (Knight 1979)[61]. The toxins cause severe inflammatory and even necrotic lesions, which could be greatly reduced by washing one's eyes with a lactose solution. Washing with saline was ineffective. These results indicate that (i) the toxin binds almost immediately to galactosyl-terminated receptors on the cell, from which it can be removed by lactose, but not by saline, and (ii) Ricin enters cells rapidly, after which washing with lactose is almost totally ineffective (Stirpe and Battelli 2006)[10].

vii) Agriculture & Crop plant Biotechnology

In agriculture, plants were transfected with RIP genes mainly barley RIP, PAP, Trichosanthin and Dianthin gave resistance to viruses and fungi (Rosenblum 2004)[58]. Many studies have been performed on the applications of RIPs in drug development and crop-plant biotechnology due to their toxicity towards viruses, tumor cells, insects and plant fungal pathogens. Many RIPs are involved in defense mechanisms in plant cells and terminate protein synthesis under appropriate physiological conditions and are involved in metabolic regulation. In addition, some RIPs accumulate in non-reproductive tissues, such as cotyledon, bark and root. Some researchers have proposed that RIP might play a role as a storage protein in these tissues (Liu et al. 2002)[62].

CONCLUSION

RIPs commonly play an important role in both biological and enzymatic activities such as RNA N-glycosidase and adenine polynucleotide glycosidase activity. RIPs were found to be more toxic to tumor cells than normal cells, and are hence used in developing antitumor drugs that selectively target tumor cells. It has vast application in medical and therapeutic field. Numerous anticancer and antiviral compounds with structural similarity to RIP can be developed and tested for their applications. A possible role of RIP, as a defense mechanism against plant pathogens could be of great biological importance. A protective effect against viral infections was also proposed. More emphasis should be given in the near future to purify RIPs in different medicinal plants. And analyzing their RIP structure together with identifying its potential activity will throw more insight into cytotoxic drug development and treatment of diseases.

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TABLE 1: TYPE 1 RIBOSOMAL INACTIVATING PROTEIN (RIP)

S. No	Family	Species	Organ	RIP
1.	*Adoxaceae*	1.*Sambucus nigra*	Fruits	Nigritin
2.	*Asparagaceae*	1.*Asparagus officinalis*	Seeds	Asparin 1, Asparin 2
3.	*Caryophyllaceae*	1.*Agrostemma githago* (Corn Cockle)	Seeds	Agrostin 2, Agrostin 5, Agrostin 6
		2.*Dianthus Caryophyllus* (Carnation)	Leaves	Dianthin 30, Dianthin 32
		3.*Dianthus barbatus*	Leaves	Dianthin 29
		4.*Saponaria officinalis* (Soap wort)	Seeds	Saporin S5, Saporin S6, Saporin S8, Saporin S9
			Leaves	Saporin L1, Saporin L2
			Roots	Saporin R1, Saporin R2, Saporin R3

		5.*Lychnis Chaledonica*	Seeds	Lychnin
4.	*Cucurbitaceae*	1.*Luffa cylindrical*	Seeds	Luffin A, Luffin B
		2.*Luffa acutangola*	Seeds	Luffaculin
		3.*Momordica charantia* (Bitter gourd)	Seeds	Momordin I, Momordin II
		4.*Momordica cochinchinesis*	Seeds	Momorcochin
		5.*Trichosanthes kirilowii* (Chinese Cucumber)	Roots	Trichosanthin, Alpha-Trichosanthin, TAP-29
			Seeds	Trichikirin
		6.*Bryonia dioica*	Leaves	Bryodin-L
			Roots	Bryodin-R
		7.*Citrullus colocynthis*	Seeds	Colocin 1, Colocin 2
		8.*Cucumis melo*	Seeds	Melonin
		9.*Cucurbita pepo*	Fruits	Pepocin
		10.*Sechim edule*	Seeds	Sechiumin
		11.*Trichosanthus anguina*	Seeds	Trichoanguin
5.	*Euphorbiaceae*	1.*Gelonium multiflorum*	Seeds	Gelonin
		2.*Jatropha curcus*	Seeds	Curcin-2
		3.*Croton tiglium*	Seeds	Crotin 2, Crotin 3
		4.*Hura creptans*	Latex	Huracrepitans RIP
		5.*Manihot palmate*	Seeds	Mapalmin
		6.*Manihot utilissima*	Seeds	Manutin 1, Manutin 2
6.	*Iridaceae*	1.*Iris hollandica*	Bulbs	Iris RIP A1, Iris RIP A2, Iris RIP A3
7.	*Lauraceae*	1.*Cinnamomum camphora* (Camphor tree)	Seeds	Camphorin, Cinnamomin
8.	*Nyctaginaceae*	1.*Mirabilis jalapa* (4'o Clock)	Root	MAP
		2.*Mirabilis expansa*	Root	ME1, ME2
		3.*Bougainvillea spectabilis*	Leaves	Bouganin
9.	*Phytolaccaceae*	1.*Phytolacca Americana* (Poke weed)	Leaves	PAP, PAP I, PAP II
			Seeds	PAP-S
			Roots	PAP-R

			Tissue culture	PAP-C
		2.*Phytolacca dodecandra*	Leaves	Dodecandrin
			Tissue culture	Dodecandrin-C
		3.*Phytolacca dioica*	Seeds	PD-S1, PD-S2, PD-S3
		4.*Phytolacca insularis*		PIP, PIP2
10.	*Pluteaceae*	1.*Volvariella volvaceae*	Fruiting bodies	Volvarin
11.	*Poaceae*	1.*Hordeum vulgare*	Seeds	Barley RIP
		2.*Triticum aestivum* (Wheat germs)	Seeds	Tritin-S
			Leaves	Tritin-L
			Germs	Tritin
		3.*Zea mays* (Maize)	Seeds	Maize RIP
		4.*Secale Cereale*	Seeds	Secale cereal RIP
12.	*Tricholomataceae*	1.*Hypsizigus marmoreus*	Fruiting bodies	Hypsin
		2.Lyophyllum shimeji	Fruiting bodies	Lyophillin

TABLE 2: TOXIC AND NONTOXIC TYPE 2 RIBOSOMAL INACTIVATING PROTEIN (RIP)

S. No	Family	Species	Organ	Toxic RIP	Non toxic RIP
1.	*Adoxaceae*	1.*Sambucus nigra* (Blue elder berry)	Bark	Nigrin-B	Nigrin B
		2.*Sambucus ebulus*	Leaves	-	Ebulin-L
		3.*Sambucus sieboldiana*	Bark	-	Sieboldin-B
2.	*Araliaceae*	*1.Aralia eleta*	Shoots	Aralin	-
3.	*Curcurbitaceae*	*1.Momordica charantia*	Seeds	-	*Momordica charantia* lectin
4.	*Euphorbiaceae*	*1.Ricinus Communis* (Castor Bean)	Seeds	Ricin	*Ricinus* agglutinin
5.	*Fabaceae*	*1.Abrus precatorius* (Rosery Pea)	Seeds	Abrin	-
6.	*Iridaceae*	*1.Iris hollandica*	Bulb	-	*Iris* agglutinin
7.	*Lauraceae*	1.*Cinnamomum camphora*	Seeds	-	Cinnamomum
		2.*Cinnamomum porrectum*	Seeds	-	Porrectin
8.	*Olacaceae*	*1.Ximenia americana*	Powder	Riproximin	-
9.	*Passifloraceae*	*1.Adenia digitata*	Roots	Modeccin	-
		2.*Adenia volkensii* (Kilyambiti)	Roots	Volkensin	-

		3.*Adenia goetzii*	Caudex	Unknown lectin like protein	-
		4.*Adenia lanceolata*	Caudex	Lanceolin	-
		5.*Adenia stenodactyla*	Caudex	Stenodactylin	-
10.	*Viscaceae*	1.*Viscum album*	Leaves	Viscumin	-
		2.*Phoradendron californium*	Leaves	*Phoradendron californ* lectin	-

TABLE 3: STRUCTURAL CLASSIFICATION OF RIBOSOMAL INACTIVATING PROTEIN (RIP)

S. No	Family	Species	RIP (Protein id)		
			Single chain	Double chain	Multi Chain
1.	*Caryophyllaceae*	1. *Dianthus caryophyllus* (Clove pink)- leaves	1 (1RL0)	-	-
		2. *Dianthus caryophyllus* (Clove pink) – Exp. Sys *E.coli*	3 (1LP8, 1LPC, 1LPD)	-	-
		3. *Lychnis chalcedonica* (Scarlet lychnis)- seeds	1 (2G5X)	-	-
2.	*Cucurbitaceae*	1. *Trichosanthus kirilkii* (Mongolian snake-gourd)	4 (1TCS, 1MRJ, 1MRK, 1QD2)	-	-
		2. *Trichosanthus kirilkii,* (Mongolian snake-gourd) Exp.Sys: *E.coli*	4 (1GIS, 1GIU, 1NLI, 2JJR)	1 (2VS6)	1 (1J4G)
		3. *Momordica balsamina* (Balsam apple)	8 (3N31, 3MY6, 3N1N, 3NFM, 3NJS, 3NX9, 3N5D, 3N1D)	1 (3N3X)	-
		4. *Momordica charantia* (Balsam pear)	7 (1AHA, 1AHB, 1AHC, 1MRG, 1MRH, 1MRI, 1F8Q)	-	-
		5. *Momordica balsamina* (Bitter gourd)	12 (3MRY, 3MRW, 3V2K, 3QJI, 3U70, 3U8F, 3U6T, 3V14, 3RL9, 3S9Q, 3SJ6, 3U6Z)	-	-
		6. *Cucurbita moschata*- sarcocarp of pumpkin	1 (3BWH)	-	-
		7. *Luffa aegyptiaca* (Smooth loofah) - Exp. Sys: *E. coli*	1 (1NIO)	-	-
		8. *Bryonia dioica* (Red bryony)- Exp. Sys: *E. coli*	-	1 (1BRY)	-

3.	*Euphorbiaceae*	1. *Ricinus communis* (Castor bean) 2. *Ricinus communis* (Castor bean) -Exp. Sys- *E.coli* 3. *Ricinus communis* (Castor bean) - host *E.coli*	4 (1RTC, 1IFS, 1IFT, 1IFU) 9 (1OBT, 1OBS, 1BR6, 1BR5, 1IL3, 1IL9, 1IL4, 1UQ5, 1UQ4) 4 (2P8N, 2R2X, 2PJO, 2R3D)	2 (2AAI, 3RTI) 1 (1IL5) -	1 (3RTJ) - -
4.	*Fabaceae*	1. *Abrus precatorius* (Indian licorice)	-	3 (1ABR, 2Q3N, 2ZR1)	-
5.	*Hyacinthaceae*	1. *Charybdis maritima* (Sea squill) –Bulb	1 (2B7U)	-	-
6.	*Nyctaginaceae*	1.*Bougainvillea spectabilis* (Great Bougainvillea) - leaves	1 (3CTK)	-	-
7.	*Phytolaccaceae*	1.*Phytolacca americana* (American pokeweed) 2. *Phytolacca dioica* (Bella sombra tree) 3. *Phytolacca dioica* (Bella sombra tree)- Exp. Sys: *E.coli* 4. *Phytolacca accinosa*- (Pokeweed seeds)	2 (1LLN, 1GIK) 2 (2QES, 2Z4U) 2 (2QET, 2Z53) 1 (2Q8W)	4 (1QCG, 1D6A, 1QCI, 1QCJ) - 2 (3H5K, 3LE7) - -	- - - -
8.	*Poaceae*	1. *Zea mays* (Maize) Exp. Sys- *E. coli*	1 (2K6H)	1 (2PQG)	2 (2PQI, 2PQJ)
9.	*Viscaceae*	1. *Viscum album* (European mistletoe)	-	9 (1MLL, 1CE7, 1M2T, 1OQL, 1PC8, 1PUM, 1PUU, 1YF8, 2R9K)	-
10.	*Hominidae*	1. Homosapiens 2. Homosapiens Exp. Sys- *E. coli*	1 (2D9L) 1 (2OLM)	- -	- -
11.	Bacteria	1. *Shigella dysenteriae* Exp. Sys: *E. coli* 2. *E. Coli*	- -	- -	2 (1DM0, 1R4Q) 1 (1R4P)
12.	Virus	1. *Enterobacteria phage* 933w	-	-	1 (2GA4)

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