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Antimicrobial Activity of Lectins from Plants

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1. Introduction

There are at least three reasons for the need in finding out new alternative antimicrobial substances from natural sources. The first reason is that people nowadays concern about toxic of synthetic substances including daily contact chemicals or even drugs used in medical or healthcare purposes (Hafidh et al., 2009). Any synthetic drugs were avoided in order to keep physiological cleans as belief. Thus, natural substances were used increasingly instead as well as any substances used for antimicrobial purposes. The second reason is that new alternative drugs are human hope for better fighting with existed diseases and pathogens. They may replace currently used drugs in points of more efficiency, more abundant, lower side-effect or safer or even lower production cost. It is fact that most alive organisms should have some mechanisms or substances fight with all time contacting pathogens so that they can be survived in nature. Although a plenty of antibiotics were discovered after first time Fleming's declaration, but they were still relatively low amounts compared with overall real natural antimicrobial substances. This mean the natural sources still flourish with novel antimicrobial substances waiting for discovered. Additional small aspect may be raised here. The natural substances are usually good leading compound sources for mostly synthetic drug from the long past due to their diversities are far from human imagination. New chemical structures are always found in natural resources as higher frequency than artificial deducing structures. The final reason is that the mechanism used to synthesize natural substances are available and they are usually can be imitated in small, medium, and even large scale production with present biotechnological knowledge which looks easier than newly designed plants.

Plants are of primary importance in the global ecosystem. They are, together with a small group of bacteria, the only living organisms which are capable of harvesting and storing solar energy by virtue of their photosynthetic apparatus which converts light energy into chemical energy through the reductive assimilation of carbon dioxide. Marine and terrestrial plants are the first link in the global food chain. Virtually all other life on earth depends on the organic molecules they synthesize. Evidently, the fact that the majority of heterotrophic organisms depends on them makes plants favorite targets of a whole variety of parasites and predators. Therefore, plants must defend themselves against their potential enemies. During the past 15 years, a large number of antimicrobial proteins (AMPs) have been identified in different plants (Broekaert et al., 1997). AMPs constitute a heterogenous class of low molecular mass proteins, which are recognized as important components of defense

system. They directly interfere with the growth, multiplication and spread of microbial organisms (Lehrer and Ganz, 1999). Different proteins with antibacterial and/or antifungal activity have been isolated from seeds, tubers, and rhizomes, where they accumulate to high levels and may also function as storage proteins. Homologous of the seed proteins have also been identified at very low concentrations in floral and vegetative tissues (Terras et al., 1995; Kheeree et al., 2010; and Charungchitrak et al., 2011). There are several classes of proteins having antimicrobial properties which include thionins, lipid transfer proteins, plant defensins, chitinases, glucanases, 2S albumins, ribosome inactivating proteins and lectin (Ye et al., 2002; and Zhang and Halaweish, 2003).

Lectins are proteins or glycoproteins of a ubiquitous distribution in nature, which have at least one carbohydrate or derivative binding site without catalytic function or immunological characteristics. They have the unique ability to recognize and bind reversibly to specific carbohydrate ligands without any chemical modification; this distinguishes lectins from other carbohydrate binding proteins and enzymes, and makes them invaluable tools in biomedical and glycoconjugate research (Peumans and Van Damme, 1995). Plants were the first discovered source of lectins and, although lectins have since been found to be universally distributed, plants remain the most frequently used source of lectin studies due to both the ease of their extraction and the relatively high yields that can be obtained. Moreover, different families of plants, as well as different tissues within the same plant, can contain different lectins with different bioactivities, including different carbohydrate-binding specificities. It has been suggested that plant lectins may have important roles according to their abundance, including in the immune defence, and also that lectins have been co-opted adapted for several functions during evolution (Sharon and Lis, 2001).

The role of lectins in the defense mechanism of plants may have evolved from the ability to lectins to agglutinate and immobilize microorganisms. The supporting evidence for this proposed role in defense against pathogens falls into two main observed categories, namely (a) the presence of lectins at potential sites of invasion by infectious agents, and (b) the binding of lectins to various fungi and their ability to inhibit fungal growth and germination. A number of studies with respect to the potential defense role of plant lectins have been reported. For example, during the imbibition of dry soybean seeds, lectin is released into the water and the presence of this lectin in the vicinity of germinating seeds hints at possible interactions of lectins with potential pathogens. The developmental pattern of the initial accumulation and final disappearance of lectin can be observed during the seed dormancy, germination and maturation, which may implicate the role of lectins in a defense mechanism necessary for plants in the initial stages of growth. Moreover, some lectins may provide some protection to plants against generalist herbivores (Howard et al., 1995). This chapter is intended to provide exposure for recent papers in details of antimicrobial activity of lectins from plants. This omission can be remedied by reading the more detailed reviews listed in the references.

2. General properties of plant lectins

Lectins are proteins or glycoproteins of non-immune origin derived from plants, animals or microorganisms that have specificity for terminal or subterminal carbohydrate residues. The main characteristic of this class of proteins is their ability to interact with carbohydrates and thus combine with glycocomponents of the cell surface, as well as with cytoplasmatic and

nuclear structures and the extracellular matrix of cells and tissues from throughout the animal and plant kingdoms, down to microorganisms (Brooks and Leathem, 1998). The availability of a large number of lectins with distinct carbohydrate specificities has resulted in the use of these proteins as tools in medical and biological research (Singh et al., 1999), and has attracted great interest because of their remarkable effects in a wide range of biological systems, including the purification and characterization of glycoconjugates and the study of cell-surface architecture. The agglutination activity of these highly specific carbohydrate binding molecules is usually inhibited by a specific simple monosaccharide, but for some lectins di-, tri-, and even poly-saccharides are required. They are classified into a small number of sugar specificity groups, such as mannose, galactose, *N*-acetylglucosamine, L-fucose and *N*-acetylneuraminic acid, according to the monosaccharide that is the most effective inhibitor of the lectin-mediated agglutination of erythrocytes (Lis and Sharon, 1986).

The lectins represent a large group of plant proteins. Lectins have been found in less than 500 species, which indicates that only a limited number of higher plants, contain detectable levels of lectins (Van Damme et al., 1998). However, the majority of the studies on lectins have been carried out on legume species (Kocourek, 1986; and Lakhtin, 1994) particularly in their seeds where they comprise up to 15% of the total protein. As a result of these studies, many plant lectins have become a very popular class of proteins because of their obvious potential in aiding researchers in other areas of the life sciences. A variety of lectins are presently envisioned to be involved in one or more at least three roles releasing to plant defense. One such defense role for some lectins may be in the recognition of oligosaccharide signals produced by the breakdown of cell wall components of the plant or pathogen upon contact with the plant. A second type of defense role may involve a direct interaction of a lectin with the infectious agent. A third defense type with a considerable support is that some lectins play role in protecting the plant animal predators (Weis and Drickamer, 1996).

Legumes and monocots are major sources of plant lectins that have been widely studied (Wood et al., 1999). Plant lectins can be classified into four major families of structurally and evolutionary related proteins: legume lectins, type 2 ribosome inactivating proteins, chitin-binding lectins, and monocot mannose-binding lectins. Three other small lectin families (Cucurbitaceae phloem lectins, amaranthins, and jacalin-related lectins) have also been characterized (Van Damme et al., 1999). Legume lectins represent the largest and most thoroughly studied family of plant lectins. They have been isolated from seeds, stem, and bark of legumes (Imberty et al., 2000). The best known legume lectins are phytohemagglutinin (PHA) from red kidney bean, soybean (SBA), jackbean (Concanavalin A), peanut lectin (PNA), and pea (PSL) (Lis and Sharon, 1998). Type 2 ribosome-inactivating proteins consist of the toxic A subunit and Gal/GalNAc binding subunit of B chain. Whereas the A chain has RNA glycosidase activity, the B chain is responsible for binding to the target cell surface and helping in the internalization of the whole protein into cell membrane (Kaku et al., 1996; and Wood et al., 1999). Ricin from seeds of *Ricinus communis*, the first plant lectin, is an example (Sphyris et al., 1995; and Lisgarten et al., 1999). Chitin-binding lectins containing hevein domains have been prevalently found in cereal. Examples are wheat germ agglutinin, pokeweed mitogen, rice, rye, and barley lectins (Lis and Sharon, 1998; and Wood et al., 1999). Monocot mannose-binding lectins were first reported from the snowdrop (*Galanthus nivalis*) (Van Damme et al., 1997). Later several lectins have been

extracted and intensively characterized from several monocot families: Alliaceae, Amaryllidaceae, Araceae, Bromeliaceae, Iridaceae, Liliaceae, and Orchidaceae (Wood et al., 1999). For example, *Narcissus pseudonarcissus* (daffodil) and *Scilla campanulata* (bluebell) lectins have been recently reported (Sauerborn et al., 1999; and Wood et al., 1999).

3. History of plant lectins

Lectin, agglutinin, and hemagglutinin are synonym of lectin's names. The first time that Lectin was descript back to 1888 by Stillmark, who study the lectin from the seeds of castor bean (about Ricin A toxic ferment from the seeds of *Ricinus communis* L. and some other Euphorbiaceae Species). He linked the toxicity of castor beans to the occurrence of a hemagglutinaing protein factor. For along time before it was definitely demonstrated that Stillmark's "ricin" was a mixture of a weakly agglutinating protein toxin (still known as ricin) and a nontoxic agglutinin (*Ricinus communis* agglutinin, or RCA). The first evidence for this came from studies by Kabet et al. during World War II. They found by immunochemical methods that the toxic and hemagglutinating properties of "ricin" were due to different substances. Only in 1960 was separation of the two substances achieved by Funatsu, and know that ricin came to the attention of the general public in 1978, following its use as a weapon in the notorious, politically motivated "umbrella murder". The dimensions of the hole, led to the conclusion that ricin was the killing agent, since very few poisons are sufficiently potent to kill a man at such a minute amount. In 1898 Elfstrand introduced for the first time the term "hemagglutinin" as a common name for all plant proteins that cause clumping of cells. The idea that toxicity is an intrinsic property of lectin was abandoned in the beginning of the century after have a report for the first time the present of nontoxic lectin in the legumes *Phaseolus vulgaris* (bean), *Pisum sativum* (pea), *Lens culinaris* (lentil), and *Vicia sativa* (vetch). Following this work more nontoxicity plant hemagglutinin has been found. Eventually, it became Evident that lectin is widespread in the plant kingdom and that toxicity is the exception rather than the rule (Van Damme et al., 1995).

The next milestone in the history of plant lectin was a term. When have been found that some hemagglutinin exhibit a clear preference toward erythrocytes of a particular human blood group within the ABO system (Boyd and Reguera, 1949; and Renkonen, 1948). The term "lectin" originally introduced to emphasize the selective agglutination behavior of some hemagglutinin, it was later applied to all proteins with agglutinating activity. "hemagglutinin" is certainly a more appropriate term than lectin because it refers to the capability of a protein to agglutinate erythrocytes but does not take into account that most lectin can also agglutinate other cells. Hence, the term agglutinin should be preferred. In the absence of a clear consensus, the term lectin is actually most commonly used, but agglutinin and hemagglutinin still persist as synonyms.

The current confusion in the terminology of lectin to a great degree is result in the fact that different names have been introduced before the mechanism causing the macroscopically visible agglutination activity was understood in molecular terms. In 1936 already observed that cane sugar inhibited the agglutination activity of Concanavalin A (Con A) (Summer and Howell, 1936). It was demonstrated in 1952 that the agglutination properties of lectin is base on a specific sugar-binding activity (Watkins and Morgan, 1952). As soon as lectin was recognized as carbohydrate-binding protein they could be distinguished from other proteins on the basis of a well-defined functional criterion. For this reason lectin is now considered initially as carbohydrate-binding proteins rather than as (hem) agglutinin.

4. Occurrence and distribution

Lectins are usually considered as a very large and heterogeneous group of proteins (Goldstein and Poretz, 1986). Although, there is no doubt indeed that numerous plant species of different taxonomic groupings contain lectins. The total number of well-documented cases is about 400. Assuming that all the close relatives of these plants also contain agglutinins and that some new lectins will be discovered in the future, the expected occurrence of lectins is still limited to a small fraction of the plant kingdom. It can be concluded, therefore, that the occurrence of at least the classical agglutinating lectins in plants is the exception rather than the rule. However, in contrast to the relative scarcity of the agglutinating lectins, chimerolectins belonging to the Class I chitinases seem to be present in almost all plant species (Collinge et al., 1993).

Lectins are widely distributed throughout the plant kingdom where they have been found in a variety of tissues of a large number of different plants. In plants, lectins are particularly localized in seeds. Howard et al., 1972, reported that seed lectins are particularly seen in cotyledons where they appear during the later stages of maturation of the seeds. In addition to cotyledons, in some cases appreciable amounts of lectins have been reported in the embryos and small amounts in the seed coats (Pueppke et al., 1978). Immunolocalization studies have revealed that lectins are primarily found in the protein bodies of the cotyledon cells (Herman and Shannon, 1984). During the early seedling growth, Weber and Neumann (1980) noticed the decrease in lectin concentration as the cotyledons are resorbed. A short survey of the occurrence and concentration of lectins in seeds as well as in different types of vegetative tissues reveals striking differences in the location and relative abundance of the individual lectins. Usually, seed lectins are confined to cotyledons (e.g. legumes) or endosperm (e.g. castor bean). Normally lectins account for up to 5% of the total seed proteins. Sometimes, they become predominant protein in the seed representing 50% of the total seed protein (e.g. Phaseolus species). The non-seed lectins are found in all kinds of vegetative tissues such as leaves, stem, bark, bulb, tubers, corns, rhizomes, roots, fruits, flowers, ovaries, phloem sap and even in nectar (Peumans and Van Damme, 1995) and are only minor, quantitatively unimportant proteins. Non-seed lectins may occur in different tissues of the same plant. The snowdrop and daffodil lectins, for instance, have been found in all vegetative tissues, although the lectin is most abundant in the bulbs (Van Damme and Peumans, 1990). Similarly, the potato lectin occurs in tubers, stems, leaves and fruits (Kilpatrick, 1980). There are exceptions also. The ground elder berry lectin is confined to the rhizome only (Peumans et al., 1985). In the case of tulip bulbs, lectins are present in large quantities in the bulb but are almost undetectable in stem and leaves (Van Damme and Peumans, 1995). Some legume lectins are found in seeds as well as in bark tissues. A thorough examination of the genes coding for these lectins revealed that the seed and bark lectins are encoded by different, though highly hommologus, genes (Van Damme et al., 1995).

5. Hemagglutinating activity by plant lectins

Lectins are a group of protein that can bind to carbohydrate (which can be in form of sugar, oligosaccharide, or polysaccharide) specifically. Binding of the lectins is differed from those enzymes, anti-lectin antibodies, and other carbohydrate specific binding protein on that they will never change any bound-carbohydrate properties, not convert such carbohydrate to other substances, not come form immune origin, and being reversible binding. In addition to

carbohydrate binding specifically, the lectins can cause cells agglutinated and glycoprotein or carbohydrate precipitated. That is why the lectins are sometimes called "agglutinin" (Sharon and Lis, 2001). Since most lectins have two or more carbohydrate binding sites in their molecules, which can make cross-linkages between cells or carbohydrate containing molecules and form solid network. However, there are also some certain lectins that presented in momovalent binding site, and thus can not agglutinate cells or precipitate carbohydrate.

Some lectins contain more than one type of acting site or one activity in single molecules so that they can bind to carbohydrate and can exhibit other behaviors such as enzymatic activity (which make this lectin called "lectzyme"), mitogenic activity, and transportation activity in the same time. From these phenomena, the lectins can be classified into three types according to their acting sites as "merolecins" (the lectins with only single carbohydrate binding domain, usually small single peptides), "hololectins" (the lectins with two resemble carbohydrate binding domains), "chimerolectins" (the lectins contains both carbohydrate binding domain and other well-defined biological active domains which act dependently of previous domain). Beside this classification, the lectins can also be classified by their ligand specificities in two manners. The first is that by sizes of binding ligand which the lectins can be divided into two group; the lectins that specifically binding to monosaccharides as well as oligosaccharides and the lectins that specifically binding to only oligosaccharides (Peumans and Van Damme, 1995). The second classification manner is relatively old style that was set up during little details of lectin's information known. Thus, they were separated by their legand specificity only in sugar types such as mannose or glucose specific lectins, galactose specific lectins, and sialic acid specific lectins. However, they were recently found that most lectins tended to recognize certain three dimension structure than monosaccharide specificity. Thus, this classification style may not up to date because many of lectins formally grouped in one class are now no longer suitable for such class. Anyways, it may be familiar to some authors and may also found in some present documents.

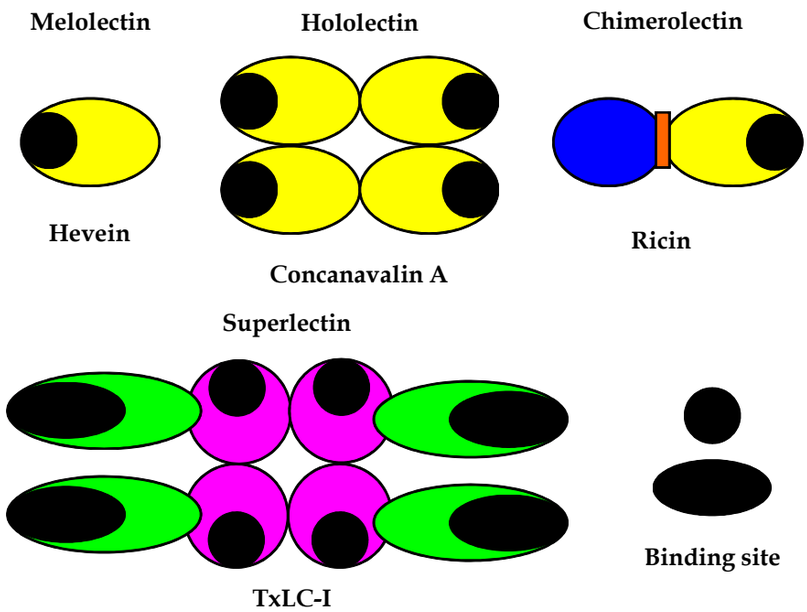


Fig. 1. Schematic representation of the three types of plant lectins: merolectins, hololectins, and chimerolectins. (Peumans and Van Damme, 1995).

Although the lectins have been found in human, animal, plant, and microorganisms, but it looks like that plant lectins were the most investigated for details (Sharon and Lis, 2001; and Chandra et al., 2006). Most lectins are present in seed cotyledons of the plant (but also found in any other parts such as roots, stems, rhizomes, and leaves in lesser amounts). In such tissues, most lectins are located within cytoplasm or protein bodies inside the cells (Moreira et al., 1991). In general, the lectins with the same ligand specificity contain different binding abilities mainly depended on their sources meant different genetic material that produce different lectins with different in three dimension structures. For instances, Tipthara et al., (2007) successfully purified a mannose specific lectin with strong rabbit hemagglutinating activity (0.017 μg of minimum amount that hemagglutination presented) of from *Curcuma Zedoaria*, Thipthara et al., (2008) also purified many lectins with weak activity (0.140 to 0.190 mg minimum amount that hemagglutination presented), Wong et al., (2008) purified mannose/glucose specific lectin with extremely strong activity (83.063 ng minimum amount that hemagglutination presented) from *Castanopsis chinensis*. Most of plant lectins become a set of important tools for glycobiology achievements. They are also applied in detection, isolation, and characterization of glycoconjugated substances mainly in glycoprotein, proteoglycan, and modified polysaccharides (Sharon and Lis, 2001). The lectins are also advantages in immunology, histochemistry, pathology, and physiology areas. One familiar instance which the lectin usage is clearly seen is ABO blood type identification using blood group specific lectin such as Concanavalin A, a lectin derived from jack bean seed (*Canavalia ensiformis*) that can specifically bind to non-reducin α-terminal mannose. This blood groups determination is based on presence or absence of specific glycoprotein on red blood cells that the lectins can bind and make red blood cells agglutination (Moreira et al., 1991) by forming network with red blood cells and then can not be collected as button like form in the U shape bottom well. From this incident, a method widely used for lectin screenings or characterizations mainly involved cells agglutination, especially red blood cells from various animals (Sharon and Lis, 2001). The lectins also have other roles in mammals. There was evidences indicated that the lectins played the important roles in cell differentiation, cell movement and phagocytosis, cell to cell and cells to matrix substances communication, cell organization in tissues, and embryo morphogenesis (Moreira et al., 1991).

On the other hand, consuming of lectins also may cause adverse results in some cases. Several lectins such as Concanavalin A and wheat germ agglutinin (WGA) are toxic to mammalian cells, but relatively low compared with other toxic substances such as approximately 1000 times lower than ricin (an toxic albumin from Caster bean). It is believed that production and accumulation of toxic lectins in some plants are a kind of defending mechanisms which plants develop for protecting them form certain plant eating organisms such as insects and mammals (Peumans and Van Damme, 1995) and plant pathogens. Aside from defense mechanisms, the lectins also have their essential roles in plant-microorganism symbiosis, cell differentiation, pollen recognition, cell wall elongation, and as a reserved protein (Moreira et al., 1991). Interestingly, some plant lectins were found to be well react with viral surface glycoprotein and were hoped to use in controlling many diseases originated from viruses which current methods are still inadequate controllable efficiencies. Balzarini et al., (2004) isolated two mannose-specific lectins from *Galanthus nivalis* (snowdrop) (GNA) and *Hippeastrum* sp. hybrid (Amaryllis) (HHA) and found that they contained *in vitro* anti-HIV virus activities ranged from 0.12 to 1.2 μg/ ml for GNA and from 0.18 to 0.70 μg/ ml for HHA depended on tested viral nature.

6. Plant lectin

Lectins have been found in a wide variety of species almost every major taxonomical classification of flowering plants (Allen and Brilliantine 1969; and Mialonier et al., 1973). Many plants and their individual tissues have been routinely screened for lectins by measuring the ability of their extracts to agglutinate erythrocytes. Although this hemagglutination assay has been of great value in detecting lectins, it is at best semiquantitative; it will not detect inactive or monovalent lectin, nor will it provide accurate estimates of lectin if an endogenous receptor for that lectin is present in the extract. The assay can at times yield false positive results because of nonspecific hemagglutination caused by lipids or by polyphenols such as tannins that are often abundant in plant tissues. It is therefore advisable to verify positive hemagglutination data by inhibiting the activity with specific sugars or by isolating the lectin (Tsivion and Sharon, 1981).

The carbohydrate specificities and structures of lectins from a large variety of plants have been studied in considerable detail. In general, lectins from plants within particular taxonomical groups have distinctive properties that distinguish them from lectins of less closely related plants. It is important to note that the lectins used in these comparisons represent the most abundant and therefore most intensively studied lectins in the plants of these families. These lectins are not all derived from homologous tissues. These differences in origin must be remembered in interpreting these comparisons since, as is discussed below, it is possible that different tissues within the same plant may contain different lectins. This reservation does not apply to comparisons of lectins obtained from homologous tissues of plants within the same family. Homologies within two of these families, the Graminaceae and Leguminoseae, are discussed in further detail below.

Graminaceae: The lectin from monocotyledonous plants is the wheat germ agglutinin, which is a 36,000 molecular weight dimer of identical protein subunits linked by interchain disulfide bonds (Nagata and Burger, 1974; and Rice and Etzler, 1974). The complete amino acid sequence of this lectin has recently been determined (Wright et al., 1984). This lectin has a specificity for oligomers of β (1-4)-*N*-acetyl-o-glucosamine (Allen et al., 1973). Lectins with similar specificities and molecular properties have been isolated from rye (Peumans et al., 1982b) and barley embryos (Mishkind et al.,1983; Peumans et al., 1982b). Indeed, these lectins are so similar that they can undergo subunit exchange to form heterodimers (Peumans et al., 1982a).

Leguminoseae: The seeds of legumes are particularly rich in lectins, and many of these lectins have been characterized extensively (Goldstein and Hayes 1978; Lis and Sharon 1986). As this review was prepared, the complete amino acid sequences of Concanavalin A (Edelman et al. 1972), favin (Cunningham et al., 1979), and lectins from lentil (Foriers et al., 1981), sainfoin (Kouchalakos et al., 1984), *Phaseolus vulgaris* (Hoffman et al., 1982), soybean (Hemperly et al., 1983), and pea (Higgins et al., 1983) have been determined. In addition, the NH_2 terminal amino acid sequences of at least 15 other legume lectins are available. Comparisons of these sequences have shown extensive homologies, particularly among those lectins from plants within the same tribes. It is clear that these lectins have been conserved during evolution of the legumes and that the homologies in their NH_2 terminal amino acid sequences reflect the taxonomical relationships of the plants in this family (Foriers et al., 1977; and Foriers et al., 1979).

7. Sugar binding activity and specificity of plant lectins

Broadly reveal, lectins can be divided into those that bind monosaccharides as well as oligosaccharides, and those that recognize oligosaccharides only (Wu et al., 2001). It is noteworthy that almost all saccharides recognized by lectins are typical constituents of animal cell surfaces. This is perhaps a reflection of the method commonly used for lectin detection (Tsivion and Sharon, 1981), as a result of which lectins recognizing sugars not present on erythrocytes might have been overlooked.

7.1 Mannose/glucose

A lectin with specificity for mannose and glucose residues has been isolated in crystalline form the fava bean (*Vicia laba*) by a procedure which included absorption to Sephadex. It has a molecular weight of 50,000 Da and appears to be a tetramer made of two subunits of 18,000 Da and two subunits of 9,000 Da. These studies determine amino acid sequence and three-dimensional structure of lectin were similar with structural features of Concanavalin A (Irvin, 1976).

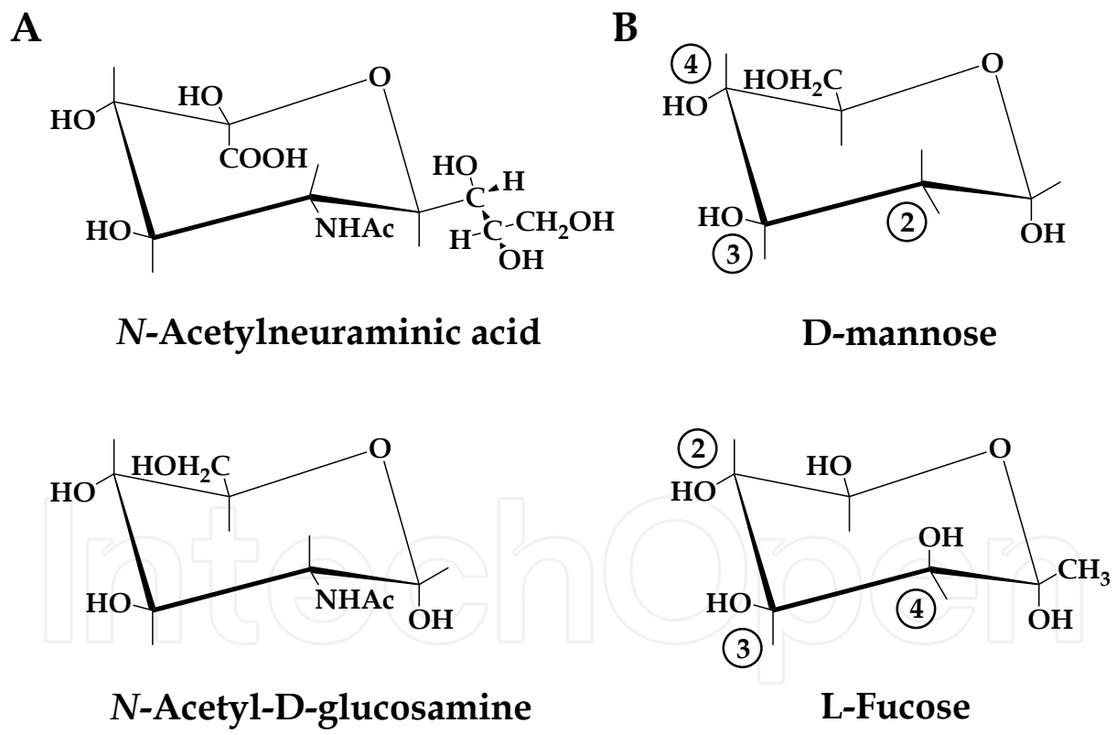


Fig. 2. Common structural features of *N*-acetylneuraminic acid and *N*-acetylglucosamine (A) and of mannose and fucose (B). Similarity of *N*-acetylglucosamine and *N*-acetylneuraminic acid at positions C-2 (acetamido) and C-3 (hydroxyl) of the pyranose ring is observed when the sialic acid molecule is suitably rotated. Rotation of the fucose molecule by 180 Å allows superimposition of its ring oxygen, 4-OH, 3-OH and 2-OH with the ring oxygen, 2-OH, 3-OH and 4-OH of mannose, respectively. Groups that thus occupy the same positions in space are underlined. (Sharon, 1993).

7.2 Galactose/*N*-acetylgalactosamine

As mentioned, lectin interacts with galactose or *N*-acetylgalactosamine such as the lectin from the corn coleoptyle. It is a glycoprotein had molecular mass under non-denaturing conditions was 88.7 kDa And had carbohydrates that constituted 12% of the total weight comprised galactose, mannose, and *N*-acetyl-D-glucosamine (Martinez-Cruz et al., 2001). In 2003 Konozy et al. were found *Erythrina speciosa* seeds can be specific with D-galactose and had two identical subunits of molecular mass was 27.6 kDa include the lectin was a neutral carbohydrate content of 5.5% (Konozy et al., 2003). *N*-acetyl-D-galactosamine-specific lectin isolation from *Glycine max* L. Merrill SA88 them were found the soybean lectin consists of four subunits it had molecular weight of each 30,000 Da in one-step purification with high purity and high yield (about 90% recovery from the crude extract) by use Poly (hydroxypropyl methacrylate-glycidyl methacrylate) beads were as an affinity matrix and *N*-acetyl-D-galactosamine (GalNAc) was as an affinity ligand (Percin et al., 2009).

7.3 Fucose

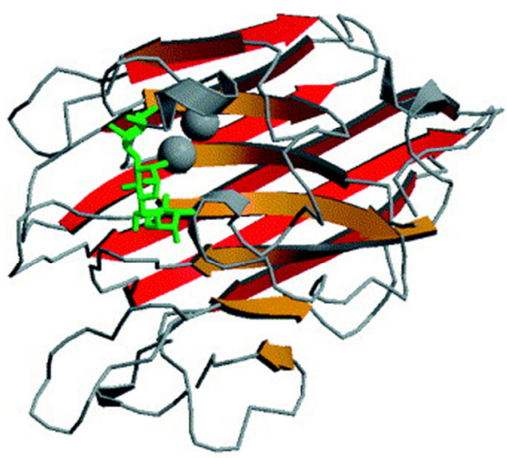
Aleuria aurantia lectin (AAL) is a commercially available lectin that is known for its high affinity for α-1, 6-fucosylated oligosaccharides and it is widely used to estimate the extent of α-1,6-fucosylation on glycoproteins and to fractionate glycoproteins. For research a novel probe for core fucose from *Aspergillus oryzae* L-fucose-specific lectin (AOL) has strongest preference for the alpha 1,6-fucosylated chain among α-1,2-, α-1,3-, α-1,4-, and α-1,6-fucosylated pyridylaminated (PA)-sugar chains. These results suggest that AOL is a novel probe for detecting core fucose in glycoproteins on the surface of animal cells (Matsumura et al., 2007). Furthermore, *Lotus tetragonolobus* lectin is a fucose-specific legume lectin. It is a homotetramer composed of four legume lectin domains was 27,800 Da (Moreno et al., 2008).

7.4 Sialic acids

Most of Sialic acid-specific lectins was found in invertebrates such as those from the Indian horseshoe crab (Mohan et al., 1982), marine crab *Scylla serrata* (Mercy and Ravindranath, 1992), lobster, tunicalase, fungus *Hericium arinaceum* (Kawagishi et al., 1994) and leaves of mulberry (Ratanapo et al., 1998). A lectin from the white shrimp *Litopenaeus setiferus* (LsL) hemolymph is a heterotetramer of two 80 kDa and two 52 kDa subunits, *N*-acetylated sugars, such as GlcNAc, GaINAc, and NeuAc, were the most effective inhibitors of the LsL hemagglutinating activity. Desialylation of erythrocytes or inhibitory glycoproteins abolished their capacity to bind LsL, confirming the relevance of sialic acid in LsL-ligand interactions (Alpuche et al., 2005). In 2009 the *Phaseolus coccineus* lectin (PCL) specificity towards sialic acid showed the molecular mass of 30 kDa consisting of homodimer subunits. Moreover the purified PCL was devoid of antifungal activity against *Candida albicans* and Penicillium *italicum*, but markedly inhibited the growth of *Hericium maydis*, *Rhizoctonia solani*, Gibberella *sanbinetti,* and Sclerotinia *sclerotiorum* while the same concentration of PCL decrease the 50% hemagglutinating activity was inhibited by sialic acid it suggesting a significant correlation between sialic acid-specific site and its bi-functional bioactivities (Chen et al., 2009).

8. Structure of plant lectin

Different lectin families are in general structurally unrelated. And even in those cases where a common fold is recruited, convergent evolution is the most likely explanation. Some lectin families such as the galectins recognize only one specific oligosaccharide, and consequently have a very conserved recognition site. On the other extreme, members of the C type lectin family span a wide variety of specificities. Consequently, their recognition sites are highly variable, and different specificities can easily be engineered by site directed mutagenesis (Iobst and Drickamer, 1994; and Kolatkar and Weiss, 2009). A general feature of binding sites of all lectins seems to be that they consist of a primary binding site that is capable of recognizing in a specific way a single monosaccharide residue, usually with a low affinity (in the millimolar range). Very often, but not always, there are further subsites that can be occupied by sugar residues connected to the one bound in the primary site. This allows for a modest increase in affinity. Folding in common between plant and animal lectins are β-sandwich fold, β-Trefoil folds and Hevein domains. The legume lectin-like β-sandwich fold found in Galectins that conserved family of β-galactosyl binding lectins that occur in both vertebrates and invertebrates (Hirabayashi et al., 2009). Except for the legume lectins, galectins and pentraxins, it is observed in a number of carbohydrate processing and other enzymes such as β-glucanase and asparagine amidase (Keitel et al., 1993; and Kuhn et al., 1994).



Concanavalin A

Fig. 3. The legume lectin-like β-sandwich folds illustrated by a member of the legume lectins (Concanavalin A in complex with the trisaccharide [Man(α-1, 3)]Man(α-1, 6)Man). In each case, only a single monomer of the multimeric protein is shown. Bound carbohydrate is shown in ball-and-stick. Metal ions are shown as grey spheres, bound ligands are shown in green ball-and-stick representation (Loris, 2002).

The β-trefoil fold was first identified as a carbohydrate recognition domain in ricin (Montfort et al., 1987). Later, it was also found to be the fold of amaranthin. The β-trefoil fold is another fairly common fold, first identified in soybean trypsin inhibitor (Sweet et al. 1974). It consists of a repeat of three subdomains, each consisting of a fourstranded antiparallel β-sheet.

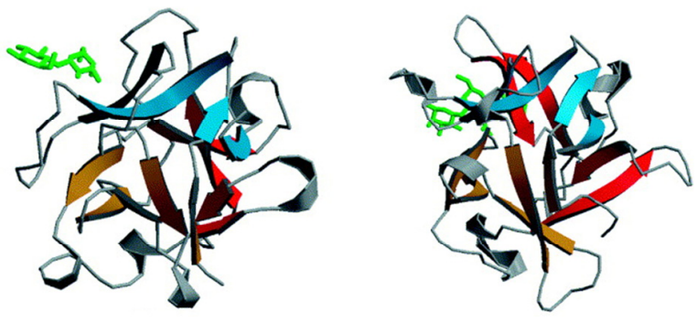


Fig. 4. The β-trefoil folds illustrated by the first domain of the subunit ricin (in complex with lactose), the first domain of amaranthin (in complex with the T-antigen) In each case, for clarity, only a single β-trefoil domain is shown in an identical orientation, although each protein is a multidomain as well as a multimeric protein. Bound carbohydrate is shown in green ball-and-stick (Loris, 2002).

Lectins comprise a structurally very diverse class of proteins characterized by their ability to bind carbohydrates with considerable specificity. Although lectins bind monosaccharides rather weakly, they employ common strategies for enhancing both the affinity and specificity of their interactions for more complex carbohydrate ligands. Members of the legume lectin family show considerable sequence and structural homology, but differences in their carbohydrate-binding specificity. The legume lectin monomer has a molecular weight of 25,000 and is composed primarily of a six- and a seven-stranded antiparallel-sheet. Concanavalin A (Con A), *Lathyrus ochrus* isolectin I (LOL I), and pea lectin all show mannose and glucose-binding specificity, and the X-ray crystal structures of their carbohydrate complexes show a monosaccharide binding- site geometry very similar to that of the LOL I-α-methyl-o-mannopyranoside complex.

The first lectin structures to be determined derived from two phylogenetically conserved families, the *Leguminoseae* (Sharon and Lis 2001; and Young and Oomen, 1992) and the *Gramineae* (Raikhel et al., 1993). Over the past ten years, major advances in X-ray crystallographic technology and the relative ease of isolation and crystallization of plant lectins have led to a rapid increase in crystal structures. The leguminous lectins clearly have dominated the field with some ten structures known today. Four of these, peanut lectin, soybean lectin, lentil lectin and hemagglutinin L, have been determined within the past three years. These lectins display diverse sugar-binding specificitiesistructures.

Lectins of the *Amaryllidaceae, Orchidaceae, Alliaceae, Araceae* and *Liliaceae* families are in the class of the mannosespecific *Liliatae* and constitute the third major structurally characterized plant lectin superfamily (Van Damme et al., 1994). They are nonseed lectins of multigene families isolated from plant bulbs (Van Damme et al., 1994) and function either as dimers or tetramers, as do the legume lectins. Their strict and exclusive specificity solely for mannose has imparted some unusual biological properties *in vitro* to this lectin family including their antiviral properties against retroviruses (e.g. HIV) (Hammaar et al., 2006). Snowdrop lectin (GNA) is a tetrameric lectin (M_r =50,000 Da) and is the first member of the *Amaryllidaceae* family crystallographically investigated.

9. Physicochemical properties of plant lectin

9.1 Composition

There are no structural features common to all lectins. Many of these proteins are relatively rich in aspartic acid, serine and threonine, which comprise as much as 30% of their amino acid content and are low in sulfur-containing amino acids. Such a pattern of amino acids is characteristic of plant proteins. In contrast, lectins such as those from wheat germ, potato and pokeweed are rich in cysteine with 20, 11.5 and 18% of the total amino acid residues respectively, most or all of which are in the form of cysteine. The high content of disulfide bonds in wheat germ agglutinin endows the protein with stability to heat (Aub et al., 1963), to proteolytic enzymes and to denaturing agents such as detergents, urea, alkali and acids (Nagata and Burger, 1972; and Rice and Etzler, 1974). The potato and *Datura stramonium* lectins are rich in hydroxyproline (Lamport, 1969). A few lectins, such as Concanavalin A, wheat germ and peanut agglutinins are devoid of covalently bound sugars. Most lectins, however, are glycoproteins with carbohydrate contents that can be as high as 50%, e.g., potato lectin. The table shown below (Table 1) is on the sugar contents of certain important glycoprotein lectins. The sugar constituents in animal glycoproteins are the same as those found in other plant glycoproteins, with the exception of L-arabinose.

Lectin	Mannose	Galactose	L-Fucose	L-Arabinose	GlcNAc	Xylose	C-P linkage	Reference
Bandeiraea simplicifolia	5.8		1		2.6	1		Lescar et al., 2007
Datura stramonium				2.8	4.5			Kilpatrick, 1978
Glycine max	4.5				1.2		GlcNAc-Asn	Lis et al., 1973
Phaseolus lunatus	3.2	3.7	0.5		1.3		GlcNAc-Asn	Mach et al., 1991
Phaseolus vulgaris	7.3				2.8		GlcNAc-Asn	Ohtani, K., and Misaki, A. (1980)
Solanum tuberosum		3		47			Ara-Hyp, Gal-ser	Matsumoto et al., 1983
Wistaria floribunda	0.77	1.63			0.65		GlcNAc-Asn	Kurokawa et al., 1976

Table 1. Well-characterized glycoprotein lectins

The molecular weight of lectins in plants ranges from 36,000 Da for wheat germ agglutinin (Nagata and Burger, 1972; and Rice and Etzler, 1974) to 265,000 for lima bean lectin (Galbraith and Goldstein, 1970). The lower limit of MW of animal lectins is found to be 14 kDa (Lis and Sharon, 1998). Some lectins exhibit a pronounced tendency to aggregate. Thus, the MW of Concanavalin A at pH below 6 is 51,000 Da and at physiological pH it is 12,000 Da (Mc Cubbin and Kay, 1971; and Wang et al., 1971). Upon storage at room temperature, soybean agglutinin and peanut agglutinin also possibly undergo irreversible self-association to high molecular weight aggregates (Lotan et al., 1975). The subunits are identical in most

lectins. But lectins comprising of non-identical subunits are known as seen in soyabean agglutinin (Lotan et al., 1975) and the lectin from *Dolichos biflorus* (Carter and Etzler, 1975) which are tetramers, consisting of two types of subunits (Wright et al., 1996). A different type of subunit heterogeneity was first demonstrated in Concanavalin A (Abe et al., 1971; and Wang et al., 1971). The anti-B lectin from *Bandeiraea simplicifolia* consists of a family of five closely related proteins, each of which is a tetramer of one or two types of subunits. One of the subunits is specific for *N*-acetyl galactosamine, whereas the specificity of the other is confined to α-galactose (Goldstein and Hayes, 1978). The structure of *Bandeiraea simplicifolia* isolectins is analogous to that of PHA isolectins. They have five tetrameric proteins comprising of varying proportions of two classes of subunits (Miller et al., 1973; Rasanen et al., 1973; and Leavitt et al., 1977). These subunits show difference in properties. It is assumed that it is due to their difference in the primary structure of subunits (Miller et al., 1973).

9.2 Metal ion requirements

With a few exceptions, all lectins examined contain metal ions and in some cases evidence has been presented for the requirement of Mn^{2+} or Ca^{2+} (Emmerich et al., 1994) for activity (Table 2). Treatment with ethylene-diamine tetra acetic acid (EDTA) at neutral pH did not remove the metal ions from Concanavalin A (Doyle et al., 1984), soybean agglutinin (Jaffe et al., 1974) or lima bean lectin (Galbraith and Goldstein, 1970). Reversible removal of metal

Lectin	Metal content (atom / mole)				References
	Mn^{2+}	Ca^{2+}	Zn^{2+}	Metal	
Bandeiraea simplicifolia I	1.2	2.0		Ca^{2+}	Lescar et al., 2002
Canavalia ensiformis	4.0	4.0		Mn^{2+}	Magnuson et al., 1983
Datura stramonium	<0.2		<0.2		Kilpatrick, 1978
Dolichos biflorus	1.6	5.4	2.0		Etzler et al., 1970
Euonymus europeus		8.0	0.7		Petryniak et al., 1977
Glycine max	1.0-1.7	3.5-4.1	0.28	Mn^{2+}	Lis and Sharon, 1973
Lens culinaris	0.64	3.8		Mn^{2+}	Westbrook et al., 1984
Marasrous oreades			0.7		Winter et al., 2002
Ononis hircina	1.0		1.0		Horejsr et al., 1978
Pisum sativum	1.0	2.5		Ca^{2+}	Reeke et al., 1986
Phaseolus coccineus	0.15	4.8	1.0		Perez-Campos et al., 1997
Phaseolus lunatus	1.0	4.0		Mn^{2+}	Mach et al., 1991
Phaseolus vulgaris	0.24	6.2		Mn^{2+}	Andrews, 1974
Ricinus communis	<0.1	<0.1	<0.1		Mandal et al.,1989
Sarothamnus scoparius	1.5		0.8		Gurtler, 1978
Ulex europeus I	0.42	2.0	0.82		Sugii and Kabat, 1982
Vicia cracca	0.9		2.4		Sitohy et al., 2007

Table 2. Metal content and metal requirements for activity of lectins

ions can be achieved under acidic conditions. The Mn^{2+} in lectins can be replaced by a variety of transition-metal ions without loss of biological activity as demonstrated for Concanavalin A (Agrawal and Goldstein, 1968; and Shoham et al., 1973). Ca^{2+} in Concanavalin A could be replaced by Cd^{2+}, but not by Ba^{2+} (Shoham et al., 1973). The metal ions confer a high degree of structural stability to Concanavalin A, protecting the lectin against heat inactivation and hydrolysis by proteolytic enzymes (Thomasson and Doyle, 1975). Ni^{2+} alone protects Concanavalin A against proteolysis at pH 7.0 but not at pH 8.2. Some lectins require metal ions for the saccharide-binding activity (Sumner and Howell, 1936). Extensive studies by NMR have revealed a complicated set of interlocking equilibrium involving the apoprotein and various complexes with metal ions and the saccharides (Brewer et al., 1983).

10. Isolate and purification of lectin

Purified lectins are essential for establish their molecular properties and are highly desirable for their many applications. In the past, lectins have been obtained solely from native sources, but they can now be produced also by recombinant techniques. Isolation of a lectin begins commonly with extraction of the tissue or organ in which it is present. This is simple in the case of plants, especially their seeds (Goldstein and Poretz, 1986; and Rudiger, 1993). The seeds are ground and the meal obtained is extracted with a neutral buffer. Often it is advisable to pre-extract the dry meal with an organic solvent, such as petroleum ether, to remove colored materials derived from the seed coat and lipids that may be present in large amounts. Animal tissues are either homogenized directly in the extraction buffer or the tissue is extracted first with acetone to remove water and lipids. The extraction buffer should preferably contain protease inhibitors to prevent degradation of the lectin during purification, and, in the case of membrane bound lectins, a detergent as well. Preliminary fractionation of the crude extract (e.g., by ammonium sulfate precipitation) is often done to obtain a protein fraction devoid of other constituents (e.g., polysaccharides in the case of plants). Final purification is achieved by affinity chromatography on a suitable adsorbent. A wide variety of affinity adsorbents, to suit any taste or purse, have been described in the literature and many of them can be purchased ready-made These include polysaccharides such as Sephadex, a polymer of glucose employed for the purification of Concanavalin A and pea lectin agarose (or Sepharose), a polymer of galactose, for the purification of the lectins from castor bean; acid-treated Sepharose for the purification of SBA; and chitin, a polymer of *N*-acetylglucosamine, for the purification of WGA. In the absence of readily available polysaccharides, use can be made of adsorbents consisting of carbohydrates or glycoproteins as such, or in the form of a synthetic derivative, that are covalently attached to an insoluble carrier. For instance, lactose coupled to Sepharose is the reagent of choice the purification of the lectins from peanut, eel electric organ or calf heartmuscle. *N*-acetylglucosamine bound to the same support serves for the purification of potato lectin and WGA, whereas immobilized porcine AH blood type substance is employed for the purification of the blood type A specific DBL and HPA. When working with lectins of an uncommon specificity, adsorbents have to be tailor made, as for example Sepharose bound asialoglycophorin for the purification of the blood type *N*-specific for lectin from *Vicia graminea*.

The lectin was purified from crude extract of mixer solution, commonly use chromatography technique such as, affinity chromatography, ion exchange

chromatography, and gel filtration chromatography. In 2004 had a research that used affinity chromatography to purify the lectin from human serum proteins by Concanavalin A sepharose column coupled to two-dimensional gel electrophoresis. The purified sample had 2 fractions before use this technique (Rodriguez-Pineiro et al., 2004). Next year, a lectin from the marine red alga *Gracilaria ornata* (*Gracilariaceae, Rodophyta*); GOL was purified by 2 steps chromatography technique consist of ion exchange chromatography on DEAE-cellulose and affinity chromatography on mucin-Sepharose 4B. The GOL significantly affected the development of *Callosobruchus maculatus* larvae, indicating the possibility of using this lectin in a biotechnological strategy for insect management of stored cowpea seeds. (Leite et al., 2005). In 2007 Shi et al. study lectin from raw and canned red kidney bean (*Phaseolus vulgaris*). They used gel filtration technique to purify. Use Affi-gel Blue gel sepharose compare to thyroglobulin-Sepharose to purify the lectin from red kidney bean. Found that the lectin from thyroglobulin more purify than Affi-gel Blue gel (Shi et al., 2007).

An alternative approach for the preparation of lectins has been made possible by the advent of recombinant DNA technology. It is based on the isolation of the cDNA or genomic DNA of the lectin, its insertion into a suitable vector and expression in an appropriate host cell. Isolation of the cDNA requires knowledge of at least part of the primary sequence of the lectin itself or of a structurally similar one. By this technique, several plant lectins, among them of pea (Stubbs et. al., 1986; and Van Eijsden et al., 1992), *Erythrina corallodendron* (Arango et al., 1993), peanut (Sharma and Surolia, 1994) and *Griffonia simplicifolia* (Zhu et al., 1996) have been expressed in *Escherichia coli*. Expression of plant lectins was also achieved in other systems, e.g. WGA in *Saccharomyces cerevisiae* (Nagahora et al., 1992), PHA and GNA in *Pichia pastoris* (Raemaekers et al., 1999), PNA in insect cells (Kumar et al., 1999) and SBA in monkey cells (Adar et al., 1997); (for a more complete listing of recombinant plant lectins) (Streicher and Sharon, 2003).

11. Lectins in edible plants

Many lectin-containing plants are common constituents of the diet of humans and farm animals. Since lectins are known to act on cells in a variety of ways, such as agglutination, mitogenic stimulation and killing, and they are often resistant to heat and proteolytic enzymes, including those of intestinal bacteria, the effects of consumption of these proteins deserve special consideration. For many years it has been known that they occur in legumes such as soybeans, kidney beans, lima beans, mung beans, lentils, garden peas and peanuts that are a major food source for humans and animals in one part of the world. Although lectin containing foods are frequently consumed in cooked or otherwise processed form, such treatments may not always be adequate to completely inactivate the lectins present. Thus, lectins have been detected in roasted peanuts (Wang et al., 1999). Slow cooking of beans, without boiling, does not always eliminate lectin activity as observed with kidney beans cooked for 11 hr at 82 ^{o}C or for 5 hr at 91 ^{o}C. The stability of plant lectins in the stomach is evidenced, for example, by the finding that when Concanavalin A, PHA or WGA were intragastrically administered into rats between 50 and 90% of the lectin was recovered after 1 hr from the stomach by homogenizing the tissue in phosphate-buffered saline containing the appropriate specific sugar. Moreover, in the few experiments with humans that ate lectin-containing foods, namely tomatoes (Kilpatrick et al., 1985), red kidney beans (Pusztai et al., 1989) or peanuts, either raw or roasted (Wang et al., 1999) the lectins have not only withstood the acidity and the proteolytic enzymes of the intestinal tract, but a

significant proportion of the amount ingested has reached the circulatory system with unimpaired hemagglutinating and immunological activities. In rodents, a diet containing lectins provoked intestinal and systemic immune responses to these proteins (Gomez et al., 1995). Furthermore, human serum was found to contain antibodies to the lectins of peanut, soybean and wheat germ (Tchernychev and Wilchek 1996).

12. Biological role

Lectins are present abundantly in many plants. Despite this abundance, their precise biological roles in the plants to which they belong, are not well understood. The available evidences suggest two main roles for them.

12.1 Mediation of symbiotic relationship between nitrogen fixing microorganisms, primarily, rhizobia and leguminous plants

Lectins localized at the root hairs are the entry sites for rhizobia. The lectins then aggregate the rhizobia in the root nodules and make them immobile (Hamblin and Kent, 1973; Bohlool and Schmidt, 1974; Diaz et al., 1989; Brewin and Kardailsky, 1997; and Hirsch, 1995). Type specificity of host-parasite interactions between leguminous plants and particular strains of rhizobia infecting them is determined by lectins. The expression of the pea lectin gene in white clover roots enabled them to be nodulated by a rhizobium strain specific for the pea plant (Van Eijsden et al., 1995).

12.2 Protection of plants from predatory animals and phytopathogens

Abrin, a type-II ribosome-inactivating protein (RIP), was the first lectin to be recognized as a defence protein (Peumans and Van Damme, 1995). Soon afterwards ricin also came to be recognized as a defence protein (Olsnes, 2004). Type-II RIPs which belong to the plant lectin family with β-trefoil fold are known to be toxic to animals and insects (Hartley and Lord, 2004; and Stirpe, 2004). Lectins from *Phaselous vulgaris* (PHA), *Robinia pseudocacia* and *Sambuscus nigra* have been reported to be toxic to higher animals (Peumans and Van Damme, 1995). Lectins from many plants, when ingested by animals, have resulted in toxic effects (Lis and Sharon, 1998), fungal growth in *Trichoderma viride* is inhibited by wheat germ aggiutinin (WGA) (Mirelman el al., 1975). Brambl and Gade (1985) have shown that eleven purified lectins, representing a wide spectrum of sugar specificity, inhibited the growth of fungal species *Neurospora crassa*, *Aspergilius amsteldomi* and *Botryodiplodia theobromae*. Known antifungal lectins include those which bind chitin (Peumans and Van Damme, 1995; Hirsch et al., 1995; Eijsden et al., 1995; Kijne, 1997; and Selitrennikoff, 2001). The anti-insect activity of many plants has been attributed to the presence of lectins in them. For example PHA (Chrispeels and Raikhel, 1991) pea nut agglutinin (PNA), WGA, *Maclura pomifera* agglutinin (MPA) and lectins from potato, thorn apple and osage orange show anti-insect activity against cowpea weevil. WGA and *Bauhinea purpurea* agglutinm are toxic to *Ostrinia nubilalis* larvae. Snow drop and garlic lectin show toxic effects on cowpea weevil and tobacco hornworm (Hilder et al., 1995; and Peumans and Van Damme, 1995).

13. Application to antimicrobial activity

The cell wall of bacteria not only precludes any interaction between the glycoconjugates on their membrane and carbohydrate-binding proteins but also prevents these proteins from

penetrating the cytoplasm. Therefore, plant lectins cannot alter the structure and/or permeability of the membrane or disturb the normal intracellular processes of invading microbes. Therefore, if lectins play a role in the plant's defense against bacteria, it must be through an indirect mechanism that is based on interactions with cell wall carbohydrates or extracellular glycans.

In 1936, a using lectin in clinical microbiology began when Summer and Howell (Summer and Howell, 1936) had a report that Concanavalin A can agglutinated various *Mycobacterium* spp. The interactions between plant lectin and microorganisms have been applied for typing of bacteria, fungi, and protozoa. It is useful for characterizing bacterial cell components and for detecting bacteriophage receptors. (Etzler, 1983; Lis and Sharon, 1986; and Nicolson, 1974). The unique property of lectin to bind non-covalently to simple sugars and polysaccharides has attracted interest in microbial taxonomy. Lectin has a role in the clinical laboratory identification and taxonomic classification of many microorganisms. Because lectins are generally monoclonal proteins and because they possess a spectrum of specificities and molecular weights, they are substantial tools for diagnostic microbiology applications. Recent observations with regard to the binding of plant lectins to components of the bacterial cell wall peptidoglycans (such as muramic acid, *N*-acetylmuramic acid, *N*-acetylglucosamine and muramyl dipeptides) revealed that seed lectins from several legume species strongly interact with these bacterial surface carbohydrates (Ajouba et al., 1994). Evidently, the observation that legume seed lectins can recognize and bind to the bacterial cell wall does not imply that such an interaction occurs *in vivo* and certainly does not prove that these lectins are involved in the protection of the seedlings against bacteria.

Lectin has been used for investigating virulence factors, surface structures, and identification of gram-positive bacteria. For example; lectin from *Dolichos biflorus* was used to confirm its specificity for identifying group C streptococci. In another test, its crude extract was coupled to polystyrene particles with a spacer arm to yield an effective lectin-latex reagent that agglutinated group C streptococcal antigens prepared as nitrous acid, autoclave,or enzyme extracts. (Slifkin and Gil, 1984) Group C streptococcal isolates from horses and cattle agglutinated with lectin from *Dolichos biflorus* and *Helix pomatia*. (Schalla et al., 1986). Concanavalin A could be precipitated various bacterial polysaccharides, with interacts specifically with bacterial cell walls containing glycosidic residues associated with teichoic acid. Accordingly, bacteria teichoic acids from cell wall containing α-glucopyranisyl residues,such as *Lactobacillus plantarum*, *Staphylococcus aureus*, and *Bacillus subtilis*. (Archibald and Coapes, 1971; Doyle et al., 1982; and Reeder and Ekstedt, 1971). Lectins from soy bean have been used to assay for detecting *Bacillus anthracis* (Cole et al., 1984). The use of soybean agglutinin (SBA) to detect very low numbers of buffered suspention of *Bacillus anthracis* vegetative cells and spores has been reported (Graham et al., 1984). The strategy was to bind the cells or spores to polystyrene plates and to detect the bound forms with horseradish peroxidase labeled soybean agglutinin (called the lectinosorbent assay).

The contrast of gram-negative bacteria and gram-positive bacteria is the cell wall of gram-negative bacteria contains to lipid but cell wall of gram-positive bacteria does not have the lipid. In 1968, Doyle et al., provided evidence that Concanavalin A reacts with macromolecules that are devoid of terminal glucopyranose or mannopyranose residues (Doyle et al., 1968). Their investigations demonstrated that Concanavalin. A precipitates lipopolysaccharide preparation derived from various strains of *Escherichia coli* as well as

from *Shigella flexneri* and *Salmonella abortivoequina*. In 1970, other investigators demonstrated that Concanavalin A can be used to detect lipopolysaccharides of various Salmonella strains as determined by gel diffusion. (Goldstein and Staub, 1970).

In 2010, Petnual et al. reported the antimicrobial activity of *Curcuma longa* lectin, expressed as the minimal inhibitory concentration (MIC), was found to inhibit the growth of all five microbial species tested, the four bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, plus the yeast *Candida albicans*, at MIC values of ≥ 0.011, 0.005, 0.092, 0.002 and 0.0046 mg/ml, respectively (Petnual et al. 2010). These results demonstrate that the *Curcuma longa* rhizome lectin is likely to be at least one of the, if not the, candidate molecule responsible for the antibacterial action observed in rhizome extracts from this plant. An outstanding feature of the antibacterial activity of the isolated lectin is it is somewhat nonselective against this fairly diverse selection of bacteria. The potentially broad effect of the *Curcuma longa* rhizome lectin on the growth inhibition of several diverse bacterial strains, confirms the important interaction between the lectin and all the strains under consideration. From the tested strains, *Pseudomonas aeruginosa* (lowest MIC) seemed to be most sensitive to the presence of lectin. Previouse studies of the binding of plant lectins to bacterial cell wall peptidoglycans indicate that several lectins of different carbohydrate specificities can recognize most of the components of the bacterial cell wall, such as muramic acid, *N*-acetylglucosamine, *N*-acetylmuramic acid and muramyl dipeptide (Ajouba et al., 1994).

Archidendron jiringa seed lectin was selected to test for antimicrobial activity with *Escherichia* coli, Pseudomonas auroginosa, Bacillus subtilis, Staphylococcus aurous, and Candida albican (Charungchitrak et al., 2011). The MIC of Archidendron jiringa seed lectin with Candida albican equal in S. aurous to be 0.0567 mg /ml and in Bacillus subtilis to be 0.2266 mg/ ml. But the MIC with *Escherichia* coli and Pseudomonas auroginosa is not detected, demonstrating stronger antimicrobial activity against gram-positive than gram-negative bacteria. Accordingly, the binding of lectins to muramic acid and N-acetylmuramic acid, carbohydrates present in the bacterial cell wall (mainly in gram-positive bacteria), has been reported (Ajouba et al., 1994). These data suggest that lectins probably play a role in plant defense, not only against phytopathogenic invertebrates, herbivores or fungi, but also against bacteria. The carbohydrate-binding site probably plays a key role in this activity, being responsible for the recognition of bacteria. Almost all microorganisms express surface-exposed carbohydrates. These carbohydrates may be covalently bound, as in glycosylated teichoic acids to peptidoglycan, or non-covalently bound, as in capsular polysaccharides (Hirmo, et al., 1997; and Caldeon, et al., 1997). Every surface-exposed carbohydrate is a potential lectin-reactive site. The ability of lectins to form complexes with microbial glycoconjugates has made it to be employed as probes and sorbents for whole cells, mutants, and numerous cellular constituents and metabolites.

The lectin from *Curcuma amarissima* inhibited 4 microbial growth consist of *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* at concentration ≥ 0.446, 0.446, 0.223, and 0.892 mg/ml respectively. But can not inhibite *Pseudomonas auroginasa* growth because at the surface of *Pseudomonas auroginosa* cell does not have polysaccharide ligands which can interact with *Curcuma amarissima* lectin. (Kheeree et al., 2011) Similar to Legume lectin from *Trinella foenumgraecum, Trifolium alexandrium, Bauhinia variegata,* and *Delonix regia* had a research that these lectins from sephadex G-150 can agglutinated both gram negative and gram positive bacteria (*Mycobacterium rhodochrous, Bacillus cercur, Bacillus megaterium,*

Bacillus sphaericus, Escherichia coli, Seratia marcescens, Corynebacterium xerosis, and *Staphylococcus aureus)* (Reda et al., 1992). Inaddition β-galactoside-binding lectin was extracted from the skin of amphibian, *Bufo arenarum*. It had an antimicrobial activity against Gram negative bacteria (*Escherichia coli* K12 4100 and wild strains of *Escherichia coli* and *Proteus morganii*) and Gram positive bacteria (*Enterococcus faecalis*) (Alicia *et al*., 2003).

Several investigators have concluded that lectins are useful reagents for the study of fungal cell surfaces and may also be of value as important aids in the classification of fungi (Barkai and Sharon, 1978). The major components of fungal cell wall is Chitin, a polymer of β-(1, 4)-*N*-acetyl-D-glucosamine (Barkai and Sharon, 1978; and Ebisu et al., 1977). The report of lecin interaction to fungal, such as fluorescein-conjugated wheat germ agglutinin has been shown to be an effective probe to detect chitin on hypha surfaces. (Barkai and Sharon, 1978; Galun et al., 1976; Galun et al., 1981; Mirelman et al., 1975; Molano et al., 1980; Tkacz and Lampson, 1972; Tracz et al., 1971; Tropchin et al., 1981). In 1975 Mirelman et al. was found wheat germ agglutinin (WGA) can be inhibits spore germination and hyphal growth of *Trichoderma viride* and interferes with the synthesis of chitin (Mirelman et al., 1975). A novel mannose-binding lectin was purified from rhizomes of *Ophiopogon japonicus* was showed antifugal activity in three phytopathogenic fungi namely *Gibberella saubinetii* and *Rhizoctonia solani* (Tian et al., 2008).

In 2010, Petnual et al. purified *Curcuma longa* lectin at a dose of 47 µg and 94 µg/0.3 cm² disc showed antifungal activity against the three tested phytopathogenic fungal species, *Exserohilum turicicum*, *Fusarium oxysporum* and *Colectrotrichum cassiicola* (Fig. 5). While the lectin dose of 47 µg/0.3 cm² disc slightly inhibited the growth of these three fungi, that at 94 µg/0.3 cm² disc showed a higher and significant degree of antifungal activity on all three isolates (Petnual et al. 2010). This effective lectin dose of around 100 µg/ 0.3 cm² disc is in accord with that reported for the lectin from *Annona muricata* seeds against the growth of *Fusarium oxysporum*, *Fusarium solani* and *Colletotrichum musae* (Damico, et al., 2003), and for the lectin from *Astragalus mongholicus* against *Fusarium oxysporum, Colletorichum* sp. and Drechslera *turcia* (Yan, et al., 2005). Other lectins, such as those from potato (Gomez, et al., 1995) and red kidney beans (Ye, et al. 2001), have also been reported to exhibit antifungal activity. However, novel non-lectin proteins with antifungal activity in plant rhizomes are also known, such as the 32 kDa protein in ginger rhizomes which exhibits antifungal activity toward *Fusarium oxysporum* at a dose of 32-160 µg/ 0.3 cm² disc of ginger rhizome (Wang and Ng, 2005).

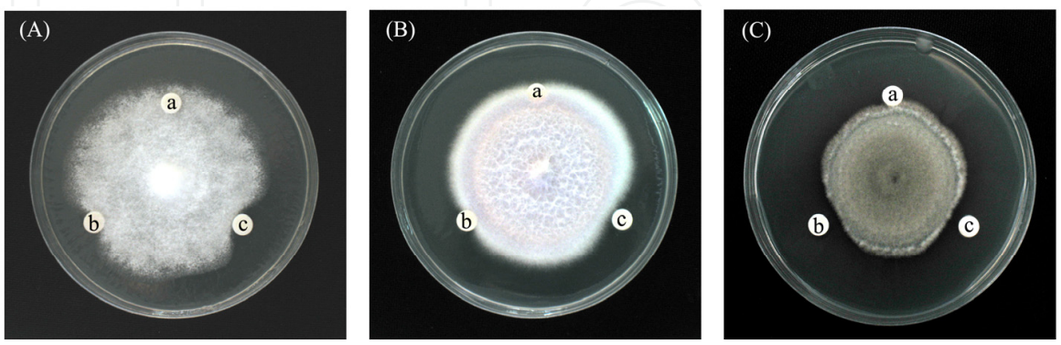


Fig. 5. Inhibitory affect of *Curcuma longa* lectin on antifungal protein toward *Exserohilum turicicum* (A), *Fusarium oxysporum* (B) and *Colectrotrichum cassiicola* (C). The negative control is 10 µl of 20 mM Tris-HCl buffer pH 7.4 (a), 47 µg *Curcuma longa* lectin. (b) and 94 µg *Curcuma longa* lectin (c) (Petnual et al., 2010).

In 2011, Kheeree et al. purified *Curcuma amarissima* lectin showed *in vitro* antifungal activity against three plant pathogenic fungal species, *Colectrotrichum cassiicola*, *Exserohilum turicicum*, and *Fusarium oxysporum*. It strongly inhibited the growth of *Colectrotrichum cassiicola* at 17.5 µg for *Fusarium oxysporum* and *Exserohilum turicicum*, which were strongly inhibited at the higher concentration of 35 µg (Fig. 6). Antifungal activity has been observed in other lectins where, for example *Astragalus mongholicus* root lectin revealed antifungal activity against various species of phytopathogenic fungi (Yan et al., 2005). Similarly with lectin from *Talisia esculenta* seeds inhibited the growth of *Fusarium oxysporum*, *Colectrotrichum lindemuthianum*, and *Saccharomyces cerevisiae*.(Freire et al., 2002) *In vitro* studies demonstrated that two novel chitin-binding lectins seeds of *Artocarpus integrifolia* inhibited the growth of *Fusarium moniliforme* and *Saccharomyces cerevisae* (Trindade et al., 2006). Many studies of plant lectins have assumed that they are implicated in host defense mechanism as antifungal proteins. However, to date only a small number of lectins have been reported to have actual antifungal activity such as lectin from the rhizomes of *Ophiopogon japonicus* showed antifungal activity against *Gibberella saubinetii* and *Rhizoctonia solani* (Tian et al., 2008). The purified *Phaseolus coccineus* Lectin (PCL) was devoid of antifungal activity against *Candida albicans* and Penicillium *italicum* (Chen et al., 2009).

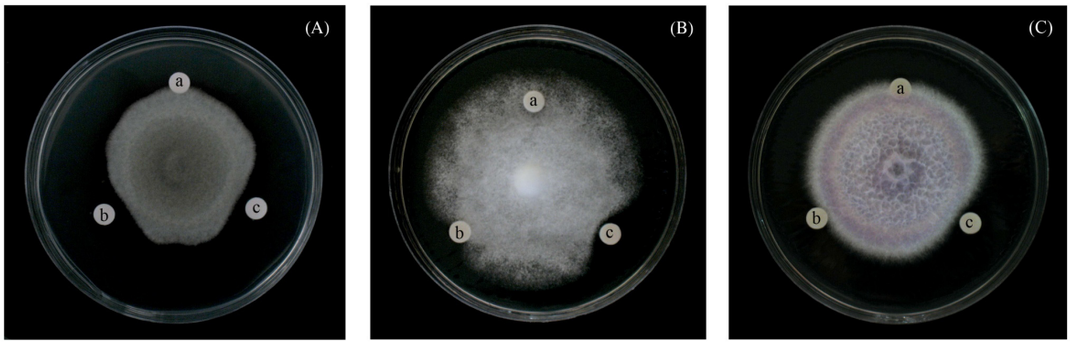


Fig. 6. Inhibitory effect of purified *Curcuma amarissima* lectin on the *in vitro* growth on PDA plates (as an antifungal activity bioassay) of; (A) *Colectrotrichum cassiicola*, (B) *Fusarium oxysporum* and (C) *Exserohilum turicicum*. For each plate, 0.625 cm diameter discs were seeded with 10 µl of TBS (a) alone as the negative control, or containing either (b) 17.5 µg/ ml or (c) 35 µg/ ml purified *Curcuma amarissima* lectin (Kheeree et al., 2011).

A large body of data exists on the interaction of lectins with a relatively broad spectrum of parasites ranging from the protozoa through the metazoa. Although Concanavalin A was used by many investigators as a lectin probe for these organisms, many other lectins have been shown to be of value in the study of cell surfaces and the identification and differentiation of the parasites. In some instances virulence of parasitic protozoa appears to be related to their surface properties, as revealed by interactions with lectins. Thus, several investigators have deemed important the comparison of surface saccharides of parasites known to differ in their virulence traits. It has been conjectured that the virulence of the trophozoite form of *Entamoeba histolytica* may depend, in part, on its surface properties. Data have been presented indicating that only strains isolated from cases of amoebic dysentery agglutinate with Concanavalin A (Martinez-Palomo et al., 1973) strains isolated from asymptomatic cases of amoebic dysentery, however, do not agglutinate with this lectin.

The unique property of lectins to bind noncovalently to simple sugars and therefore to polysaccharides and glycoconjugates has attracted the interest of virologists. In virology, lectins have been used for detection of viral glycoproteins in purified and infected cells, as well as for viral purification. Lectin studies have revealed information about the structure of viral glycoproteins, structures important in their pathogenicity. A significant contribution of lectin use in virology has been in the development of unique diagnostic methods that yield specific identification of viral agents. Purified influenza virus yields macroscopically visible flocculation when mixed with Concanavalin A. (Klenk et al., 1984) When influenza virus is treated with a proteolytic enzyme, the glycoprotein spikes of the virus are released. These treated viral particles no longer agglutinate with this lectin, but will flocculate in the presence of *N*-acetylgalactosamine-associated lectins, such as *Dolichos biflorus* or *Helix pomatia*. Other viruses, including arboviruses, vesicular stomatitis virus, paramyxoviruses, leukoviruses, and hepatitis B virus, also agglutinate with Concanavalin A. Concanavalin A was shown to block specifically adsorption of the bacteriophage binding sites of *Bacillus subtilis* possessing α-glucosylated teichoic acids in the cell walls associated with teichoic acids. It was suggested that the application of this lectin might be useful as a means to correlated bacteriophage and serologic typing of staphylococci. (Archibald and Coapes, 1972).

14. Conclusion

Biochemical and molecular studies of numerous lectins eventually demonstrated that only a limited number of carbohydrate-binding motifs evolved in plants (Peumans et al. 2000). Since the specificity of these binding motifs is primarily directed against foreign glycans, it is generally accepted now that many plant lectins are involved in the recognition and binding of glycans from foreign organisms, and accordingly play a role in plant defense (Peumans and Van Damme 1995; and Van Damme et al. 1998). Most plant lectins are probably involved in the plant's defense. Whereas direct interference with viruses and microorganisms are rather exceptional, the deleterious effects of plant lectins on predatory invertebrates and higher animals are obvious. Considering the abundance of lectins in storage organs and their storage protein-like behavior, we believe that plants accumulate part of their nitrogen reserve in the form of carbohydrate-binding proteins, which can be used as passive-defense proteins. Although low antimicrobial activity could be obtained from plant lectins, the information was still promising important for future research because nowadays extraction of bioactive compounds directly from their natural source is not the only way for the investigation. If the structure of the bioactive compound was elucidated, using knowledge on recombinant DNA technology could possibly produce a synthetic compound. And since antimicrobial-resistant organisms have been the major problem in medical treatment, searching for new antimicrobial compounds are still is interested.

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