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Review Article

CONCANAVALIN - A POTENTIAL GLYCOPROTEIN

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Abstract: Concanavalin is a widely acclaimed lectin present in *Canavalia* spp. known for broad applications. It is a homotetramer with glucose or mannose selective and require metal ion for its function. The structure of concanavalin A has been studied widely and interpreted for cell surface interaction, lymphocyte mitogenesis, anti-proliferative activity and apoptosis. Concanavalin agglutinates erythrocytes and possesses defensive role in host plant. Concanavalin from different species of *Canavalia* have high genetic homology but differ in their quaternary structure, which diversify functional properties. This review provides a comprehensive picture on the biogenesis, structure and function of concanavalin.

Keywords: *Canavalia*; concanavalin; homology; haemagglutination; mitogenicity.

Note - Coloured Figures are available on Journal Website in "Archives" Section

1. Introduction

Concanavalin is a lectin of genus *Canavalia* in the legume family (Sumner, 1919). *Canavalia* is a wild legume with tropical and subtropical distribution. It is tolerant to severe environmental conditions and commonly prevails in coasts, mangroves and interior habitats. It is useful as sand binder, green manure and forage crop in agriculture. It is used as feed for livestock while the tender pods and ripened beans are consumed by fisher folk (Bhagya and Sridhar, 2009). It consists of four sub-genera with 48-50 species and some of the species like *C. cathartica* Thouars [Syn.: *C. virosa* (Roxb.) Wight & Arn. (mangrove bean)], *C. ensiformis* (L.) DC. (jack-bean), *C. gladiata* (Jacq.) DC. (sword bean), *C. rosea* (Sw.) DC. [Syn.: *C. maritima* Thouars; *C. obtusifolia* (Lam.) DC. (bay bean)], *C. brasiliensis* Benth. (Brazilian jackbean), *C. grandiflora* Benth. and *C. boliviana* Piper are valued legume crops owing to their high protein content (Smartt, 1990; Bhat, 2014) (Fig. 1). Seeds of *Canavalia* contain antinutritional components like concanavalin, canavanine, urease, canatoxin, trypsin inhibitors, chymotrypsin inhibitor, α-amylase inhibitor, phytic acid, phytin, phytin phosphorus, saponins, tannins, condensed tannins, canavalin, total phenols, L-DOPA, hydrogen cyanide and so on, which make them unpleasant and unpalatable for consumption (Faye *et al*., 1986; Yamauchi and Minamikawa, 1987; Barcellos *et al*., 1993; Sato *et al*., 1993; Mohan and Janardhanan, 1994; Siddhuraju and Becker, 2001; Agbede and Aletor, 2005). However, several studies have reported various methods of processing to eliminate the antinutritional properties (Carlini and Udedibie, 1997; Seena *et al*., 2006; Bhagya *et al*., 2009; D'Cunha *et al*., 2009). Concanavalin A (Con A) from *C. ensiformis* (Jack bean) is widely studied for its structure, function and applications. Con A was first isolated and crystallized by Sumner and Howell (1936) from the seeds of jack beans, where it represents 20% of seed proteins (Dalkin and Bowles, 1983). Henceforth, concanavalin C (Con C) from *C. cathartica*, concanavalin M (Con M) from *C. maritima*, concanavalin Br (Con Br) from *C. brasiliensis*,

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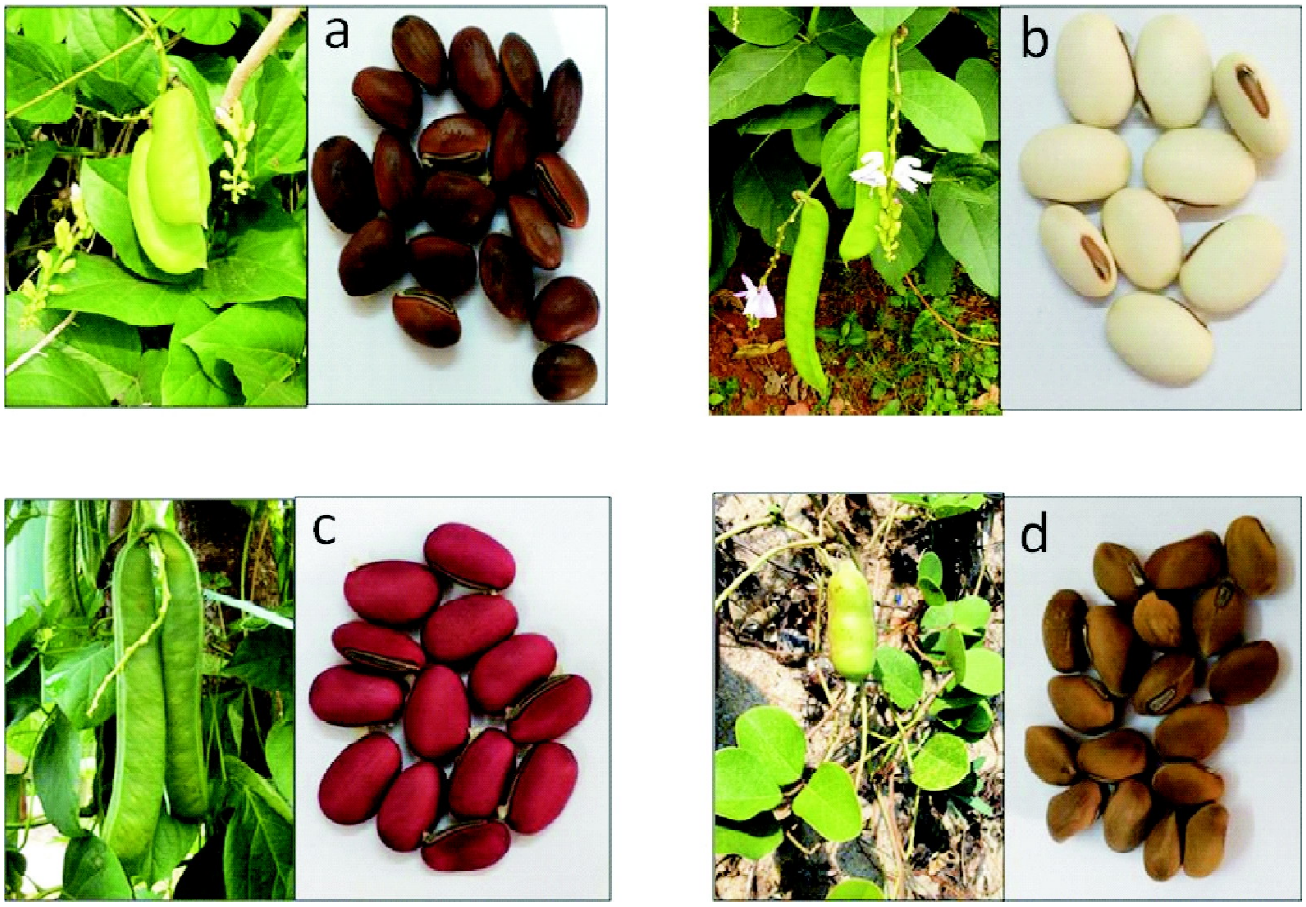


Figure 1: Canavalia **spp. (a)** ***C. cathartica*** **(b)** ***C. ensiformis*** **(c)** ***C. gladiata*** **(d)** ***C. maritima***

concanavalin G (Con G) from *C. gladiata*, concanavalin Bol (Con Bol) from *C. boliviana* and concanavalin GF (Con GF) from *C. grandiflora* have been reported.

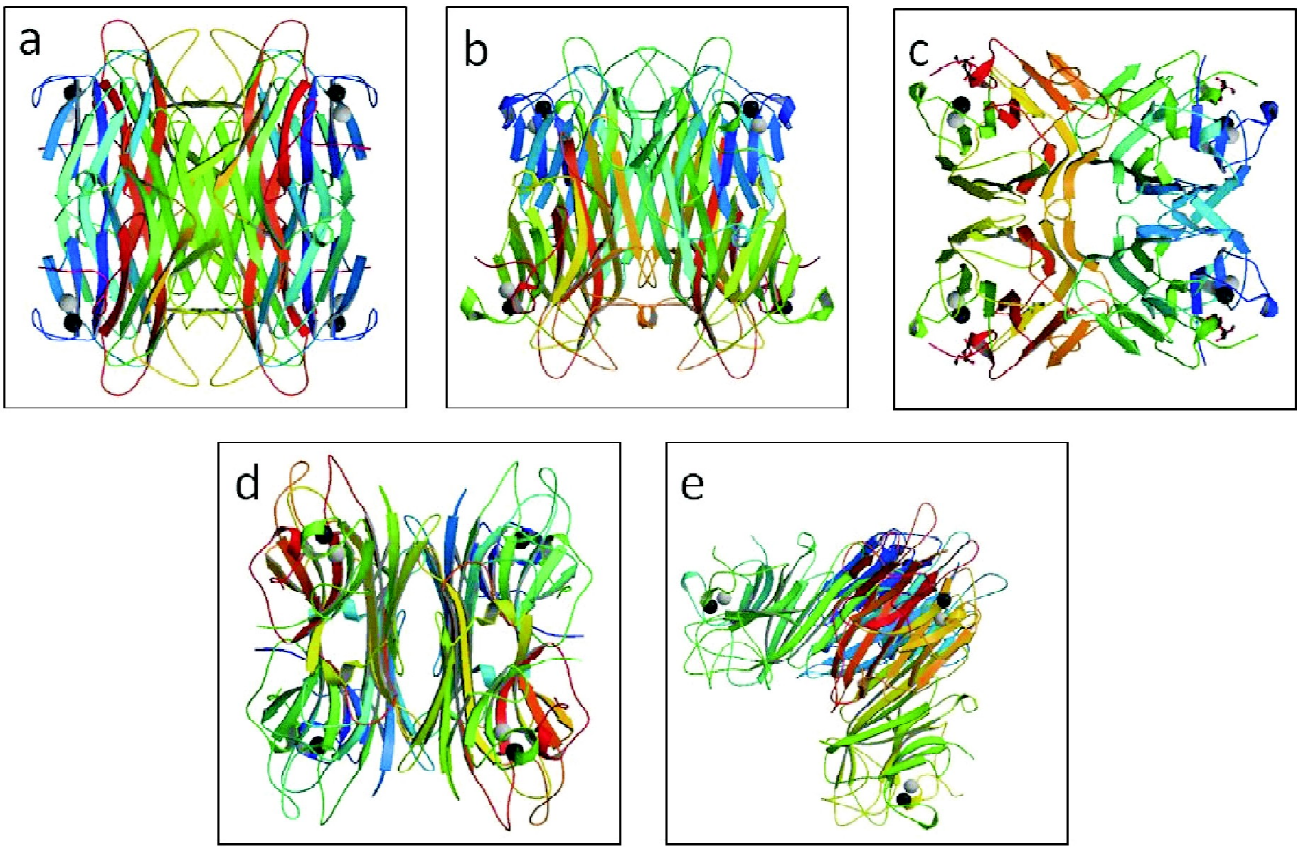
Lectins are predominant in parenchyma cells of the cotyledons and are also located in storage vacuoles (Liener, 2012). They play an important role in plant defense (Chrispeels and Raikhel, 1991) and carbohydrate transport during seedling growth (Ensgraber, 1958). Many reports confirm legume lectin binding with symbiotic rhizobia to form nodules (Chen and Phillips, 1976; Wolpert and Albersheim, 1976; Law and Strijdom, 1977). Wong (1980) detected Con A binding to symbiotic and non-symbiotic strains of rhizobia. He opined that lectin - rhizobium interaction depends on physical characteristics of lectin in addition to its sugar specificity. During symbiosis lectins distributed in root hair tip attaches to cell membrane polysaccharides of rhizobia, which facilitates binding and concentrating the rhizobia at particular sites on root hair. This interaction results in release of Nod factors which initiates infection thread and nodule formation (Hirsch, 1999). *In vitro* and *in vivo* studies of concanavalin report a variety of biological activities including heamagglutination, insecticidal, antiviral, antitumor, immunomodulatory, antimicrobial, mitogenic, antiproliferative and other applications in agriculture, medicine and therapeutics.

Like most legume lectins, concanavalin is devoid of introns (Carrington *et al.*, 1985; Yamauchi and Minamikawa, 1990; Grangeiro *et al.*, 1997). The gene comprises of 870 bp and three gene sequences have been deposited in the GenBank database of NCBI. They are - Con A (Accession # EU233458.1), Con G (Accession # X16041.1) and Con Br (Accession # Y13904.1). Synthesis of Con A initiates after 30 days of flowering and continues till seed maturation (Yamauchi and Minamikawa, 1986, 1987). Con A is synthesized as pro-Con A, which is further processed by the excision of a small glycosylated segment from the center, which is catalyzed by N-glycanase and then ligated (Faye and Chrispeels, 1987; Sheldon *et al.*, 1998). Large amount of functional mRNA for Con A are found

in the early stages of seed development (Raychaudhuri *et al*., 1987). The functional mRNA translates into a precursor protein of 290 amino acid residues. This is further processed during seed development to form a functional protein with 237 amino acid residues. Post-translational processing involves: deglycosylation, peptide cleavage and re-ligation (Carrington *et al*., 1985; Bowles *et al*., 1986, 1988). The quaternary structure at neutral pH is a homotetramer, where each monomer has one sugar binding site and two sites for metal ions (Kalb and Levitzki, 1968; Yariv *et al*., 1968; Sanz-Aparicio *et al*., 1997; de Almeida Gadelha *et al*., 2005; Osterne *et al*., 2017). The sedimentation equilibrium of Con A did not show any monomer, trimer or oligomer greater than tetramer (Light-Wahl *et al*., 1993). Con A exists in two conformations (Brown *et al*., 1982) i.e., it is dimeric at pH below 5 and tetrameric at pH above 7 (McCubbin *et al*., 1971). In the pH range of 5 to 6, it shows dimer-tetramer equilibrium with high affinity for α-D-glucose and α-D-mannose (Dani *et al*., 1981). Monomers are joined base to base to form dimers which in turn pair across additional crystallographic 2-fold axes resulting in tetramers (Becker, 1975). The Mn^{2+}, Mg^{2+} and Ca^{2+} metal ion binding is essential for structural stability and sugar binding in concanavalin (Kaushik *et al*., 2009). The metal ions offer structural stability to Con A by protecting against heat inactivation and hydrolysis by proteolytic enzymes (Thomasson and Doyle, 1975). Loss of Ca^{2+} ions results in destabilization of protein backbone. The Ala207-Asp208 peptide bond in the β-strand adjacent to the metal binding site, undergoes a *cis* to *trans* isomerisation. The *cis* form for this bond is highly conserved in legumes and maintains Ca^{2+} ions in Con A. This change in conformation inhibits the sugar binding ability of concanavalin. Thus demetalization reduces the inter-dimer interaction affecting the structural stability (Bouckaert 1995). The crystallographic studies show concanavalin belongs to orthorhombic space group (Hardman *et al*., 1971; Delatorre *et al*., 2007; Bezerra *et al*., 2011). Each subunit of Con A is a flat-based dome with overall dimensions of 42 × 40 × 39 Å. The polypeptide chain is arranged as antiparallel β sheets. The two metal binding sites are located on the surface of the dome, while the saccharide binding site is deeper between the two β sheets (Becker *et al*., 1975). Quaternary structure of concanavalins is shown in Fig. 2 (Berman *et al*., 2000). Cultured embryo and cotyledon tissues of *C. ensiformis* showed the presence of Con A, whereas it was absent in root cultures (Sato *et al*., 1993). Similar results were seen in callus cultures of *C. cathartica* (Jayavardhanan *et al*., 1996). In mature seeds of *C. ensiformis*, Con A is accumulated in storage vacuoles (Herman and Shannon, 1984). Marcus *et al*., (1984) opined that several molecular form of lectins occur during the development of the *C. ensiformis* seedling. Con A-like lectin was detected in tissues of cotyledons and embryos of *C. gladiata*, which declined in cotyledons with growth (Ghosh *et al*., 1985).

2. Concanavalin homology

Lectins from red kidney bean (PHA), soybean (SBA), jackbean (Con A), peanut (PNA) and pea (PSL) showed homologies at the N-terminal amino acid sequence, which indicates their conservation during evolution. Although they show sequence and structural homology, they differ in sugar specificity and biological function (Lis and Sharon, 1998). Concanavalins have similarity in their N-terminal amino acid sequence (ADTIVAVELDTYPNTDI) (de Almeida Gadelha *et al*., 2005; Osterne *et al*., 2017). However, complete amino acid sequence of Con A when compared to Con Br and Con C showed 99 % homology (Grangeiro *et al*., 1997; Osterne *et al*., 2017) and 98% with Con M (de Almeida Gadelha *et al*., 2005). Structural properties of different concanavalin are shown in Table 1. Carbohydrate recognition domain (CRD) volume in concanavalins depends on the distance between amino acid residues and it plays an important role in carbohydrate recognition thereby eliciting different biological activities (Arruda *et al*., 2013). A study of the toxicity of concanavalins on Brine Shrimp shows LC_{50} is inversely proportional to CRD volume (Table 1). According to LC_{50} value, concanavalins are ordered in the following sequence: Con A (376.48 µg/mL) > Con Bol (218.13 µg/mL) > Con M (146.55 µg/mL) > Con GF (110.51 µg/mL) > Con Br (54.38 µg/mL) (Arruda *et al.,* 2013). Lectins elicit vasodilatory effects in endothelial cells by inducing nitric oxide production. The production of nitric oxide was least in Con Br due to its small CRD volume compared with Con A and Con M (Bezerra *et al*., 2011). Despite high degree of similarity, concanavalins differ in their carbohydrate specificity thereby demonstrate difference in biological functions.



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2CNA:A|PDBID      ADTIVAVELDTYPNTDIGDPSYPHIGIDIKSVRSKKTAKWNMQDGKVGTAHIIYNSVDKR   60
1AZD:A|PDBID      ADTIVAVELDTYPNTDIGDPSYPHIGIDIKSVRSKKTAKWNMQNGKVGTAHIIYNSVGKR   60
1WUV:A|PDBID      ADTIVAVELDTYPNTDIGDPNYPHIGIDIKSVRSKKTAKWNMQNGKVGTAHIIYNSVGKR   60
5F5Q:A|PDBID      ADTIVAVELDTYPNTDIGDPSYPHIGIDIKSVRSKKTAKWNMQNGKVGTAHIIYNSVGKR   60
2CWM:A|PDBID      ADTIVAVELDTYPNTDIGDPSYPHIGIDIKSVRSKKTAKWNMQNGKVGTAHIIYNSVGKR   60
                  ********************.**************************:*************.**

2CNA:A|PDBID      LSAVVSYPNADATSVSYDVDLNDVLPEWVRVGLSASTGLYKETNTILSWSFTSKLKSNST   120
1AZD:A|PDBID      LSAVVSYPNGDSATVSYDVDLDNVLPEWVRVGLSASTGLYKETNTILSWSFTSKLKSNST   120
1WUV:A|PDBID      LSAVVSYPNGDSATVSYDVDLDNVLPEWVRVGLSASTGLYKETNTILSWSFTSKLKSNST   120
5F5Q:A|PDBID      LSAVVSYPNGDSATVSYDVDLDNVLPEWVRVGLSASTGLYKETNTILSWSFTSKLKSNST   120
2CWM:A|PDBID      LSAVVSYPNGDSATVSYDVDLDNVLPEWVRVGLSASTGLYKETNTILSWSFTSKLKSNST   120
                  *********.*:::*******::************************************

2CNA:A|PDBID      HQTDALHFMFNQFSKDQKDLILQGDATTGTDGNLELTRVSSNGSPEGSSVGRALFYAPVH   180
1AZD:A|PDBID      HETNALHFMFNQFSKDQKDLILQGDATTGTEGNLRLTRVSSNGSPQGSSVGRALFYAPVH   180
1WUV:A|PDBID      HETNALHFMFNQFSKDQKDLILQGDATTGTDGNLELTRVSSNGSPQGSSVGRALFYAPVH   180
5F5Q:A|PDBID      HETNALHFVFNQFSKDQKDLILQGDATTGTDGNLELTRVSSNGSPQGSSVGRALFYAPVH   180
2CWM:A|PDBID      HETNALHFVFNQFSKDQKDLILQGDATTGTDGNLELTRVSSNGSPQGSSVGRALFYAPVH   180
                  *:*:****:**********************:***.**********:**************

2CNA:A|PDBID      IWESSATVSAFEATFAFLIKSPDSHPADGIAFFISNIDSSIPSGSTGRLLGLFPDAN     237
1AZD:A|PDBID      IWESSAVVASFEATFTFLIKSPDSHPADGIAFFISNIDSSIPSGSTGRLLGLFPDAN     237
1WUV:A|PDBID      IWESSAVVASFDATFTFLIKSPDSHPADGIAFFISNIDSSIPSGSTGRLLGLFPDAN     237
5F5Q:A|PDBID      IWESSAVVASFDATFTFLIKSPDSHPADGIAFFISNIDSSIPSGSTGRLLGLFPDAN     237
2CWM:A|PDBID      IWESSAVVASFDATFTFLIKSSDSHPADGIAFFISNIDSSIPSGSTGRLLGLFPDAN     237
                  ******.*::*:***:***** ************************************
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Figure 2: **Quaternary structure of concanavalins: (a) Con A (PDB code -2CNA); (b) Con G (PDB code -1WUV); (c) Con C (PDB code -5F5Q); (d) Con M (PDB code -2CWM); (e) Con Br (PDB code -1AZD) (Retrieved from *www.rcsb.org*); (f) Alignment of amino acid sequence of concanavalins using Clustal Omega tool (http://www.ebi.ac.uk/Tools/msa/clustalo/). Amino acid residues comprising CRD region are highlighted in bold letters (Tyr12, Asn14, Leu99, Tyr100, Asp208 and Arg228)**

Table 1
Structural comparison of concanavalins

Concanavalin	*Subunit mass (kDa)*	*Molecular mass (kDa)*	*CRD volume (Å)*[k]
Con A	25.5[a]	104[a]	151
Con G	30[b]	110[h]	-
Con M	25.5[c]	103.8[i]	122
Con C	25.48[d]	-	-
Con Br	30[e]	102.7[j]	105
Con Bol	25.57[f]	105.2[f]	134
Con GF	25.61[g]	-	121

- Not determined;

[a] Sumner *et al*., 1938; [b] Wong and Ng, 2005; [c] Perez *et al*., 1991; [d] Osterne *et al*., 2017; [e] Moreira and Gavada, 1984; [f] Bezerra *et al*., 2011; [g] Barroso-Neto *et al*., 2014; [h] Laija *et al*., 2010a; [i] de Almeida Gadelha *et al*., 2005; [j] Sanz-Aparicio *et al*., 1997; [k] Arruda *et al*., 2013

3. Haemagglutination activity

Concanavalin is known for its agglutinin activity. It precipitates glycogen, amylopectin (Smith *et al*., 1968), yeast mannan (Cifonelli *et al*., 1956), bacterial teichoic acid (Reeder and Ekstedt, 1971), α-mannans of several microorganisms (So and Goldstein, 1968), fat emulsions and starch granules (Sumner and Howell, 1936). Concanavalins are predominantly found in globulin fraction and are heat labile (Sumner, 1919). Various types of processing like dry heat, cooking and sprouting showed decreased haemagglutination activity, which is proportional to decreased globulin fraction (Bhagya *et al*., 2006; Seena *et al*., 2006; Bhagya *et al*., 2009; D'Cunha *et al*., 2009; Bhagya *et al*., 2010). Mohan and Janardhanan (1994) showed that the globulin fraction of *C. gladiata* seeds strongly agglutinates all types of human erythrocytes, but albumin weakly agglutinates blood groups A and O. However, albumin and globulin fractions of *C. ensiformis* showed only a weak agglutination with no specificity in blood group (Mohan and Janardhanan, 1994). The mechanism of agglutination is a result of interaction between the erythrocyte stromata and lectin (Sumner and Howell, 1936). The hydrophilic reactive compound on erythrocyte combines with Con A resulting in a hydrophobic colloid, which on neutralization by salts results in clumping or agglutination (Sumner and Howell, 1936). Lectin forms multiple cross bridges between RBC cells to achieve agglutination (Liener, 2012). Binding of metal ions play an important role in saccharide interaction and agglutinating activity. Concanavalin is a homotetramer and each subunit has two metal binding sites and one sugar binding site (Fig. 3). It binds to Mn^{2+} and Ca^{2+} with specificity to mannose/ glucose. One saccharide is bound to each subunit and four sugar molecules occupy symmetrically equivalent positions on the tetrameric Con A molecule (Becker *et al*., 1975). Transition metal ions such as Mn^{2+} is necessary for the binding of Ca^{2+}, which brings about an alteration in the former metal binding site influencing binding of saccharides (Kalb and Levitzki, 1968). The activity of Con A is influenced by pH, temperature and modifications in chemical structure. Acidic pH, low temperature and acylation causes Con A to form dimer, which fails to form cross-linkage necessary for agglutination (Huet, 1975). This confirms the necessity of tetrameric form of Con A for agglutination activity.

Concanavalins are also known for agglutination of erythrocytes of different animals including humans (Table 2). Con A and Con M demonstrated strong haemagglutination activity against rat erythrocytes and weak activity against cattle and human erythrocytes (Grant *et al*., 1991). Agglutination activity of Con G was prominent in A blood group erythrocytes compared to B and O groups (Tresina and Mohan, 2012). It showed strong agglutination activity towards cattle red blood cells

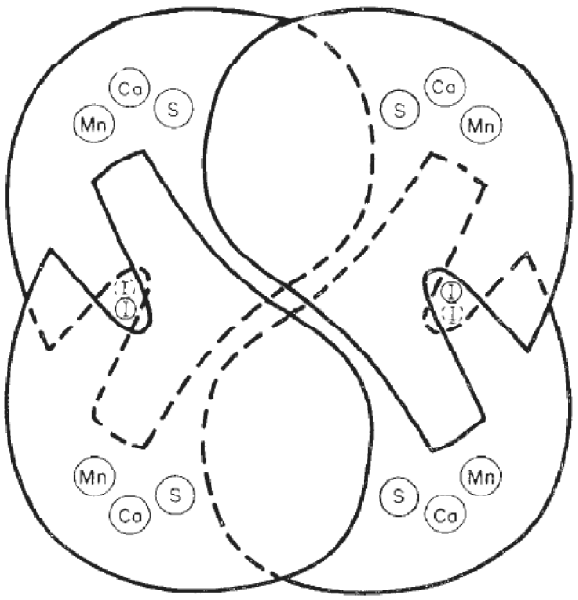

Figure 3: **Schematic representation of Con A tetramer. Ca, Mn and S indicate the positions of Ca $^{2+}$, Mn^{2+} and carbohydrate binding sites respectively. (Adapted from Becker *et al*., 1975)**

Table 2
Haemagglutination activity (HU/mg) of concanavalins

Erythrocyte		*Con A (HU/mg)*	*Con G G(HU/mg)*	*Con C (HU/mg)*	*Con M (HU/mg)*
Rat		333[a]	-	-	666[a]
Rabbit		167[a]	-	2.34[d]	5.1[a]
Cattle		2.6[a]			
		163[c]	164[c]	163[c]	1.2[a]
Human	A	-	138[b]	256[d]	-
	B	-	94[b]	64[d]	-
	AB	1.2[a]	-	-	1.2[a]
	O	1.2[a]	36[b]	256[d]	1.2[a]
		40.6[c]	81.9[c]	40.8[c]	

- Not determined; [a]Grant *et al*., 1991; [b]Tresina and Mohan, 2012; [c]Siddhuraju and Becker, 2001; [d]Suseelan *et al*., 2007.

(Siddhuraju and Becker, 2001). Purified Con C exhibited high haemagglutination activity towards A and O human blood groups compared to B group at pH 7.2. (Suseelan *et al*., 2007). Con A, Con G and Con C also showed agglutination in Bombay blood group (Rodrigues and Torne, 1990). Quantitative inhibition studies are used to determine the sugar binding specificity of lectins and these studies showed that mannose is the strong inhibitor of haemagglutination activity of concanavalins confirming its mannose specificity (Debray *et al*., 1981; Ramos *et al*., 1996; Suseelan *et al*., 2007; Laija *et al*., 2010a). Other than mannose, some sugars and glycoproteins also demonstrated inhibition of haemagglutination. Human transferrin, lactotransferrin and α-Methylmannose showed greater inhibition of Con A activity compared to mannose. Con C activity was also known to be inhibited by methyl-α-D-glucopyrannoside and glycoproteins. Glucose, fructose, N-acetyl glucosamine inhibited Con G activity. Regarding Con M, trehalose and maltose showed stronger inhibition than mannose (Table 3). Such diversity in haemagglutination inhibitors can be attributed to difference in CRD volume, which is specific in recognition of carbohydrates.

4. Mitogenicity

Plant lectins like Con A and PHA (phytohaemagglutinin) are used as mitogen to induce lymphocyte division for the purpose of karyotyping. Lectin triggers multitude of reactions involving different signal transduction pathways by binding to the glycoconjugates on cell surface

Table 3
Inhibition of haemagglutination of concanavalins by sugars

Sugar	*Minimum inhibitory concentration (mg/mL)*			
	Con A[a]	*Con C*[b]	*Con G*[c]	*Con M*[d]
D-glucose	0.9	>180.16	11.53	-
α-D(+)glucose	-	-	-	0.76
L-glucose	-	>36.03	-	-
D-mannose	0.23	<18.02	0.72	-
α-D(+)mannose	-	-	-	0.38
D-galactose	-	>180.16	>36.03	-
Fructose	0.23	-	22.52	-
β-D(-)fructose	-	-	-	0.76
L(-)sorbose	-	-	-	1.50
L-Fucose	>0.41	-	-	-
α-D-(+)fucose	-	>32.83	-	-
Arabinose	-	-	>30.01	-
D-Xylose	-	>30.01	-	-
Sucrose	-	-	-	2.84
Lactose	-	>68.46	>68.46	-
Maltose	-	-	-	0.34
D(+)trehalose	-	-	-	0.34
Melibiose	-	-	>68.46	-
D-raffinose	-	>100.88	>100.88	8.58
Stachyose	-	>133.32	-	-
Methyl D-pyranoside	-	-	> 38.84	-
Methyl-α-D-glucopyranoside	0.24	< 19.42	-	-
Methyl-β-D-glucopyranoside	-	>38.84	-	-
Methyl-α-D-mannopyranoside	0.06	-	-	-
D(+)glucosamine	-	> 35.83	-	-
N-acetyl-D-glucosamine	-	> 44.24	27.65	-
D(+)galactosamine	-	>35.83	-	-
N-acetyl-D-galactosamine	-	> 44.24	> 44.24	-
N-acetylmuramic acid	-	-	-	1.23
N-acetylneuraminic acid	-	-	-	2.57
Human transferrin	0.16	-	-	-
Human lactotransferrin	0.16	-	-	-
Glycerol	-	>18.42	-	-
BSA	-	>100	-	-
Ovalbumin	-	<6.25	-	-
Mucin	-	<3.125	-	-
Fetuin	-	<6.25	-	-
Asialofetuin	-	<3.13	-	-
Thyroglobulin	-	<0.195	-	-

- Not determined; [a]Debray *et al*., 1981; [b]Suseelan *et al*., 2007; [c]Laija *et al*., 2010a; [d]Ramos *et al*., 1996

receptor, which ultimately results in cell proliferation (Liener, 2012). Commercially available PHA is widely used for cytogenetic studies. Con A induces mitotic division of human leucocytes up to 10 µg/1.5 million cells (Powell and Leon, 1970). Con Br showed highest mitogenic potential and stimulated maximum interferon-gamma production compared to Con A in peripheral blood mononuclear cells and also as compared to other Diocleae tribe legume lectins (Barral-Netto *et al*., 1992). Con C induced mitogenesis in spleen cells at 2.5 µg/mL concentration (Suseelan *et al*., 2007). Crude lectin extract from *C. gladiata* showed mitogenic stimulation of human lymphocyte culture at a concentration of 10 µg/mL (Laija *et al*., 2010b). Con M showed mitogenicity towards human peripheral blood mononuclear cells (Karnboj *et al*., 1992). In a pulse chase experiment, Con G stimulated [methyl-^{3}H] thymidine uptake in mouse splenocytes at lower concentration compared to Con A (Wong and Ng, 2005). Con A increased telomere length and replication capacity of human peripheral blood mononuclear cells in young males (20-25 years) compared to older males (60-65 years) (Murillo-Ortiz *et al*., 2013). Con A enhanced osteogenesis in human bone marrow mesenchymal stem cell cultures by increasing osteocalcin, RUNX2, BMP-2, BMP-4, and BMP-6 mRNA expression levels (Sekiya *et al*., 2008). Concanavalins can be used to study the molecular pathways, which stimulate cell division.

5. Antimicrobial property

Many reports on lectins possessing antibacterial, antifungal and antiviral activities are demonstrated. Concanavalin is shown to directly interfere with growth and multiplication of microorganisms. At 250 mg/mL concentration, Con A showed bacteriostatic activity against *Staphylococcus aureus* (76%), *Streptococcus mutans* (66%) and *Bacillus subtilis* (56%) (Kulkarni and Tayade, 2013). Con M, Con Br and Con Bol prevented growth and biofilm formation by *S. mutans*, whereas Con G and Con A was shown to have reverse effect (Cavalcante *et al*., 2011). This can be attributed to its ability to inhibit virulent genes involved in biofilm formation (Cavalcante *et al*., 2013). Some studies showed anti-adhesion property of concanavalins to prevent the adherence of bacteria to host cells. Con A inhibited both the adherence and biofilm formation by *S. mutans* on saliva-coated surfaces in a concentration dependent manner. The presence of Con A in the growth media possibly competes with the bacterial glucan binding lectin for the attachment sites on host cell surface (Islam *et al*., 2009). Con A and Con Br attached to enamel pellicle with high intensity, inhibited the adherence of oral pathogenic bacteria like *Streptococcus oralis, S. sanguinis, S. mitis, S. mutans* and *S. sobrinus* (Teixeira *et al*., 2006). Con Br inhibited the growth of yeasts (*Candida, Rhodotorula, Trichosporon* and *Kloeckera* spp) isolated from vaginal secretion of pregnant and non-pregnant women with and without vulvo-vaginal yeast infection (Gomes *et al*., 2012). It was also effective against *Candida parapsilosis* with minimum fungicidal concentration of 3.90 µg/mL (Klafke *et al*., 2013). Succinylated Con A efficiently inhibited human immunodeficiency virus (HIV) type 1 infection by interfering with cell fusion process (Matsui *et al*., 1990). Con A was able to bind gp120 envelope protein from HIV I and II, thus inhibiting fusion of HIV-infected cells with CD4 cells (Hansen *et al*., 1989). Host cells of sendai, herpes and polio viruses developed resistance when Con A was adsorbed on their cell surface. These viruses turned non-infectious when directly treated with Con A (Okada and Kim, 1972).

6. Insecticidal property

Lectins play a role in plants defence against pathogens. Legume lectins are of current interest for their broad insecticidal potential. Most toxicity has been demonstrated among mannose/glucose lectins. Interaction of lectins with different glycoproteins of insect gut epithelia interferes with physiological processes and affects digestion (Lagarda-Diaz *et al*., 2017). Concanavalins are shown to harm the developmental stages of insects and increase mortality. Bioassays performed to evaluate the toxicity of Con A showed resistance to major insect pests. Con A is reported to delay larval development and affected survival of hemipterans such as *Acyrthosiphon pisum, Macrosiphon albifrons, Aphis gossypii, Myzus persicae, Macrosiphum euphorbiae* and *Aulacorthum solani* (Rahbé *et al*., 1995; Sauvion *et al*., 2004). Semi-artificial diet containing Con A was highly toxic to *Lacanobia oleracea* (tomato moth) larvae, where the lectin severely affected survival, delayed larval development and decreased growth and consumption. Con A gets bound to brush border membrane of the larva, which later is absorbed and released into the haemolymph (Fitches *et al*., 2001). In a similar experiment to study toxicity of Con A against bird

cherry-oat aphid (*Rhopalosiphum padi*) (Sprawka *et al*., 2014) and grain aphid (*Sitobion avenae*) (Sprawka *et al*., 2015), the gut extract of these insects confirmed caspase-3 activity along with DNA fragmentation, thereby inducing apoptotic pathway. Transgenic potato expressing Con A showed retarded larval development of tomato moth (*Lacanobia oleracea*) and decreased the fecundity of peach-potato aphids (*M. persicae*). The insecticidal activity of Con A was higher in transgenic potato compared to feeding pest with Con A incorporated artificial diet, thereby suggesting that transgenic approaches are much more effective in pest management (Gatehouse *et al*., 1999). The use of lectins as a biopesticide is well documented; further understanding the mechanism of action of toxicity and effect on non-pathogenic insects needs to be established.

7. Antitumor activity

Concanavalin is the widely studied legume lectin in cancer research. Con A (IC_{50}- 3 µg/mL) and Con Br (IC_{50}- 20 µg/mL) showed antiproliferative activity in MOLT-4 and HL-60 cells by producing a rate of DNA damage which exceeded 80% (Faheina-Martins *et al*., 2012). Con G was more effective in inhibition of L1210 leukaemia cells compared to Con A (Wong and Ng, 2005). Con Br reduced cell viability and induced apoptosis, which resulted in decreased cell migration (de Oliveira Silva *et al*., 2014). Con A has shown cell death through apoptotic and autophagy pathways. Con A induced apoptosis by p73 regulated Akt-Foxo1a-Bim pathway in p53 deficient cells (Amin *et al*., 2007). A Chinese group showed that Con A induces apoptosis in human breast carcinoma MCF-7 cells through p53 dependent pathway by reducing NF-κB, ERK, JNK levels, and increasing p53 and p21 levels, which was determined using western blotting (Shi *et al*., 2014). Con A induced autophagy through BNIP3-mediated pathway in hepatoma cells (Chang *et al*., 2007) and MEK/Extracellular signal-regulated kinases (ERK) pathway in human cervical cancer (HeLa) cells (Roy *et al*., 2014). Administration of Con A in MCF-7 bearing nude mice decreased the subcutaneous tumor mass volume and weight (Shi *et al*., 2014). $CD8^+$ cells assisted anti-hepatoma activity of Con A was investigated using an *in situ* hepatoma model (Chang *et al*., 2007). Lectins are widely studied to differentiate malignant tumors from benign cells by their degree of glycosylation.

8. Applications in research and diagnostics

Lectins are also used as a diagnostic and therapeutic tool in bacteriology. Con A conjugated with amoxicillin trihydrate (an antibiotic used in the treatment of *Helicobacter pylori*), when used as a drug carrier resulted in increased muco-adhesiveness and controlled release of drug in simulated GI fluid (Jain *et al*., 2014). The potential of Con A as a drug delivery carrier to oral cavity was studied, where it showed $0.82x10^9$ molecules of Con A binding to buccal cells *in vitro* and retention of lectin for 60 min in buccal cavity of rats (Smart *et al*., 2002). Lectin histochemistry is proved to be very useful in detecting and distinguishing different types of adenomas and carcinomas adding to their treatment therapy (Sherwani *et al*., 2003). A microfluidic device in combination with Con A was utilized for the separation of metastatic K562 cells in whole blood samples. The separation efficiency reached 84%, which was much higher than that of microfluidic device experiment devoid of Con A (Li *et al*., 2010).

Affinity chromatography with Con A as ligand has been effective in separation and analyzing different glyconjugates from clinical samples (Hage, 1999). Con A along with pea lectin and PHA immobilized column was useful to fractionate and analyze asparagine-linked oligosaccharides synthesized by BW5147 mouse lymphoma cell line. This serial lectin affinity chromatography technique has the capacity to retain 85% of glycopeptides (Cummings and Kornfeld, 1982). Repeatability and efficiency of glycoprotein enrichment from complex mixture like blood serum was studied using Con A immobilized column (Madera *et al*., 2008). Tandem lectin affinity chromatography monolithic columns with surface immobilised Con A, WGA and *Ricinus communis* agglutinin identified a panel of 23 candidate protein markers from breast cancer and disease-free human sera (Selvaraju and El Rassi, 2012).

Administration of Con A at a dose 10 µg/g body weight for 70 days inhibited the development of insulin-dependent diabetes mellitus by polyclonal T cell activation in non-obese diabetic mice (Pearce and Peterson, 1991). A ^{125}I-labelled Con A showed high binding affinity towards RBCs from diabetic patients than control, which was correlated to glycosylated state of haemoglobin (Okada *et al*., 1982). Fluorescence labelled Con A and dextran was incorporated as sensor to monitor glucose level in

eye fluid (Müller *et al*., 2012) and abdominal subcutaneous tissue (Müller *et al*., 2013) of diabetic patients. The device showed good stability and longevity for a study period of two weeks. Con A along with a panel of lectins was employed as a probe to detect glycoconjugate distribution in environmental model biofilms grown within river water (Neu *et al*., 2001).

9. Conclusions

Concanavalins from underutilized wild legumes of *Canavalia* spp. have wide range of biological properties. Specificity of these proteins to carbohydrate moieties on cell surface makes it a potential diagnostic tool or marker in biological research. Even though *Canavalia* lectins are structurally similar, slight modifications in their three dimensional structure causes significant differences in their biological activities and thus helps in specific diagnosis. A detailed structural analysis in response to its biological activity could provide valuable insight on their mechanism of action.

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Conflict of Interest

The authors do not have any conflict of interest with the contents of this manuscript.

Abbreviations

Con A, concanavalin A; Con Br, concanavalin Br; Con C, concanavalin C; Con M, concanavalin M; Con G, concanavalin G; CRD, carbohydrate recognition domain; LC_{50}, lethal concentration 50; PHA, phytohaemagglutinin; RUNX2, Runt-related transcription factor 2; BMP-2, bone morphogenetic protein-2; BMP-4, bone morphogenetic protein-4; BMP-6, bone morphogenetic protein-6; HIV, human immunodeficiency virus; gp120, envelope glycoprotein GP120; CD4, cluster of differentiation 4; IC_{50}, half maximal inhibitory concentration; MOLT-4, human acute lymphoblastic leukemic cell line; HL-60, human promyelocytic leukemic cell line; L1210, mouse lymphocytic leukemic cell line; MCF-7, human breast adenocarcinoma cell line; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; ERK, extracellular signal–regulated kinases; JNK, c-Jun N-terminal kinases; $CD8^+$, cluster of differentiation 8 which binds to the constant portion of the class I MHC molecule.

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