

# Lectins from red algae and their biomedical potential

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**Abstract** Lectins are unique proteins or glycoproteins of non-immune origin that bind specifically to carbohydrates. They recognise and interact reversibly to either free carbohydrates or glycoconjugates, without modifying their structure. Lectins are highly diverse and widely distributed in nature and have been extensively reported from various red algae species. Numerous red algae species have been reported to possess lectins having carbohydrate specificity towards complex glycoproteins or high-mannose *N*-glycans. These lectin-glycan interactions further trigger many biochemical responses which lead to their extensive use as valuable tools in biomedical research. Thus, owing to their exceptional glycan recognition property, red algae lectins are potential candidate for inhibition of various viral diseases. Hence, the present report integrates existing information on the red algae lectins, their carbohydrate specificity, and characteristics of purified lectins. Further, the review also reports the current state of research into their anti-viral activity against various enveloped viruses such as HIV, hepatitis, influenza, encephalitis, coronavirus and herpes simplex virus and other biomedical activities such as anti-cancer, anti-microbial, anti-inflammatory, anti-nociceptive and acaricidal activities.

**Keywords** Rhodophyceae · Lectins · Haemagglutination · Carbohydrate specificity · Anti-viral

## Introduction

Lectins (haemagglutinins) are carbohydrate-binding proteins/glycoproteins of non-immune origin which agglutinate cells or precipitate glycoconjugates (Dixon, 1981). Lectins bind reversibly to carbohydrates and interact specifically with glycans linked to membrane bound or soluble glycoconjugates without altering their covalent structure (Liener et al. 1986). Various non-covalent forces such as hydrogen bonding, hydrophobic interactions and van der Waal's forces are involved in lectin-sugar interactions (Mirelman, 1986). Proteins are considered as lectins if they fulfill the following conditions (Rüdiger and Gabius, 2001): (a) should bind carbohydrates, (b) should not modify the carbohydrates they bind to and (c) should vary from immunoglobulins. Further, the “carbohydrate recognition domain” of lectins resides in their polypeptide sequence which is the underlying basis of carbohydrate-binding property of most lectins, as revealed by their amino acid sequence analysis (Drickamer, 1988). Lectins have potential to agglutinate erythrocytes, lymphocytes and microbial cells based on their carbohydrate specificity (Sharon and Lis 2004). High amounts of sialoglycoproteins are present on the surface of red blood cells (Furthmayr 1977). Agglutination results in cross-linking of several blood cells as lectins interact specifically to terminal sugar residues on the surface of erythrocytes (Khan et al. 2002). Thus, erythrocyte agglutination is a characteristic feature of lectins which plays a role in the determination of lectin activity based on haemagglutination assays (Ambrosi et al. 2005). In nature, lectins are present amongst wide variety of organisms like animals, higher plants, algae, fungi, protozoa, yeast, mushroom, corals, prokaryotes, invertebrates and vertebrates (Singh et al. 1999). Plant lectins have been extensively explored for their various biological applications such as anti-cancer, immunomodulatory and pro-healing (Teixeira et al. 2012). Apart from them, lectins from microfungi (Singh et al.

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2011), mushrooms (Singh et al. 2010, 2016) and algae (Singh et al. 2015) have also been explored for various biomedical applications. Amongst algae, agglutinins have been most extensively reported from the Rhodophyceae, Chlorophyceae, Cyanophyceae and Phaeophyceae (Akkouh et al. 2015; Singh et al. 2017; Teixeira et al. 2012).

Recently, there has been increased interest in lectins from various marine species such as algae, sponge, mollusk, fish and arthropod, owing to their potential value in various medical applications (Rabelo et al. 2012; Cheung et al. 2015). Marine algae are excellent sources of novel lectin molecules for research and have wide applications in various fields such as pharmaceutical science, medicine, food science, glycobiology and biochemistry (Praseptianga 2015, 2017). Over the years, marine algae from varied regions such as Britain (Boyd et al. 1966; Blunden et al. 1975), USA (Shiomi et al. 1979), Japan (Shiomi et al. 1980) and Brazil (Ainouz et al. 1992) have been screened for the presence of haemagglutinins. Algal lectins, particularly from red algae, share some common characteristics of monomeric forms, low molecular weight, thermostability and divalent cation-independent hemagglutination, along with affinity only for glycoproteins but not for monosaccharides (Hori et al. 1990; Rogers and Hori, 1993). Thus, algal lectins are molecules with low molecular weight and may be less antigenic when used in biological models as depicted by several studies on biological applications of lectins from algae (Teixeira et al. 2012).

Amongst red marine algae, lectin from *Griffithsia* sp. (GRFT) has been widely studied (Mori et al. 2005). Owing to high expression level, potency, stability and current indication for topical delivery, GRFT acts as best-case scenario for non-vaccine plant made pharmaceuticals; however, there are many challenges associated with its bulk production (Fuqua et al. 2015). Lectins from various other red marine algae such as *Eucheuma serra* (Fukuda et al. 2006), *Bryothamnion seaforthii* and *Bryothamnion triquetrum* (Pinto et al. 2009), *Acrocystis nana* (Anam et al. 2017a, 2017b) and *Solieria filiformis* (Chaves et al. 2017) have anti-cancer potential and also used as drug delivery agents owing to their carbohydrate specificity. Thus, studies conducted on various algal lectins bring in a new element of research in biomedical applications.

Even though there has been increasing interest towards algal lectins, still limited information is available on its structural characteristics. Thus, the current review compiles lectins particularly from red algae along with their haemagglutination activity and carbohydrate specificity. It also focuses on physiochemical characteristics of lectins from red algae along with their structural studies and their biomedical potential against various diseases.

### Haemagglutination activity of lectins from red algae

Agglutination is a remarkable property of lectins which occurs owing to polyvalent nature of protein along with its affinity

towards specific carbohydrates on cell surface. Based on their ability to agglutinate human blood type erythrocytes, lectins are classified as specific or non-specific (Sharon and Lis 1972). Agglutination preferences of crude lectins and lectins purified from various red algae species are tabulated in Tables 1 and 2, respectively. Around 800 algal species have been analysed for the presence of lectins and approximately 60% of analysed species showed haemagglutination activity (Teixeira et al. 2012). Crude lectin extract from *Porphyra* sp. specifically agglutinates human blood type B erythrocytes only (De Souza et al. 2007). Crude lectins from *Acanthophora spicifera* (Ainouz et al. 1992), *Bossiella cretacea*, *Nemalion vermiculare*, *Rhodomela munita* and *Tichocarpus crinitus* (Chernikov et al. 2007) specifically agglutinate human blood type O erythrocytes. Purified lectin from *Vidalia obtusiloba* preferentially agglutinates human blood type O erythrocytes followed by A erythrocytes (Melo et al. 2004), whereas *Enantiocladia duperreyi* purified lectin exhibits slight preference for bromelain-treated human blood type O erythrocytes followed by B and A erythrocytes (Benevides et al. 1998). *Amansia multifida* (Ainouz et al. 1992) crude lectin specifically agglutinates human blood type O and A erythrocytes, whereas *Gracilaria tikvahiae* G-3, *Gracilaria verrucosa* G-16S and *Eucheuma nudum* crude lectins agglutinate human blood type A and B erythrocytes only (Chiles and Bird 1989).

Crude lectins from *Gracilaria ferox* and *Hypnea musciformis* exhibit non-specific agglutination of human erythrocytes (Ainouz and Sampaio 1991). Purified lectin from *Ptilota serrata* is non-specific towards human blood groups and agglutinates all native and enzyme (papain)-treated erythrocytes to similar extent (Sampaio et al. 1999). Human red blood cell membrane sialoglycoprotein (glycophorin) consists of 15-O-glycosidically linked chains attached to the sialylated T-antigen structure (Marchesi et al. 1976) along with one biantennary complex carbohydrate chain which is N-glycosidically linked to asparagines (Yoshima et al. 1980). *Tichocarpus crinitus* purified lectin agglutinates various erythrocytes such as human ABO and rat erythrocytes but has highest agglutination titre with rabbit erythrocytes (Molchanova et al. 2010). Human erythrocytes possess only Neu5Ac sialic acid linked to glycoproteins (Reuter et al. 1980), whereas rabbit erythrocytes express high concentration of Neu5Ac and Neu5,9Ac<sub>2</sub> along with Neu9Ac5Gc linked to glycoprotein on their cell surface (Pfeil et al. 1980).

Most of the red algal lectins preferentially agglutinate rabbit and sheep erythrocytes. *Acanthophora spicifera* crude lectin exhibits haemagglutination activity with only rabbit erythrocytes (Chiles and Bird 1989). Crude lectin extracts from *Agardhiella ramosissima*, *Halymenia agardhii* and *Halymenia floresia* also exhibit specific agglutination towards rabbit and sheep erythrocytes (Chiles and Bird 1989). Crude lectins from *Agardhiella tenera*, *E. nudum*, *Gymnogongrus griffithsiae*, *G. tikvahiae*, *G. verrucosa*, *H. agardhii*, *H. floresia* and

**Table 1** Biological action spectrum of crude lectins from red algae

Red algae	Haemagglutination activity with erythrocyte type/s	Reference(s)
<i>Acanthophora spicifera</i>	Rabbit <sup>a,b,c,d</sup> , goat <sup>b</sup> , chicken <sup>a,d</sup> , pig <sup>b,c</sup> and human O <sup>b,d</sup> erythrocytes	Ainouz et al. (1992)
<i>A. spicifera</i>	Rabbit erythrocytes	Chiles and Bird (1989)
<i>A. spicifera</i>	Rabbit <sup>a,b</sup> , sheep <sup>a,b</sup> and chicken <sup>a,b</sup> erythrocytes	Dinh et al. (2009)
<i>Acrocystis nana</i>	Rabbit <sup>a</sup> erythrocytes	Anam et al. (2017a)
<i>Agardhiella ramosissima</i>	Rabbit and sheep erythrocytes	Chiles and Bird (1989), Bird et al. (1993)
<i>A. tenera</i>	Rabbit and sheep erythrocytes	Bird et al. (1993)
<i>Amansia multifida</i>	Rabbit <sup>a,b,c,d</sup> , goat <sup>b,d</sup> , chicken <sup>a,c,d</sup> , pig <sup>b,c,d</sup> , human A <sup>a</sup> and human O <sup>b</sup> erythrocytes	Ainouz et al. (1992)
<i>Bossiella cretacea</i>	Human O erythrocytes	Chemikov et al. (2007)
<i>Bryothamnion seaforthii</i>	Rabbit <sup>a</sup> , chicken <sup>a</sup> and cow <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991)
<i>B. triquetrum</i>	Rabbit <sup>a,b,c,d,e</sup> , goat <sup>b,c,d</sup> , pig <sup>a,b,c,d,e</sup> , human A <sup>b,c,d</sup> , human B <sup>a,b,c,d</sup> , human O <sup>a,b,c,d,e</sup> and human AB <sup>b,c,d</sup> erythrocytes	Ainouz et al. (1992)
<i>Callophyllis crispata</i>	Horse <sup>a,c,f</sup> erythrocytes	Hori et al. (1988b)
<i>C. japonica</i>	Sheep <sup>a,e,f</sup> erythrocytes	Hori et al. (1988b)
<i>Campylaephora crassa</i>	Sheep <sup>a,f</sup> erythrocytes	Hori et al. (1988b)
<i>Carpopeltis flabellata</i>	Rabbit <sup>a</sup> erythrocytes	Hori et al. (1986a)
<i>Chondria crassicaulis</i>	Sheep <sup>a,f</sup> erythrocytes	Hori et al. (1988b)
<i>Chondrus ocellatus</i>	Sheep <sup>a,f</sup> erythrocytes	Hori et al. (1988b)
<i>Chrysomenia wrightii</i>	Sheep <sup>a,f</sup> erythrocytes	Hori et al. (1988b)
<i>Cryptonemia crenulata</i>	Rabbit <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991)
<i>Dictyurus occidentalis</i>	Rabbit <sup>a,b,c,d</sup> erythrocytes	Ainouz et al. (1992)
<i>Enantiocladia duperreyi</i>	Rabbit <sup>c</sup> , human A <sup>b,c,d</sup> , human B <sup>b,c,d</sup> , human O <sup>a,b,c,d,e</sup> and human AB <sup>b,c,d</sup> erythrocytes	Ainouz et al. (1992)
<i>E. denticulatum</i>	Rabbit <sup>a,b</sup> and sheep <sup>a,b,c</sup> erythrocytes	Dinh et al. (2009)
<i>E. nudum</i>	Rabbit, sheep, human A and human B erythrocytes	Chiles and Bird (1989)
<i>E. nudum</i>	Rabbit and sheep erythrocytes	Bird et al. (1993)
<i>Galaxaura fastigiata</i>	Sheep <sup>a,f</sup> erythrocytes	Hori et al. (1988b)
<i>G. filamentosa</i>	Rabbit <sup>a,b</sup> , sheep <sup>a,b</sup> and chicken <sup>a,b</sup> erythrocytes	Dinh et al. (2009)
<i>Gelidiella acerosa</i>	Goat <sup>c,d,e</sup> and chicken <sup>a,b,c,e</sup> erythrocytes	Ainouz et al. (1992)
<i>Gelidiopsis gracilis</i>	Rabbit <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991)
<i>Gelidium amansii</i>	Sheep <sup>a,f</sup> erythrocytes	Hori et al. (1988b)
<i>G. coarctatum</i>	Rabbit <sup>d</sup> erythrocytes	Ainouz et al. (1992)
<i>G. divaricatum</i>	Rabbit erythrocytes	Zheng and Lai-Sheng (2002)
<i>Georgiella confluens</i>	Chicken <sup>a,b,c,d,e</sup> and sheep <sup>a,b,c,d,e</sup> erythrocytes	De Souza et al. (2007)
<i>Gigartina skottsbergii</i>	Rabbit <sup>a</sup> , chicken <sup>d</sup> , goat <sup>b,c,d</sup> , sheep <sup>a,b,c,d,e</sup> , human A <sup>a,b,c,d</sup> , human B <sup>b,d</sup> and human O <sup>a,c,d</sup> erythrocytes	De Souza et al. (2007)
<i>Gloiopeltis furcata</i>	Rabbit <sup>a</sup> , sheep <sup>a,e,f</sup> , human A <sup>c</sup> , human B <sup>c</sup> and human O <sup>c</sup> erythrocytes	Hori et al. (1988b)
<i>G. furcata</i>	Rabbit, sheep, chicken, human A, human B and human AB erythrocytes	Zheng and Lai-Sheng (2002)
<i>Gracilaria bursa-pastoris</i>	Rabbit <sup>a,c,f</sup> , sheep <sup>a,e,f</sup> , horse <sup>f</sup> , chicken <sup>a,f</sup> and human A <sup>f</sup> erythrocytes	Hori et al. (1988b)
<i>G. bailiniaie</i>	Rabbit <sup>a,b,c</sup> , sheep <sup>b</sup> and chicken <sup>a,b,e</sup> erythrocytes	Dinh et al. (2009)
<i>G. bangmeiana</i>	Chicken <sup>a,b</sup> erythrocytes	Dinh et al. (2009)
<i>G. bursa-pastoris</i>	Rabbit <sup>a</sup> erythrocytes	Hori et al. (1986a)
<i>G. cearensis</i>	Rabbit <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991)
<i>G. cervicornis</i>	Rabbit <sup>a</sup> and chicken <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991)
<i>G. changii</i>	Sheep <sup>a,b</sup> and chicken <sup>b</sup> erythrocytes	Dinh et al. (2009)

**Table 1** (continued)

Red algae	Haemagglutination activity with erythrocyte type/s	Reference(s)
<i>G. cornea</i>	Chicken <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991), Lima et al. (2005)
<i>G. eucheumatoides</i>	Rabbit <sup>a,b</sup> , sheep <sup>a,b,c</sup> , chicken <sup>a,b</sup> , human A <sup>a,b</sup> , human B <sup>b</sup> and human O <sup>b</sup> erythrocytes	Dinh et al. (2009)
<i>G. ferox</i>	Sheep <sup>a</sup> , rabbit <sup>a</sup> , chicken <sup>a</sup> , cow <sup>a</sup> , human A <sup>a</sup> , human B <sup>a</sup> , human AB <sup>a</sup> and human O <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991)
<i>G. fisheri</i>	Rabbit, sheep and goose erythrocytes	Boonsri et al. (2017)
<i>G. salicornia</i>	Rabbit <sup>a,b,c</sup> , sheep <sup>a,b,c</sup> and chicken <sup>a,b,c</sup> erythrocytes	Dinh et al. (2009)
<i>G. sjoestedtii</i>	Sheep <sup>a</sup> and chicken <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991)
<i>G. textorii</i>	Rabbit <sup>a,e,f</sup> , sheep <sup>a,e,f</sup> , horse <sup>a,e,f</sup> , human A <sup>a,f</sup> , human B <sup>a,f</sup> and human O <sup>a,f</sup> erythrocytes	Hori et al. (1988b)
<i>G. tikvahiae</i>	Rabbit and sheep erythrocytes	Bird et al. (1993)
<i>G. tikvahiae</i>	Rabbit, sheep, human A and human B erythrocytes	Chiles and Bird (1989)
<i>G. verrucosa</i>	Rabbit <sup>f</sup> , sheep <sup>f</sup> , horse <sup>a,e,f</sup> , chicken <sup>a,f</sup> , human A <sup>a,f</sup> , human B <sup>a,f</sup> and human O <sup>a,f</sup> erythrocytes	Hori et al. (1988b)
<i>G. verrucosa</i>	Horse, cow, sheep, rabbit, guinea pig, carp and chicken erythrocytes	Shiomi et al. (1981)
<i>G. verrucosa</i>	Rabbit and sheep erythrocytes	Bird et al. (1993)
<i>G. verrucosa</i>	Rabbit, sheep, human A and human B erythrocytes	Chiles and Bird (1989)
<i>Gracilariopsis lemaneiformis</i>	Sheep erythrocytes	Bird et al. (1993)
<i>Grateloupia filicina</i>	Sheep <sup>a,e,f</sup> erythrocytes	Hori et al. (1988b)
<i>G. filicina</i>	Rabbit erythrocytes	Zheng and Lai-Sheng (2002)
<i>G. filicina</i>	Rabbit <sup>a,b</sup> , sheep <sup>a,b</sup> , chicken <sup>a,b</sup> , human A <sup>a,b</sup> , human B <sup>a,b</sup> and human O <sup>a,b</sup> erythrocytes	Dinh et al. (2009)
<i>Gymnogongrus flabelliformis</i>	Sheep <sup>a,f</sup> erythrocytes	Hori et al. (1988b)
<i>G. griffithsiae</i>	Rabbit and sheep erythrocytes	Bird et al. (1993)
<i>G. turquetti</i>	Chicken <sup>d</sup> and sheep <sup>d</sup> erythrocytes	De Souza et al. (2007)
<i>Halymenia</i> sp.	Rabbit <sup>a</sup> and human B <sup>a,e</sup> erythrocytes	De Souza et al. (2007)
<i>H. agardhii</i>	Rabbit and sheep erythrocytes	Chiles and Bird (1989), Bird et al. (1993)
<i>H. floresia</i>	Rabbit and sheep erythrocytes	Chiles and Bird (1989), Bird et al. (1993)
<i>H. maculata</i>	Rabbit <sup>a,b,c</sup> , sheep <sup>a,b,c</sup> , chicken <sup>a,b,c</sup> , human A <sup>a,b</sup> , human B <sup>a,b</sup> and human O <sup>a,b</sup> erythrocytes	Dinh et al. (2009)
<i>Hypnea boergesenii</i>	Rabbit <sup>a,b,c</sup> , sheep <sup>a,b</sup> and chicken <sup>a,b</sup> erythrocytes	Dinh et al. (2009)
<i>H. cervicornis</i>	Rabbit <sup>a</sup> and cow <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991)
<i>H. japonica</i>	Rabbit <sup>a</sup> erythrocytes	Hori et al. (1986a, 2000)
<i>H. japonica</i>	Rabbit <sup>a,e,f</sup> , sheep <sup>a,e,f</sup> , horse <sup>a,e,f</sup> , human A <sup>a,f</sup> , human B <sup>a,e,f</sup> and human O <sup>a,e,f</sup> erythrocytes	Hori et al. (1988b)
<i>H. musciformis</i>	Sheep <sup>a</sup> , rabbit <sup>a</sup> , cow <sup>a</sup> , human A <sup>a</sup> , human B <sup>a</sup> , human AB <sup>a</sup> and human O <sup>a</sup> erythrocytes	Ainouz and Sampaio (1991)
<i>H. musciformis</i>	Rabbit and sheep erythrocytes	Bird et al. (1993)
<i>H. musciformis</i>	Rabbit erythrocytes	Melo et al. (1997)
<i>H. nidulans</i>	Rabbit <sup>a,b</sup> , sheep <sup>a,b</sup> , human A <sup>a,b</sup> , human B <sup>a,b</sup> and human O <sup>a,b</sup> erythrocytes	Dinh et al. (2009)
<i>H. valentiae</i>	Rabbit <sup>a,b</sup> , sheep <sup>a,b</sup> , human A <sup>b</sup> , human B <sup>b</sup> and human O <sup>b</sup> erythrocytes	Dinh et al. (2009)
<i>Kappaphycus alvarezii</i>	Rabbit <sup>a,b,c</sup> and sheep <sup>a,b</sup> erythrocytes	Dinh et al. (2009), Le et al. (2009)
<i>K. alvarezii</i>	Rabbit <sup>a</sup> erythrocytes	Hung et al. (2009), Hirayama et al. (2016)
<i>K. striatum</i>	Rabbit <sup>a,b,c</sup> and sheep <sup>a,b,c</sup> erythrocytes	Dinh et al. (2009), Hung et al. (2011)

**Table 1** (continued)

Red algae	Haemagglutination activity with erythrocyte type/s	Reference(s)
<i>Laurencia dichotoma</i>	Rabbit <sup>a</sup> erythrocytes	Ainouz et al. (1992)
<i>L. microcladia</i>	Rabbit <sup>a,c</sup> erythrocytes	Ainouz et al. (1992)
<i>L. obtusa</i>	Rabbit <sup>a,b</sup> and chicken <sup>b</sup> erythrocytes	Dinh et al. (2009)
<i>L. undulata</i>	Rabbit <sup>a,e,f</sup> , sheep <sup>a,e,f</sup> , horse <sup>f</sup> , human A <sup>e</sup> , human B <sup>e</sup> and human O <sup>e</sup> erythrocytes	Hori et al. (1988b)
<i>Meristiella echinocarpa</i>	Rabbit <sup>a,b,c,d</sup> , goat <sup>b,d</sup> , chicken <sup>a,b,d</sup> and pig <sup>b,c,d</sup> erythrocytes	Ainouz et al. (1992)
<i>Nemalion vermiculare</i>	Human O erythrocytes	Chemikov et al. (2007)
<i>Palmaria decipiens</i>	Rabbit <sup>a,b,c,d,e</sup> , chicken <sup>a,b,c,d</sup> , goat <sup>b,d</sup> , sheep <sup>a,b,c,d,e</sup> , human A <sup>a,b,c,d</sup> , human B <sup>b,d</sup> and human O <sup>a,b,c,d</sup> erythrocytes	De Souza et al. (2007)
<i>P. palmata</i>	Rabbit <sup>a,b,e,f</sup> , horse <sup>a,b,e,f</sup> , sheep <sup>b,f</sup> , human A <sup>b,f</sup> , human B <sup>b,f</sup> and human O <sup>b,f</sup> erythrocytes	Kamiya et al. (1982)
<i>Pantoneura plocamioides</i>	Rabbit <sup>b,c</sup> and chicken <sup>b,c</sup> erythrocytes	De Souza et al. (2007)
<i>Polysiphonia</i> sp.	Rabbit <sup>a</sup> and sheep <sup>a,e,f</sup> erythrocytes	Hori et al. (1988b)
<i>Porphyra</i> sp.	Human B erythrocytes	De Souza et al. (2007)
<i>Rhodomela munita</i>	Human O erythrocytes	Chemikov et al. (2007)
<i>R. subfusca</i>	Sheep <sup>a,f</sup> , human A <sup>f</sup> and human B <sup>f</sup> erythrocytes	Hori et al. (1988b)
<i>Solieria filiformis</i>	Rabbit <sup>a,b,c,d</sup> , goat <sup>b,d</sup> , chicken <sup>b,c,d</sup> and pig <sup>b,c,d</sup> erythrocytes	Ainouz et al. (1992)
<i>S. robusta</i>	Rabbit <sup>a,e,f</sup> , sheep <sup>a,e,f</sup> , horse <sup>a,e,f</sup> , chicken <sup>a,f</sup> and human O <sup>f</sup> erythrocytes	Hori et al. (1988b)
<i>Tichocarpus crinitus</i>	Human O erythrocytes	Chemikov et al. (2007)
<i>Vidalia obtusiloba</i>	Rabbit <sup>b,c</sup> and human A <sup>a</sup> erythrocytes	Ainouz et al. (1992)
<i>V. volubilis</i>	Rabbit <sup>d</sup> erythrocytes	Ainouz et al. (1992)

<sup>a</sup> Trypsin-treated erythrocytes  
<sup>b</sup> Papain-treated erythrocytes  
<sup>c</sup> Bromelain-treated erythrocytes  
<sup>d</sup> Subtilisin-treated erythrocytes  
<sup>e</sup> Native erythrocytes  
<sup>f</sup> Pronase-treated erythrocytes  
<sup>g</sup> Neuraminidase-treated erythrocytes

*H. musciformis* specifically agglutinate rabbit and sheep erythrocytes, whereas *Gracilariopsis lamaneiformis* crude lectin agglutinates only sheep erythrocytes (Bird et al. 1993). Purified lectin from red algae *A. tenera* (abundant on northeast coast of the USA) exhibits high activity with guinea pig and rabbit erythrocytes as compared to other erythrocytes (Shiomi et al. 1979). *Gelidium divaricatum* and *Grateloupia filicina* crude lectin extracts exhibit agglutination only with rabbit erythrocytes (Zheng and Lai-Sheng 2002). Crude protein extract from *Gracilaria fisheri* (GPE) strongly agglutinates rabbit erythrocytes with agglutination titre of 1:512 followed by sheep and goose erythrocytes with haemagglutination titre of 1:128 and 1:64, respectively (Boonsri et al. 2017). However, GPE is unable to agglutinate rat, mouse and hamster erythrocytes (Boonsri et al. 2017).

A lectin purified from *Aglaothamnion callophyllidicola* specifically agglutinates horse erythrocytes (Shim et al. 2012). *Callophyllis crispata* crude lectin agglutinates only native and

enzyme-treated horse erythrocytes (Hori et al. 1988b). Low molecular weight *G. verrucosa* haemagglutinin (L-GVH) has low haemagglutination activity than high molecular weight *G. verrucosa* haemagglutinin (H-GVH) but strongly agglutinates horse and rabbit erythrocytes as compared to other erythrocytes (Kakita et al. 1999). *Serraticardia maxima* purified lectin agglutinates non-treated horse erythrocytes more strongly as compared to other animal erythrocytes (Shiomi et al. 1980). However, purified lectin from *A. tenera* and *C. purpureum* exhibits weak activity towards horse erythrocytes (Shiomi et al. 1979; Kamiya et al. 1980). Pig, sheep and horse erythrocytes consist of high proportion of Neu5Gc as parent sialic acid (Cabezas 1973) along with traces of Neu5Ac in pig and horse erythrocytes (Cabezas and Cabezas 1973).

*Gracilaria verrucosa* purified lectin exhibited high agglutination activity with chicken erythrocytes as compared to various other red algae agglutinins (Shiomi et al. 1981). *Gracilaria cornea* crude lectin agglutinates trypsin-treated chicken

**Table 2** Biological action spectrum of purified lectins from red algae

Red algae	Haemagglutination activity with erythrocyte type/s	Reference(s)
<i>Agardhiella tenera</i>	Guinea pig, rabbit, mouse, horse, sheep and human A, B and O erythrocytes	Shiomi et al. (1979)
<i>Aglaothamnion callophyllidicola</i>	Horse erythrocytes	Shim et al. (2012)
<i>Bryothamnion triquetrum</i>	Rabbit <sup>a,b</sup> erythrocytes	Nascimento et al. (2015)
<i>Carpopeltis flabellata</i>	Rabbit <sup>a</sup> erythrocytes	Hori et al. (1986a), Matsubara et al. (1996)
<i>C. flabellata</i>	Rabbit <sup>a,c</sup> , mouse <sup>a,c</sup> , horse <sup>a,c</sup> , chicken <sup>a</sup> and human <sup>c</sup> erythrocytes	Hori et al. (1987)
<i>Cystoclonium purpureum</i>	Rabbit, guinea pig, mouse, horse, human A, human B and human O erythrocytes	Kamiya et al. (1980)
<i>Enantiocladia duperreyi</i>	Human A <sup>c</sup> , human B <sup>c</sup> and human O <sup>c</sup> erythrocytes	Benevides et al. (1998)
<i>Eucheuma amakusaensis</i>	Sheep <sup>a,c</sup> and rabbit <sup>a</sup> erythrocytes	Kawakubo et al. (1997), Kawakubo et al. (1999)
<i>E. cottonii</i>	Sheep <sup>a,c</sup> and rabbit <sup>a</sup> erythrocytes	Kawakubo et al. (1997, 1999)
<i>Georgiella confluens</i>	Chicken <sup>a,c</sup> erythrocytes	Souza et al. (2010)
<i>Gracilaria ornata</i>	Rabbit <sup>a</sup> and chicken <sup>a</sup> erythrocytes	Leite et al. (2005)
<i>G. tikvahiae</i>	Rabbit, sheep, human A and human B erythrocytes	Chiles and Bird (1990)
<i>G. verrucosa</i>	Horse, cow, sheep, rabbit, guinea pig, carp and chicken erythrocytes	Shiomi et al. (1981)
<i>G. verrucosa</i>	Rabbit, guinea pig and sheep <sup>f</sup> erythrocytes	Kakita et al. (1997)
<i>G. verrucosa</i>	Horse, rabbit and sheep <sup>f</sup> erythrocytes	Kakita et al. (1999)
<i>Hypnea cervicornis</i>	Rabbit <sup>a</sup> erythrocytes	Nascimento et al. (2006)
<i>H. japonica</i>	Rabbit <sup>a</sup> erythrocytes	Matsubara et al. (1996), Hori et al. (2000)
<i>H. japonica</i>	Rabbit, horse, sheep, chicken and human A, human B and human O erythrocytes	Hori et al. (1986b)
<i>H. musciformis</i>	Rabbit <sup>a,b,c,d,e</sup> , cow <sup>a</sup> , sheep <sup>a</sup> , human A <sup>a,b,c,d</sup> , human B <sup>a,b,c,d</sup> and human O <sup>a,b,c,d,e</sup> erythrocytes	Nagano et al. (2002)
<i>Pterocladia capillacea</i>	Rabbit <sup>a,c,d</sup> erythrocytes	Oliveira et al. (2002)
<i>P. capillacea</i>	Rabbit <sup>a</sup> erythrocytes	Silva et al. (2010)
<i>Ptilota filicina</i>	Human A <sup>b,c</sup> , human B <sup>b,c</sup> and human O <sup>b,c</sup> erythrocytes	Sampaio et al. (1998)
<i>P. plumosa</i>	Human A <sup>b</sup> , human B <sup>b,c</sup> and human O <sup>b</sup> erythrocytes	Sampaio et al. (2002)
<i>P. serrata</i>	Human A <sup>b,c</sup> , human B <sup>b,c</sup> and human O <sup>b,c</sup> erythrocytes	Sampaio et al. (1999)
<i>Serraticardia maxima</i>	Horse <sup>a,b,e</sup> , cow <sup>a,b,e</sup> , sheep <sup>a,b,e</sup> , rabbit <sup>a,b,e</sup> , guinea pig <sup>a,b,e</sup> , mouse <sup>a,b,e</sup> and chicken <sup>a,b,e</sup> erythrocytes	Shiomi et al. (1980)
<i>Solieria filiformis</i>	Rabbit <sup>a</sup> erythrocytes	Chaves et al. (2017)
<i>S. chordalis</i>	Human A <sup>b,c,e,g</sup> , human B <sup>b,c,e,g</sup> and human O <sup>b,c,e,g</sup> erythrocytes	Rogers and Toplis (1983)
<i>S. robusta</i>	Rabbit <sup>a,c,f</sup> erythrocytes and human A <sup>a</sup> erythrocytes	Hori et al. (1988a)
<i>S. robusta</i>	Rabbit <sup>a</sup> erythrocytes	Matsubara et al. (1996)
<i>Tichocarpus crinitus</i>	Rabbit, rat, human A, human B and human O erythrocytes	Molchanova et al. (2010)
<i>Vidalia obtusiloba</i>	Rabbit <sup>c</sup> , human O <sup>c</sup> and human A <sup>c</sup> erythrocytes	Melo et al. (2004)

<sup>a</sup> Trypsin-treated erythrocytes<sup>b</sup> Papain-treated erythrocytes<sup>c</sup> Bromelain-treated erythrocytes<sup>d</sup> Subtilisin-treated erythrocytes<sup>e</sup> Native erythrocytes<sup>f</sup> Pronase-treated erythrocytes<sup>g</sup> Neuraminidase-treated erythrocytes

erythrocytes but is unable to agglutinate rabbit and human erythrocytes (Lima et al. 2005). Chicken erythrocytes possess high proportion of *N*-acetylneuraminic acid (95–100%) along with traces of *N*-glycolylneuraminic acid (0–5%) (Eylar et al. 1962). As compared to chicken, horse and calf erythrocytes, the sialic acid content is largest amongst human erythrocyte which is about twice their amount (Eylar et al. 1962).

Lectins from marine red algae (specially from the order Gigartinales) are more sensitive to enzyme (trypsin or pronase)-treated rabbit and sheep erythrocytes as compared to other animal and human erythrocytes (Hori et al. 1988b). Mild enzymatic treatment of erythrocytes can expose hidden sites on/within erythrocytes surface and allow access to certain lectins. Proteases such as bromelain and pronase digest outer structures made up of protein and glycoprotein and in turn expose antigens that are normally hidden (Cunliffe and Cox 1979). However, haemagglutination titre of purified *S. maxima* lectin decreases with proteolytic enzyme (trypsin and pronase)-treated horse erythrocytes (Shiomi et al. 1980). Carnin (purified agglutinin from *Carpopeltis flabellata*) does not show any agglutination with trypsin-treated sheep and human ABO erythrocytes (Hori et al. 1987). Upon enzymatic treatment of erythrocyte, agglutination titre increases, if lectin receptors become mobile on membrane surface or decreases upon removal of membrane receptors (Shiomi et al. 1980). Thus, upon treatment of erythrocytes with proteolytic enzymes, the susceptibility of agglutination varies.

De novo exposure of underneath crypt antigens occurs upon protease treatment of erythrocytes as it removes glycol coat from its surface. Trypsin treatment of erythrocytes alters the sialic acid content of erythrocyte sialoglycoproteins (Okamura et al. 2007). Solnins, agglutinins from *Solieria robusta*, strongly agglutinate rabbit erythrocytes with an enhanced sensitivity towards enzyme (trypsin/pronase)-treated rabbit erythrocytes (Hori et al. 1987). Solnin A, isolectin purified from *S. robusta*, specifically agglutinates trypsin-treated human blood type A erythrocytes (Hori et al. 1988a). A lectin purified from *Gracilaria ornata* strongly agglutinates trypsin-treated rabbit erythrocytes but is unable to agglutinate enzyme-treated human erythrocytes (Leite et al. 2005). Lectins from *Hypnea japonica*, *C. flabellata* and *S. robusta* strongly agglutinate trypsin-treated rabbit erythrocytes (Hori et al. 1990). *Kappaphycus alvarezii* (earlier called *Eucheuma cottonii*) crude lectin strongly agglutinates enzyme (trypsin and papain)-treated sheep and rabbit erythrocytes as compared to native erythrocytes and is unable to agglutinate chicken and human erythrocytes (Le et al. 2009). Proteolytic enzymes such as papain or bromelain hydrolyse proteins such as membrane sialoglycoprotein (glycophorin A) on the erythrocyte surface leading to reduction in cell surface sialic acid (Lambert et al. 1977; Rogers and Topliss 1983). Neuraminidase treatment of erythrocytes leads to reduction in lectin activity of *Solieria chordalis* extract; thus, lectin receptor might be sialic acid as

neuraminidase treatment of erythrocyte surface can lead to removal of surface sialic acid (Rogers and Topliss 1983). Purified recombinant *B. triquetrum* lectin (rBTL) agglutinates only papain and trypsin-treated rabbit erythrocytes indicating that its ligands are not directly accessible at the cell surface (Nascimento et al. 2015). Crude lectin from *A. nana* exhibits a high haemagglutination titre with trypsin-treated rabbit erythrocytes (Anam et al. 2017a). Further, carbohydrate specificity of these lectins is the underlying cause for their varied agglutination preferences towards different erythrocytes.

## Carbohydrate specificity of lectins from red algae

Depending on the originating algal species, the hemagglutination-inhibition profiles of crude lectins and lectins purified from red algae are diverse with unique carbohydrate-binding specificities and tabulated in Tables 3 and 4, respectively. Haemagglutination activity of lectins from Rhodophyceae species is strongly inhibited by various glycoproteins and glycopeptides which may recognise various membrane-associated complex carbohydrates. Thus, based on their binding preferences towards glycoproteins, lectins from red algae can be categorised as complex-type specific (complex *N*-glycan or complex *O*-glycan or both), high-mannose type specific or both complex and high-mannose glycan specific. However, lectins from few red algae also show specificity towards monosaccharides and their derivatives.

## Glycoprotein-specific lectins

Majority of lectins from red algae are not inhibited by simple sugars constituting the carbohydrate moiety of the glycoprotein; however, they particularly recognise complex carbohydrate structure in the glycoprotein (Tables 3 and 4).

### Complex carbohydrate specific

Some lectins exhibit specificity towards sugar chains of *N*-glycosidic types (Hori et al. 1986a), whereas a few are *O*-glycan specific (Dinh et al. 2009). Carbohydrate specificity of purified lectins from *E. duperreyi* (Benevides et al. 1998), *Ptilota filicina* (Sampaio et al. 1998), *Pterocladia capillacea* (Oliveira et al. 2002), *G. ornata* (Leite et al. 2005) and *G. cornea* (Lima et al. 2005) is complex and is strongly inhibited by porcine stomach mucin (PSM). PSM is an *O*-linked glycoprotein having terminal GalNAc residues along with galactose and fucose as internal residues (Sampaio et al. 1999). Crude protein extract of *Gracilaria fisheri* (GPE) has specificity towards porcine stomach mucin, fetuin and bovine albumin with a minimum inhibitory concentration (MIC) value of 1.56, 3.12 and 6.25  $\mu\text{g mL}^{-1}$ , respectively (Boonsri et al. 2017). L-GVH exhibits specificity

**Table 3** Carbohydrate specificity of crude lectins from red algae

Red algae	Carbohydrate/glycoprotein specificity	Reference(s)
<i>Acanthophora spicifera</i>	Fetuin, asialofetuin, yeast mannan, porcine stomach thyroglobulin, bovine submaxillary mucin and asialo bovine submaxillary mucin	Dinh et al. (2009)
<i>Carpopeltis flabellata</i>	Complex and high-mannose <i>N</i> -glycans	Hori et al. (1990)
<i>C. flabellata</i>	L-rhamnose, fetuin, $\alpha_1$ -acid glycoprotein and yeast mannan	Hori et al. (1986a)
<i>Eucheuma denticulatum</i>	Transferrin, asialotransferrin, fetuin, asialofetuin, yeast mannan, porcine stomach thyroglobulin, bovine submaxillary mucin and asialo bovine submaxillary mucin	Dinh et al. (2009)
<i>E. nudum</i>	Fetuin and lactoferrin	Chiles and Bird (1989)
<i>Gigartina skottsbergii</i>	Fetuin and bovine submaxillary mucin	De Souza et al. (2007)
<i>Gracilaria bursa-pastoris</i>	Fetuin, $\alpha_1$ -acid glycoprotein and ovomucoid	Hori et al. (1986a)
<i>G. euchematoides</i>	<i>N</i> -acetyl-D-glucosamine, <i>N</i> -acetyl-D-galactosamine, transferrin, asialotransferrin, fetuin, asialofetuin, porcine stomach thyroglobulin, bovine submaxillary mucin and asialo bovine submaxillary mucin	Dinh et al. (2009)
<i>G. fisheri</i>	Porcine stomach mucin, bovine albumin and fetuin	Boonsri et al. (2017)
<i>G. salicornia</i>	Asialotransferrin, fetuin, asialofetuin, porcine stomach thyroglobulin, bovine submaxillary mucin and asialo bovine submaxillary mucin	Dinh et al. (2009)
<i>Halymenia agardhii</i>	Fetuin and lactoferrin	Chiles and Bird (1989)
<i>Hypnea boergesenii</i>	Transferrin, asialotransferrin, fetuin, asialofetuin, porcine stomach thyroglobulin, bovine submaxillary mucin and asialo bovine submaxillary mucin	Dinh et al. (2009)
<i>H. japonica</i>	Fetuin, $\alpha_1$ -acid glycoprotein and ovomucoid	Hori et al. (1986a)
<i>H. nidulans</i>	Transferrin, asialotransferrin, fetuin, asialofetuin, porcine stomach thyroglobulin, bovine submaxillary mucin and asialo bovine submaxillary mucin	Dinh et al. (2009)
<i>H. valentiae</i>	Asialotransferrin, fetuin, asialofetuin, porcine stomach thyroglobulin, bovine submaxillary mucin and asialo bovine submaxillary mucin	Dinh et al. (2009)
<i>Kappaphycus alvarezii</i>	Transferrin, asialotransferrin, fetuin, asialofetuin, yeast mannan, porcine stomach thyroglobulin, bovine submaxillary mucin and asialo bovine submaxillary mucin	Dinh et al. (2009)
<i>K. alvarezii</i>	Fetuin, porcine thyroglobulin, asialo-porcine thyroglobulin, bovine thyroglobulin, asialo-bovine thyroglobulin, yeast mannan, bovine submaxillary mucin and asialo-bovine submaxillary mucin	Le et al. (2009)
<i>K. alvarezii</i>	High-mannose type <i>N</i> -glycans	Sato et al. (2011)

towards complex-type glycoproteins (asialofetuin, fetuin and thyroglobulin), with highest affinity towards desialylated oligosaccharide chain of asialofetuin as revealed by inhibition assays (Kakita et al. 1999). Asialofetuin and fetuin consist of triantennary *N*-linked glycans possessing terminal galactose and sialic acid, respectively (Sampaio et al. 1999). Ovomucoid consists of highly complex *N*-linked glycans, ovalbumin possesses heterogenous *N*-linked glycans and thyroglobulin exhibits both high-mannose and complex-type *N*-glycans (Sampaio et al. 1999). Purified lectin from *Hypnea cervicornis* is inhibited only by glycoprotein PSM at an MIC value of  $19 \mu\text{g mL}^{-1}$  (Nascimento et al. 2006). *Tichocarpus crinitus* (Molchanova et al. 2010) and *Georgiella confluens* (Souza et al. 2010) purified lectins exhibit complex carbohydrate-binding specificity as its activity is inhibited by glycoprotein fetuin and PSM only. The hemagglutinating activity of the *A. callophyllidicola* purified lectin is inhibited by complex glycoproteins, fetuin and asialofetuin with an MIC value of 19 and  $62 \mu\text{g mL}^{-1}$ , respectively (Shim et al. 2012).

*Hypnea japonica* crude lectin exhibits specificity only towards complex *N*-glycans, as revealed by haemagglutination-inhibition tests (Hori et al. 1986a). Similar to hypnin A, iso-haemagglutinins (hypnin A-1 and A-2) from *H. japonica* do not recognise high-mannose *N*-glycans but exhibit specificity towards some glycoproteins bearing complex-type *N*-glycans (transferrin, fetuin and  $\alpha_1$ -acid glycoprotein) or *O*-glycans (fetuin and mucin), their desialylated forms and glycopeptides prepared from asialofetuin (Hori et al. 2000). Isolectin (hypnin A3) purified from *H. japonica* exhibits unique and strict specificity towards core ( $\alpha 1-6$ ) fucosylated *N*-glycans as revealed by SPR analysis and hapten inhibition assay (Okuyama et al. 2009). Crude lectins from *Hypnea valentiae*, *H. boergesenii*, *H. nidulans* and *Gracilaria salicornia* are specific for *O*-glycans as their lectin activity was inhibited by asialofetuin bearing both complex *N*-glycans and *O*-glycans as well as by bovine submaxillary mucin (BSM) and its asialo-derivative bearing *O*-glycans (Dinh et al. 2009). BSM consists of *N*-acetyl neuraminic acid as

**Table 4** Carbohydrate specificity of purified lectins from red algae

Red algae	Carbohydrate/glycoprotein specificity	Reference(s)
<i>Aglaothamnion callophyllidicola</i>	Fetuin and asialofetuin	Shim et al. (2012)
<i>Bryothamnion seaforthii</i>	Fetuin, mucin and avidin	Ainouz et al. (1995)
<i>B. triquetrum</i>	Fetuin, mucin and avidin	Ainouz et al. (1995)
<i>B. triquetrum</i>	<i>N</i> -glycans and core $\alpha$ 1,6-fucosylated octasaccharide	Nascimento et al. (2015)
<i>Carpopeltis flabellata</i>	Transferrin, fetuin, $\alpha$ 1-acid glycoprotein, asialofetuin and yeast mannan	Hori et al. (1987)
<i>Enantiocladia duperreyi</i>	<i>N</i> -acetyl-D-galactosamine, D-galactosamine, <i>o</i> -nitrophenyl- $\beta$ -D-galactopyranoside, lactulose, lactose, $\beta$ -lactose, D-melibiose, <i>p</i> -nitrophenyl- $\alpha$ -D-galactopyranoside, <i>p</i> -nitrophenyl- $\beta$ -D-galactopyranoside, D-fucose, methyl- $\alpha$ -D-galactopyranoside, D-galactose, D-raffinose, methyl- $\beta$ -D-galactopyranoside and porcine stomach mucin	Benevides et al. (1998)
<i>Eucheuma amakusaensis</i>	$\alpha$ 1-Acid glycoprotein, BSA, mucin, $\gamma$ -globulin (bovine), fetuin, asialofetuin, ovalbumin, IgM (mouse), thyroglobulin and yeast mannan	Kawakubo et al. (1997), Kawakubo et al. (1999)
<i>E. cottonii</i>	$\alpha$ 1-Acid glycoprotein, BSA, mucin, $\gamma$ -globulin (bovine), fetuin, asialofetuin, ovalbumin, IgM (mouse), thyroglobulin and yeast mannan	Kawakubo et al. (1999)
<i>E. serra</i>	High-mannose type (HM) <i>N</i> -glycans	Hori et al. (2007)
<i>Georgiella confluens</i>	Fetuin and porcine stomach mucin	De Souza et al. (2007), Souza et al. (2010)
<i>Gracilaria cornea</i>	Fetuin and porcine stomach mucin	Lima et al. (2005)
<i>G. ornata</i>	Asialofetuin, lactotransferrin, porcine stomach mucin, bovine thyroglobulin and porcine thyroglobulin	Leite et al. (2005)
<i>G. tikvahiae</i>	<i>N</i> -acetylneuraminic acid, $\alpha$ -acid glycoprotein, asialofetuin, fetuin, lactoferrin, ovomucoid, asialo bovine submaxillary mucin, bovine submaxillary mucin, human transferrin, fetuin glycopeptide A, glycopeptide B and lactoferrin glycopeptide I	Chiles and Bird (1990)
<i>G. verrucosa</i>	Asialofetuin, fetuin and thyroglobulin	Kakita et al. (1997)
<i>Hypnea cervicornis</i>	D-glucose, D-mannose, D-galactose, methyl- $\alpha$ -D-galactopyranoside, L-fucose, <i>N</i> -acetyl D-galactosamine, <i>N</i> -acetyl D-glucosamine, lactulose, carrageenan, fucoidan, human serotransferrin, desialylated human serotransferrin, $\alpha$ 1 acid glycoprotein, desialylated $\alpha$ 1 acid glycoprotein, human lactotransferrin, desialylated human lactotransferrin, hen ovomucoid, hen ovalbumin, porcine thyroglobulin, desialylated porcine thyroglobulin, bovine lactotransferrin, desialylated bovine lactotransferrin, bovine fetuine, bovine asialofetuin, bovine submaxillary mucin, porcine stomach mucin, ovine submaxillary mucin and desialylated ovine submaxillary mucin	Nagano et al. (2005)
<i>H. cervicornis</i>	Porcine stomach mucin	Nascimento et al. (2006)
<i>H. japonica</i>	Fetuin, $\alpha$ 1-acid glycoprotein and ovomucoid	Hori et al. (1986a)
<i>H. japonica</i>	Glycoproteins bearing complex-type <i>N</i> -glycans (transferrin, fetuin and K1-acid glycoprotein) or <i>O</i> -glycans (fetuin and mucin), their desialylated forms and the glycopeptide prepared from asialofetuin	Hori et al. (1996b, 2000)
<i>H. japonica</i>	Core $\alpha$ 1-6 fucosylated <i>N</i> -glycans and glycoprotein containing core ( $\alpha$ 1-6) fucose	Okuyama et al. (2009)
<i>H. musciformis</i>	D-glucose, D-mannose, D-galactose, methyl- $\alpha$ -D-galactopyranoside, L-fucose, <i>N</i> -acetyl D-galactosamine, <i>N</i> -acetyl D-glucosamine, lactulose, carrageenan, fucoidan, human serotransferrin, desialylated human serotransferrin, $\alpha$ 1 acid glycoprotein, desialylated $\alpha$ 1 acid glycoprotein, human lactotransferrin, desialylated human lactotransferrin, hen ovomucoid, hen ovalbumin, porcine thyroglobulin, desialylated porcine thyroglobulin, bovine lactotransferrin, desialylated bovine lactotransferrin, bovine fetuine, bovine asialofetuin, bovine submaxillary mucin, porcine stomach mucin, ovine submaxillary mucin and desialylated ovine submaxillary mucin	Nagano et al. (2005)
<i>Kappaphycus striatum</i>	Fetuin, yeast mannan, porcine thyroglobulin, asialo-porcine thyroglobulin, bovine thyroglobulin, asialo-bovine thyroglobulin, bovine submaxillary mucin and asialo-bovine submaxillary mucin	Hung et al. (2011)
<i>Palmaria palmata</i>	<i>N</i> -acetylneuraminic acid and D-glucuronic acid	Kamiya et al. (1982)
<i>Pterocladia capillacea</i>	Avidin and porcine stomach mucin	Oliveira et al. (2002)

**Table 4** (continued)

Red algae	Carbohydrate/glycoprotein specificity	Reference(s)
<i>P. capillacea</i>	Mucin	Silva et al. (2010)
<i>Ptilota filicina</i>	<i>p</i> -Nitrophenyl- <i>N</i> -acetyl- $\alpha$ -D-galactoside, <i>p</i> -nitrophenyl- <i>N</i> -acetyl- $\beta$ -D-galactoside, <i>o</i> -nitrophenyl- <i>N</i> -acetyl- $\alpha$ -D-galactoside, <i>o</i> -nitrophenyl- $\beta$ -D-fucoside, <i>p</i> -nitrophenyl- $\alpha$ -D-galactoside, <i>p</i> -nitrophenyl- $\beta$ -D-fucoside, <i>p</i> -nitrophenyl- $\beta$ -D-galactoside, <i>o</i> -nitrophenyl- <i>N</i> -acetyl- $\beta$ -D-galactoside, <i>o</i> -nitrophenyl- $\alpha$ -D-galactoside, <i>o</i> -nitrophenyl- $\beta$ -D-galactoside, lactose, <i>N</i> -acetyl-galactosamine, melibiose, D-galactose, methyl- $\alpha$ -D-galactoside, galactosamine-HCl, methyl- $\beta$ -D-galactoside, raffinose, 2-deoxy-D-galactose, D-fucose, lactulose, fucoidan, porcine stomach mucin, asialo bovine mucin and bovine submaxillary gland mucin	Sampaio et al. (1998)
<i>P. plumosa</i>	<i>p</i> -nitrophenyl- $\alpha$ -D-galactoside, <i>p</i> -nitrophenyl- $\alpha$ -D-glucoside, <i>p</i> -nitrophenyl- $\beta$ -D-fucoside, D-glucose, D-fucose, methyl- $\alpha$ -D-galactoside, <i>p</i> -nitrophenyl- $\beta$ -D-galactoside, <i>p</i> -nitrophenyl- $\beta$ -D-glucoside, D-galactose, L-fucose, <i>o</i> -nitrophenyl- $\alpha$ -D-galactoside, <i>o</i> -nitrophenyl- $\beta$ -D-fucoside, 2-deoxy-D-glucose, methyl- $\beta$ -D-galactoside, D-arabinose, melibiose, raffinose, $\alpha$ -lactose, <i>o</i> -nitrophenyl- $\beta$ -D-galactoside, lactulose, glucosamine-HCl, rhamnose and <i>N</i> -acetyl-glucosamine	Sampaio et al. (2002)
<i>P. serrata</i>	<i>o</i> -Nitrophenyl- <i>N</i> -acetyl- $\alpha$ -D-galactoside, <i>p</i> -nitrophenyl- <i>N</i> -acetyl- $\beta$ -D-galactoside, lactose, <i>o</i> -nitrophenyl- <i>N</i> -acetyl- $\beta$ -D-galactoside, <i>p</i> -nitrophenyl- <i>N</i> -acetyl- $\alpha$ -D-galactoside, <i>p</i> -nitrophenyl- $\beta$ -D-fucoside, <i>o</i> -nitrophenyl- $\beta$ -D-fucoside, <i>N</i> -acetyl-galactosamine, methyl- $\alpha$ -D-galactoside, methyl- $\beta$ -D-galactoside, D-galactose, melibiose, D-fucose, <i>o</i> -nitrophenyl- $\alpha$ -D-galactoside, <i>p</i> -nitrophenyl- $\beta$ -D-galactoside, galactosamine-HCl, raffinose, lactulose, <i>o</i> -nitrophenyl- $\beta$ -D-galactoside, <i>p</i> -nitrophenyl- $\alpha$ -D-galactoside, 2-deoxy-D-galactose, fucoidan, porcine stomach mucin, asialo bovine mucin, asialofetuin and bovine submaxillary gland mucin	Sampaio et al. (1999)
<i>Solieria chordalis</i>	Fetuin, bovine submaxillary gland mucin, porcine mucin, $\alpha$ -D(+)-melibiose and human MN sialoglycoprotein	Rogers and Toplis (1983)
<i>S. filiformis</i>	Mannose oligosaccharides	Chaves et al. (2017)
<i>S. robusta</i>	Fetuin, asialotransferrin, asialofetuin, asialo- $\alpha$ -acid glycoprotein, yeast mannan, ovalbumin, glycopeptide-fraction from fetuin and <i>N</i> -glycopeptide from yeast mannan	Hori et al. (1988a)
<i>S. robusta</i>	Complex and high-mannose <i>N</i> -glycans	Hori et al. (1996b)
<i>Tichocarpus crinitus</i>	Porcine stomach mucin and fetuin	Molchanova et al. (2010)
<i>Vidalia obtusiloba</i>	<i>N</i> -acetyl-galactosamine, D-galactosamine, D-galactose, $\alpha$ -lactose and porcine stomach mucin	Melo et al. (2004)

terminal residue linked to GalNAc (Sampaio et al. 1999). As fetuins are carrier proteins in bloodstream (Bies et al. 2004) and asialofetuin-labelled liposomes are useful for receptor-mediated transfer of DNA sequence to mouse liver cells (Hara et al. 1995), thus fetuin/asialofetuin-specific lectins could be useful in drug delivery studies. A rare specificity is exhibited by *S. chordalis* purified agglutinin towards receptor incorporated in O (2  $\rightarrow$  6) glucoside of sialic acid and 2-acetamido-2-deoxy-D-galactopyranose as revealed by haemagglutination inhibition by sialoglycoproteins fetuin, BSM and PSM (Rogers and Toplis 1983). Preference of *S. chordalis* purified lectin towards subterminal GalNAc instead of Gal residue suggests the role of *N*-acetyl group in conferring rigid conformation on penultimate sugar, thus leading to stabilizing influence on terminal residue linkage (Rogers and Toplis 1983).

#### High-mannose specific

The genus *Euclidean* is a valuable source of lectin proteins as its several species possess high yields of lectins which are homologous between species (Kawakubo et al. 1999). *Euclidean serra* purified agglutinin, ESA-2, is the only lectin that has strict oligosaccharide binding specificity towards high-mannose *N*-glycans along with terminal GlcNAc residues without binding to monosaccharides and core pentasaccharide (Hori et al. 2007). Carbohydrate inhibition studies revealed that *K. alvarezii* (earlier called *E. cottonii*) and *Euclidean denticulatum* crude lectins are specific for high-mannose *N*-glycans as yeast mannan bearing high-mannose *N*-glycans was most inhibitory along with porcine stomach thyroglobulin (PTG) bearing both high-mannose and complex *N*-glycans was also inhibitory (Dinh et al. 2009).

*Kappaphycus alvarezii* crude agglutinin (KAA) preferentially recognises high-mannose type *N*-linked glycoproteins (Le et al. 2009). Consistent with native KAA, recombinant KAA (His-rKAA-1, rKAA-1) lectins from *Kappaphycus alvarezii* also shows specificity towards high-mannose glycoproteins such as yeast mannan, PTG, asialo-PTG, BSM, asialo-BSM, fetuin, asialofetuin, transferrin and asialo-transferrin (Hirayama et al. 2016).

Purified isolectins from *Kappaphycus striatum*, KSA-1, KSA-2 and KSA-3 (Hung et al. 2011), and *K. alvarezii*, KAA-1 and KAA-2 (Le et al. 2009), have special affinity towards high-mannose *N*-glycans as revealed by haemagglutination inhibition analysis. High-mannose type *N*-glycan possessing molecules such as yeast mannan, porcine and bovine thyroglobulin and their asialo-derivatives are highly inhibitory towards haemagglutination activity of *Kappaphycus* isolectins (Le et al. 2009; Hung et al. 2011). Moderate inhibition of *Kappaphycus* isolectins by *O*-linked glycan glycoproteins (fetuin and BSM) is due to non-specific interaction between lectin and *O*-linked glycoproteins (Le et al. 2009; Hung et al. 2011). KSA-2 isolectin specifically interacts with extended carbohydrate structure with a minimal length of tetrasaccharide, Man( $\alpha$ 1-3) Man( $\alpha$ 1-6)Man( $\beta$ 1-4)GlcNAc (Hung et al. 2011). GRFT purified from *Griffithsia* sp. exhibits specificity towards  $\alpha$ (1,2)mannobiose (Moulaei et al. 2010, 2015). The hemagglutination activity of isolectins from *E. denticulatum* (EDA-1, EDA-2 and EDA-3) is commonly inhibited by glycoproteins bearing high-mannose *N*-glycans but not by monosaccharides such as mannose (Hung et al. 2015). In a direct binding experiment with pyridylaminated oligosaccharides, an isolectin EDA-2 exclusively binds to high-mannose type *N*-glycans but not to other glycans that include complex types and a core pentasaccharide of *N*-glycans, indicating that it recognises the branched oligomannoside moiety (Hung et al. 2015). Molecular docking calculations and favourable binding energies reveal that purified isolectins (SfL-1 and SfL-2) from *S. filiformis* specifically bind to 3 $\alpha$ ,6 $\alpha$  mannopentose oligosaccharides, thus exhibits specificity towards high-mannose oligosaccharides (Chaves et al. 2017).

#### Both high-mannose and complex glycoprotein specific

*Carpopeltis flabellata* (Hori et al. 1986a, 1987) and *S. robusta* (Hori et al. 1988a) purified lectins recognise both high-mannose *N*-glycans and complex *N*-glycans. Purified agglutinins from marine red algae *E. serra* (ESA-1 and ESA-2), *E. amakusaensis* (EAA-1, EAA-2 and EAA-3) and *E. cottonii* (ECA-1 and ECA-2) inhibit complex specific glycans but have preferential affinity for glycoproteins bearing high-mannose type *N*-glycans such as thyroglobulin and yeast mannan (Kawakubo et al., 1997, 1999).

#### Simple sugar-specific lectins

Mostly red algae agglutinins have affinity towards various glycoconjugates, whereas lectin-induced agglutination is usually unaffected with monosaccharides as inhibitors. However, a few lectins from red algae act as an exception by exhibiting specificity towards varied monosaccharides. Crude haemagglutinin from *C. flabellata* is inhibited by L-rhamnose (Hori et al. 1986a). Purified lectins from marine algae *P. serrata*, PSL (Sampaio et al. 1999), *P. filicina*, PFL (Sampaio et al. 1998) and *V. obtusiloba* (Melo et al. 2004) are strongly inhibited by simple sugar (galactose) and their derivatives and by glycoprotein PSM. A well-defined monosaccharide binding site is possessed by PFL and PSL which recognise specific arrangement in more complex glycans where galactose occurs (Sampaio et al., 1998, 1999). *Enantiocladia duperreyi* purified lectin-induced agglutination is inhibited by simple sugars (glucose and fucose) and their derivatives (Benevides et al. 1998). *Enantiocladia duperreyi* lectin exhibits preference for bromelain-treated human blood group O erythrocytes which has D-fucose as its sugar determinant (Benevides et al. 1998). *Enantiocladia duperreyi* lectin thus has H antigen preference as also indicated by inhibitory effect of PSM, which is a fucose-carrying glycoprotein (Benevides et al. 1998). Inhibition of purified lectins from *E. duperreyi* (Benevides et al. 1998), *P. filicina* (Sampaio et al. 1998) and *P. serrata* (Sampaio et al. 1999) by nitrophenyl-galactosides is stronger than methyl-D-galactosides; thus, these lectins possess hydrophobic region in vicinity of its carbohydrate-binding site (Mo and Goldstein 1994). Purified lectin from *G. tikvahiae* also exhibits affinity towards *N*-acetylneuraminic acid along with its glycoconjugate, *N*-acetylneuraminic acid-(2  $\rightarrow$  3)-lactose (Chiles and Bird 1990). Purified lectins from few algal species such as *P. serrata* (Sampaio et al. 1999) and *P. filicina* (Sampaio et al. 1998) react with more extended structures/polysaccharides (such as fucoidan) than monosaccharides (L-fucose). Purified rBTL is the first lectin that exhibits strict specificity towards core 6-fucose; however, it shows no binding to ABO, Lewis epitopes or to 3-fucosylated *N*-glycan core (Nascimento et al. 2015).

#### Characteristics of lectins from red algae

Marine macroalgae lectins have been widely isolated and characterised particularly the members of Rhodophyceae (Hori et al. 1990). A panorama of lectins from red algae, purified and characterised over decades, has been tabulated in Table 5.

**Table 5** Characteristics of lectins from red algae

Red algae	Purification method/s	Characteristics					Reference(s)		
		Molecular weight/subunits	pI	pH optima	Temperature optima	Carbohydrate content		EDTA/divalent ion requirement	Amino acids
<i>Agardhiella tenera</i>	Sephadex gel filtration	Monomer, 13 kDa	6.10	4–10	50 °C	2.7%	N.R.	Gly, Ser, Thr rich	Shiomi et al. (1979)
<i>Aglaohammon calophyllicicola</i>	Fetuin-agarose affinity chromatography	Dimer, 50 and 14 kDa	–	–	–	–	–	–	Shim et al. (2012)
<i>Acrocystis nanai</i> <sup>a</sup>	–	–	–	6–7	30 °C	–	Ca <sup>2+</sup> and Mn <sup>2+</sup>	–	Anam et al. (2017a)
<i>Bryothammon seaforthii</i>	DEAE-cellulose chromatography	Monomer, 4.5 kDa	–	–	90 °C	3.2%	N.R.	–	Ainouz et al. (1995)
<i>B. triquetrum</i>	DEAE-cellulose chromatography	Monomer, 3.5 kDa	–	–	90 °C	3.8%	N.R.	–	Ainouz et al. (1995)
<i>Carpopeltis flabellata</i>	Sephadex gel filtration	–	–	7–9	30 °C	–	N.R.	–	Hori et al. (1986a)
<i>C. flabellata</i>	Yeast mannan-Sepharose 4B affinity chromatography, gel permeation HPLC	Monomer, 25 kDa	6.60–6.80	7–10	30 °C	–	N.R.	Gly, Asx rich	Hori et al. (1987, 1990)
<i>Cystoclonium purpureum</i>	Gel filtration, DEAE Sephadex column chromatography	Dimer, 6.2 kDa	5.60	4–10	50 °C	5.6%	N.R.	Gly, Ser rich	Kamiya et al. (1980)
<i>Enantocladia dupeireyi</i>	DEAE-Cellulose, $\alpha$ -lactose-agarose affinity chromatography	Monomer, 16 kDa	–	–	50 °C	6.5%	Ca <sup>2+</sup> and Mn <sup>2+</sup>	–	Benevides et al. (1998)
<i>Eucheuma amakusaensis</i>	Superdex gel filtration, DEAE-Toyopearl ion exchange	Monomer, 29 kDa	(4.95, 5.20, 5.50) <sup>b</sup>	–	–	Nil	–	Glx, Asx, Gly, Ser rich	Kawakubo et al. (1999)
<i>E. cottonii</i>	Superdex gel filtration, DEAE-Toyopearl ion exchange	Monomer, 29 kDa	(5.05, 5.10) <sup>b</sup>	–	–	Nil	–	Glx, Asx, Gly, Ser rich	Kawakubo et al. (1999)
<i>E. serra</i>	Superdex gel filtration, DEAE-Toyopearl ion exchange	Monomer, 29 kDa	(4.75, 4.95) <sup>b</sup>	2.5–10.5	60 °C	Nil	N.R.	–	Kawakubo et al. (1997)
<i>Georgiella confluens</i>	DEAE-Sepharose ion exchange, affinity chromatography	Monomer, 21.5 kDa	–	–	80 °C	–	N.R.	–	Souza et al. (2010)
<i>Gracilaria bursa-pastoris</i>	Sephadex gel filtration	–	–	7	30–40 °C	–	N.R.	–	Hori et al. (1986a)
<i>G. cornea</i>	Phenyl-Sepharose CL-4B hydrophobic chromatography, affinity chromatography	Monomer, 60 kDa	4.30	–	40 °C	52.5%	N.R.	–	Lima et al. (2005)
<i>G. ornata</i>	DEAE-cellulose ion exchange, mucin-Sepharose 4B affinity chromatography	Monomer, 17 kDa	5.40	–	50 °C	2.9%	N.R.	Asx, Glx, Ser, Glu, Ala, Cys rich	Leite et al. (2005)
<i>G. fisheri</i> <sup>a</sup>	–	–	–	–	50 °C	–	Mn <sup>2+</sup>	–	Boonsri et al. (2017)
<i>G. tikvahiae</i>	DEAE-trisacryl ion exchange chromatography, sephadex gel filtration and hydroxylapatite chromatography	Dimer, 29.7 and 24.9 kDa	–	–	–	–	N.R.	–	Chiles and Bird (1990)
<i>G. verrucosa</i>	DEAE-cellulose chromatography, sephadex chromatography	Tetramer, 2 subunits, 12 and 10.5 kDa	4.80	4–12	40 °C	–	N.R.	Asp, Glu rich	Shiomi et al. (1981)

**Table 5** (continued)

Red algae	Purification method/s	Characteristics				Reference(s)			
		Molecular weight/subunits	pI	pH optima	Temperature optima	Carbohydrate content	EDTA/divalent ion requirement	Amino acids	Reference(s)
<i>G. verrucosa</i> <sup>e</sup>	DEAE-Toyopearl ion exchange, gel filtration	76 to 359 kDa, diffused band	–	5–10	100 °C	6.2 <sup>c</sup>	N.R.	–	Kakita et al. (1997)
<i>G. verrucosa</i> <sup>d</sup>	DEAE-Toyopearl ion exchange, gel filtration	71 kDa, diffused band	–	5–10	100 °C	6.2 <sup>c</sup>	N.R.	–	Kakita et al. (1999)
<i>Griffithsia</i> sp.	Hydrophobic chromatography, anion exchange chromatography, reversed-phase chromatography, and size exclusion chromatography	Monomer, 13 kDa	–	–	–	–	–	–	Mori et al. (2005)
<i>Hypnea cervicornis</i>	DEAE-Sephacel ion exchange, reversed-phase high-performance liquid chromatography	Monomer, 9 kDa	–	–	–	–	–	Gly, Cys rich	Nascimento et al. (2006)
<i>H. japonica</i> <sup>a</sup>	–	–	–	3–9	50–90 °C	–	N.R.	–	Hori et al. (1986a)
<i>H. japonica</i>	Reversed-phase and gel permeation high-performance liquid chromatography	(Monomer, 4.2 kDa, dimer, 4.2 kDa, monomer, 12 kDa) <sup>f</sup>	4.30	–	–	–	N.R.	Ser, Gly rich	Hori et al. (1986b, 1990)
<i>H. japonica</i>	Toyopearl gel filtration, reverse phase HPLC	Monomer, 8.5–9.5 kDa	–	–	–	–	–	Ser, Gly, Pro rich, 4 Cys residues	Hori et al. (2000)
<i>H. musciformis</i>	DEAE-Cellulose ion exchange chromatography, RP-HPLC	Monomer, 9.3 kDa	–	–	–	–	–	–	Nagano et al. (2002)
<i>Kappaphycus alvarezii</i>	Ion exchange chromatography	Monomer, 28 kD	–	3–10	50 °C	–	N.R.	–	Le et al. (2009)
<i>K. striatum</i>	Ion exchange chromatography	Monomer, 28 kDa	–	3–10	60 °C	–	N.R.	–	Hung et al. (2011)
<i>Palmaria palmata</i>	DEAE-Sephacel ion exchange, sephadex gel filtration, hydroxyapatite chromatography	Dimer, 20 kDa	4.60	6–10	60 °C	–	N.R.	Ser, Gly, Asp rich	Kamiya et al. (1982)
<i>Pterocladella capillacea</i>	Guar gum affinity chromatography	Monomer, 5.8 kDa	–	7–10	60 °C	Nil	N.R.	–	Oliveira et al. (2002)
<i>Ptilota filicina</i>	Affinity chromatography	Trimer, 19.3 kDa	–	4–9	50 °C	–	Ca <sup>2+</sup> , Mg <sup>2+</sup> or Mn <sup>2+</sup>	Thr, Asp, Gly rich	Sampaio et al. (1998)
<i>P. plumosa</i>	Affinity chromatography	Trimer, 17.4 kD	–	–	–	–	–	Ala, Gly, Val rich	Sampaio et al. (2002)
<i>P. serrata</i>	Affinity chromatography	Trimer, 18.3 kDa	–	6–8	80 °C	–	Ca <sup>2+</sup> , Mg <sup>2+</sup> or Mn <sup>2+</sup>	Ala, Val, Leu, Glu rich	Sampaio et al. (1999)
<i>Serraticardia maxima</i>	Sephaeryl gel filtration	Monomer, 25 kDa	–	5–9	–	–	N.R.	–	Shiomi et al. (1980)
<i>Solitaria chordalis</i>	Affinity adsorption	Monomer, 35 kDa	–	–	–	–	–	–	Rogers and Topliss (1983)
<i>S. filiformis</i>	DEAE-Sephacel ion exchange	28 kDa	–	–	–	–	–	–	Chaves et al. (2017)
<i>S. robusta</i>	Gel filtration, ion exchange HPLC	Monomer, 29 kDa	(4.30, 4.20, 4.10) <sup>b</sup>	6–10	40 °C	–	N.R.	Gly, Asx, Glx rich	Hori et al. (1988a, 1990)

**Table 5** (continued)

Red algae	Purification method/s	Characteristics					Reference(s)		
		Molecular weight/subunits	pI	pH optima	Temperature optima	Carbohydrate content		EDTA/divalent ion requirement	Amino acids
<i>Tichocarpus crinitus</i>	Phenyl-Sepharose hydrophobic chromatography, Superdex gel filtration	Monomer, 41 kD	4.93	7–8	50 °C	6.9%	N.R.	Asp, Glu, Ser, Thr, Pro, Ala rich	Molchanova et al. (2010)
<i>Vidalia obtusiloba</i>	DEAE-cellulose ion exchange, guar gum affinity chromatography	Dimer, 59.6 and 15.2 kDa	4.00–5.00	–	50 °C	43.2%	Ca <sup>2+</sup> or Mn <sup>2+</sup>	Glu, Asp, Leu rich	Melo et al. (2004)

NR not required

<sup>a</sup> Characteristics of crude extract

<sup>b</sup> pI of different isolectins

<sup>c</sup> High molecular weight *Gracilaria verrucosa* haemagglutinin (H-GVH)

<sup>d</sup> Low molecular weight *Gracilaria verrucosa* haemagglutinin (L-GVH)

<sup>e</sup> 6.2 mg hexose in 9 mg lectin

<sup>f</sup> Molecular weight of hypnins A, B, C and D

## Purification methods

Lectins from red algae can be purified by either multistep chromatographic techniques or single-step affinity chromatography (Table 5). Owing to carbohydrate specificity of lectins, they are mostly purified by single-step affinity chromatography. Lectins from *P. filicina* (Sampaio et al. 1998) and *P. serrata* (Sampaio et al. 1999) have been isolated by affinity chromatography onto a cross-linked guar gum and 95-fold and 1.8-fold purification, respectively, was achieved. *Pterocladia capillacea* lectin has been purified by affinity chromatography onto a guar gum column with a purification fold of 14.5 and recovery yield of 27.4% (Oliveira et al. 2002). *Ptilota plumosa* lectin (PPL) has been purified from algal extracts by affinity chromatography using Sephadex G-200 column and a purification fold of 212 and high specific activity of 46,282 was obtained (Sampaio et al. 2002). Fetuin-specific lectin from *A. callophyllidicola* has been purified by agarose-bound fetuin affinity chromatography (Shim et al. 2012).

A lectin has been purified from the seaweed *Gracilaria cornea* by combination of phenyl-Sepharose CL-4B hydrophobic interaction chromatography followed by affinity chromatography using immobilised mucin (Lima et al. 2005). Lectin from red marine algae *V. obtusiloba* has been purified by combination of ion exchange chromatography on DEAE-cellulose and affinity chromatography onto a cross-linked guar gum column (Melo et al. 2004). *Gracilaria ornata* lectin has been purified by DEAE-cellulose ion exchange chromatography and mucin-Sepharose 4B affinity chromatography (Leite et al. 2005). A lectin from Antarctic seaweed *G. confluens* has been purified by ion exchange followed by affinity chromatography using immobilised PSM (Souza et al. 2010). *Tichocarpus crinitus* lectin has been purified by phenyl-Sepharose hydrophobic chromatography and gel exclusion chromatography (Molchanova et al. 2010). Recently, isolectins from *S. filiformis* have been purified by DEAE-Sepacel ion exchange chromatography (Chaves et al. 2017).

## Isoelectric point and amino acid content

Lectins from red algae have low isoelectric points (pIs), usually in the range of 4–6 (Table 5). Red algal lectins usually have high contents of acidic (aspartic acid and glutamic acid) and hydroxyl (serine and threonine) amino acids and low levels of basic amino acids such as arginine, histidine and lysine (Hori et al. 1990; Okamoto et al. 1990; Sampaio et al. 1999). The large proportion of acidic amino acids accounts for the acidic pI in lectins from some red algae species such as *S. filiformis* (Benevides et al. 1996) and *V. obtusiloba* (Melo et al. 2004). *Vidalia obtusiloba* lectin is rich in acidic amino acids (glutamic and aspartic acids) and in the hydrophobic amino acid (leucine) but has a low content of cystine and

methionine (Melo et al. 2004). *Tichocarpus crinitus* lectin has high proline content along with acidic and hydroxyl amino acids (Molchanova et al. 2010).

Amino acid composition of *H. cervicornis* lectin (Nascimento et al. 2006) revealed high content of cysteine (15.5 mol%) and low serine (4.4 mol%), similar to the *H. musciformis* lectin (15.2 Cys, 5.4 Ser residues per mol). However, *H. japonica* isolectins contained lower Cys (4.4 mol%) and higher Ser (15.5 mol%) (Hori et al. 2000). Similarly, *B. triquetrum* lectin contained lower Cys (3.6 mol%) and higher Ser (16.3 mol%) (Calvete et al. 2000). It seems that amino acid content varies in red algae lectins. Further, *H. cervicornis* lectin is involved in formation of seven intramolecular disulphide bonds as lectin consists of 14 cysteines per molecule (Nascimento et al. 2006). Amino acid sequence of hypnins revealed that hypnin A-1 and A-2 polypeptides are composed of 90 amino acid residues including four half cysteines which is involved in formation of two intrachain disulphide bonds (Hori et al. 2000). Reduction and S-pyridylethylation of haemagglutinin (hypnin A-1 and A-2) result in loss of activity, which indicates the involvement of disulphide bonds in maintaining haemagglutination activity of each haemagglutinin (Hori et al. 2000). The sequence of both the agglutinins varies at three positions, with Leu19, Ser31 and Tyr52 of hypnin A-2 as compared to Pro19, Arg31 and Phe52 of hypnin A-1 (Hori et al. 2000). *Gracilaria ornata* lectin also consists of high amount of cysteine, 7.79 mol% (Leite et al. 2005). However, disulphide bonds might not be involved in subunit association of *Cystoclonium purpureum* (Kamiya et al. 1980), *G. verrucosa* (Shiomi et al. 1981) and *Palmaria palmata* (Kamiya et al. 1982) agglutinins as indicated by the absence of half cysteine or cysteine in their amino acid content.

### Molecular weight

Lectins from red algae are usually low molecular weight proteins and exist in monomeric form such as lectins from *C. flabellata*, *S. robusta* (Hori et al. 1990), *B. seaforthii*, *B. triquetrum* (Ainouz et al. 1995), *P. capillacea* (Oliveira et al. 2002), *H. musciformis* (Nagano et al. 2002), *G. ornata* (Leite et al. 2005), *G. cornea* (Lima et al. 2005), *Griffithsia* sp. (Mori et al. 2005; Moulaei et al. 2010, 2015), *K. alvarezii* (Le et al. 2009), *T. crinitus* (Molchanova et al. 2010), *G. confluens* (Souza et al. 2010) and *K. striatum* (Hung et al. 2011). However, some marine red algal lectins have dimeric structures, such as *P. palmata* (Kamiya et al. 1982) and *V. obtusiloba* (Melo et al. 2004); trimeric, such as *P. filicina* (Sampaio et al. 1998), *P. serrata* (Sampaio et al. 1999), *Ptilota plumosa* and *Ptilota gunneri* (Sampaio et al. 2002); or tetrameric structures, such as *G. verrucosa* (Shiomi et al. 1981). Lectin from *A. callophyllidicola* is dimeric with subunits having molecular weights of 50 and 14 kDa (Shim et al. 2012). Isolectins, Sfl-1

and Sfl-2, from *S. filiformis* have a molecular mass of 27,552 and 27,985 Da, respectively (Chaves et al. 2017).

On the basis of their biochemical properties, Rogers and Hori (1993) categorised red algae lectins into three types: (a) glycoprotein-specific low molecular weight proteins with no requirement of divalent cations, (b) monosaccharide and related small molecule-specific intermediate molecular weight proteins with no requirement of divalent cations and (c) monosaccharide-specific large lectins (MW > 64,000) with requirement of divalent cations. Amongst all red algae lectins reported till now, agglutinins from *B. seaforthii* and *B. triquetrum* have lowest molecular masses 3500 and 4500 Da, respectively (Ainouz et al. 1995). A 41-kDa lectin (GVA-1) has been reported from *G. verrucosa*, which is a key species in Japan as food source or raw material of agar-agar (Shiomi et al. 1981). H-GVH has large molecular size (480 kD) as revealed by gel filtration analysis and is not a classical lectin but a sulfated polysaccharide hemagglutinin and the sulfated polysaccharide moiety of it plays a role in hemagglutination activity (Kakita et al. 1997).

### pH and temperature

Haemagglutination activity of lectins from marine red algae such as *K. alvarezii* (Le et al. 2009), *A. tenera* (Shiomi et al. 1979), *C. purpureum* (Kamiya et al. 1980) and *G. verrucosa* (Shiomi et al. 1981) lectins is stable over a broad pH range. *Eucheuma serra* isolectins also exhibit lectin activity over broad pH range of 2.5–10.5 (Kawakubo et al. 1997). Lectins from few red algae species such as *P. serrata* (Sampaio et al. 1999), *Gracilaria bursa-pastoris* (Hori et al. 1986a), *T. crinitus* (Molchanova et al. 2010) and *A. nana* (Anam et al. 2017a) exhibit activity near neutral pH range (6–8) with loss of activity beyond/above this range.

Haemagglutination activity of lectins from *P. capillacea* (Oliveira et al. 2002), *G. ornata* (Leite et al. 2005), *K. alvarezii* (Le et al. 2009) and crude protein extract from *G. fisheri* (Boonsri et al. 2017) is stable till 50 °C temperature. Lectins from *C. flabellata* (Hori et al. 1986a, 1990) and *A. nana* (Anam et al. 2017a) are stable only up to 30 °C temperature. Haemagglutinins from several red algae species such as *A. tenera*, *C. purpureum*, *C. flabellata* and *V. obtusiloba* are thermosensitive with total or almost total activity loss when heated to temperatures equal to or above 60 °C (Shiomi et al. 1979; Kamiya et al. 1980; Hori et al. 1987; Melo et al. 2004). *Pterocladia capillacea* lectin activity is unstable at high temperature, as its activity is lost at 60 °C (Fabregas et al. 1992). However, a few red algae lectins are thermostable or thermoresistant, such as haemagglutinins from *B. seaforthii*, *B. triquetrum* (Ainouz et al. 1995), *G. verrucosa* (Kakita et al. 1997, 1999), *H. japonica* (Hori et al. 1986b), *P. serrata* (Sampaio et al. 1999) and *G. confluens* (Souza et al. 2010).

## Effect of metal ions

Red algae lectins usually do not require divalent cations for maintenance of their haemagglutination activity; however, a few lectins isolated from the red algae such as *Plumaria elegans*, *P. serrata* (Rogers et al. 1990), *P. filicina* (Sampaio et al. 1998), *E. duperreyi* (Benevides et al. 1998), and *V. obtusiloba* (Melo et al. 2004) are dependent on metals, like  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Mg}^{2+}$ . Crude lectin extract from *A. nana* requires divalent cations  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  for its haemagglutination activity (Anam et al. 2017a). Thus, metal requirement is not a general characteristic of red algae lectins; however, a few lectins are metalloproteins.

## Carbohydrate content

A few Rhodophyceae lectins are glycoproteins with varied carbohydrate content; however, some lectins possess no carbohydrate content (Table 5). Lectins from *C. purpureum* (Kamiya et al. 1980), *G. bursa-pastoris* (Okamoto et al. 1990), *E. duperreyi* (Benevides et al. 1998), *G. ornata* (Leite et al. 2005) and *T. crinitus* (Molchanova et al. 2010) are glycoproteins. Carbohydrate content of lectins from red algae can be as low as 2.7% in *A. tenera* lectin (Shiomi et al. 1979) or 2.9% in *G. ornata* lectin (Leite et al. 2005) or as high as 43% in *V. obtusiloba* lectin (Melo et al. 2004) or 52.5% in *G. cornea* lectin (Lima et al. 2005).

## Structure of red algal lectins

The structural analysis of lectins helps in investigation of their potential applications in various fields. Amongst lectins from red marine algae, the primary structure of *B. triquetrum* lectin (BTL) was the first to be determined (Calvete et al. 2000). Structural characterization of *B. triquetrum* lectin interaction with the octasaccharide depicts an extended epitope for recognition which includes the fucose residue, the distal GlcNAc and one mannose residue as revealed by STD-NMR (Nascimento et al. 2015). Amongst lectins from other red algal species, crystal structure of griffithsin lectin has been thoroughly investigated which is a stable homodimer where each subunit contains 121 amino acids (Ziolkowska et al. 2006, 2007a, 2007b). Crystal structure of carbohydrate free lectin from *Griffithsia* sp. and its interaction with specific glycan molecules such as mannose, *N*-acetylglucosamine, 1 → 6 $\alpha$ -mannobiose and maltose have been determined through X-ray crystallography, isothermal titration calorimetry and molecular modeling (Ziolkowska et al. 2006; 2007a, b). The domain-swapped dimer of griffithsin molecule involves that first two  $\beta$ -strands of one chain are connected with ten strands of the other chain and vice versa (Ziolkowska et al. 2006). GRFT-glycan complex analysis revealed that three almost

identical carbohydrate-binding sites (separated by approximately 15 Å) occur on each monomer of GRFT, each interacting with a monosaccharide and thus in turn six principal sites occur in the dimer (Ziolkowska et al. 2006). GRFT-glucose (Ziolkowska et al. 2006) and GRFT-mannose (Ziolkowska et al. 2007a) interaction studies revealed the difference in the orientation of the O1 atom. The interaction heats for glucose (1.5-fold decrease) and *N*-acetylglucosamine (2-fold decrease) are comparable to that of mannose as revealed by isothermal titration calorimetry binding experiments with GRFT (Ziolkowska et al. 2007a, 2007b). However, the saturation of binding does not occur in case of GRFT-glucose and GRFT-*N*-acetylglucosamine (Ziolkowska et al. 2007b), whereas GRFT-mannose binding is fully saturable (Ziolkowska et al. 2007a). GRFT-oligosaccharide interaction studies revealed that it preferentially interacts with terminal sugar and all binding sites have an aspartic acid (Asp) residue which makes extensive contacts with the sugar (Ziolkowska et al. 2007a). Hydrogen bonding interactions occur amongst aspartic acid residue (Asp30, Asp70 and Asp112) and O5 and O6 of mannose (Ziolkowska et al. 2006).

Further, Moulaei et al. (2010) created a monomeric version of GRFT (mGRFT), and mGRFT-Man9 complex studies revealed that two of the three arms of nonamannoside bound to two of the three mannose binding sites in which the terminal mannose units on the D1 and D2 arms are bound to sites 3 and 1, respectively, and further in a transient interaction, third mannose binding site (site 2) is occupied by the D2 arm of another nonamannoside. However, Ziolkowska et al. (2007a) proposed that all three arms of Man9GlcNAc<sub>2</sub> could interact with GRFT with a one-to-one stoichiometry and thus these interaction studies were inconsistent with Moulaei et al. (2010). Xue et al. (2013) constructed a series of mutants wherein two monomers were connected by an amino acid linker and expressed to form an obligate dimer to understand the importance of having three binding sites on each monomer. They compared a mutant (with three of the binding sites in one of the subunits mutated to remove binding to mannose) and another mutant (with all six mannose binding sites mutated) to a construct having all six binding sites, three from each monomer (Xue et al. 2013). Binding studies revealed that the mutant lacking all mannose binding sites did not bind gp120; however, the obligate dimer and construct containing mannose binding sites on only one subunit bound to gp120 with comparable affinities (Xue et al. 2013). The structural and biochemical studies involving mode of action and construction of lectin analog from *Griffithsia* sp. further help in laying emphasis over its therapeutic potential (Lusvardi and Bewley 2016).

The primary structure of lectin from *K. alvarezii* (KAA-2) has been revealed through peptide mapping and complementary DNA (cDNA) cloning which reveals four internal tandem-repeat domains in a molecule (Hirayama et al. 2016). Similar to native KAA lectin, His-tagged rKAA-1

and rKAA-1 specifically bind to high-mannose *N*-glycans with an exposed  $\alpha$ 1-3 mannose in the D2 arm as revealed by oligosaccharide binding analysis by a centrifugal ultrafiltration-HPLC method with 27 pyridylaminated oligosaccharides (Hirayama et al. 2016). Primary structure of two isolectins from *S. filiformis* (SfL-1 and SfL-2) consisting of four tandem-repeat domains with approximately 67 amino acids has been recently determined (Chaves et al. 2017). Both isolectins exhibit 82% sequence identity towards each other and are composed of two  $\beta$  barrel-like domains formed by five anti-parallel  $\beta$ -strands, which are connected by a short peptide linker (Chaves et al. 2017). Molecular docking calculations suggest that amino acid residues W<sub>152</sub>G<sub>153</sub>G<sub>154</sub>, R<sub>236</sub> and E<sub>264</sub>G<sub>265</sub>P<sub>266</sub> are present in SfL-1 and SfL-2 and are involved in carbohydrate-binding (Chaves et al. 2017).

### Biomedical potential of red algal lectins

Carbohydrate specificity of Rhodophyceae lectins is the underlying basis for their varied activities such as anti-viral, anti-cancer and anti-inflammatory and thus has potential for diagnosis and selective treatment of these diseases. The percentile potential data of red algae lectins against various targets is shown in Fig. 1 which signifies maximum reports on its anti-HIV activities. The biomedical applications of red algal lectins are given in Table 6.

#### Anti-human immunodeficiency virus

Human immunodeficiency virus (HIV) has exerted an enormous toll on global human health, and with no vaccine currently available, the focus lies on development of alternate effective measures targeting virus entry point and virus-host interaction stages, to halt HIV infections. Griffithsin (GRFT), a lectin derived from *Griffithsia* sp., has high affinity towards

mannose-rich *N*-linked glycans and has strong anti-viral activity against HIV with inhibitory values in the low picomolar range (Mori et al. 2005; Emau et al. 2007; O’Keefe et al. 2009). Grifonin-1 (GRFN-1), an 18-residue peptide derived from  $\beta$ -sheet core of griffithsin, exhibits an anti-viral activity and low toxicity in vitro, which suggests it a potent topical or systemic agent against HIV (Micevicz et al., 2010). Mechanism of action (MOA) of GRFT involves binding to high-mannose oligosaccharides on gp120 and thus targeting viral entry. GRFT prevents gp120 interaction with HIV co-receptors but does not impede binding of HIV to CD4 (Alexandre et al. 2011). HIV aggregation via multivalent interactions between GRFT and gp120 oligosaccharides occurs owing to dimeric nature of GRFT, with three carbohydrate-binding sites per monomer (Moulaei et al. 2010). GRFT inhibits HIV gp120 binding to DC-SIGN and inhibits DC-SIGN-mediated HIV transfer to CD4+ T lymphocytes along with removal of gp120 from gp120-DC-SIGN complex (Hoorelbeke et al. 2013). GRFT can act as a systemic anti-viral therapeutic agent against enveloped viruses as experimental studies reveals that subcutaneous GRFT administration in rodent species (mouse and guinea pig) accumulates to relevant therapeutic conditions tolerated with minimal toxicity (Barton et al. 2014). Further, in vitro assessment of the toxicological effects of GRFT on isolated mouse peripheral blood mononuclear cells (mPBMCs) and in vivo murine model reveals that GRFT is an outstanding microbicide candidate as no toxicities are reported (Kouokam et al. 2016). Thus, an excellent in vitro and in vivo safety profile for GRFT in the murine model suggests it as a powerful weapon in the fight against human immunodeficiency virus-1 (HIV-1) and other blood-borne enveloped viruses (Kouokam et al. 2016). Thus, GRFT is a potent and broad-spectrum anti-viral agent, which can act as a promising microbicide candidate to prevent HIV acquisition. Recently, Barton et al. (2016) indicated that the pharmacokinetic profile of GRFT depends on the route of

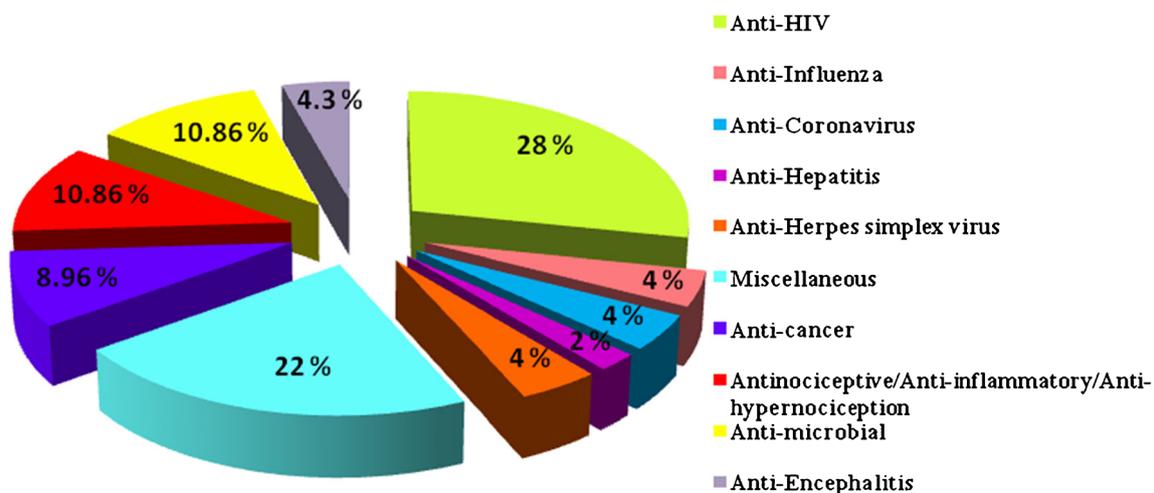


Fig. 1 Percentile data on the biomedical potential of Rhodophyceae lectins against various diseases. Data survey from various internet sources

**Table 6** Biomedical potential of red algae lectins

Red algae	Biomedical application/s	Reference(s)
<i>Acrocystis nana</i>	Anti-cancer	Anam et al. (2017b)
<i>Amansia multifida</i>	Anti-nociceptive	Neves et al. (2007)
<i>Bryothamnion seaforthii</i>	Anti-cancer	Conrado et al. (2012)
	Pro-healing	Nascimento-Neto et al. (2012)
<i>B. triquetrum</i>	Anti-cancer	Nascimento et al. (2015)
<i>Carpopeltis flabellata</i>	Mitogenic	Hori et al. (1987)
<i>Eucheuma serra</i>	Anti-influenza	Sato et al. (2015)
	Anti-cancer	Sugahara et al. (2001), Hayashi et al. (2012)
	Anti-bacterial	Liao et al. (2003)
	Mitogenic	Kawakubo et al. (1997)
<i>Galaxaura marginata</i>	Anti-bacterial	Liao et al. (2003)
<i>Gracilaria bursa-pastoris</i>	Mitogenic	Okamoto et al. (1990)
<i>G. cornea</i>	Acaricidal	Lima et al. (2005)
<i>G. fisheri</i>	Anti-bacterial	Boonsri et al. (2017)
<i>G. tikvahiae</i>	Mitogenic	Bird et al. (1993)
<i>G. verrucosa</i>	Mitogenic	Bird et al. (1993)
<i>Griffithsia</i> sp.	Anti-human immunodeficiency virus	Mori et al. (2005), Emau et al. (2007), O'Keefe et al. (2009), Micevicz et al. (2010), Moulaei et al. (2010), Alexandre et al. (2011), Ferir et al. (2011), Kouokam et al. (2011), Xue et al. (2012), Hoorelbeke et al. (2013), Nixon et al. (2013), Xue et al. (2013), Barton et al. (2014), Moulaei et al. (2015), Barton et al. (2016), Kouokam et al. (2016)
	Anti-herpes simplex virus-2	Nixon et al. (2013), Levendosky et al. (2015)
	Anti-human papillomavirus	Levendosky et al. (2015)
	Anti-hepatitis C virus	Meuleman et al. (2011), Takebe et al. (2013)
	Anti-Japanese encephalitis activity	Ishag et al. (2013), Ishag et al. (2016)
	Anti-Middle East respiratory syndrome coronavirus	Millet et al. (2016)
	Anti-SARS coronavirus	O'Keefe et al. (2010)
<i>Halosaccion glandiforme</i>	Anti-cancer	Perdhana (2017)
<i>Hypnea cervicornis</i>	Anti-nociceptive and anti-inflammatory potential	Nascimento et al. (2006), Bitencourt et al. (2008)
	Anti-hypernociceptive potential	Figueiredo et al. (2010)
	Anti-cancer	Okuyama et al. (2009)
<i>H. musciformis</i>	Anti-fungal	Melo et al. (1997)
<i>Kappaphycus alvarezii</i>	Anti-influenza	Sato et al. (2011), Sato et al. (2015)
	Anti-human immunodeficiency virus	Hirayama et al. (2016)
<i>Pterocladia capillacea</i>	Anti-nociceptive and anti-inflammatory	Silva et al. (2010), Abreu et al. (2012)
<i>Solieria filiformis</i>	Anti-nociceptive and anti-inflammatory	Abreu et al. (2012), Abreu et al. (2016)
	Antibacterial	Holanda et al. (2005)
	Anti-cancer	Chaves et al. (2017)
<i>S. robusta</i>	Mitogenic	Hori et al. (1988a)
<i>Tichocarpus crinitus</i>	Mitogenic	Molchanova et al. (2010)

administration as it is readily bioavailable in rats after intravenous and subcutaneous treatments. They also suggested the multi-use of GRFT for treatment of systemic and enteric

infections thus suggesting its role for anti-viral therapy and prevention of rectal transmission of HIV-1 and other susceptible viruses. Another lectin from red algae *K. alvarezii* (KAA)

and its recombinant form (His-tagged rKAA-1) interacts with gp120 glycoprotein of HIV owing their high-mannose specificity and are able to inhibit its entry into host cell with an  $IC_{50}$  value of 7.3–12.9 nM (Hirayama et al. 2016).

### Anti-hepatitis virus

Belonging to the Hepacivirus genus in the *Flaviviridae* family, hepatitis C virus (HCV) is a small positive single-stranded RNA virus which infects hepatocytes and causes serious liver diseases in humans (Simmonds, 2013). HCV chronically infects around 170 million people worldwide and about one third of them are at risk of developing liver fibrosis, cirrhosis and hepatocellular carcinoma in the coming decades (Dienstag and McHutchison 2006). HCV entry into hepatocytes is a complex multistep process that involves viral envelope glycoproteins along with several other cell entry factors (Dubuisson and Cosset 2014). Two heavily *N*-glycosylated envelope glycoproteins (E1 and E2) are present on the surface of viral particles and these *N*-glycans act as a promising target for new anti-viral strategies (Vieyres et al. 2010). GRFT specifically interacts with *N*-linked high-mannose oligosaccharides that are present on the viral envelope and can prevent HCV infection to hepatocytes in vitro and mitigate HCV infection in vivo (Meuleman et al. 2011). Thus, GRFT treatment has important clinical relevance as it prevents reinfection of the new liver graft which commonly occurs following liver transplantation in HCV-infected patients (Meuleman et al. 2011; Takebe et al. 2013).

### Anti-herpes simplex virus and anti-human papilloma virus

Griffithsin (GRFT) is a promising broad-spectrum microbicide as it blocks herpes simplex virus 2 (HSV-2) infection and also inhibits human papilloma virus (HPV) (Levendosky et al. 2015). Mechanism of action of GRFT against HSV-2 involves entry inhibition (Nixon et al. 2013) along with prevention of cell-to-cell spread (Levendosky et al. 2015). Four glycoproteins (gD, gB, gH and gL) play a role in viral entry and amongst them HSV gD is essential both for viral entry and for cell-to-cell spread and explains mechanism of action of GRFT (Levendosky et al. 2015). GRFT-gD binding occludes the receptor contact site of gD, thus inhibits the critical role that gD plays in HSV-2 entry (Levendosky et al. 2015). Thus, viral entry inhibition in enveloped viruses by GRFT emphasises the role of *N*-glycosylation in viral entry and the coincident susceptibility to anti-viral lectins as GRFT has high affinity for mannose-rich *N*-linked glycans (Levendosky et al. 2015). Further, GRFT also inhibits non-enveloped virus HPV by targeting  $\alpha_6$  integrin (secondary receptor) or HPV-integrin-GRFT complex is internalised without leading to infection thus suggesting involvement of secondary HPV receptor

internalization (Levendosky et al. 2015). Thus, mechanism of action of GRFT against HSV2 is comparable to its action against HIV by interfering with virus entry and not involving attachment (Alexandre et al. 2011).

### Anti-influenza virus

Various viral envelope glycoproteins are glycosylated, which play role in attachment and fusion of influenza viral envelope glycoprotein (haemagglutinin) to host endosomal membranes (Neumann and Kawaoka 2011). These influenza viruses cause annual epidemics or occasional global pandemics such as H1N1 subtypes which emerged in 2009 and spread pandemics all over world (Neumann and Kawaoka 2011). High-mannose binding lectin from *K. alvarezii* exhibits anti-influenza activity (Sato et al. 2011). *Kappaphycus alvarezii* (KAA-2) lectin inhibits influenza strains with  $EC_{50}$  of low nanomolar levels by interfering with virus entry into host cell (Sato et al. 2011). Owing to high-mannose binding specificity of ESA-2 lectin from *E. serra*, it inhibits influenza virus infection with an  $EC_{50}$  value of 12.4 nM with no cytotoxicity up to 1000 nM (Sato et al. 2015). ESA-2 lectin directly binds to HM glycans on envelope glycoprotein and thus inhibits influenza virus propagation (Sato et al. 2015).

### Anti-coronavirus

Griffithsin acts as a potent inhibitor of SARS-CoV infection (in vitro and in vivo) with very low toxicity (O'Keefe et al. 2010). Middle East respiratory syndrome coronavirus (MERS-CoV) epidemic emerged in Arabian Peninsula in 2012 and is still ongoing with 35% of mortality rate (Zaki et al. 2012; Alhagbani 2016). Coronavirus are enveloped viruses having single-stranded, positive sense RNA genome and possess large number of spike proteins on their surface which mediate virus entry (binding and fusion) into host cell (Millet and Whittaker 2014). The MERS-CoV protein is highly glycosylated with 19 predicted glycosylation sites and thus acts as target for carbohydrate-binding proteins (lectins). Spike proteins mediate binding to the host cell surface along with fusion of the viral envelope with host cell membrane and griffithsin inhibits spike protein function during entry thus inhibits MERS-CoV infection at the entry step (Millet et al. 2016). Thus, owing to mannose specificity of griffithsin, it acts as potent anti-coronavirus candidate and inhibits in vitro MERS-CoV infection and production with minimal effect on cell viability (Millet et al. 2016).

### Anti-encephalitis virus

Belonging to the genus of Flavivirus, Japanese encephalitis virus (JEV) is a mosquito-borne virus and a leading cause of high morbidity and mortality rate in Southeast Asia and the

Western Pacific region (Chung et al. 2007). The JEV genome contains three structural genes: capsid protein (C), pre-membrane (prM) and envelope (E) (Sumiyoshi et al. 1987). The E protein contains two potential glycosylation sites (Dutta et al. 2010), whereas prM contains only one glycosylation site (Kim et al. 2008). E protein is involved in virus attachment, fusion, penetration, cell tropism, virulence and attenuation (Lindenbach et al. 2007), whereas prM plays role in virus release and pathogenesis (Kim et al. 2008). Griffithsin (GRFT) is a broad-spectrum anti-viral protein and possesses anti-viral activity against Japanese encephalitis virus (JEV) infection (Ishag et al. 2013). The dimeric structure of GRFT having six binding sites is the underlying cause of its high affinity to interact with the viral glycosylated proteins and prevent its entry into cells (Ziolkowska et al. 2006). Its mechanism of action involves binding of GRFT to the JEV glycosylated viral proteins, specifically E and prM; however, increasing concentrations of mannose inhibit GRFT binding to JEV as revealed by in vitro experiments using Pull-down assay and Co-immunoprecipitation (CO-IP) assay (Ishag et al. 2016). Thus, the binding of GRFT to the glycosylated viral proteins contributes to its anti-JEV activity and has potentials in the development of therapeutics against JEV or other flavivirus infection (Ishag et al. 2016).

#### Anti-nociceptive, anti-inflammatory and anti-hypernociceptive

Lectin from *A. multifida* (LEC) has potential as an analgesic drug as it is a potent central and peripheral natural anti-nociceptive compound (Neves et al. 2007). Oral or intraperitoneal administration of lectin in Swiss mice inhibits acetic acid-induced abdominal writhings in dose-dependant manner (Neves et al. 2007). Anti-nociceptive action of LEC involves D-mannose as a pretreatment with this carbohydrate abolishing lectin activity (Neves et al. 2007). *Pterocladia capillacea* lectin (PcL) exhibits peripheral action with anti-nociceptive and anti-inflammatory activity (Silva et al. 2010). Intravenous administration of lectin (PcL) to Swiss mice significantly reduces writhings in a dose-dependant manner and reduces neutrophil migration in Wistar rats (Silva et al. 2010).

*Solieria filiformis* (SfL) and *P. capillacea* (PcL) lectins have anti-inflammatory effects (Abreu et al. 2012). SfL and PcL lectins are effective in controlling inflammatory processes as they are not cytotoxic, promotes increased cell viability and induces Th2 immune response in mouse splenocytes (Abreu et al. 2012). Lectin from *S. filiformis* can also act as a therapeutic agent owing to its anti-nociceptive and anti-inflammatory potential (Abreu et al. 2016). Anti-nociceptive and anti-inflammatory activity of *S. filiformis* lectin was evaluated in Swiss mice and Wistar rats, respectively, which showed reduced number of abdominal writhings, reduced paw licking

times and reduced neutrophil migration in a peritonitis model along with no signs of systemic damage (Abreu et al. 2016).

*Hypnea cervicornis* agglutinin (HCA), along with anti-nociceptive and anti-inflammatory effects (Nascimento et al. 2006; Bitencourt et al. 2008), also exhibits anti-hypernociceptive effect in rats (Figueiredo et al. 2010). Intravenous administration of HCA to rats inhibits carrageenan and antigen-induced hypernociception, inhibits neutrophil migration and leads to an increase in nitric oxide production (Figueiredo et al. 2010). Further carbohydrate-binding sites play an important role as lectin effect was inhibited, when combined with mucin (Figueiredo et al. 2010).

#### Anti-cancer

Amongst all the malignant tumors, osteosarcoma has worst prognosis with low survival rate of treated patients (Link et al. 1991). Carbohydrate structures vary amongst normal and different cancer cell lines, with carcinomas and sarcomas having common surface bound sugar structures (Stastny and Gupta 1994). Specific interactions between unique sugar chains of high-mannose type on carcinoma cell surface and *E. serra* agglutinin lead to specific binding of agglutinin to carcinoma cells and induce apoptotic cell death (Sugahara et al. 2001). At concentration above  $1.2 \mu\text{g mL}^{-1}$ , ESA induces cell death against human colon cancer (Colo201) and human cervix cancer (HeLa cells) (Sugahara et al. 2001). Binding of *E. serra* agglutinin to sarcoma cells also induces apoptosis with higher anti-proliferative activity of lectin than in carcinoma cells (Hayashi et al. 2012).

Owing to exceptional carbohydrate specificity of red algal lectins, they can distinguish between membrane structures and act as a useful tool in typing of malignant cells (Shiomi et al. 1979; Kamiya et al. 1980; Kamiya et al. 1982; Pinto et al. 2009). Owing to unique carbohydrate specificity of Hypnin A-3 towards core ( $\alpha$ 1-6) fucosylated *N*-glycans, it might act as a potential candidate for cancer diagnosis as core ( $\alpha$ 1-6) fucose is a critical marker for lung and liver cancer (Okuyama et al. 2009).

Lectin from *B. seaforthii* has potential as prognostic marker in primary central nervous system (CNS) tumor (Conrado et al. 2012). Owing to strict specificity of *B. triquetrum* lectin for the core  $\alpha$ 1,6-fucosylation, which is an important marker for cancerogenesis, rBTL is a potent candidate as a marker to detect core-fucosylation of animal cell surface glycoproteins and as cancer diagnosis tool (Nascimento et al. 2015). *Halosaccion glandiforme* lectin inhibits HeLa cervical cancer cell growth and have no cytotoxic effect against MCF-7 as well as HeLa cells (Perdhana 2017). *Solieria filiformis* isolectins have specificity towards high-mannose oligosaccharide present in the MCF-7 thus significantly inhibits 50%

of MCF-7 breast cancer cell viability at a concentration of  $125 \mu\text{g mL}^{-1}$  (Chaves et al. 2017).

### Anti-microbial

Various algal lectins can act as anti-microbial agents and interfere in biofilm formation owing to their carbohydrate specificity (Vasconcelos et al. 2014). *Hypnea musciformis* lectin exhibits anti-fungal activity against the dermatophyte fungus *Trichophyton rubrum* and plant pathogen *Colletotrichum lindemuthianum* (Melo et al. 1997). Purified lectins from *E. serra* and *Galaxaura marginata* has inhibitory activity against *Vibrio vulnificus* (Liao et al. 2003). *Soliera filiformis* lectin has an inhibitory effect over Gram-negative bacterial species such as *Serratia marcescens*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus* sp. and *Pseudomonas aeruginosa* (Holanda et al. 2005). *Soliera filiformis* lectin exhibits bacteriostatic effect owing to its interaction with glycoconjugate mannan or its hapten over surface walls of Gram-negative bacteria (Holanda et al. 2005). However, non-inhibitory effect of *S. filiformis* lectin over Gram-positive bacteria or some Gram-negative bacteria such as *Escherichia coli* and *Salmonella typhimurium* is due to non-accessibility of specific carbohydrates on cell wall of these bacteria or due to the absence of these carbohydrates (Holanda et al. 2005). Perdhana et al. (2017) determined the cytotoxicity and anti-bacterial effect of crude lectin fractions from red macro algae collected from the southern coast of Java Island, Indonesia. Crude protein extract from *G. fisheri* (GPE) has an anti-bacterial activity against *Vibrio parahaemolyticus* (Boonsri et al. 2017). Thus, owing to its anti-bacterial potential, *G. fisheri* could be used as a feed supplement in shrimp culture to protect against or prevent acute hepatopancreatic necrosis disease (Boonsri et al. 2017).

### Miscellaneous applications

Owing to unique carbohydrate specificity of red algae lectins, which identify glycans attached to cell surfaces, glycoconjugates or free sugars, they can detect abnormal cells and biomarkers related to diseases and thus play a significant role in treatments of various immunological diseases, wounds, etc. Selective adsorption of salivary molecules to the teeth leads to formation of an organic and acellular film known as acquired enamel pellicle (Yin et al. 2005). Dental caries involve bacterial adhesion to this acquired pellicle as initial stage of plaque formation (Scheie 1994). Thus, an effective solution towards this problem involves anti-adhesion lectin therapy which interferes with bacterial adhesion and aggregation (Teixeira et al. 2006). Through competitive mechanism, *B. seaforthii* lectin (BSL) blocks oral streptococci adhesion to their mucin receptors in acquired pellicle and presents great stability (Teixeira et al. 2007).

A major problem in public health in many countries is disruption of skin integrity which leads to cutaneous wounds (Schreml et al. 2010; Chen et al. 2012). Highly specialised cells interact with extracellular matrix during complex healing process which leads to growth and tissue repair (Diegelmann and Evans, 2004; Goldberg and Diegelmann, 2010). Owing to potential of lectins for promoting healing, they act as a promising candidate in treatment of skin wounds (Chahud et al. 2009; Nascimento Neto et al. 2011). A lectin from *B. seaforthii* has a pro-healing activity as it accelerates healing of skin wounds by exerting activity on migration of polymorphonuclear cells towards injured site (Nascimento-Neto et al. 2012).

Lectin from *G. cornea* has acaricidal activity as exposure of lectin to female cattle tick (*Boophilus microplus*) significantly reduces cattle tick weight after the oviposition period, egg mass weight, hatching period and mean larvae survival time (Lima et al. 2005).

*Carpopeltis flabellata* (Hori et al. 1987), *S. robusta* (Hori et al. 1988a) and *G. bursa-pastoris* (Okamoto et al. 1990) lectins exhibit mitogenic potential towards mouse splenic lymphocytes from BALB/c mice. *Eucheuma serra* lectins (ESA-1 and ESA-2) stimulate mitogenesis in mouse lymphocytes (Kawakubo et al. 1997). *Gracilaria tikvahiae* (HBOI Strain G5) and *G. verrucosa* (HBOI Strain G-16S) lectins are highly mitogenic for murine splenocytes; however, *G. tikvahiae* (McLachlan HBOI Strain G-3) lectin is mitogenic for human lymphocytes (Bird et al. 1993). *Tichocarpus crinitus* lectin also has a weak mitogenic effect on human lymphocytes in vitro and has the ability to stimulate synthesis of pro-inflammatory cytokines by human whole blood cells (Molchanova et al. 2010).

### Conclusions

Robust preventive measures are required to deal with worldwide spread of enveloped viruses such as HIV, HSV, HCV and SARS-CoV. Owing to carbohydrate specificity of red algae lectins towards glycoproteins present on enveloped viruses and their broad-spectrum activity against susceptible viruses, these lectins are being advocated for further development as anti-viral agents. Lectins from red algae have specificity towards complex glycoproteins or high-mannose glycans with a few lectins having affinity towards monosaccharides. Owing to their sugar specificity, further their agglutination preferences are understood. Thus, based on their carbohydrate specificity, red algae lectins have potential biomedical application like anti-viral, anti-cancer and anti-microbial agents. Owing to anti-nociceptive and anti-inflammatory effects of red algae lectins, they act as promising tools to discover safe and efficient agents for pain therapy. However, owing to lectin stability and toxicity concerns, there has been limited clinical translation of major research findings conducted in lectinology field.

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### Compliance with ethical standards

**Conflict of interest** Authors declare that they have no conflict of interest.

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