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Lamiaceae Lectins

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ABSTRACT

The Lamiaceae family is widely distributed throughout the world and some species have been used in folk medicine, gastronomy and as a source of essential oils and aromas since ancient times; secondary metabolites from many species from Eurasia and North-America have been characterised during the last 5-6 decades. By contrast, species from the neotropical *Calosphace* Benth. subgenus have been little explored, despite their great diversity. Studies on seeds have been limited to mucilage distribution, chromosome patterns and, since the 1970s, to the presence of lectins, mainly in *Salvia* and *Sclarea* Benth. subgenera from the temperate zone. This review initially summarises the available information concerning the presence of lectins in both temperate and neotropic genera, the former being the subject of the pioneering work of Bird's group in the 1970s which revealed the presence of seed lectins able to recognise the Tn antigen (GalNAcα Ser/Thr). Later work has contributed to further our knowledge regarding neotropic genera lectins. The various detection methods used for assessing the presence of lectins in Lamiaceae seeds and vegetative tissues are discussed. The biochemical characteristics of lectins isolated from five genera (*Salvia*, *Lepechinia*, *Moluccella*, *Glechoma* and *Clerodendron*), their structural features and fine specificity are compared. Recent findings concerning their interactions with cell lines and Tn antigen-containing tumour cells are presented. An overview is given about their potential applications and those research lines which would help us in better understanding these lectins.

Keywords: carbohydrate specificity, characterization, *Clerodendron trichotomum*, *Glechoma hederacea*, *Lepechinia bullata*, *Moluccella laevis*, *Salvia bogotensis*, *Salvia sclarea*, Tn

Abbreviations: **aBSM**, asialo bovine submaxillary mucin; **aOSM**, asialo ovine submaxillary mucin; **CELISA**, Cell enzyme-linked immunosorbent assay; **ELLSA**, Enzyme-linked lectinosorbent assay; **ECL**, *Erythrina corallodendron* lectin; **Gleheda**, *Glechoma hederacea* lectin; **GPs**, glycoproteins; **LBL**, *Lepechinia bullata* lectin; **MLL**, *Moluccella laevis* lectin; **RBCs**, red blood cells; **SBoL**, *Salvia bogotensis* lectin; **SPR**, surface plasmon resonance; **SSL**, *Salvia sclarea* lectin; **VVB4**, *Vicia villosa* B4 isolectin

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INTRODUCTION

The Lamiaceae family is widely distributed throughout the world, encompassing some 3,185 species following traditional circumscription. Due to the proposals of Cantino (1992), Cantino *et al*. (1992) and Wagstaff *et al*. (1998), this family has recently incorporated a substantial number of species formerly located in the Verbenaceae family, bringing the total species' number close to 7,170 (Harley *et al*. 2004). Most studies on this family have been centred on analysing secondary metabolites, such as essential oils, terpenoids , flavonoids and, more recently, antioxidants and compounds having pharmacological activity (for a review, see Harley *et al*. 2004).

Reviewing databases from 1970 to the present has revealed an increasing interest in the family, as about 570 articles out of the 2,000 to date have been published during the last three years. Most studied species are from Eurasia and North-America; during the previously-mentioned period only about 27 papers (up to July 30th 2007) dealt with

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Invited Review

species from Africa and Central- and South-America. It is worth noting that despite its great diversity, species from the neotropical *Calosphace* Benth. subgenus have been little explored.

Contrasting with the numerous studies on secondary metabolites from aerial parts, little work has been done on nutlets (seeds), dealing mainly with myxocarpy (mucilage presence) (Ryding 1992a, 1992b, 2001), morphology (Paton 1992) and chromosome number (for references see Harley and Heywood 1992). Knowledge regarding constituents such as proteins, carbohydrates or nucleic acids remains virtually a "no man's land".

Amongst the proteins, lectins represent a group whose definition has evolved from purely factual criteria, such as "…a sugar-binding protein or glycoprotein of non-immune origin which agglutinates cells and/or precipitates glycoconjugates," (Goldstein *et al*. 1980) to structural/functional criteria defining them as being, "…proteins possessing at least one non-catalytic domain, which binds reversibly to a specific mono- or oligosaccharide" (Peumans and van Damme 1995). The feature acknowledged by both definitions is the specific recognition of carbohydrate moieties without modifying them. Lectins have widespread occurrence as they are found in animals, plants, micro-organisms and viruses. Although the earliest report on lectins appeared in 1888, the major impetus for studying them started in the late 1960s, playing a key role in developing glyocobiology. For an overview of the field, the reader is referred to Liener *et al*. (1986), van Damme *et al*. (1998), and Sharon and Lis (2003). Most of the work has been carried out on animal and legume lectins; however, Lamiaceae lectins have been little studied despite their unique features. This review focuses on the presence, characterisation, structural and functional properties and potential uses of lectins from Lamiaceae, isolated both from seeds and vegetative tissues, highlighting these proteins' importance as tools in a variety of biological studies.

LECTIN DETECTION METHODS

Lectins' ability to specifically recognise a carbohydrate moiety forms the basis for several methods leading to detecting their presence; the more common methods (see **Box 1**) include erythroagglutination, polysaccharide precipitation or binding to a ligand linked to a support (solid or gel). Immunochemical methods are a valuable option, but they do require the availability of specific anti-lectin antibodies. In the case of crude Lamiaceae lectin extracts, special attention should be given to the almost always omnipresent pigments (mainly polyphenols) and pectins in seeds and to low lectin content as they may hinder detection or lead to incorrect results. Pigments interfere with most protein assays and reduce protein solubility due to insoluble complex formation; in some cases, they may even haemolyse erythrocytes or irreversibly inactivate the supports used for purification. For their part, pectins markedly increase solution viscosity, thereby reducing extraction efficiency and detection assay sensitivity.

Several methods can be used for eliminating or reducing pigments and pectins; for example thiourea in extraction buffer, treating extracts with DEAE-cellulose and digestion with pectinases have been revealed to be very effective in improving lectin detection whilst keeping them soluble (Fernández-Alonso *et al*. 2003).

Erythrocyte agglutination has been by far the most widely used method for lectin detection; however, most Lamiaceae lectins recognise, among naturally-occurring human red blood cells (RBCs), those cells present in individuals suffering from the "polyagglutinability syndrome" which is very rare; this severe restriction may be overcome by enzymatically treating A-type erythrocytes (Hirohashi *et al*. 1985) or rabbit erythrocytes (Peumans *et al*. 1996), thereby exposing the Tn antigen (GalNAcα Ser/Thr) which is specifically recognised by many Lamiaceae lectins. Erythroagglutination qualitatively assesses lectin presence and, if specific titre is determined, at best a comparative semi-quantitative estimation is achieved. It should be kept in mind that agglutination is affected by factors such as ligand accessibility on cell membrane, the cell's metabolic state and experimental conditions (lectin:cell concentration ratio, temperature, mixing, eventual presence of haemolytic agents, etc.). Very little work has been done on animal RBC erythroagglutination. Interestingly, the presence of a polyagglutinable RBC phenotype in porcine RBCs has been described recently (Swanson and Cooling 2005); it was shown that these erythrocytes were agglutinated by *Salvia sclarea* and *Salvia horminum*, thereby demonstrating the presence of exposed Tn antigen. This finding will likely eliminate the requirement for enzymatic treatment of human or rabbit RBCs, as mentioned above.

Enzyme-linked lectinosorbent assay (ELLSA), a modification of the common ELISA method, has gradually become the standard method for lectin detection as it can be designed for quantitative purposes, allows a substantial number of assays to be run simultaneously and has inherently increased sensitivity compared to erythroagglutination. Applying ELLSA to Lamiaceae lectins requires the binding of a carbohydrate bearing the Tn antigen (usually desialylated mucin) to a polystyrene plate, using biotinylated *Vicia villosa* isolectin B4 as control and streptavidine-peroxidase as detection system (Duk *et al*. 1994).

As opposed to classical legume lectin detection (e.g. Concanavalin A) by polysaccharide precipitation, to our knowledge, Lamiaceae lectins have not been detected by this procedure.

Box 1 Methods for lectin detection

1. **Erythroagglutination.** A 2% PBS suspension of erythrocytes washed three times is employed. To 50 ul of red blood cells (RBCs) per well, in a 96-well plate, 50 ul of sample in PBS or isotonic solution is added, gently shaken and after 2 hours the formation of a layer covering the bottom of the well is observed. Blanks are simultaneously prepared adding 50 ul of PBS instead of sample. If needed, RBCs can be treated enzymatically to enhance agglutination (Pérez 1984; Vega and Pérez 2006).
2. **ELLSA.** The volume of each reagent applied to the plate is 100 ul/well and all incubations are performed at 37°C. The reagents are diluted with phosphate buffer saline (50 mM phosphate buffer; 150 mM NaCl pH 7.4) (PBS) containing 1.3% BSA and PBS containing 0.1% Tween 20 is used for washing the plate (three times) between incubations. The 96 –well microtiter plates (NUNC, F16 Maxisorp) are sensitised overnight with asialo ovine submaxillary mucin (0.14 ug/ml) in 0.1 M carbonate buffer pH 9.6. After washing the plate, samples (crude extracts) are added to the wells and incubated for 1 hour; after washing the plate, biotinylated *Vicia villosa* B4 isolectin (1 mg/ml, diluted 1:2000) is added, incubated for 1 hour and the plate is washed. In control wells the lectin samples are replaced by PBS-BSA. Streptavidine-peroxidase (1.3 ug/ml) is added and after incubation (1 hour) Abs_{410} is read in an ELISA reader. Activity (%) is calculated as (100- (Abs_{410} sample/ Abs_{410} control)* 100) (Duk *et al*. 1994; Vega 1997).
3. **Polysaccharide precipitation.** Lectin samples (5 mg protein) are mixed with various levels of polysaccharide (0-10 mg) in a total volume of 1.5 ml 50 mM phosphate buffer pH 7.0. After 2 hours at room temperature, the insoluble complex is removed by centrifugation and washed twice with 1-ml aliquots of buffer; the insoluble residue is dissolved in 1 ml of 0.1 M NaOH and analyzed for carbohydrate and protein (Olson and Liener 1967).

EARLY WORK ON LECTIN PROSPECTING IN CRUDE EXTRACTS

In the course of Bird's search for new sources of lectins useful for blood serotyping, this investigator (together with Wingham) examined seed extracts from several Lamiaceae species from the *Salvia* and *Sclarea* Benth. subgenera from Eurasia and North-America. The first report concerning the presence of lectins in Lamiaceae dates as far back as 1960 when Bird detected anti-A "agglutinins" (a term used at that time for lectins) in *Hyptis suaveolens* seeds (Bird 1960). Other agglutinins were subsequently found in *Clerodendrum trichotomum* (Verbenaceae) (Bird and Wingham 1968), *Moluccella laevis* (Bird and Wingham 1969, 1970a), *Phlomis fruticosa* (Bird and Wingham 1970b) and *Salvia sclarea* seeds (Bird and Wingham 1973), the latter being identified as specific for the Tn antigen. Although *Phlomis fruticosa* seed lectin was characterised as anti(A+B)-specific by Bird and Wingham (1970b), these authors noticed strong activity against, "group O polyagglutinable cells 'Ba' " which, according to Gunson *et al*. (1970), were characterised as Tn RBCs; this lectin is therefore considered as being anti-Tn.

Further work, mainly on *Salvia* by Bird's group, detected lectins in a significant number of species and assessed the degree of affinity for several antigens. **Table 1** summarises the information obtained between 1960 and 1986; the lectins were detected by erythroagglutination in all cases and the method's limitations should therefore be kept in mind. During this period, *Clerodendrum trichotomum* was the only species from which lectin had been detected (besides seeds) in vegetative tissues such as fruits and leaves.

Bird's pioneering studies drew attention to several aspects which more recent research has had to take account of and, in appropriate cases, devise procedures for solving the problems so identified. Difficulties regarding lectin extraction due to abundant pectins in *Hyptis suaveolens* and several Salvia species (markedly increasing extract viscosity) were early noted and likely led to failure in lectin detection in some species. Later work, as discussed below, illustrates this point. The detection method was based on erythroagglutination and was thereby qualitative; it frequently used erythrocytes obtained from patients suffering from polyagglutinability or related syndromes which implied a very limited availability of RBCs but, on the other hand, led to identifying lectins capable of recognising rare antigens. These findings in particular were very valuable as they provided hints for later work with some species; the observation of erythroagglutination inhibition by *N*-acetyl-α-D-galactosamine (GalNAc) in very early stages of research on Lamiaceae lectins was also relevant (Bird and Wingham 1960, 1970b, 1973, 1974; Moore and Marsh 1975; Bird and Wingham 1982) as it became one of the criteria for the identification of these lectins.

The fact that most examined species belonged to the *Salvia* genus was likely due to early detection of an anti-Tn specific lectin in *Salvia sclarea* which was very useful for characterising the Tn-syndrome. Alternatives to *S. sclarea* as a lectin source were thus systematically searched. As discussed below, a few of those species listed in **Table 1** have now been reinvestigated and their lectins isolated and characterised.

No lectin was detected in some *Salvia* species such as *Salvia microphylla*, S. *officinalis*, *S. patens*, *S. splendens* (Bird and Wingham 1974), *S. aegyptiaca*, *S. apiana*, *S. carduacea*, *S. coccinea*, *S. columbaria*, *S. hispanica*, *S. mellifera* (Bird and Wingham 1976) and *Marrubium vulgare*

Table 1 Early work (1960-1986) on Lamiaceae species, showing seed lectins directed against a given antigen.

Species	Main antigen	Other antigens [1]	Reference
Hyptis suaveolens	A_1	--	Bird 1960
Moluccella laevis	A+N	---	Bird and Wingham 1969, 1970a
Phlomis fruticosa	Tn	(A+B)	Bird and Wingham 1970b
Salvia sclarea	Tn	---	Bird and Wingham 1973
Salvia farinacea	Tn	Cad	Bird and Wingham 1974
Salvia haematodes	Tn	T	Bird and Wingham 1974
Salvia horminum	Tn, Cad	--	Bird and Wingham 1974
S. sclarea var. *turkestanica*	Tn	--	Bird and Wingham 1974
Salvia argentea	Tn	--	Bird and Wingham 1974
Salvia pratensis	Tn	--	Bird and Wingham 1974
Salvia horminum	Sd(a^{++})	--	Moore and Marsh 1975
Salvia aethiopsis	Tn	T	Bird and Wingham 1976
Salvia nemorosa	Tn		Bird and Wingham 1976
Salvia sclareoides	Tn	T	Bird and Wingham 1976
Salvia taraxacifolia	Tn	T	Bird and Wingham 1976
Salvia verbenaca	Tn	T	Bird and Wingham 1976
Salvia glutinosa	T	--	Bird and Wingham 1976
Salvia aurea	Tn	--	Bird and Wingham 1977
Salvia candelabrum	Tn	--	Bird and Wingham 1977
Salvia forskohlei	Tn	--	Bird and Wingham 1977
Salvia grandiflora	Tn	--	Bird and Wingham 1977
Salvia japonica	Tn, T	Cad	Bird and Wingham 1977
Salvia nilotica	Tn	T, Cad	Bird and Wingham 1977
Salvia sylvestris var *superba*	Tn	--	Bird and Wingham 1977
Salvia transylvanica	Tn (1)	--	Bird and Wingham 1977
Salvia verticillata	Tn	T, Cad	Bird and Wingham 1977
Leonorus cardiaca	Cad		Bird and Wingham 1979
Marrubium candidissimum	Tn		Bird and Wingham 1981
Marrubium supinum	Tn (1)	---	Bird and Wingham 1981
Hyptis sp. *Chan*	Tn, A	--	Bird and Wingham 1982
Salvia lyrata	Tn	--	Bird and Wingham 1982
Marrubium velutinum	Tn	--	Bird and Wingham 1982
Clerodendrum trichotomum	N.D.	---	Bird and Wingham1968
Clerodendrum trichotomum	N.D.		Kitagaki *et al*. 1985[2], 1986[3]

[1] Weak activity
[2] Lectin isolated from fruits
[3] Lectin isolated from fruits and leaves

(Bird and Wingham 1981). However, no further comments were made by these investigators concerning the reasons for their absence. It still remains unclear whether there is a gene encoding the protein for some of them, whether the level of expression is too low for the detection methods used or whether some seed constituents hamper detection.

RECENT WORK ON LECTIN PROSPECTING

A search of the literature has shown that no additional Lamiaceae lectins have been detected in endemic Old World species during the last 20 years, with one exception (*Glechoma hederacea*); a recent systematic survey has been conducted on species belonging to the neotropical *Calosphace* Benth. subgenus (Fernández–Alonso *et al*. 2003; Pérez *et al*. 2006). A certain number of species naturalised in the New World have also been investigated (Vega *et al*. unpublished results), some having commercial value. As a significant number of species have been examined (*c*. 90) **Table 2** only gives results where data obtained by ELLSA are presented (% lectin activity). **Appendices 1** and **2** list the studied species with respective pectin, protein content and lectin activity data.

Erythroagglutination of enzymatically-modified RBCs was also carried out in many cases, corroborating ELLSA results. It is noteworthy that anti-Tn lectin is widely distributed in Lamiaceae as in 21 not previously examined genera the protein has consistently been found by Fernández–Alonso *et al*. (2003), Pérez *et al*. (2006) and Vega *et al*. (unpublished results) to have high activity towards asialo bovine submaxillary mucin (aBSM) employed in the detection system. It is likely that lectins will be found in related species in those cases where just one species has been tested. It is worth noting that *Stachys pusilla* and one species of the *Leonotis* genera seem to be devoid of lectin activity amongst the genera tested so far.

ISOLATED AND CHARACTERISED LECTINS

Several factors have to be considered when choosing a given species with the aim of isolating and characterising a detected lectin. Some species have shown restricted geographical distribution and the starting material (seeds) is frequently scarce and, since no phenological studies have been carried out, such factors affecting availability have severely limited further work on lectin characterisation. One solution, which has proved to be effective in our hands, has been to cultivate those species which appear to be promising as a lectin source or are menaced by extinction; again, virtually nothing is known about seed viability or the factors involved in plant growth. Genera such as *Lavandula*, *Mentha*, *Origanum* or *Thymus*, which include species of economic interest, have shown good levels of lectin activity and therefore present themselves as candidates for carrying out detailed studies on seed lectin molecular and physiological properties.

Table 2 Recent work on Lamiaceae genera in which seed lectins are present.

Genus	Species analysed	Lectin activity (%) [1,2]
Aegiphyla	2	32.6-61.5
Agastache	1	37.4
Ballota	1	75.4
Hyptis	11	58.3-96.0
Hyssopus	1	76.2
Lavandula	1	70.5
Leonorus	1	90.3
Lepechinia	6	42.5-88.6
Melissa	1	67.6
Mentha	1	77.9
Minthostachys	1	45.8
Ocimum	3	45.0-64.1
Origanum	2	52.0-74.7
Rosmarinus	1	82.3
Salvia	41	39.5-99.0
Satureja	1	70.3
Scutellaria	4	63.1-72.4
Sideritis	1	72.8
Solenestemum	1	70.8
Stachys	5	68.1-90.2
Thymus	1	59.9

[1] Range of most frequent values determined by ELLSA
[2] % activity calculated as follows:
A = Abs $_{410}$ control VVB4; B= Abs $_{410}$ sample; 100- ((B/A)*100) = % activity

The presence of mucilage (myxocarpy) is very common in *Salvia* and *Ocimum* genera seeds and in species such as *Melissa officinalis* and *Rosmarinus officinalis*; the crude extracts are consequently very viscous, thereby hampering lectin detection. This can be resolved by digesting the extracts with Pectinex® (a mixture of polygalacturonases); as a result, the extract can be easily handled and lectin assay sensitivity becomes consistently and markedly increased (Fernández–Alonso *et al*. 2003; Pérez *et al*. 2006; Vega *et al*. unpublished). Species such as *Salvia officinalis*, *Salvia hispanica* and *Salvia coccinea*, which had previously been reported to be devoid of lectin (Bird and Wingham 1974, 1976), have shown activity levels as high as 80% by ELLSA, after Pectinex digestion; it is therefore advisable to reduce extract pectin content before testing for lectin presence. We have remarked that, in some cases, high viscosity can be misleading when erythroagglutination assays are performed. Data regarding myxocarpy in Lamiaceae can be found in Hedge (1970), Grubert (1974), Ryding (1992a, 1992b, 2001), Oran (1997), Fernández-Alonso *et al*. (2003) and Pérez *et al*. (2006).

Only a small number of Lamiaceae lectins have been isolated and (to a variable extent) characterised (**Table 3**) perhaps because a researcher is faced with more complex starting material, as compared to Leguminosae lectins, thereby encountering the difficulties mentioned above. A synthesis of the aspects related to isolation will first be presented in this section, followed by the available data concerning the proteins' biochemical characterisation.

The starting material for most of the listed species consisted of seeds, with the exception of *G. hederacea* and *C. trichotomum*; the lectin was detected in and isolated from leaves in the former and, in the latter, although the lectin was initially detected in seed extracts (Bird and Wingham 1968), the protein was isolated and characterised from fruits and leaves (Kitagaki *et al*. 1985; Kitagaki-Ogawa *et al*. 1986). There is consequently no experimental evidence supporing identity or even similarity between seed and vegetative tissue lectins from the same species; this point is relevant not only for fundamental considerations but also for practical purposes concerning which starting material is appropriate as a lectin source. The purification procedures described to date vary considerably, having an affinity chromatography step in common for which many types of support have been used. With a few exceptions, little attention has been given to removing pigments which are commonly present in high quantities or to the extracts' considerable viscosity (frequently observed), due to pectin-like polysaccharides. Both factors can, in our experience, markedly influence the success of a given isolation procedure (as discussed above) and should thus be taken into account. Affinity chromatography supports are generally prepared taking advantage of Lamiaceae lectin recognition of Gal or GalNAc and, in several cases, just include these sugar moieties as sole ligand; however, as will be discussed below, lectin's affinity constants for these monosaccharides are lower than those shown for oligosaccharides or complex sugars having *O*-glycosidic bonds.

Most studies have used haemoagglutination as the detection method for assaying type O, enzyme-treated or in one instance, *S. sclarea* lectin (SSL), Tn erythrocytes; this again hampers comparing relative lectin strength amongst different isolates. As the ELLSA method uses easily-prepared desialylated mucin, with the technique's inherent ad-

Table 3 Lamiaceae lectins isolated to date.

Species	Abbreviation	Reference
Clerodendrum trichotomum	CTL	Kitagaki *et al*. 1985; Kitagaki-Ogawa *et al*. 1986
Glechoma hederacea	Gleheda	Wang *et al*. 2003a
Lepechinia bullata	LBL	Pérez G, Sanabria E, Rojas V, Quintero S,Vega N unpublished results
Moluccella laevis	MLL	Lis *et al*. 1988; Alperin *et al*. 1992
Salvia bogotensis	SBoL	Vega and Pérez 2006
Salvia sclarea	SSL	Piller *et al*. 1986; Medeiros *et al*. 2000

Table 4 Molecular properties of *Salvia bogotensis*, *Salvia sclarea*, *Lepechinia bullata* and *Moluccella laevis* seed lectins.

	S. bogotensis[1]	***S. sclarea***[2, 3]	***L. bullata***[4]	***M. laevis***[5]
M_r subunits (kDa)	38.8; 35.7[6]	26-28[2]; 32[3]	26	26
M_r protein (kDa)	38.702 Da	59[2]; 60-61[3]	65.4[7]; 58[8]	67; 130
Bands in SDS-PAGE (kDa)	72.6; 38.8; 35.7[6]	50; 35[2] 72; 32[3]	65.4[7]; 39[6,7]; 26[7]	46[7]; 26[7]; 67[8], 42[8], 26[8]
Neutral sugars (%)	16	15[2]	13.4	10[9]
pI	8.6-8.8	8.8, 8.0[2,5]; 5.5[3]	8.5	N.D.
GalNAc inhibition	37.5 mM	0.1 mM[2]	33.6	0.017 mM[9]
aOSM inhibition	0.2 ug	0.02 mM[2]	N.D.	0.003 ug/ml
Interchain S-S	No	Yes	No	Yes

[1] Vega and Pérez 2006; [2] Piller *et al*. 1986; [3] Medeiros *et al*. 2000; [4] Pérez, Sanabria, Rojas, Quintero, Vega (unpublished results); [5] Alperin *et al*. 1992; [6] Minor band; [7] Reduced protein; [8] Non-reduced protein; [9] Lis and Sharon 1994.

vantages, it is more suitable for comparing results from different laboratories.

To round off these initial considerations, we would like to draw attention to purification-fold and lectin yield (mg/100 g starting material) estimates; values range from 90 to 2800-fold for the former (Piller *et al*. 1986; Vega and Pérez 2006) and from 1 to 40 mg/100 g starting material for the latter (Kitagaki-Ogawa *et al*. 1986; Wang *et al*. 2003a).

Seed lectins

Comparing the molecular properties of the lectins isolated from nutlets (seeds) has shown that they share some common features (**Table 4**):

- They appear as dimers/tetramers close to neutral pH, but studies regarding the factors leading to associated forms have yet to been carried out. It is worth noting that *Moluccella laevis* lectin (MLL) appears to possess a unique structural arrangement of three 67, 42 and 26 kDa subunits present in the absence of reducing agents, of which only the 26 kDa subunit was active after affinity chromatography in the presence of 8 M urea (Alperin *et al*. 1992). The role of 67 and 42 kDa subunits remains to be established;
- They show a relatively high carbohydrate content (10-16%). N-linked oligosaccharide (2-3 glycan/polypeptide chain) structure has been elucidated for SSL (Medeiros *et al*. 2000) and corresponds to the Manα6(Manα3)(Xylβ2) Manβ4GlcNAcβ4(Fucα3)GlcNAc heptasaccharide, characteristic for plant *N*-glycans. Preliminary evidence has revealed a similar structure for the MLL oligosaccharide (Lis and Sharon 1994) and it has been proposed that an analogous structure is present in *Salvia bogotensis* lectin (SBoL) (Vega and Pérez 2006) having 3-4 oligosaccharide units per monomer. Two glycoforms are found in MLL, SSL and SBoL, one of them being the predominant species;
- All are basic proteins, as shown by pI values;
- GalNAc is the most potent inhibitor amongst monosaccharides, although a wide range of sugar concentrations is needed to inhibit erythroagglutination; and
- They show high affinity for asialomucins, such as asialo ovine submaxillary mucin (aOSM) or asialo bovine submaxillary mucin (aBSM), which are the best erythroagglutination inhibitors and have thus been used efficiently as ligands in affinity chromatography.

Only MLL from the lectins shown in **Table 4** has been able to recognise both A+N blood types and the 26 kDa subunit agglutinated A^{MM} erythrocytes (10-20 units/mg specific activity) and O^{NN} erythrocytes (40-80 units/mg specific activity) (Alperin *et al*. 1992). *Salvia* and *Lepechinia* lectins only agglutinate Tn erythrocytes; minimum required lectin concentration has been 0.17 ug/ml for SBoL (Vega and Pérez 2006) and 25 ug/ml for *Lepechinia bullata* lectin (LBL) (Pérez *et al*. unpublished results). When these lectins have been tested against rabbit, cow, horse or dog RBCs they were unable to agglutinate them (Vega and Pérez 2006; Pérez *et al*. unpublished results).

Legume lectin activity generally depends on the presence or addition of Ca^{+2} and Mn^{+2} ions as they contribute towards the carbohydrate binding site. In the case of Lamiaceae lectins, the effect of cations has been studied with SSL, SBoL and LBL for which adding Ca^{+2}, Mg^{+2} or Mn^{+2}, in various concentrations, did not alter the haemoagglutinating titre. Removing cations by EDTA did not reveal any metal requirement for the former lectin, whereas activity became markedly reduced for the last two but not abolished and recovery of activity was minimal after re-equilibration with 0.1 M $CaCl_2$ or 0.1 M $MnCl_2$ (Vega and Pérez 2006; Pérez *et al*. unpublished results). The lectin's activity therefore depended upon Ca^{+2} and Mn^{+2} being bound to native protein; current demetallisation procedures (EDTA or EGTA addition, dialysis versus 1 M AcOH) are probably unable to completely remove the bound cations.

SBoL thermal stability is remarkable as it retains 50% of its activity at 56ºC, this being similar to *Glechoma hederacea* lectin (Gleheda) (Wang *et al*. 2003a); on the contrary, SSL appears to be very labile as it is almost inactive at 37ºC (Piller *et al*. 1986). Unfortunately, no data are available for MLL or LBL in this respect. Stability under strong dissociating conditions has been well documented for MLL which retains its anti-A+N activity in 8 M urea at room temperature (Alperin *et al*. 1992). Erythroagglutinating activity has generally been seen to be better at pH values close to 8.0-8.5 for SSL and SBoL, which (as well as Gleheda) are stable in a 3 to 12 pH range.

Lectins from vegetative tissues

The molecular properties of lectins isolated from vegetative parts of *Clerodendrum trichotomum* (fruits, leaves) and *Glechoma hederacea* (leaves) are shown in **Table 5**.

It has been found (Wang *et al*. 2003b) that *G. hederacea* lectin content varies widely amongst different clones, showing values from <0.5 ug/g leaf (virtually no lectin) to 2,700 ug/g leaf; except from calyces, no lectin was detected in other vegetative tissue. As nutlets (seeds) were not assayed for lectin presence, it remains to be established whether Gleheda is restricted to leaves, which would make it an exception regarding the rest of the Lamiaceae species (about 130) hitherto examined.

The three lectins are dimers linked by disulphide bridges but differing in their association in solution, as Gleheda is predominantly tetrameric and *C. trichotomum* lectins are

Table 5 Molecular properties of *Clerodendrum trichotomum* and *Glechoma hederacea* lectins.

	C. trichotomum[1, 2]	*G. hederacea*[3]
M_r subunits (kDa)	28[1]	26[4]; 28[4]; 25, 723[5]
M_r protein (kDa)	56[1]	80-93
Bands in SDS-PAGE (kDa)	56[1,6]; 28[1,4] 26[2,4]; 28[2,4]; 30[2,4]	60[6]; 26[4]; 28[4]
Neutral sugars (%)	16[1]	10
Hexosamines (%)	4[1]	N.D.
pI	N.D.	6.11
GalNAc inhibition (mM)	0.1[2,7]; 0.53[2,8]	25
aOSM inhibition	N.D.	Yes
Interchain S-S	Yes	Yes

[1] Kitagaki *et al*. 1985; [2] Kitagaki-Ogawa *et al*. 1986; [3] Wang *et al*. 2003a; [4] Reduced protein; [5] Calculated from sequence data (Wang *et al*. 2003a); [6] Non-reduced protein; [7] Leaf lectin; [8] Fruit lectin.

dimeric as revealed by ultracentifugal analysis. They differ widely in some respects (expression level, carbohydrate content, GalNAc affinity) as well as in lectin content despite their common source (leaves) as *C. trichotomum* lectin level is more constant than that of Gleheda (30 mg/100 g leaves *cf* 1-50 mg/100 g leaves, respectively). The structure of the oligosaccharide bound to fruit and leaf lectins was determined by Kitagaki-Osawa *et al*. (1986), this being identical to that determined in SSL by Medeiros *et al*. (2000). Taking their data into account, Wang *et al*. (2003a) have assumed that the size of the N-glycan present in Gleheda is similar to that found in SSL and have concluded that each Gleheda subunit contains about 1.5 *N*-glycans. It thus appears that, taking the data as a whole, *N*-glycan structure is the same in all Lamiaceae lectins; however, different proportions are present not only amongst the lectins but also in a given lectin, due to the presence of glycoforms.

STRUCTURAL ASPECTS

Table 6 gives a comparison of amino acid composition of hitherto analysed lectins. Each lectin's amino acid composition has been calculated as residues per polypeptide chain to facilitate comparison, taking carbohydrate content and the most reliable data on molecular weight (preferably MS or ultracentrifugation data) into consideration. Surprisingly, SBoL overall amino acid composition is more similar to that of *C. trichotomum* leaf lectin than that of SSL, with the exception of Glx, Gly, Lys and Trp content which are considerably higher than in leaf lectin. About 40% of the amino acids for Gleheda (whose composition was calculated from the cDNA sequence) have values far from those shown by any other Lamiaceae lectin. The reduced number of Lamiaceae lectins analysed thus far hinders further interpretation of the dissimilar data regarding amino acid composition.

As the amount of ½ Cys was only determined in one instance (*S. bogotensis* lectin), after performic oxidation and free CySH were analysed, there is no way of comparing this data with the interchain linkage proposed as a molecular feature of some Lamiaceae lectins.

It is worth noting that ornithine was present in *C. trichotomum* leaf lectin (5.1 res/mol) and mature fruit lectin (2.1 res/mol); Kitagaki-Ogawa *et al*. (1986) attributed this to post-transcriptional processing of Arg by an arginase-like enzyme during fruit ripening, as only trace amounts were present in immature fruits.

The complete polypeptide sequence (deduced from a cDNA clone) is only available for *G. hederacea* lectin and, according to Wang *et al*. (2003a), shares 30% identity and about 50% similarity with legume lectins. Gleheda 26 and 28 kDa polypeptide N-terminal sequences were identical (KTTHFAVPPADAFDPNDTSFIRLT). Attempts to establish N-terminal sequences by standard methods have been unsuccessful for SSL and SBoL, because their N-terminus' are blocked (Osinaga, pers. comm.; Vega and Pérez 2006).

Gleheda sequence data alignment has indicated that both the residues delineating the monosaccharide binding site and those binding the Ca^{2+} ions in *Erythrina corallodendron* (a legume lectin) are highly conserved in Gleheda; this added to the already-mentioned high identity/similarity led Wang *et al*. (2003a) to propose a model for the tertiary structure of the *G. hederacea* lectin which is very similar to the well-established 3D one for legume lectins. However, generalising this model to Lamiaceae lectins requires structural data (both at primary and tertiary levels) obtained with seed lectins. It has to be kept in mind that it has been shown in legumes that seed lectins very often do not have their counterparts in vegetative tissue lectins.

FINE CARBOHYDRATE SPECIFICITY AND COMBINING SITES

The available information regarding lectins' fine specificity and the type, or approximate morphology (broad structure), of their carbohydrate binding site is discussed in some detail in this section to enable the reader to appreciate the

Table 6 Amino acid composition of Lamiaceae lectins. Data expressed as calculated residues/polypeptide chain.

Amino acid	*Salvia bogotensis*[1]	*S. sclarea*[2]	*C. trichotomum*[3]		*G. hederacea*[4]
			leaf	fruit	
Asx	15.3	27.6	16.5	18.5	31
Thr	12.8	31.2	16.4	21.5	19
Ser	59.8	34.4	42.2	35.8	20
Glx	41.7	14.7	19.8	15.6	10
Gly	91.5	59.5	44.8	34.1	20
Ala	36.3	35.7	29.3	27.1	16
Val	9.7	20.6	15.6	15.8	20
Met	0[5]	0.6	N.D	N.D.	4
Cys	3.9[5]	0.6	N.D.	N.D.	3
Ile	6.4	9.6	9.5	10.9	16
Leu	9.9	12.7	12.7	13.5	15
Tyr	4.6	5.9	2.6	2.2	4
Phe	4.0	17.2	8.8	9.4	12
His	3.7	4.4	1.8	2.5	9
Lys	21.0	3.7	5.9	5.2	14
Arg	2.7	6.3	1.6	3.7	6
Pro	12.7	8.3	10.6	10.6	11
Trp	8.9[6]	N.D.	1.3[6]	1.0 [6]	5

[1] Vega and Pérez 2006. Calculations are based on Mr = 38,702 with 16% carbohydrate.
[2] Calculated from Medeiros *et al*. 2000. Mr =30,000 with 5.9% carbohydrate
[3] Calculated from Kitagaki *et al*. 1985. Mr = 28,000 with 20% carbohydrate.
[4] Calculated from sequence data (Wang *et al*. 2003a). Mr = 25,723.
[5] Determined as $MetSO_2$ and $CySO_3$.
[6] Spectrophotometrically determined.

extent of this type of proteins' similar and dissimilar features.

Moluccella laevis lectin

Duk *et al*. (1992) studied *Moluccella laevis* lectin interaction with a variety of compounds, including blood group substances, oligosaccharides and glycoproteins (GPs), to clarify its demonstrated unusual anti-A+N specificity (Bird and Wingham 1970). Agglutination assays of untreated and enzymatically-modified A^{MM} and O^{NN} erythrocytes confirmed the dual blood type specificity and different localisation of A and N blood-type determinants. Further work by Krotkiewski *et al*. (1997), with MLL, demonstrated that higher expression of non-galactosylated GalNAc in glycophorin A-N than in Glycophorin A-M is restricted to the GalNAc residues located on amino acid residues 2-4 of the protein.

One can speculate that the uniquely dual specificity shown by MLL is somehow related to the unusual structure conferred by the association of its three subunits. ELLSA assays (formerly called ELISA) with glycophorin A, human blood group substances (N, M, A_1, A_2, Le^a, Le^b) and various blood group substances from horse, bovine or pig gastric mucosa indicated that MLL recognised an internal sugar structure, absent in glycophorin, suggesting the presence of an O-linked oligosaccharide. The haemagglutinating inhibitory activity of a wide range of Gal derivatives (about 30) and Gal-containing di- and tri-saccharides revealed that the best was methyl 2-(4-azidosalicylamino)-2-deoxy-α-D-galactopyranoside (α-GalpN(4Azsal)OMe), this being 15,000 times more potent than Gal and 30 times more potent than GalNAc. This suggested a strong hydrophobic interaction involving the OH-2. ELISA assays confirmed the inhibition results obtained by erythroagglutination and stressed the relevance of hydrophobic forces in the lectin-carbohydrate interaction.

The asialoagalacto derivatives from human or horse origin were the strongest inhibitors amongst the native and enzymatically-treated glycophorins tested (GPA^M, GPA^N) (ELISA assay). On the other hand, aOSM was the most potent inhibitor (0.003 ug/ml compared to 0.025 ug/ml asialoagalacto glycophorin A). The set of results with GPs derivatives led the authors to define MLL specificity as being, "anti-Tn, cross-reactive with blood types A and N, and with sialosyl-Tn" (Duk *et al*. 1992).

Teneberg *et al*. (1994) used thin layer chromatography and ELLSA binding assays to establish the specificity of lectin binding to 38 glycosphingolipids. The highest activity was found for both linear glycosphingolipids having α-terminal linked GalNAc (the Forssman antigen (GalNAcα1-3 GalNAcβ1-3Galα1-4Galβ1-4GlcβCer) being one of the more strongly bound) and for branched structures such as the A blood group determinant (GalNAcα3(Fucα2)Galβ). By contrast, glycosphingolipids having β-terminal linked GalNAc or Gal were completely inactive.

The set of results regarding MLL carbohydrate specificity and combining sites demonstrate that, besides its unusual "dual" anti-A, anti-N specificity, the protein is able to strongly recognise the Tn antigen, an ensemble of properties distinguishing MLL from the rest of the Lamiaceae lectins.

Salvia sclarea lectin

As this was the first Lamiaceae lectin to be isolated, several studies have been carried out concerning its specificity. The first attempt to characterise the lectin's carbohydrate binding properties (Piller *et al*. 1986) used several GalNAc-containing oligosaccharides, a few (5) glycopeptides and BSM and asialoBSM. The results obtained by haemoagglutination inhibition assays revealed the protein's ability to recognise the Tn antigen. Piller *et al*. (1990) then used seven GalNAc-containing oligosaccharides to compare ^{125}I-labelled SSL binding properties to those of several lectins known to be GalNAc-specific. SSL was revealed to be the most specific lectin for the Tn structure; this was confirmed by inhibition of glycoprotein and glycopeptide binding, aBSM being the most potent inhibitor. Amongst the tested oligosaccharides, the Forssman disaccharide (GalNAcα1-3GalNAc) strongly interacted with the lectin. Medeiros *et al*. (2000) assayed three synthetic glycopeptides (containing one to three Tn groups) and concluded that SSL binding was independent of Tn density.

Wu (2005) used the ELLSA technique and a wide range of GalNAc-containing oligosaccharides and mammalian glycoproteins to undertake a systematic study of the protein's glyco-recognition factors. His main findings can be summarised as follows:

- By the use of a panel of glycoproteins, including mammalian salivary mucins of armadillo, ovine, bovine, porcine and hamster, both native and desialylated, as well as blood group A, B, H, Le^a, Le^b antigens, it was found that SSL only recognises exposed high density Tn-glycoproteins (native armadillo GPs, aOSM). The lectin was unable to bind to sialyl Tn glycoproteins or forms masked by other constituents. It is worth noting that such high Tn antigen specificity is also shared by the *S. bogotensis* lectin which only binds to exposed Tn GPs (Vega and Pérez 2006);
- The cluster effect on binding was examined by inhibiting the SSL-aOSM interaction with a set of 30 glycoproteins/glycan encompassing Tn, T and blood group determinants. The binding was strongly inhibited by high-density polyvalent Tn GPs, the best ihibitor being aOSM as it was 36 times more potent that GalNAc. The results led to considering the carbon-3 configuration in GalNAc and N-acetamido at carbon-2 of Gal as being crucial for SSL binding to GPs; and
- The inhibitory effect of monosaccharides, their derivatives and oligosaccharides (forty compounds) was established; amongst the most potent were the Forsmann pentasaccharide (GalNAcα1-3 GalNAcβ1-3Galα1-4Galβ1-4Glc) and the GalNAcα1-3Galβ1-4Glc trisaccharide (indicating that GalNAcα1 at the non-reducing end is required for binding) and pNO2-phenyl-β-GalNAc, suggesting that hydrophobic interactions contribute to binding.

SSL binding to the Forsmann antigen led Wu (2005) to suggest that the combining site in SSL is a shallow groove capable of accommodating an extended structure where the exposed GalNAc is likely taking part in a hydrogen bond network provided by the amino acid residues.

SSL ability to bind the Forssman antigen, which is found in colon, gastric and lung cancers, may be advantageously used as a potential tool for diagnosing colon cancer (Wu 2005).

Glechoma hederacea lectin

The initial data on the carbohydrate specificity of the lectin were obtained by Wang *et al*. (2003a) who found that only Gal derivatives inhibited rabbit trypsin-treated erythrocyte agglutination; they subsequently used surface plasmon resonance (SPR) to show that several plant and animal GPs having terminal Gal/GalNAc and O-linkages (i.e. asialomucin, asialofetuin) strongly interacted with the lectin. Detailed studies with SPR, using mono and oligosaccharides, showed that GalNAc, Me-α-D-Gal and *p*-nitrophenyl-α-D-Gal were the best inhibitors of the asialofetuin-Gleheda interaction. A molecular model of Gleheda was constructed based on the similarity of the lectin's deduced primary structure to that of *Erythrina corallodendron* lectin (ECL); docking experiments revealed (Wang *et al*. 2003a) that Gal interacted with Asp^{73}, Gly^{93}, His^{115}, AsN^{117}, Thr^{2O1} and AsN^{202}, amino acid residues analogous to those present in the ECL carbohydrate binding site (Elgavish and Shaanan 1998).

The recent work of Singh *et al*. (2006) has shed light on Gleheda's fine specificity by examining its interaction with a wide array of glycans (human blood group GPs, sialo and asialo GPs, OSM-derived glycopeptides (≤3000 Da), Gal-

containing mono and oligosaccharides). The main findings revealed that:

i) the dominant factor for strong binding is a high-density exposed Tn ensemble;

ii) Tn and human blood group precursor GPs cryptoforms having Gal β1-3/4 GlcNAc as major glycotopes and some Tn and T glycotopes are also, albeit less strongly, bound;

iii) Gal configuration is essential for binding and the presence of the *N*-acetamido group at C-2 of Gal strongly enhances the interaction; and

iv) the lectin combining site is of a cavity type where hydrogen bond-mediated interactions (involving Asp^{73}, Gly^{93}, AsN^{117}, Thr^{201}, AsN^{202}) are strengthened by a hydrophobic interaction with the imidazole ring of His^{115}.

It is worth noting that blood group A trisaccharide (GalNAcα1-3(L-Fucα1-2)Gal) and Forssman disaccharide (GalNAcα1-3GalNAc) were weakly bound, revealing that replacing the free hydroxy group at C-2 of the penultimate Gal group with an acetamido group reduced lectin binding.

Comparing the available data concerning the three lectins' fine specificity has shown that they all bind with high affinity to multivalent Tn structures, such as those present in asialo mucins and in proteins having several *O*-glycosidic links. They also share features such as hydrophobic interactions playing a role in carbohydrate binding, *N*-acetamido group as an interaction enhancer and the importance of the Gal configuration for sugar recognition.

On the other hand, a close analysis of the data provides evidence that the carbohydrate-combining site of each lectin has its own characteristics, especially those regarding size and conformation; it is likely that more accurate comparisons will be possible as more structural data (primary and tertiary structures) becomes available. It is intriguing that, given the sharp differences observed in the six Lamiaceae lectins hitherto analysed regarding subunit structure, size and covalent (and non-covalent) linkage between subunits, all of them display a strong affinity for the Tn determinant, especially when it is present in a high-density arrangement.

INTERACTIONS WITH CELLS

The available data regarding *in vitro* or *in vivo* lectin effects is very scarce, perhaps due to the fact that only recently have pure and well-characterised lectins been at researchers' disposal. Effects concerning cytotoxicity, mitogenic and insecticidal action are summarised below, pointing out the relevance of the findings.

Cell binding and cytotoxic activity

Most Lamiaceae constituents' biological activities which have been described to date refer to essential oils or diterpenes which are non-water soluble and very often present in crude extracts. An interested reader can find a review of the type of activities and type of compounds responsible for them in Badisa *et al*. (2004). As far as lectins are concerned, evidence has only recently begun to appear revealing interesting features regarding their biological activities.

The Tn antigen is also specifically recognised by the *Vicia villosa* (Leguminosae) B4 isolectin (VVB4) (Tollefsen and Kornfeld 1983); similar behaviour towards Tn-containing structures can therefore be expected for VVB4 and many Lamiaceae lectins. VVB4 binding to pancreatic and gastric cancer-associated mucins (Kawa *et al*. 1991, 1992) and this lectin's binding (without causing cytotoxicity) to ovarian, epidermal and breast carcinoma cells (Avichezer and Arnon 1996) has suggested that similar results would be obtained with the hitherto isolated Lamiaceae lectins. This line of research will probably prove very fruitful. Thurnher *et al*. (1993) assayed MLL binding to two T-lymphocyte cell lines stably expressing the Tn antigen (one being derived from an individual having the idiopathic form of the Tn syndrome and the other from a Tn^+ Jurkat cell line) in an attempt to delineate its specificity. Flow cytometry experiments showed that MLL bound strongly to both cell lines. Interestingly, comparing mAb5F4 monoclonal antibody binding suggested that 5F4 epitopes and MLL binding sites were not identical.

Taking into account SBoL's ability to specifically recognise the Tn antigen, Vega (2004) demonstrated this lectin's binding to MCF-7 (breast cancer), HeLa (cervix cancer) and BHK (kidney fibroblasts) cell lines and periodontal tissue and mice skin fibroblast primary cultures. The assays (direct cytochemistry and indirect immunocytochemistry) revealed strong specific binding to the centrosomal complex (centrosome, centriole and Golgi) and transformed cell lines' perinuclear region which was also observed with VVB4 isolectin; Tn antigen presence in these cell lines was thus demonstrated for the first time with a lectin from the Lamiaceae family.

Unexpectedly, the primary cultures were also capable of interacting with SBoL, albeit less strongly than transformed cells, suggesting the existence of small amounts of Tn antigen which had been hitherto considered to be absent in primary cultures. SBoL cytotoxicity assays (Vega 2004) with HeLa, MCF-7, BHK and T47D (breast cancer) cells revealed marked differences amongst the cell lines, HeLa being the most susceptible to the lectin (LC 50% 6 ug/ml) and MCF-7 being the most resistant (LC 50% 300 ug/ml). These results showed (for the first time for a Lamiaceae lectin) that binding and cytotoxicity occur through the participation of different specific receptors.

Preliminary evidence of Gleheda's insecticidal activity against Colorado potato beetle larvae (*Leptinotarsa decemlineata*) has been obtained by Wang *et al*. (2003b) using a single dose of lectin; it would have been very interesting to carry out dose-response experiments and to assay several insect pests to elucidate whether the lectin were insect-specific.

Nevertheless, Gleheda's insecticidal activity stresses the importance of this unusual lectin, begging the question of whether such activity is shared by other Lamiaceae lectins.

A general cytotoxic effect was ruled out by Wang *et al*. (2003b) because they found that the lectin below 200 ug/ml was not cytotoxic for L1210 (murine leukaemia) and CEM (human T-lymphocytes) cell lines and moderately toxic (LC 50% 155 and 188 ug/ml) for FM3A (murine mammary carcinoma) and Molt4CI8 (human T-lymphocyte) cells. As lectin binding to cells was not assayed, these results cannot be compared with those obtained with SBoL.

The set of results pertaining to lectin cell binding and cytotoxic activity provides several hints for further studies of these activities. Cell lines' different susceptibility for a given lectin suggests the presence/absence of particular cell receptors and perhaps of different cytoplasmatic mediators leading to cell death; apoptotic mechanisms are very likely involved as indicated by some preliminary evidence (Vega, unpublished results). As lectin binding does not necessarily result in cytotoxicity, the question arises as to which protein(s) leads to the latter. It is also interesting, and maybe has implications from a practical point of view, that the Lamiaceae lectins and monoclonal antibodies have a similar, but not identical, specificity which may be due to different Tn antigen density requirements.

It has been well established for several non-Lamiaceae lectins, such as those from *Viscum album* (mistletoe), *Abrus precatorius* (abrin) and *Ricinus communis* (ricin), that their cytotoxic properties are explained by their function as type 2 ribosome-inactivating proteins (RIPs) (Hartley and Lord 2004) which are characteristically heterodimeric proteins (A and B chains) in which the β chain has cell-binding properties. Given the homomeric nature of Lamiaceae lectins (excepting MLL), it is thus unlikely that the cytotoxic properties described above are due to RIP activity.

Mitogenic activity

Aqueous crude extracts from aerial parts of *Salvia officinalis* (mainly constituted by polysaccharide but having 9%

protein) have revealed mitogenic, comitogenic (in the presence of phytohaemoagglutinin, PHA) and high adjuvant effects on rat thymocytes (Ebrigerová *et al*. 2006). However, it is unlikely that these effects were due to lectin from the aerial part as the amount of lectin in these tissues was probably very low except in leaves from some clones, according to results provided by Wang *et al*. (2003b).

Evidence for human lymphocyte stimulus has been obtained by Vega (2004) who observed metaphases and abundant blasts with 26-56 ug/ml SBoL; these results were corroborated by lymphocyte proliferation, determined by the method described by Mosmann (1983), similar to that obtained by phytohemagglutin (PHA) with 0.15 ug of both SBoL and PHA. It would be interesting to assay other Lamiaceae lectins to ascertain whether such biological activity is a feature common to all of them.

LECTINS AS TOOLS FOR TN ANTIGEN DETECTION IN TUMOUR CELLS

The Tn antigen was initially recognised as the factor responsible for human RBC polyagglutinability when blood-typing was carried out; it was first identified by Dausset *et al*. (1959) in RBCs from a patient suffering from haemolytic anaemia and its presence was explained by the absence of 3-β-D- galactosyltransferase which uses GalNAcα-Ser/Thr as substrate and yields the T-structure (Galβ1-3GalNAcα–Ser/Thr) (Cartron *et al*. 1987). This deficiency was named the "Tn syndrome," presenting very low clinical incidence, and was routinely diagnosed in patients' RBCs with the aid of crude *Salvia sclarea* seed extracts and those from other *Salvia* species (Bird and Wingham 1973, 1974, 1976, 1977).

As discussed at the beginning of this review, many Lamiaceae lectins specifically recognise the Tn antigen which is of paramount importance as this antigen is commonly expressed (in an exposed form) in a large number of human carcinomas (Springer 1984) and is considered to be specifically associated with transformed cells (Hakomori 1990).

It is surprising that, despite the early acknowledgement of Tn antigen–containing structures in tumour cells, few studies have been carried out to assess anti-Tn lectin interaction with tumour cells. By contrast, anti-Tn monoclonal antibodies (mAbs) have been widely used as tools for this purpose. However a word of caution should be given as accumulating evidence has shown that mAbs and lectins do not interact with Tn-containing structures in an identical manner. The observed differences have been ascribed to different Tn-density requirements for the interaction to occur.

Several studies have used VVB4 as a detection tool for Tn antigen in various cancer types. Tn expression in human colon cancer has been analysed by Itzkowitz and coworkers, who consistently found this antigen expressed in 72-81% of colon cancer cases and in all colorectal polyps (Itzkowitz *et al*. 1989, 1992), thereby concluding that Tn is a useful tumour marker. Sherwani *et al*. (2003) have pointed out VVB4's ability to specifically recognise cancer-associated mucins in pancreatic and gastric cancers in their appraisal of the lectins' histochemical specificity in carcinomas. Nishiyama *et al*. (1987) examined the presence of Tn antigen with VVB4 in 53 cases of urinary bladder transitional carcinomas during various stages; these authors found a significant number of Tn-positive cases showing invasive recurrence and concluded that the combined examination of T and Tn expression was useful for estimating malignancy and a patient's clinical course. As far as we are aware, the above cited studies are the only ones which have used VVB4 for Tn detection in cancer cells.

Tn presence in bladder cancer tissue sections (Nishiyama *et al*. 1987) led to assaying SBoL for the histochemical detection of Tn antigen in descamated cells from 33 urinary bladder cancer patients; it was found (Vega 2004) that the antigen was present in all stages of the neoplasia, detection levels ranging from 50% to 88%, depending upon the stage of the malignancy. No Tn was detected in control samples from healthy individuals; positive control assays with VVB4 showed no differences with SBoL. These results are the first example of a Lamiaceae lectin being used as a tool for cancer marker detection, having the additional advantage that the technique is a non-invasive one.

FINAL REMARKS

There is a need for systematic studies in view of the increasing number of Lamiaceae species in which lectins have been detected, as a relatively small number of species has been surveyed and ampler data can provide clues for solving taxonomic issues. In this regard, information on habitat, growth habit and seed collection periods may be correlated with myxocarpy or the presence of secondary metabolites. As opposed to Leguminosae lectins, there is no information whatsoever about lectin levels during seed formation, germination or in vegetative tissues, intracellular localisation or the presence of vacuoles analogous to protein bodies where the lectin would be stored. In short, basic knowledge is lacking on almost every aspect.

More than a century after their discovery, perhaps the major and still unresolved question lies in determining lectins' physiological role; despite the abundant studies which have been carried out with Leguminoseae lectins, none of the several roles which have been proposed for them (storage proteins, pest control, growth regulators, symbiotic nitrogen-fixation mediators) have been firmly established. The situation is complicated as comparing *G. hederacea* leaf lectin's physiological features with those of legume lectins led Wang *et al*. (2003b) to claim that *Gleheda* is unique regarding temporal and spatial expression and clone, tissue and cell localisation. Similar studies on well-characterised Lamiaceae seed lectins will prove/disprove this assertion and will be of great value in establishing whether there is a general physiological pattern for these proteins.

It is foreseeable that several types of study will be carried out in the near future to broaden our knowledge of Lamiaceae lectins. Detailed analysis of seed lectin primary structure is still lacking which hampers tertiary structure determinations either by X-ray crystallography, which requires crystallization of the lectins, or by 2D-NMR; the latter will require the purification of substantial amounts of protein. Cell interaction studies, both with transformed and non-transformed cell lines, will shed light on pathways leading to apoptosis, cell proliferation, signal transduction and cell-cell interactions. Specific recognition of Tn-containing glycans will likely lead to structural studies on O-rich glycoproteins such as mucins whose role now appears to be more complex than initially thought. An important and emerging aspect regards Lamiaceae lectins' potential biomedical applications in detecting and assessing Tn antigen in tumour cells.

Detailed studies of lectin insecticidal activity, preferably by assaying a set of lectins, will undoubtedly contribute towards assessing their potential in biotechnological applications.

A lot of ground has been covered in this field since the discovery of these particular proteins but it is clear that there is still a long way to go towards understanding and using Lamiaceae lectins.

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Appendix 1 Mucilage, protein content and lectin activity in seeds of Lamiaceae species.

Species	Mucilage	Protein (mg/ml)	Lectin activity (%)	
			− Pectinex	+ Pectinex
Aegiphila bogotensis	No	1.16	38.8	61.5
A. cuatrecasana	No	0.20	38.2	32.6
Ballota nigra	No	0.79	75.0	75.4
Hyptis brachyata	No	0.33–3.45[1]	16 - 24.1	42.8-60.8
H. capitata	No	0.82-3.16	5.0-67.7	79.8-96.0
H. colombiana	No	0.45	17.6	30.0
H. mutabilis	No	0.90-1.98	63.4-89.5	58.3-95.7
H. pectinata	0.3	0.36	33.8	34.8
H. perbullata	No	2.06	42.0	68.8
H. personata	No	0.26	45.9	70.6
H. recurvata	No	0.35	45.0	79.7
H. sinuata	0.1	0.29-1.12	63.4-85.8	65.6-89.1
H. suaveolens	1.0	0.26-0.62	12.0-54.2	7.4-85.3
Lavandula vera	No	2.95	12.2	70.5
Leonorus japonica	No	4.05	47.9	90.3
Leonotis nepetifolia	No	0.23	4.1	8.4
Marsipianthes chamaedris	0.25	3.00	23.2	N.D.
Ocimum basilicum	1.5	0.76	54.9	64.1
O. campechianum	0.7	0.19-1.31	5.2-16.4	8.8-51.8
O. gratissimum	0.2	1.16	3.3	45
Scutellaria incarnata	No	7.33	44.0	71.6
S. purpurescens	No	2.36	3.8	6.3
S. racemosa	No	0.02	0.0	63.1
S. ventenatii	No	19.08	2.0	8.4
Sideritis hirsuta	No	7.55	71.3	72.8
Solenestemon scutellaroides	0.1	0.70	12.2	70.8
Stachys arvensis	No	1.92	64.1	96.4
S. bogotensis	No	4.50	49.4	70.2
S. calcicola	No	0.87	0.0	68.1
S. micheliana	0.2	0.76	33.6	71.4
S. pusilla	No	1.08	2.6	0
S. radicans	No	2.84	18.0	90.2

[1] A range of values is shown for samples from different locations.

Appendix 2 Mucilage, protein content and lectin activity in seeds of Lamiaceae species. Tribe *Mentheae*.

Species	Mucilage	Protein (mg/ml)	Lectin activity (%)	
			− Pectinex	+ Pectinex
Agastache rugosa	No	0.83	22.3	37.4
Hyssopus officinalis	No	0.92	52.1	76.2
Lepechinia betonicifolia	No	2.48	88 .0	88.6
L. bullata	No	0.91-3.95[1]	34.9-90.4	63.9-88.5
L. conferta	No	1.41-2.50	43.9-49.3	42.5-57.2
L. salvifolia	No	0.77-3.40	8.0-78.3	46.1-78.8
L. velutina	No	0.20	13.5	55.3
L. vulcanicola	No	2.25	40.3	57.2
Melissa officinalis	2.3	0.53	0	67.6
Mentha spicata	No	0.83	49.7	77.9
Minthostachys mollis	No	0.20	17.8	45.8
Origanum vulgare	No	1.28	0	74.7
O. mejorana	No	1.38	48.3	52
Rosmarinus officinalis	1.8	1.38	13.8	82.3
Salvia aethiopis	0.8	2.47	54.1	77.8
S. amethystina	0.5	3.8	71.3	N.D.
S. ametystyina ssp. *ametisthyna*	0.5	0.13	3.4	14.2
S. aratocensis ssp. *suratensis*	0.7	0.31	22.5	11.0
S. bogotensis ssp. *bogotensis*	1.0	3.0	96.7	98.0
S. bogotensis ssp. *aratocensis*	2.0	2.4	3.0	48.7
S. bogotensis ssp.*sochensis*	1.5	3.4	0.0	26.0
S. carnea var. *carnea*	1.0	3.5	19.7	55.9
S. chicamochae	0.8-1.0	3.0-6.6	33.0-36.3	67.2-79.0
S. chicamochae × *S. rubescens*	0.8	0.1	2.8	10.0
S. coccinea	1.1	1.16	33.1	39.5
S. cocuyana	1.3	1.25	20.5	80.0
S. cuatrecasana	0.6	0.2	4.3	49.5
S. gachantivana ssp. *gachantivana*	0.5	4.0	31.0	83.7
S. hispanica	2.3	0.84	16.3	79.7
S. horminum	1.8	2.67	69.6	86.9
S. lavandulaefolia	No	0.59	47.4	50.4
S. lasiocephala	1.3	0.19	41.2	99.0
S. melaleuca ssp. *totensis*	No	0.9	70.0	85.0
S. misella	1.0	0.4	53.9	65.4
S. officinalis	No	2.99	16	68
S. orthostachys ssp. *gachantivana*	1.0	10.55	81.4	81.3
S. orthostachys ssp. *nov.*	0.8	1.83	42.1	52.5
S. orthostachys ssp. *orthostachys*	0.6	0.2	4.3	49.5
S. orthostachys ssp. *soatensis*	2.0	7.50	28.0	87.4
S. palifolia	No	4.0	73.4	74.7
S. pauciserrata ssp. *pauciserrata*	0.5	3.0	86.7	95.0
S. rubescens	0.7	0.35-0.51	41.8-88.5	40.8-88.5
S. rubescens ssp. *colombiana*	0.5	7.50	40.3	45.3
S. rubescens ssp. *dolychothrix*	1.0	7.50	28.3	52.4
S. rubescens ssp. *rubescens*	1.3	4.00	98.2	99.0
S. rubriflora	No	3.6	34.3	68.9
S. rufula ssp. *rufula*	1.5	2.85	7.7	68.0
S. sagitata	0.8	2.11	92.9	95.9
S. sordida	0.5	2.6	50.0	52.1
S. sphaceloides ssp. *pax fluminensis*	0.8	0.36-2.94	6.8-50.5	11.0-55.5
S. splendens	1.5	2.1	87.0	87.5
S. tillifolia	0.25	0.9	25.8	43.9
S. tortuosa	1.0-3.0	0.57-0.68	42.3-0.68	87.0-75.2
S. uribei	0.50	3.6	0.0	51.3
S. verbenaca	0.8	0.79	7.6	45.1
S. xeropapillosa	1.0-1.5	0.73-3.45	0.0-65.1	28.0-81.8
Satureja momntana	No	3.01	21.9	70.3
Thymus vulgaris	0.5	1.51	20.4	59.9

[1] A range of values is shown for samples from different locations.