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A serotaxonomic study of *Acacia* gum exudates

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Abstract

A simple immunological analysis of plant cell wall component epitopes present in 16 gum exudates obtained from 13 species of *Acacia* trees has shown that each of the gum samples studied had a unique composition. These analyses demonstrated, that in some cases gums harvested from species in the same taxonomic group exhibited wider differences in their composition of cell wall component epitopes as compared to gums from taxonomically unrelated species. Furthermore, gums obtained from different sub-species of a single species (e.g. *A. nilotica* and *A. nilotica* sspp. *nilotica*) were shown to have very distinct chemical compositions. The results also demonstrated for the first time, the presence of hydroxyproline-rich glycoprotein (HRGP) epitopes in the gum exudates of *A. tortilis*, *A. nilotica*, *A. nilotica* sspp. *nilotica*, *A. microbotrya*, *A. pycnantha* and *A. campylacantha*. These clearly demonstrate the utility of such immunological tests in chemotaxonomic analyses of this economically important class of plant gum exudates.

Keywords: *Acacia*; Immuno-dot blots; Arabinogalactan-protein; Hydroxyproline-rich glycoprotein; Pectin

1. Introduction

To date about 1200 *Acacia* species have been identified (Ross, 1979) and the commercial grades of gum exudates obtained from these species vary considerably in quality (Anderson & De Pinto, 1980). The best commercial grade gums are currently defined as being "clean" i.e., they are highly water-soluble and give colourless or pale yellow aqueous solutions. In the "gum trade", the gum obtained from *A. senegal* (L.) Willdenow has the greatest commercial value and is recognised as the best in quality. Primarily because of this, over the past 60 or so years, most research concerned with *Acacia* plant gum exudates has focused on the gum obtained from *A. senegal* which is generally known as gum arabic and is defined by the FAO/WHO Joint Expert Committee on Food Additives (JECFA), as "a dried exudation obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or closely related species of *Acacia* (family Leguminosae)" (FAO, 1995) which grow naturally throughout the Sahelian regions of Africa. Currently, the principal source of this gum is the Kordofan province of Sudan which produces over 90% of the worlds supply (Joseleau & Ullmann, 1990).

Gum arabic has a wide range of applications in food; for example, it is used to inhibit sugar crystallization in confectionary products and in the production of soft drinks as an emulsifier/stabilizer of essential oils (Osman, Williams, Menzies & Phillips, 1993a). It is also used to clarify wine, as an adhesive (Anderson & De Pinto, 1980) and to encapsulate pharmaceuticals (Joseleau & Ullmann, 1990). Inferior grades of gum are darker in colour and are less readily water-soluble. These lower grades of gum are used in non-food related industries such as printing and textiles and in the production of explosives (Anderson & De Pinto, 1980).

From the intensive study of the chemical and physicochemical properties of gum arabic, it has been shown that the gum harvested from *A. senegal* trees consists mainly of polysaccharides, with galactopyranose (Gal*p*) and arabinofuranose (Ara*f*) being the major monosaccharides present. According to Anderson and Stoddart (1966) gum arabic also contains a small proportion of nitrogenous material that is difficult to remove. Akiyama, Eda and Kato (1984)

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have suggested that gum arabic could be considered as "a kind of arabinogalactan-protein (AGP)". More recently, Vandevelde and Fenyo (1985) and Randell, Phillips and Williams (1988, 1989) have proposed that gum arabic consists of at least three "fractions" namely an AGP fraction (10.4% of the total gum) containing 11.8% protein, an arabinogalactan (AG) fraction (88.4% of the total) containing 0.35% protein and a glycoprotein (GP) fraction (ca. 1% of the total) containing 47.3% protein. All three fractions were shown to share similar branched structures composed of a β(1–3) linked galactose backbone with branches of β(1–6) linked galactose (Gal) containing arabinose (Ara), rhamnose (Rha), uronic acids and their derivatives (Randell et al., 1988, 1989).

In 1991, Qi, Fong and Lamport (1991) isolated two major fractions from gum exudates of *A. senegal* namely a gum arabic glycoprotein (GAGP) of high Mr containing ~90% carbohydrate (mainly Ara and Gal) and a gum arabic polysaccharide (GAP) which was shown to be a glucorhamnoarabinogalactan with a low Mr and little or no protein. They also observed that while the GAGP polypeptide backbone closely resembled that of extensins, the polysaccharide moiety was similar to that of the AGPs (Qi et al., 1991).

Fractionation of gum arabic by size exclusion chromatography further demonstrated the polydisperse nature of *A. senegal* gum components with respect to their molecular mass and chemical composition (Osman et al., 1993a; Osman, Menzies, Williams, Phillips & Baldwin, 1993b). This confirmed the heteropolymolecular nature of the gum originally proposed by Anderson and Stoddart (1966). Whilst most studies of gum arabic and related *Acacia* gum exudates (and seed proteins) have focused on the chemical and physicochemical properties of these substances, a number of immunological investigations have also been reported. Intravenous injections of *Acacia* gum were used for the treatment of wound shock during the First World War (Bayliss, 1918) and the first record of gum arabic's antigenicity was reported by Maytum and Magath (1932) and later confirmed by Bohner, Sheldon and Trenis (1941). In 1953, Whistler and Smart (1953) showed that gum arabic free from nitrogenous impurities was unable to induce antibodies in laboratory animals, Heidelberger (1955) found that type XIV antipneumococcal horse sera precipitated gum arabic and other galactose-containing gums (e.g., *A. pycnantha* gum, gum karaya, larch arabinogalactan and carob mucilage). Further studies indicated that gum arabic reacted strongly with antibodies directed against type II Pneumococcal polysaccharides which possess terminal D-glucuronic acid units as immunodeterminants (Heidelberger, Adams & Dische, 1956; Heidelberger, 1956). Subsequentely, in the 1960s Matsuyama (Matsuzawa, 1963; Matsuzama, 1967) raised anti-gum arabic antisera to identify blood group antigens.

Anderson and De Pinto (1980) was the first to suggest that biochemical and biophysical data on *Acacia* gum exudates could be used to augment the classical botanical classification of *Acacia* based solely on external morphological features such as in the monograph of Bentham (1875). The first report to follow up on Anderson's suggestions was published by El Tinay, Karamalla, El Amin, Shigidi and Ishag (1979). In this study, seed proteins harvested from 22 species of *Acacia* collected from Northern, Central and Western Sudan were compared by serological methods in an attempt to classify Sudanese *Acacia* species. From the results, the seed proteins were divided into two main groups and six sub-groups. In general, the phytochemical groupings established, agreed with the botanical classification for the main groups but not in all cases.

More recently, Brain (1987) reported a study using immunological techniques to study *Acacia* phylogeny, whereby the seed proteins of 37 species of *Acacia* were tested serologically by double diffusion and immunoelectrophoresis using rabbit antisera raised against whole seed contents of *A. karroo*, *A. ataxacantha* and *A. mearnsii* (Brain, 1987). Identity and absorption tests showed remarkable homogeneity in the Gummiferae series. The seed proteins of *Acacias* from Africa and Australia were analysed and were found to have virtually identical reactions with the antisera. In terms of the evolution and diversification of the Gummiferae *Acacias*, it was remarkable that despite geographical separation for millions of years that there was so little difference in the seed proteins of these species (Brain, 1987). The African Vulgares species studied were found to be serologically quite different from the various Gummiferae, which supports Pedley's view (Pedley, 1986) that the Gummiferae arose independently from the Vulgares and therefore should not be included in the same genus.

The first detailed description of the use of anti-gum arabic antibodies in an immunoassay was described by Pazur, Miskiel, Burdett and Docherty (1986). Since which time several similar reports have also been published (Pazur, Miskiel, Witham & Marchetti, 1991; Miskiel & Pazur, 1991; Williams, Menzies, Phillips & Smith, 1992; Menzies, Osman, Phillips & Williams, 1992; Osman et al., 1993a). Recently, a sensitive and specific ELISA for *Acacia senegal* gum which could differentiate this gum from other commonly used food hydrocolloids including other plant exudates such as gums ghatti, tragacanth and karaya has been reported (Williams et al., 1992; Menzies et al., 1992). Further studies of *Acacia* gums using this ELISA in combination with a range of chemical and physicochemical techniques indicated that the interaction with the anti-

gum arabic antisera could be correlated with differences in the molecular composition of the gums. These studies suggested the usefulness of such an immunoassay in chemotaxonomic studies of *Acacia* (Osman et al., 1993a). The only major drawback of this technique was that polyclonal antisera were used, of which, only a finite quantity was available. In the same year Osman et al. reported the use of a panel of anti-arabinogalactan/arabinogalactan-protein (AG/AGP) monoclonal antibodies in conjunction with a range of chemical/physicochemical analyses to study the molecular composition of gum arabic (Bayliss, 1918). This and later studies (Osman, Menzies, Albo Martin, Williams, Phillips et al., 1995) demonstrated the utility of anti-plant cell wall monoclonal antibodies in the analysis of the composition of gum arabic and fractions derived from it.

More recently and of particular relevance to the current study, was the report of the use of a panel of anti-AG/AGP monoclonal antibodies in conjunction with a range of other analytical techniques, to compare the physicochemical and immunological properties of gum arabic (harvested from *A. senegal*) and gum tahla (harvested from *A. seyal*) (Menzies, Osman, Malik & Baldwin, 1996). This study demonstrated that a quick and simple immunoassay namely immuno-dot blots could be used to distinguish between gum tahla and gum arabic.

Based on these earlier findings, the current study has made use of an existing panel of monoclonal antibodies which recognise epitopes present on a range of plant cell wall components namely AGs/AGPs, pectins and hydroxyproline-rich glycoproteins (HRGPs), to perform a serotaxonomic study of *Acacia* gum exudates. The objective of which was to provide serotaxonomic information on these species to augment the "classical" botanical taxonomic data and thereby to assist in the taxonomic classification of the species in question.

2. Results and discussion

All the gum exudate samples were tested for the relative abundance of several plant cell wall components namely AGs/AGPs, pectins and HRGPs via immuno-dot blot analyses using an existing panel of monoclonal antibodies which recognise epitopes present on these molecules (See Table 1).

3. Samples A, B, C, D & E (series I—Phyllodineae)

Gum samples A (*A. microbotrya*), B (*A. pycnantha*), C (*A. mabellae*), D (*A. saligna*) and E (*A. longifolia*) were all shown to contain a wide variety of AG/AGP terminal sugar epitopes. Sample D was also shown to contain moderate levels of both unesterified and methyl-esterified pectin epitopes (as detected by JIM 5 and JIM 7 respectively), whereas the other samples contained little or none of either of these epitopes. The results obtained with the *A. saligna* (sample D) gum were unusual in comparison with the other Phyllodineae gum samples in that it interacted quite strongly with JIM 4 and with the anti-unesterified and methyl-esterified pectin antibodies.

The presence of HRGP epitopes in sample B as shown in Table 1 is of significance, since it represents the first report of the presence of HRGP epitopes and presumably HRGP molecules in the gum exudate of *A. pycnantha* (sample B). These HRGP molecules are indeed quite likely to be similar in structure to those previously shown to be present in the gum of *A. senegal* (gum arabic) (Qi et al., 1991).

The gum from *A. mabellae* (sample C) was atypical of the other Phyllodineae samples in general since it demonstrated no apparent interaction with any of the antibodies used! The results obtained for the Phyllodineae gum samples, in the main, support earlier chemotaxonomic studies based upon differences in polysaccharide parameters (sugar composition, optical rotation and structural patterns) (Anderson, Bell & McNab, 1972) and amino acid composition (Anderson & McDougall, 1985). These studies which compared the gums obtained from *A. saligna* (sample D) and *A. pycnantha* (sample B), demonstrated that these two species were not as closely related taxonomically as was originally believed based purely on morphological considerations. It has been suggested that *A. saligna* (sample D) be re-classified within the subseries Juliferae due to its close chemical similarities with *A. longifolia* (sample E) (Churms, Merrifield & Stephen, 1980). The results of the current study demonstrated that the molecular composition of samples B (*A. pycnantha*) and D (*A. saligna*) were very different even though they were placed next to each other in Bentham's classification scheme, whereas the composition of samples D (*A. saligna*) and E (*A. longifolia*) were shown to be very similar.

However, Anderson, Gill, McNab and De Pinto (1984a) have suggested that *A. saligna* (sample D) cannot be seen as an anomalous member of the series Phyllodineae and so, should not be re-classified within the subseries Juliferae, the reason being that there were indications that, other species with close affinities also have widely differing gum compositions. For example, *A. saliciformis* is close taxonomically to *A. mabellae* (sample C), yet studies of the gum exudates of these species (Anderson et al., 1972) revealed considerable differences in several of the analytical parameters used.

According to Anderson, Gill, Jeffry and McDougall (1985), *A. microbotrya* gum (sample A) was shown to

Table 1
Results of immuno-dot blot of gum samples. The interaction of gum samples with anti AG/AGP, anti-pectin and anti-HRGP monoclonal antibodies. (Each result presented is the mean value of three replicates of each immuno-dot blot experiment)

	Phyllodineae (Series I)					Botryocephalae (Series II)	Gummiferae (Series IV)							Vulgares (Series V)		
MAb	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
MAC 207	+ +	+	–	+ + + +	+ + + +	+ + + + +	+ + + +	+ + + +	–	+ + + +	+	+ + +	–	–	–	+ +
JIM 4	–	–	–	+ + + +	+	–	+	+	+ + + +	–	–	–	–	+	+ + +	+ + +
JIM 8	–	+ +	–	+ + + +	+ + + + +	+ + + + +	+ + +	+ +	+ + + +	–	+ + +	+ +	+	+ + +	+ + + + +	+ + +
				+ +	+ +				+ +							+ +
JIM 13	+ + +	+ + + +	–	+ + + +	+ + + +	+ + + + + +	+ + + +	+ + + +	+ + + +	+ + +	+ + + + +	+ + + +	+ + +	+ + +	+ + + +	+ + +
		+ +		+ +	+ + +		+ +		+ + +				+ +	+ +	+ +	+ + +
JIM 14	–	+	–	–	–	–	+	–	+	–	+	+ + + +	–	–	–	–
												+ +				
JIM 15	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
JIM 16	+ +	+ + +	–	–	–	+	+ +	–	+	–	–	+ +	–	–	+	+ +
JIM 5	–	–	–	+ + +	+	–	+	–	+ + +	–	–	–	–	–	–	–
JIM 7	–	+	–	+ + + +	+	–	+	+	+ + + +	–	–	–	+	+ +	+ +	+
JIM 12	–	+ +	–	–	–	–	+	–	+ +	+ +	–	–	–	+ + +	+ + + +	+ + + +

MAb: Monoclonal antibody.
Scale of intensity for the reactions: + ⇒ + + + + + + +
List of samples: A, *A. microbotrya* Benth; B, *A. pycnantha* Benth; C, *A. mabellae*; D, *A. saligna*; E, *A. longifolia*; F, *A. mearnsii* De Willd; G, *A. tortilis* Hayne; H, *A. seyal* Del; I, *A. nilotica* (L.) Willd. Ex Del; J, *A. nilotica* sspp. *nilotica* (L.) Willd. Ex Del. ssp. nilotica; K, *A. karroo*; L, *A. karoo*; M, *A. karroo*; N, *A. campylacantha*; O, *A. verek*; P, *A. senegal* (L.) Willd;

share chemical similarities with the gum exudate of *A. mabellae* (sample C) as judged by their sugar composition. However, the results of the current study do not support this claim.

The observed differences in the cell wall epitope composition of the gum samples harvested from *Acacia* species previously assigned to the Phyllodineae (samples A, B and C) suggest that these species may not be as closely taxonomically related as previously determined using botanical morphological characteristics. In the light of these results it is suggested that further taxonomic techniques be used in order to determine whether or not *A. saligna* (sample D) should be re-grouped within the same subseries Juliferae as *A. longifolia* (sample E) as suggested by Churms et al. (1980).

4. Sample F (series II—Botryocephalae)

Sample F (*A. mearnsii*) was shown to contain relatively high levels of several of the AG/AGP epitopes recognised by the panel of anti-cell wall antibodies. Pectin and HRGP epitopes however were not detected in sample F by the method used.

5. Samples G, H, I, J, K, L & M (series IV—Gummiferae)

As can be seen from Table 1, most of the Gummiferae gum samples studied contained a number of AG/AGP sugar epitopes. Some of these samples (G and I) were also shown to contain both unesterified and methyl-esterified pectin epitopes. Samples H and M however, exhibited only trace levels of the methyl-esterified pectin epitope, as recognised by JIM 7; whilst the remainder (J, K and L) apparently contained neither esterified or unesterified pectic epitopes. When tested against the anti-HRGP antibody (JIM 12), three samples (G, I and J) gave low signals, whilst the remaining samples (H, K, L and M) had no apparent interaction with this antibody.

According to Stephen (1987), gums from *A. seyal* (sample H) and *A. nilotica* (sample I) display many common features (sugar composition and low nitrogen content). The current study however, found that the AG/AGP epitopes of samples H and I were quite different; sample I did not contain the arabinose containing epitope recognised by MAC 207 (Pennell, Knox, Schofield, Selvendran & Roberts, 1989) but sample H contained quite high levels of this epitope. Conversely, sample I contained sugar epitopes recognised by JIM 4, JIM 14, JIM 15 and JIM 16 but sample H contained none of these epitopes. Moreover, as mentioned earlier moderate levels of unesterified and methyl-esterified pectin were detected in sample I, whereas sample H was shown to contain only a very low level of methyl-esterified pectin epitopes. From these results it is clear that at least two gum exudates from the Gummiferae species, *A. seyal* and *A. nilotica*, show considerable differences in their molecular composition.

A comparison of samples I (*A. nilotica*) and J (*A. nilotica* sspp. *nilotica*) revealed major differences in the AG/AGP terminal sugar epitopes present. Sample J contained only the sugar epitopes recognised by MAC 207 and JIM 13 whereas sample I contained most of the epitopes recognised by the panel of antibodies used with the exception of MAC 207. Furthermore, moderate levels of pectic epitopes were observed in sample I, whereas sample J did not apparently contain any pectin epitopes. The most probable explanation for the observed differences in the AG/AGP epitopes is that they are due to genetic differences between these two sub-species.

The cell wall epitope compositions of samples K (*A. karroo*, S. Africa) and M (*A. karroo*, Zimbabwe) however, were shown to be quite similar in terms of the AG/AGP, HRGP and pectin epitopes present.

As mentioned earlier, the hydrolysed *A. karoo* gum (sample L) (essentially all the peripheral sugar groups have been removed) was shown to contain a slightly different AG/AGP terminal sugar epitope profile than sample K (unhydrolysed sample of *A. karoo* gum). The most likely explanation for this result is that the removal of peripheral sugar groups from AGs/AGPs present in the "native" gum consequently exposed previously concealed non-terminal sugars present in sample L, which were recognised by the antibodies concerned.

In summary, the current study of the cell wall epitope composition of gum exudate samples from the Gummiferae (subseries *medibracteatra*) has shown that samples collected from the same species, samples I (*A. nilotica*) and J (*A. nilotica* sspp. *nilotica*), rather surprisingly demonstrated broader differences as compared to samples harvested from different species, for example samples G (*A. tortilis*) and H (*A. seyal*). These results in general support the conclusions drawn by earlier studies on gums from the series Gummiferae (Anderson & De Pinto, 1980; Anderson, Bridgeman & De Pinto, 1984b), that gums from this series show wide variation in their chemical composition (sugar composition and nitrogen content).

6. Samples N, O & P (series V—Vulgares)

From Table 1 it can be seen that the Vulgares gum samples N (*A. campylacantha*), O (*A. verek*/*A. senegal*, Nigeria) and P (*A. senegal*) contain a wide variety of

AG/AGP epitopes. Methyl-esterified pectin epitopes were also detected in all three samples, whereas unesterified pectin epitopes were not shown to be present in any of these samples. The relatively high levels of the HRGP epitope detected in all three of the Vulgares gum samples, are the most significant difference between these samples and the other exudates tested. These results in general support the broad similarity in chemical components previously reported to be present in gum samples of *A. senegal* (Anderson, Miller & Wang, 1991a).

When the results of the current analysis of the gum exudates of *A. senegal* (gum arabic) and *A. seyal* (gum tahla) are compared to the results obtained by Menzies et al. (1996) the data presented tally very well indeed. The only notable difference between these two sets of data, is that in the present investigation the level of interaction of the *A. senegal* samples with JIM 16 was noticeably lower than that previously observed by Menzies et al. In addition, the current study used three antibodies not used in the earlier study i.e. JIM 5, JIM 7 and JIM 12. In the light of the results obtained with this additional panel of antibodies, we suggest that the "best" antibodies to use in conjunction with β-glucosyl Yariv reagent for industrial screening purposes in order to distinguish between gum arabic and gum tahla are JIM 12 and MAC 207.

In conclusion, although the results of the current study, in general support those of earlier investigations, a number of novel observations were also recorded. A possible explanation for the observed differences in the results obtained from the present study when compared to those obtained in earlier investigations, may in part be due the different experimental methodologies used. The method employed in the majority of previous investigations was to compare the results of gross amino acid and sugar composition analyses performed on gum samples obtained from various *Acacia* species. In the current study however, the *Acacia* gum exudate samples were tested for the presence/absence of specific terminal sugar epitopes present on a range of plant cell wall components. This immunological approach, as such, may be regarded as providing a more "detailed" comparison between the gum samples analysed. In a number of the previous investigations, gum samples which had the same gross sugar and/or amino acid compositions may well have contained very different proportions of various cell wall macromolecules, as is suggested by the results presented.

As stated by Anderson and De Pinto (1980) "Gum exudates are the most complex of the polysaccharide group of natural products" and in the 20 years since this statement was made, the observed heteropolymolecular nature of these substances has become yet more complex (Osman et al., 1993a; Bayliss, 1918)! Therefore, it is clear that a holistic approach to the study of these natural products is the most likely to yield conclusive results. Unfortunately, due to the extremely high carbohydrate content of *Acacia* gum exudates it is unlikely that the development of DNA based taxonomic techniques on such samples would be feasible. Thus, the development of quick, simple, reliable immunological tests based on monoclonal antibodies used in conjunction with "traditional" chemical and physicochemical analyses, would probably be the best experimental approach for both industrial (Menzies et al., 1996) and academic purposes (Anderson & De Pinto, 1980).

As mentioned earlier the current study has demonstrated for the first time the presence of HRGP epitopes and presumably the corresponding HRGP molecules in the gum exudates obtained from one member of the series Phyllodineae, *A. pycnantha*, from three members of the series Gummiferae, *A. tortilis*, *A. nilotica* and *A. nilotica* sspp. *nilotica* and from one member of the series Vulgares, *A. campylacantha*. It will be of considerable interest in future investigations to determine, how similar purified HRGPs from these gum samples are in terms of their biochemical structure and composition to those previously reported in the gum exudate of *A. senegal* (Qi et al., 1991). It would also be of interest to compare the results of the current study and earlier chemical/physicochemical and morphological analyses with the results of a molecular systematic analysis of plant tissues harvested from each of these species, the results of which would hopefully provide sufficient data for final "definitive" taxonomic assignments to be made on the species which comprise this fascinating and agronomically important genus.

7. Experimental

7.1. Materials

All *Acacia* gum samples used in the current study were botanically authenticated and details of their origin and form are given where known.

7.2. Series and sub-series assignments

Series and sub-series assignments of the *Acacia* species from which the gum exudates were harvested according to Bentham (1875).

7.2.1. Series I: Phyllodineae

Subseries: Univerves (Racemosae)
A. microbotrya Benth. (sample A)

A. pycnantha Benth. (sample B)
A. mabellae (sample C)
A. saligna (sample D)—also known as *A. cyanophylla*
Subseries: Juliferae (Tetramerae)
A. longifolia (sample E)

7.2.2. Series II: Botryocephalae

A. mearnsii De Willd. (sample F)

7.2.3. Series IV: Gummiferae

Subseries: Medibracteatae
A. tortilis Hayne. (sample G)
A. seyal Del. (sample H) roller-dried.
A. nilotica (L.) Willd. ex Del. (sample I)—Zimbabwe, 1991.
A. nilotica sub. spp. *nilotica* (L.) Willd. ex Del. ssp. *nilotica* (sample J).
A. karroo (sample K) freeze dried—South Africa
A. karroo (sample L) autohydrolysed and freeze dried—South Africa
A. karroo (sample M) ground—Zimbabwe 1991

7.2.4. Series V: Vulgares

Subseries: Gerontogeae spiciflorae
A. campylacantha (sample N)—Sudan 1937
A. verek (sample O)—Nigeria 1931—also known as *A. senegal*
A. senegal (L.) Willd. (sample P)

7.3. Immuno-dot blots

Immuno-dot blots were performed as previously described (Osman et al., 1993b) using a panel of anti-cell wall monoclonal antibodies. Each dot blot experiment was performed in triplicate using a standardised colour development period for the blots of 20 minutes. The results presented in Table 1. represent the mean value of these three sets of results. The panel of anti-cell wall monoclonal antibodies used in these experiments comprised of a set of anti-AGP/AG antibodies which bind to carbohydrate epitopes present on these molecules (MAC 207, JIM 4, JIM 8, JIM 13, JIM 14, JIM 15 and JIM 16) (Knox, Linstead, Peart, Cooper & Roberts, 1991); a pair of antibodies which recognise methyl-esterified and unesterified pectin respectively (JIM 5 and JIM 7) (Knox, Linstead, King, Cooper & Roberts, 1990); and an antibody raised against an HRGP epitope (JIM 12) (Smallwood, Bevan, Donovan, Neill, Peart et al., 1994).

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