



Journal of
Plant Sciences

ISSN 1816-4951



Academic
Journals Inc.

www.academicjournals.com

Comparison and Temperature Study of Lectin Activities in Texas Live Oak (*Quercus fusiformis*) Crude Extracts*

R.A. Ynalvez, L.M. Fuentes and C.V. Sanchez

Texas A and M International University, Laredo, TX 78041, USA

Corresponding Author: R.A. Ynalvez, Department of Biology and Chemistry, Texas A and M International University, 5201 University Blvd., Laredo, TX 78041, USA

ABSTRACT

Lectins are proteins that contain at least one non-catalytic carbohydrate binding domain. In plants, these proteins are hypothesized to play a critical role in plant defense functions. The extant literature shows that lectins have diverse applications, including medicinal and therapeutic ones. This study examines the presence and the level of lectin activity in the Texas live oak (*Quercus fusiformis* Small), a plant native to and replete in the South Texas region. To detect and compare lectin activity among selected plant parts of *Q. fusiformis*, agglutination and protein assays were conducted. The influence of four factors on lectin activity was investigated. These factors are: plant part (leaf, stem and fruit), tree section (A, B and C), temperature (0, 50 and 100°C) and time duration (1, 2 and 3 h) at the different temperature levels. Analysis of variance (ANOVA) associated with a factorial experiment, mean comparisons by way of a Tukey's test, trend analysis and regression analysis comprised the analytical strategy. Results indicated that lectin activity is present in each of the selected plant parts and varies significantly across these parts. ANOVA revealed that lectin activity is significantly linked to temperature level. Although relatively stable from 0 to 50°C, trend and regression analyses indicated significant linear and quadratic effects of temperature on lectin activity. These analyses indicated that maximum activity is predicted to occur at about 31°C. No interaction effect was detected between and among the four factors examined.

Key words: Lectins, defense proteins, thermostable, agglutination, Texas live oak

INTRODUCTION

Lectins are proteins possessing at least one noncatalytic carbohydrate binding domain with carbohydrate binding described to be specific and reversible (Van Damme *et al.*, 1998). Lectins are a class of cell-agglutinating proteins also known for their ability to agglutinate erythrocytes *in vitro*. These proteins are widely distributed in living organisms such as algae, animals, microorganisms, fungi and plants (Van Damme *et al.*, 2008; Fujii *et al.*, 2009; Bashir *et al.*, 2010; Han *et al.*, 2010; Molchanova *et al.*, 2010; Khan and Khan, 2011). Plants are the most frequently utilized sources of lectins due to (1) the relative ease of plant lectin extraction and (2) the high yields of lectins that can be obtained as compared to other sources (Charungchitrak *et al.*, 2011).

Plant lectins have mostly been found in seeds and in almost all kinds of vegetative tissues, including fruits, bulbs, leaves, stems and roots (Lamb *et al.*, 1983; Peumans *et al.*, 2000; Singh *et al.*, 2004; Kaur *et al.*, 2006; Echemendia-Blanco *et al.*, 2009; Raja *et al.*, 2011).

*Results of a preliminary study were published in an abstract for a poster presented at the Plant Biology 2010. Meeting sponsored by the American Society of Plant Biologists held in Montreal, Canada on July 31-August 4, 2010

Furthermore, genome-wide sequence analyses of soybean, rice and *Arabidopsis* have revealed that there are a total of 309, 267 and 199 members of lectin superfamilies in these plants, respectively (Jiang *et al.*, 2010). Most lectins from the same species have the same carbohydrate specificity. However, lectins from different tissues and cellular localizations from the same plant have exhibited different carbohydrate specificities and biological activities (Rocha *et al.*, 2009; Charungchitrak *et al.*, 2011). Lectins are hypothesized to evolve as one of the principal communication molecules involve in plant protection (Fokunang and Rastall, 2003). Likewise, numerous studies support the hypothesis that plant lectins play a vital role in plant defense functions, protecting them against diseases brought by bacteria, fungi and insects (Freire *et al.*, 2002; Oliveira *et al.*, 2008; De Hoff *et al.*, 2009; Tanaka *et al.*, 2009; Vandenberg *et al.*, 2010; Charungchitrak *et al.*, 2011). In some plants, such as *Oryza sativa* and *Triticum aestivum*, lectins are reported to be involved in plants' responses to environmental stress such as high salinity and hypothermia (Zhang *et al.*, 2000; Jiang *et al.*, 2010; Timofeeva *et al.*, 2010). Lectins are also known to play a role in symbiosis (Vershina *et al.*, 2010; Jimbo *et al.*, 2010).

Lectins provide a diverse and an increasing number of applications. Studies have shown that lectins serve as useful probes for structural investigations of polysaccharide and complex carbohydrates on cell surfaces. For example, the ability of lectins to bind selectively to carbohydrate moieties of glycoproteins makes these proteins useful as differentiating markers to study cancer cells (Arab *et al.*, 2010; De Lima *et al.*, 2010; Matsumoto *et al.*, 2010) and characterizing differentiating stem cells (Wearne *et al.*, 2006). Several lectins are reported to possess different biological activities which include anti-bacterial (Oliveira *et al.*, 2008; Raja *et al.*, 2011), anti-fungal (Freire *et al.*, 2002), anti-HIV (Balzarini *et al.*, 2004; Shi *et al.*, 2007; Hoorelbeke *et al.*, 2010), immunomodulative effects (Reis *et al.*, 2008), anti-proliferative and mitogenic for specific cell types (Peumans *et al.*, 2000; Kaur *et al.*, 2005; De Almeida Buranello *et al.*, 2010). Lectins are also being studied as tools for drug deliveries (Li *et al.*, 2008; Poiroux *et al.*, 2011). All these highlights lectins' great potential for pharmacological and therapeutic applications (De Mejia and Prisecaru, 2005). With the numerous and increasing applications of lectins, it is therefore imperative and relevant to identify novel plant sources of lectins.

A number of plant species native to the South Texas region has not yet been investigated for their potential as lectin sources and *Q. fusiformis* is one of those not yet studied. *Quercus fusiformis*, the Texas live oak which belongs to the family Fagaceae is also known as Escarpment live oak, Plateau live oak, Scrub live oak and West Texas live oak (Simpson, 1999). It is a thicket-forming shrub (or a large, spreading tree) that is nearly identical to the live oak, *Quercus virginiana* Mill, in appearance. However, *Q. fusiformis* can be thought of as a smaller but a more resistant variant of *Q. virginiana*; it is also more cold-hardy and drought-tolerant than *Q. virginiana* (Garrett, 1996). Texas live oaks' leaves are evergreen, firm textured, ovate to elliptic, 1 to 6 inches (2.5-15 cm) long and usually without lobes except on young plants. Their acorns are 1/2 to 1 inch (1.2-2.5 cm) long, rather elongate and spindle-shaped and narrow at the base (Tull and Miller, 1999). Texas live oaks are deciduous trees that grow on well-drained soils from alkaline to slightly acidic conditions. They can be found in Central, North Central and South Texas (Simpson, 1999; Tull and Miller, 1999). In order to explore new lectin sources, this study aims to (1) investigate the presence of and compare lectin activity among the different plants parts, leaf, stem and fruit of *Q. fusiformis* and if indeed lectin activity is present and (2) to examine its lectin's thermostability which is an important property that is advantageous for future commercial production.

MATERIALS AND METHODS

This study was conducted from May 2010-May 2011.

Sample collection: The plant part, leaf, stem and fruit (acorn), samples used in this study were collected from three *Q. fusiformis* trees at three different locations (N27°34'24.3" W99°26'00.3", N27°34'23.4" W99°26'12.3", N27°34'22.3" W99°26'17.3") on the Texas A and M International University campus. Leaf, stem and fruit samples were collected from three different sections of each tree; A, B and C. Section A covered that section of the tree towards the north, section B the southeast direction and section C towards the southwest direction. These samples were stored in appropriately labeled, large Ziploc™ bags in a -40°C refrigerator until they were used for protein extraction.

Crude extraction: The frozen plant part samples were homogenized using a mortar and pestle and added with ice-cold 0.01 M phosphate buffer saline (0.15 M NaCl), pH 7.2 (1:8 w/v). Additional homogenization for 1 min was done for each respective sample using a Waring™ laboratory blender. The homogenates were filtered using cheese cloths and centrifuged at 4,000 rpm at 10°C for 30 min using an Avanti JE Centrifuge. Crude extracts obtained from the previous step were either stored in a -40°C freezer for later assays or used for agglutination assays immediately after.

Agglutination and protein assays: The crude extracts were assayed for lectin activities expressed as Hemagglutinating Activities (HAs) using Corningware™ 96-well microtiter U-plates. The human red blood cells (RBCs) obtained from the Laredo Medical Center (LMC) Laboratory Department used in these assays were postpartum blood samples screened negative for both HIV and blood-borne communicable diseases. RBCs were washed with 0.01 M phosphate buffered saline, 0.15 M NaCl, pH 7.2 (PBS) until these washes were clear. Serial two-fold dilutions of *Q. fusiformis* crude extracts in PBS (50 µL) were incubated with 2% suspension RBCs (50 µL) at room temperature for about an hour or until a red button on the microtiter plate's well was observed in the negative control (PBS only). The positive result, agglutination of the RBCs, was indicated by a carpet layer formation on the microtiter plate's well. HA was defined as the reciprocal of the lowest sample dilution showing full agglutination. Specific activity, SA, was calculated by dividing HA by protein concentration (µg). Protein content determination was done using the Bio-Rad™ Bradford Protein Assay Kit with γ -globulin as protein standard. One milliliter of Bradford reagent was added to each 20 µL of the sample and was mixed thoroughly using a vortex mixer and incubated for 15 min at room temperature. The absorbance was read at 595 nm using Spectronic 20 GeneSys spectrophotometer.

Temperature study: The effect of temperature on lectin activity was determined by incubating the different crude extracts (leaf section A, leaf section B, leaf section C, stem section A, stem section B, stem section C, fruit section A, fruit section B and fruit section C) at three levels of temperature. These crude extracts were exposed to temperatures of 0, 50 and 100 for 3 h. At 30 min intervals (six intervals in all for the entire three hours) the test tubes containing the extracts were shaken. After every hour, 200 µL of these extracts were pipetted-out from each test tube into a 0.6 mL Eppendorf tube and kept in an ice box until ready for agglutination assays. Eppendorf tubes with the 200 µL of sample were centrifuged for 5 min at 13,200x g in an Eppendorf 3415 D table top centrifuge. The resulting supernatants were used to perform the agglutination assays.

Statistical analysis: An Analysis of Variance (ANOVA) associated with a 3×3×3 factorial experiment in randomized complete blocks was employed using the PROC GLM facility of the statistical computing software, Statistical Analysis System (SAS, Raleigh, NC) version 9.2 (Dowdy and Wearden, 1983; Quinn and Keough, 2002; Field and Miles, 2010). The experiment comprised of four factors, each of which had three levels: plant part (leaf, stem and fruit), tree section (A, B and C), incubation temperature (0, 50 and 100°C) and time duration (1, 2 and 3 h) which translates to 81 treatment combinations in each complete block. Each of the three different trees comprised one block. Graphical (i.e., histogram, residual plots) and analytical (i.e., the Kolmogorov-Smirnov and the Shapiro-Wilks tests of normality) analyses of the residuals revealed that the response variable, Specific Activity (SA), significantly departed from the ANOVA requirements of normality and of homoscedastic (equal) variances (Field and Miles, 2010). To normalize and to stabilize variances, the natural logarithm of SA was calculated and used for statistical analysis (i.e., ANOVA, mean comparisons, trend analysis and regression analysis). To identify which of the factor means were and were not significantly different, a Tukey test was used (Field and Miles, 2010). Given the significant effect of temperature at the 0.1% level, its corresponding sum of square was partitioned using orthogonal polynomials of the form -1, 0, +1 and +1, -2, +1 to test for linear and quadratic trends, respectively (Dowdy and Wearden, 1983; Field and Miles, 2010; Neter *et al.*, 1996). The linear and the quadratic trends were significant at the 1 and 5% levels, respectively.

RESULTS AND DISCUSSION

This study is an investigation of lectin activity in *Q. fusiformis*. Extracts from different plant parts namely leaves, stems and fruits (acorns) of *Q. fusiformis* contain detectable levels of lectin activity as shown by the ability of these extracts to agglutinate erythrocytes. Lectins' ability to bind to the sugars on the surface of erythrocytes allows the erythrocytes in a buffer to remain in suspension. In the presence of lectins, the erythrocytes are prevented from interacting among themselves. On the other hand, in the absence of lectin, erythrocytes are left to interact among themselves and thus erythrocytes aggregate and form a discrete button at the bottom of the microtiter plate's well.

Comparison of lectin activities: As mentioned earlier, Hemagglutination Activity (HA) was expressed as the reciprocal of the lowest dilution showing agglutination. Results of agglutination assays showed that HAs differed among the different plant part extracts of *Q. fusiformis*. The lowest dilutions of the stem, fruit and leaf extracts that produced agglutinations were on the average, 1/16 (HA = 16), 1/64 (HA = 64) and 1/128 (HA = 128), respectively. Leaf extracts with an HA of 128 had the highest agglutination activity from among the plant part extracts tested. Specific Activity (SA) values expressed as the HA per microgram protein were calculated and used to compare lectin activities among the different plant parts being tested. Table 1 presents ANOVA results indicating that SAs from different plant parts differ significantly at the 0.1% level. In addition to plant parts, the amount of sunlight received by each section of the tree could be a factor that might affect lectin activity the influence of this factor has not been previously studied. Hence, in this study the hypothesis that tree section affects lectin activity is tested. However, ANOVA results showed no significant differences among tree sections (A, B and C) (Table 1). Furthermore, although samples were collected from oak trees that were almost the same height and maturity, ANOVA results indicated that block II (tree #2) was significantly different from blocks

Table 1: Analysis of variance results for the natural logarithm of lectin specific activity, ln (SA)

Sources	dF	SS	MS	Fc
Block	2	27.70	13.85	8.99***
Section (SE)	2	0.34	0.17	0.11
Plant Part (PP)	2	132.94	66.47	43.13***
SE×PP	4	3.77	0.94	0.61
Temperature (TE)	2	25.10	12.55	8.14***
SE×TE	4	1.96	0.49	0.32
PP×TE	4	2.41	0.60	0.39
SE×PP×TE	8	2.55	0.32	0.21
Time (TI)	2	8.44	4.22	2.74
SE×TI	4	0.63	0.16	0.10
PP×TI	4	9.24	2.31	1.50
SE×PP×TI	8	0.66	0.08	0.05
TE×TI	4	2.43	0.61	0.39
SE×TE×TI	8	0.41	0.05	0.03
PP×TE×TI	8	6.25	0.78	0.51
SE×PP×TE×TI	16	3.37	0.21	0.14
Error	160	246.56	1.54	

***Denotes significance at the 0.001 level

I (tree #1) and III (tree #3) (Table 1, 2). Differences among trees could be attributed to extraneous factors such as differences in soil properties (e.g., moisture, type and pH) and/or the actual age of the trees.

Comparison of means by way of a Tukey test (Table 2) showed that SAs from different plant parts differ significantly from each other with leaf extracts containing the highest activity at $10.2 \text{ HA } \mu\text{g}^{-1}$ and stem extracts exhibiting the lowest activity at $1.7 \text{ HA } \mu\text{g}^{-1}$. The results obtained from the present study, support previous findings that the level of lectin activities across different plant parts within species varies. For example, in *Cycas revoluta*, agglutination activity was detected mainly from leaf extracts and was very low in seed and root extracts (Yagi *et al.*, 2002). In *Musa acuminata* (banana) and *Musa* spp. (plantain), lectins are abundantly present in the pulp of mature fruits. No activity was detected in extracts from peels, leaves and corms; while extracts from roots and pulp showed low agglutination activity (Peumans *et al.*, 2000).

The possible roles of *Q. fusiformis* lectins: The functional role of lectins in plants is still inconclusive, yet there are several hypotheses pertaining to the presence and role of lectins. A hypothesis as to why lectin activity is only observed or high in a particular plant part is linked to the idea that plants use lectins as defense proteins. A strong supporting evidence of the role of lectin as a defense protein has been provided in the study of the *Nicotiana tabacum* lectin discovered in the leaves of tobacco (Chen *et al.*, 2002). Under normal environmental conditions, tobacco lectin is undetected in the leaves but upon treatment of certain jasmonates (or insect herbivore), tobacco lectin however started to accumulate locally at the sites of attack (Lannoo *et al.*, 2007; Vandenberg *et al.*, 2009). An additional set of evidence in support of the lectin as defense protein thesis is presented in the following studies. Feeding experiments with *Spodoptera littoralis* larvae using transgenic *N. attenuata* (a species that lacks a functional *N. tabacum* lectin gene) with ectopically expressed *N. tabacum* lectin, have shown a reduction in mass gain and

Table 2: Mean comparison of different blocks, plant parts and temperature levels with respect to lectin specific activity, ln (SA)

Blocks	ln (SA)	SA (HA μg^{-1})	Plant part	ln (SA)	SA (HA μg^{-1})	Temperature ($^{\circ}\text{C}$)	ln (SA)	SA (HA μg^{-1})
I	1.014 ^a	2.7579	Leaf	2.323	10.203 ^a	0	1.507 ^a	4.5131
II	1.802 ^b	6.0595	Stem	0.543	1.7212 ^b	50	1.613 ^a	5.0175
III	1.188 ^a	3.2815	Fruit	1.139	3.1226 ^c	100	0.884 ^b	2.4216

In a column, means with the same letters are not significantly different at the 0.05 level by Tukey's test (Field and Miles, 2010)

slower development of *S. littoralis*. In contrast, using transgenic *N. tabacum* plants with RNAi silenced lectin expression showed an enhanced larval performance of *S. littoralis* (Vandenborre *et al.*, 2010).

Plants protect themselves from predators and from adverse conditions by employing different constitutive and induced defense mechanisms (Howe and Jander, 2008; Sharma *et al.*, 2011). Constitutive defense is described as the basal line of defense needed to survive the first encounter of an attack, while inducible defense results from the accumulation of newly synthesized biochemical compounds during an attack (Lawrence *et al.*, 2006). For example, the lectin in banana serves as an inducible defense protein. It was reported to be induced when banana plants were treated with methyl jasmonate (Peumans *et al.*, 2000). Another example of inducible defense protein is the lectin in *Oryza sativa* (rice) leaves which was detected only in plants grown under saline conditions (Zhang *et al.*, 2000). Since the lectin activities from the present study were detected from samples that have not been treated with factors known to trigger lectin induction, *Q. fusiformis* lectins could be hypothesized to serve as constitutive defense proteins.

Higher levels of lectin activity may be seen in plant parts that are more attractive to other organisms and which are important for plants' survival. In a previous study, banana fruit lectin was shown to have the capability to stimulate animal lymphocytes and might potentially exhibit a differential effect depending on the sugars present on the surface of the immunocompetent cells in the gut of different animals (Peumans *et al.*, 2000). The banana lectin may be selectively toxic for species that do not contribute to the dissemination of banana seeds. On the other hand, the fruits will be non-toxic and are edible to animals that are likely to disseminate the seeds and hence contribute to the survival of that particular plant species in its natural habitat (Peumans *et al.*, 2000).

In the case of *Q. fusiformis*, the leaves are likely to be the most attractive part to other organisms i.e., insects, fungi, etc. This could possibly explain why the leaves exhibit the highest lectin activity. Relative to the stems which are hard and to the fruits (acorns) which are protected with a tough leathery shell, the leaves are more succulent, attractive and readily accessible to other organisms. It can also be hypothesized that *Q. fusiformis* lectins could serve as a line of defense against fungal attack. Oak wilt, a known aggressive fungal disease caused by the fungus *Ceratocystis fagacearum*, is an aggressive disease that affects many species of oak (Anderson *et al.*, 2000). Thus an anti-fungal activity in response to fungal attack could be present in *Q. fusiformis* lectins similar to those in *Talisia esculenta* seed and *Urtiga dioca* rhizomes lectins which were known to bind to chitin. It is suggestive that these chitin binding proteins cross-linked chitin preventing cell expansion at the tip of the growing hyphae which will slow down hyphal growth thus protecting the plants against fungal attack (Chrispeels and Raikhel, 1991; Freire *et al.*, 2002).

Temperature study of *Q. fusiformis*' lectins: Literature indicates that the thermal stability of plant lectins across species varies. In this study, the thermal stability of *Q. fusiformis* lectins was investigated at different temperature levels and time durations across plant parts. There were

neither significant interactions between plant parts and temperature levels, nor between plants parts and time durations (Table 1). From among the temperature levels, 0, 50 and 100°C, results have shown that lectin activity (SA) was highest at 50°C with SA, 5.02 HA μg^{-1} which was not significantly different at 0°C with SA, 4.51 HA μg^{-1} (Table 2). On the other hand, SA was lowest at 100°C with SA, 2.42 HA μg^{-1} and is significantly different from 0 and 50°C (Table 2). The lectin activity of *Q. fusiformis* is comparable to *Pisum sativum* and *Momordica charantia* lectins which have maximum activities at 60 and 55°C, respectively (Sitohy *et al.*, 2007; Toyama *et al.*, 2008). When compared to lectins from *Artocarpus camansi*, *Archidenron jiringa* and *Aegle marmelos* whose thermal stability is below 40°C (Occena *et al.*, 2007; Charungchitrak *et al.*, 2011; Raja *et al.*, 2011), *Q. fusiformis*' lectin activity is relatively more stable. On the other hand, lectins from *Ganoderma capense* and *Eugenia uniflora* that had complete retention of lectin activity even after exposure at 100°C for 1 h (Ngai and Ng, 2004; Oliveira *et al.*, 2008) are more stable than *Q. fusiformis*' lectin activity. The latter, on the other hand retained approximately 50% of its activity (2.4 HA μg^{-1}) even at a high temperature of 100°C and after 3 h of incubation thus *Q. fusiformis* lectin can be described as a thermostable lectin. The effect on lectin activity at 3 h was not significantly different from the 1 and 2 h incubation (Table 2).

Since temperature was shown to be a significant factor affecting lectin activity at the 0.1% level (Table 1), the trend of lectin activity across temperature levels was examined. Based on the results of the trend analysis (or the partitioning) of temperature sum of squares shown in Table 3, it is clear that a significant linear and a significant quadratic effect of temperature on lectin activity exist at the 1 and 5% level, respectively. A regression analysis of the linear and the quadratic term of temperature on the natural logarithm of SA predicted maximum lectin activity to be at about 31°C (Fig. 1). Hence, future experiments will include determination of lectin activity of

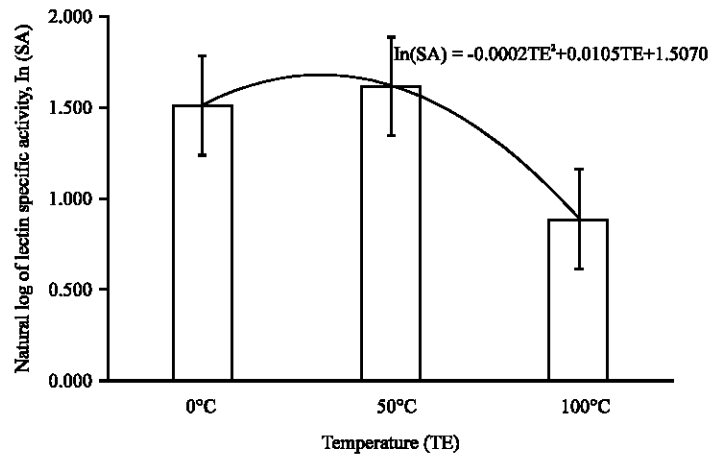


Fig. 1: Trend of ln(SA) across different temperature levels. Error bars are 95% confidence intervals

Table 3: Partitioning of the effect of temperature on the natural logarithm of lectin specific activity, ln(SA)

Sources	dF	SS	MS	Fc	Sig.
Temperature (TE)	2	25.10	12.55	8.14	***
Linear trend	1	15.70	15.70	10.19	**
Quadratic trend	1	9.40	9.40	6.10	*
Error	160	246.56	1.54		

*, **, ***Denote significance at the 0.05, 0.01, 0.001 levels

Q. fusiformis at the 20-100°C range at 5 or 10° intervals in order to accurately estimate (1) the temperature at which maximum activity occurs and (2) the temperature at which its lectin activity starts to destabilize. Mean comparison via a Tukey test indicated significant difference in activities between 50 and 100°C, while no significant difference was observed between 0 and 50°C (Table 2). Environmental changes such as temperature change are known to affect protein's conformation (Yeasmin *et al.*, 2007). Thus the observed decrease in activity at 100°C could be attributed to heat-induced lectin denaturation which may have weakened the non-covalent interactions between the lectin and the carbohydrate ligand leading to decreased lectin activity. The three sets of samples, only samples exposed at 100°C were observed to have protein precipitation, an indication that denaturation had taken place.

CONCLUSIONS

It was shown that lectin activities were present and were significantly different in the leaf, stem and fruit of *Q. fusiformis*. Lectin activity was (1) most stable at the 0-50°C range (2) predicted to be highest at about 31°C (3) still able to retain 50% of its activity at 100°C. The demonstration of lectin activity by *Q. fusiformis* extracts has provided a scientific basis for the potential of the Texas live oak as a practical and promising source of lectin. Specifically, results of this preliminary study have shown that *Q. fusiformis* leaves, among other plant parts examined, maybe the most practical and promising source of lectin. Thus, the purification and characterization of *Q. fusiformis* lectin from leaves, including the determination of this lectin's biological activities, needs to be carried out.

ACKNOWLEDGMENTS

The Avanti JE Centrifuge was acquired through a U.S. National Science Foundation Major Research Instrument grant award (DBI 0959395). This research was funded by a Texas A and M International University Research Development Award to the first author. The authors would like to thank Marcus Antonius Ynalvez for the statistical analysis; Carolina Gonzalez, an undergraduate research volunteer, for her assistance in the temperature studies; Manuel Montes, Sandra Prado and Luis Mares for their work in the preliminary studies on the identification and characterization of Texas live oak lectin activity.

REFERENCES

- Anderson, L.J., P.C. Harley, R.K. Monson and R.B. Jackson, 2000. Reduction of isoprene emissions from live oak (*Quercus fusiformis*) with oak wilt. *Tree Physiol.*, 20: 1199-1203.
- Arab, M.R., S. Salari, M. Karimi and H. Mofidpour, 2010. Lectin histochemical study of cell surface glycoconjugate in gastric carcinoma using *Helix pomatia* agglutinin. *Acta Med. Iran*, 48: 209-213.
- Balzarini, J., S. Hatse, K. Vermeire, K. Princen and S. Aquaro *et al.*, 2004. Mannose-specific plant lectins from the Amaryllidaceae family qualify as microbicides for prevention of human immunodeficiency virus infection. *Antimicrob. Agents Chemother.*, 48: 3858-3870.
- Bashir, H., T. Khan, A. Masood and R. Hamid, 2010. Isolation, purification and characterization of a lectin from a local kashmiri variety of soybean (*Glycine max*). *Asian J. Biochem.*, 5: 145-153.
- Charungchitrak, S., A. Petsom, P. Sangvanich and A. Karnchanatat, 2011. Antifungal and antibacterial activities of lectin from the seeds of *Archidendron jiringa* Nielsen. *Food Chem.*, 126: 1025-1032.

- Chen, Y., W.J. Peumans, B. Hause, J. Bras and M. Kumar *et al.*, 2002. Jasmonic acid methyl ester induces the synthesis of a cytoplasmic/nuclear chito-oligosaccharide binding lectin in tobacco leaves. *J. Fed. Am. Soc. Exp. Biol.*, 8: 905-907.
- Chrispeels, M.J. and N.V. Raikhel, 1991. Lectins, lectin genes and their role in plant defense. *Plant Cell.*, 3: 1-9.
- De Almeida Buranello, P.A., M.R. Moulin, D.A. Souza, M.C. Jamur, M.C. Roque-Barreira and C. Oliver, 2010. The lectin ArtinM induces recruitment of rat mast cells from the bone marrow to the peritoneal cavity. *PLoS One*, 5: 1-11.
- De Hoff, P.L., L.M. Brill and A.M. Hirsch, 2009. Plant lectins: The ties that bind in root symbiosis and plant defense. *Mol. Genet. Genomics*, 282: 1-15.
- De Lima, A.L.R., C.C.B. Cavalcanti, M.C.C. Silva, P.M.G. Paiva, L.C.B.B. Coelho, E.I.C. Beltrao and M.T.D.S. Correia, 2010. Histochemical evaluation of human prostatic tissues with *Cratylia mollis* seed lectin. *J. Biomed. Biotech.*, 2010: 1-6.
- De Mejia E. and V. Prisecaru, 2005. Lectins as bioactive plant proteins: A potential in cancer treatment. *Crit. Rev. Food Sci. Nutr.*, 45: 425-445.
- Dowdy, S. and S. Wearden, 1983. *Statistics for Research*. Wiley, New York, pp: 537.
- Echemendia-Blanco, D., E.V. Driessche, I. Ncube, J.S. Read and S. Beeckmans, 2009. Stability, subunit interactions and carbohydrate-binding of the seed lectin from *Pterocarpus angolensis*. *Prot. Pept. Lett.*, 16: 1120-1134.
- Field, A. and J. Miles, 2010. *Discovering Statistics Using SAS*. SAGE Publication, Thousand Oaks, CA, USA, pp: 719.
- Fokunang, C.N. and R.A. Rastall, 2003. Phytohaemagglutinins in membrane signalling, biomedical and genetic engineering research. *Biotechnology*, 2: 162-177.
- Freire, M.D.G.M., V.M. Gomes, R.E. Corsini, O.L.T. Machado and S.G. de Simone *et al.*, 2002. Isolation and partial characterization of a novel lectin from *Talisia esculenta* seeds that interferes with fungal growth. *Plant Physiol. Biochem.*, 40: 61-68.
- Fujii, Y., S.M.A. Kawsar, R. Matsumoto, H. Yasumitsu, N. Kojima and Y. Ozeki, 2009. Purification and characterization of a d-galactoside-binding lectin purified from bladder moon shell (*Glossaulax didyma* Roding). *J. Biol. Sci.*, 9: 319-325.
- Garrett, H., 1996. *Howard Garrett's Plants for Texas*. University of Texas Press, Texas, USA., ISBN-13: 9780292727885, Pages: 182.
- Han, J.W., M.G. Jung, M.J. Kim, K.S. Yoon, K.P. Lee and G.H. Kim, 2010. Purification and characterization of a D-mannose specific lectin from the green marine alga, *Bryopsis plumosa*. *Phycol. Res.*, 58: 143-150.
- Hoorelbeke, B., D. Huskens, G. Ferir, K.O. Francois and A. Takahashi *et al.*, 2010. Actinohivin, a broadly neutralizing prokaryotic lectin, inhibits HIV-1 infection by specifically targeting high-mannose-type glycans on the gp120 envelope. *Antimicrob. Agents Chemother.*, 54: 3287-3301.
- Howe, G.A. and G. Jander, 2008. Plant immunity to insect herbivores. *Ann. Rev. Plant Biol.*, 59: 41-66.
- Jiang, S.Y., Z. Ma and S. Ramachandran, 2010. Evolutionary history and stress regulation of the lectin superfamily in higher plants. *BMC Evol. Biol.*, 10: 79-79.
- Jimbo, M., H. Yamashita, K. Koike, R. Sakai and H. Kamiya, 2010. Effects of lectin in the scleractinian coral *Ctenactis echinata* on symbiotic zooxanthellae. *Fish. Sci.*, 76: 355-363.
- Kaur, A., J. Singh, S.S. Kamboj, A.K. Sexana, R.M. Pandita and M. Shamnugavel, 2005. Isolation of an N-acetyl-D-glucosamine specific lectin from the rhizomes of *Arundo donax* with antiproliferative activity. *Phytochemistry*, 66: 1933-1940.

- Kaur, A., S.S. Kamboj and J. Singh, 2006. Isolation of a new lectin from the bulbs of *Crinum latifolium* (L.). *J. Biological Sci.*, 6: 9-14.
- Khan, F. and M.I. Khan, 2011. Fungal lectins: Current molecular and biochemical perspectives. *Int. J. Biol. Chem.*, 5: 1-20.
- Lamb, J.E., S. Shibata and I.J. Goldstein, 1983. Purification and characterization of *Griffonia simplicifolia* leaf lectins. *Plant Physiol.*, 71: 879-887.
- Lannoo, N., G. Vandenborre, O. Miersch, G. Smagghe, C. Wasternack, W. Peumans and E.M. Van Damme, 2007. The jasmonate-induced expression of the *Nicotiana tabacum* leaf lectin. *Plant Cell Physiol.*, 48: 1207-1218.
- Lawrence, S.D., C. Dervinis, N. Novak and J.M. Davis, 2006. Wound and insect herbivore responsive genes in poplar. *Biotechnol. Lett.*, 28: 1493-1501.
- Li, J., H. Wu, J. Hong, X. Xu and H. Yang *et al.*, 2008. Odorranalectin is a small peptide lectin with potential for drug delivery and targeting. *PLOS One*, 3: e2381-e2381.
- Matsumoto, H., S. Shinzaki, M. Narisada, S. Kawamoto and K. Kuwamoto *et al.*, 2010. Clinical application of a lectin-antibody ELISA to measure fucosylated haptoglobin in sera of patients with pancreatic cancer. *Clin. Chem. Labor. Med.*, 48: 505-512.
- Molchanova, V., O. Chernikov, I. Chikalovets and P. Lukyanov, 2010. Purification and partial characterization of the lectin from the marine red alga *Tichocarpus crinitus* (Gmelin) rupestris (Rhodophyta). *Bot. Mar.*, 53: 69-78.
- Neter, J., M.H. Kutner, C.J. Nachtsheim and W. Wasserman, 1996. *Applied Linear Statistical Models*. 4th Edn., McGraw Hill, Boston, Massachusetts, ISBN-13: 9780130410207.
- Ngai, P.H. and T.B. Ng, 2004. A mushroom (*Ganoderma capense*) lectin with spectacular thermostability, potent mitogenic activity on splenocytes and antiproliferative activity toward tumor cells. *Biochem. Biophys. Res. Commun.*, 314: 988-993.
- Occena, I.V., E.R.E. Mojica and F.E. Merca, 2007. Isolation and partial characterization of a lectin from the seeds of *Artocarpus camansi* Blanco. *Asian J. Plant Sci.*, 6: 757-764.
- Oliveira, M.D.L., C.A.S. Andrade, N.S. Santos-Magalhaes, L.C.B.B. Coelho, J.A. Teixeira, M.G. Carneiro-da-Cunha and M.T.S. Correia, 2008. Purification of a lectin from *Eugenia uniflora* L. Seeds and its potential antibacterial activity. *Lett. Appl. Microbiol.*, 46: 371-376.
- Peumans, W.J., W. Zhang, A. Barre, C.H. Astoul and P.J. Balint-Kurti *et al.*, 2000. Fruit-specific lectins from banana and plantain. *Planta*, 211: 546-554.
- Poiroux, G., M. Pitie, R. Culerrier, B. Segui and E.J.M. Van Damme *et al.*, 2011. Morniga G: A plant lectin as an endocytic ligand for photosensitizer molecule targeting toward tumor-associated T/Tn antigens. *Photochem. Photobiol.*, 87: 370-377.
- Quinn, G.P. and M.J. Keough, 2002. *Experimental Design and Data Analysis for Biologists*. 1st Edn., Cambridge University Press, Cambridge, pp: 537.
- Raja, S.B., M.R. Murali, N.K. Kumar and S.N. Devaraj, 2011. Isolation and partial characterisation of a novel lectin from *Aegle marmelos* fruit and its effect on adherence and invasion of *Shigellae* to HT29 cells. *PLoS One*, 6: e16231-e16231.
- Reis, E.A.G., D.A. Athanazio, B.S. Cavada, E.H. Teixeira and V. de Paulo Teixeira Pinto *et al.*, 2008. Potential immunomodulatory effects of plant lectins in *Schistosoma mansoni* infection. *Acta Trop.*, 108: 160-165.
- Rocha, B.A.M., F.B.M.B. Moreno, P. Delatorre, E.P. Souza and E.S. Marinho *et al.*, 2009. Purification, characterization and preliminary x-ray diffraction analysis of a lactose-specific lectin from *Cymbosema roseum* seeds. *Appl. Biochem. Biotechnol.*, 152: 283-293.

- Sharma, N., K.P. Sharma, R.K. Gaur and V.K. Gupta, 2011. Role of chitinase in plant defense. *Asian J. Biochem.*, 6: 29-37.
- Shi, J., S.J. Xue, Y. Kakuda, S. Ilic and D. Kim, 2007. Isolation and characterization of lectins from kidney beans (*Phaseolus vulgaris*). *Process Biochem.*, 42: 1436-1442.
- Simpson, B.J., 1999. *A Field Guide to Texas Trees*. Gulf Publishing Company, Texas, USA., ISBN-13: 9780877193579, Pages: 488.
- Singh, J., J. Singh and S.S. Kamboj, 2004. A novel mitogenic and antiproliferative lectin from a wild cobra lily, *Arisaema flavum*. *Biochem. Biophys. Res. Comm.*, 318: 1057-1065.
- Sitohy, M., M. Doheim and H. Badr, 2007. Isolation and characterization of a lectin with antifungal activity from Egyptian *Pisum sativum* seeds. *Food Chem.*, 104: 971-979.
- Tanaka, H., J. Toyama and R. Akashi, 2009. Molecular characterization of a galactose-binding lectin from *Momordica charantia* seeds and its expression in tobacco cells. *Asian J. Plant Sci.*, 8: 544-550.
- Timofeeva, O.A., Y.Y. Nevmerzhitskaya and M.A. Moscowkina, 2010. Lectin activity and composition in the wheat cell walls under the effects of low temperature and inhibitors of calcium signaling pathway. *Russian J. Plant Physiol.*, 57: 198-204.
- Toyama, J., H. Tanaka, A. Horie, T. Uchiyama and R. Akashi, 2008. Purification and characterization of Anti-H lectin from the seed of *Momordica charantia* and the inter-specific differences of hemagglutinating activity in *Cucurbitaceae*. *Asian J. Plant Sci.*, 7: 647-653.
- Tull, D. and G.O. Miller, 1999. *A Field Guide to Wildflowers, Trees and Shrubs of Texas*. Gulf Publishing Company, Texas, USA., ISBN-13: 9780877193586, Pages: 344.
- Van Damme, E.J.M., W.J. Peumans, A. Barre and P. Rouge, 1998. Plant lectins: A composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Crit. Rev. Plant Sci.*, 17: 575-692.
- Van Damme, E.J.M., N. Lannoo and W.J. Peumans, 2008. Plant lectins. *Adv. Bot. Res.*, 48: 107-209.
- Vandenborre, G., O. Miersch, B. Hause, G. Smaghe, C. Wasternack and E.J. Van Damme, 2009. *Spodoptera littoralis*-induced lectin expression in tobacco. *Plant Cell Physiol.*, 50: 1142-1155.
- Vandenborre, G., K. Groten, G. Smaghe, N. Lannoo, I.T. Baldwin and E.J.M. Van Damme, 2010. *Nicotiana tabacum* agglutinin is active against Lepidopteran pest insects. *J. Exp. Bot.*, 61: 1003-1014.
- Vershinina, Z.R., A.K. Baimiev and A.V. Chemeris, 2010. Symbiotic reactions of sea buckthorn roots transformed with the pea lectin gene. *Russian J. Plant Physiol.*, 57: 101-109.
- Wearne, K.A., H.C. Winter, K. O'Shea and I.J. Goldstein, 2006. Use of lectins for probing differentiated human embryonic stem cells for carbohydrates. *Glycobiology*, 16: 981-990.
- Yagi, F., T. Iwaya, T. Haraguchi and I.J. Goldstein, 2002. The lectin from leaves of Japanese cycad *Cycas revoluta* Thunb. (gymnosperm) is a member of the jacalin-related family. *Eur. J. Biochem.*, 269: 4335-4341.
- Yeasmin, T., A.K. Tang, A. Hossain and N. Absar, 2007. Effects of physico-chemical agents on the biological activities of the mulberry seed lectins. *Asian J. Biochem.*, 2: 111-117.
- Zhang, W., W.J. Peumans, A. Barre, C.H. Astoul and P. Rovira *et al.*, 2000. Isolation and characterization of a jacalin-related mannose-binding lectin from salt stressed rice (*Oryza sativa*) plants. *Planta*, 210: 970-978.