WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 1, 1707-1719. Research Article ISSN 2277– 7105

IMMUNOMODULATORY ACTIVITY OF LECTIN EXTRACTED FROM BARK OF THE BLACK MULBERRY (*MORUS NIGRA*)

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Article Received on
08 Nov 2014,

Revised on 03 Nov 2014,
Accepted on 28 Dec 2014

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ABSTRACT

A lectin present in the *Morus Nigra* was extracted by soluble proteins (crude extract) in phosphate buffer (0.1M, pH 7.2). The lectin agglutinated specifically rabbit erythrocytes. The hemagglutinating activity assay showed that the lectin was shown an agglutination to A blood human group. Extracted lectin of *Morus Nigra* showed thermo stability in more than 100°C. However, the lectin was stable in the PH ranged between 3 to12 and was showen to be inhibited by the glycoproteins fetuin and casein and by the sugars D-glucosamine, xylose and galacto-pyranose. Immunomodulatory activity of extracted lectins from *Morus Nigra* was evaluated on phagocytic activity by carbon clearance test. Adult Albinos Wistar mice randomly divided into four groups, were the first was served as a control, while the remaining groups respectively treated with extracted lectins from *Morus Nigra* at dose of: 25, 50 and 100 mg/kg by intra-peritoneal injection (IP). Change in phagocytic activity was determined after 48 h injection of carbon ink suspension. In carbone clearance test, extracted lectins from *Morus Nigra* exhibited significantly phagocytic index dose-dependent against control group, indicating stimulation of the reticulo-endothelial system. Present study thus reveals that extracted lectins from *Morus Nigra* holds promise as immunomodulatory agent, which act by stimulating dose dependent phagocytic function.

KEYWORDS: Immunomodulatory, Carbon Clearance rate, *Morus Nigra*.

INTRODUCTION

Lectins constitute a group of proteins or glycoproteins of non-immune origin, which bind reversibly to carbohydrates and usually agglutinate cells or precipitate polysaccharides and glycoconjugates.[1] The lectins were redefined by Peumans & Van Damme (1995)[2] as proteins possessing at least one non-catalytic domain, which binds reversibly to a specific mono or oligosaccharide. However, according to Cummings (1997),[3] antibodies and proteins with enzymatic activity related to carbohydrates can not be considered as lectins. As a consequence of their chemical properties, they have become a useful tool in several fields of biological research (immunology, cell biology, membrane structure, cancer research and genetic engineering). Lectins are present in a wide range of organisms from bacteria to animals, being present in all classes and families, although not in all the kinds and species.[4] Many flowering plants from diverse taxonomic groups accumulate large quantities of so-called vegetative storage proteins (VSPs) in various vegetative storage organs. These VSPs play a primary role in nitrogen accumulation, storage, and distribution in biennial and perennial plants, and, accordingly, are believed to contribute to the survival of the plant in its natural environment.[5] Moreover, some VSPs with a particular enzymatic or other biological activity act as aspecific defense proteins against herbivorous animals or phytophagous invertebrates, for example, and hence may play a dual storage/defense role.[6,7] The concept of functional "vegetative" homologs of the classic seed storage proteins was originally developed for two proteins, called VSP_ and VSP_, that accumulate in large quantities in soybean (*Glycine max*) leaves, seed pods, and hypocotyls forced to act as a nitrogen sink.[8,9] However, it is evident that the term VSP also applies to the previously identified major tuber proteins from potato (*Solanum tuberosum*) patatin,[10] and sweet potato *Ipomoea batatas*; sporamin,[11] as well as to all other abundant proteins found in various vegetative storage organs like bulbs, tubers, and rhizomes. VSPs are also common in the bark of deciduous trees. Studies with poplar *Populus deltoids*,[12] elderberry *Sambucus nigra*,[13] and several legume trees not only demonstrated the occurrence of abundant bark-specific VSPs, but also led to the identification of some of these bark VSPs. Thereby, it was observed that the bark of the black mulberry (*Morus nigra*) tree accumulates high concentrations of a Gal- and a Man-specific JRL. It should be mentioned here that at present, the family of JRLs is subdivided into Gal- and Manspecific agglutinins.[14,15] Jacalin and its Gal-specific homologs are made up of four identical protomers each consisting of a heavy (α_) and a light (_β) polypeptide chain. Both chains are derived from a large preproprotein through a complex co- and posttranslational processing, but they remain together by noncovalent interactions. Jacalin

follows the secretory pathway and eventually accumulates in storage protein vacuoles.[15] in the present work we describe the extracted of a new lectin from the *Morus Nigra* collected from Algeria and investigated the immunomodulatory effect by using phagocytic activity by carbon clearance test in vivo experimental model mice.

MATERIALS AND METHODS

The lectins extracted from *Morus Nigra* used in this work originated from Algerian. Human blood group A, B and O erythrocytes were collected from healthy donors. Rabbit was obtained by venous puncture of healthy animals.

Preparation of Extracts

Seeds of *Morus Nigra* were grounder to be a fine powder using blender to top speed. The dry powder was incubated in phosphate buffer (0.1M, pH 7.2) for approximately 24h at 4°C. The mixture was then centrifuged at 6000 rpm for 30 min, the remaining debris was removed by passing the supernatant through filter paper.[16] The supernatant was applied to a gel chromatography on dextran G-75. Following that, the fractions contained lectins were dialyzed against distilled water and then lyophilized, the lyophilized extracts were dissolved in 0. 9% NaCl and injected interperitoneally into mice at concentrations of 25, 50 and 100 mg/Kg body weight for determination of phagocytic activity.

Preparation of Sephadex G75

4g of dephadex G75 was suspended in 100ml of phosphate buffer (0.1M, PH: 7.2). The mixture was then incubated for 48h at room temperature. Finally it was packed into a 12x1.2 column for used for extracted lectin of *Morus Nigra.*

Extracted lectin from *Morus Nigra* by dephadex G75

Supernatant sample of *Morus Nigra* were loaded into sephadex G75 column equilibrated with phosphate buffer (0.1M, PH7.2). The absorbance at 280 nm was used to estimate protein content in column eluates.

Hemagglutinin Assay

The experiment was performed in microtiter plates, according to Correia and Coelho (1995)17. Agglutination activity was measured in micro-titer plates using serial two fold dilutions of lectins. Each well contained 50µl of rabbit red blood cells (3%) and 50µl of extracted lectins at room temperature the results were read after one hour.

Inhibition Tests

Inhibition tests were carried out using stock solutions (in 0.9% NaCl) of sugars and glycoproteins. A two-fold dilution series was prepared for each substance in 0.9% NaCl with a final volume of 50 µL. Aliquots of the diluted lectin were added to each tube of the diluted inhibitor series. The mixture was incubated at room temperature for 1 h, before the addition of the erythrocytes suspension (50µL). The hemagglutination inhibition activity was recorded as the highest sugar dilution which inhibited the agglutinating activity.

PH Test

The buffers used to study the stability of *Morus Nigra* lectin under different conditions of pH were phosphate buffer (0.1M) at different PH (1 to 12).

Heat Stability Test

The heat stability of the hemagglutinating activity of *Morus Nigra* lectin was determined by incubation of aliquots of lectin solution at different temperatures (40, 60, 80 or 100°C) for 1h and the remaining hemagglutinating activity determined.

Phagocytic Activity

Animals *Albinos Wistar* mice were housed under hygienic conditions in the departmental animal house. Animals were housed under standard conditions of temperature (21±1°C), and up to 12h of light daily, fed with standard pellet diet, and had free acess to water. All the experiments were performed in accordance with the institutional animal ethics committee.

Phagocytic activity index was determined as per the method reported by Cheng *et al*., 2005.[19] Phagocytic activity of reticulo-endothelial system was assayed by carbon clearance test. Phagocytic index was calculated as a rate of carbon elimination of reticulo-endothelial system by clearance test. In this test four groups of animals were used. GroupI was kept as a control, while animals of treatment group: II, III and VI were administrated extracted lectins from *Morus Nigra* at dose of: 25, 50and 100mg/kg by interperitoneally injection respectively. After 48 h, phagocytic activity was determined. Mice were injected with Carbon ink suspension at a dose 0.1 ml/100g via tail vein, the mixture consisted of black carbon ink 3ml, saline 4ml and 3% gelatine solution 4ml. Blood samples were taken from the retro orbital vein by using glass capillaries, at 5 and 15 min. Blood sample drops[14] were mixed with 0.1% sodium carbonate solution (4ml) for the lysis of erythrocytes and the absorbance measured at 675 nm using a spectrophotometer.

The phagocytic activity is expressed by the phagocytic index K which measures all the reticulo-endothelial system function in the contact with the circulating blood. The clearance rate is expressed as the half-life period of the carbon in the blood ($t_{1/2}$, min). These are calculated by means of the following equations.[20]

$$K = \frac{lnOD1 - lnOD2}{t2 - t1}, \quad t_{1/2} = \frac{0.963}{k}$$

Where OD_1 and OD_2 are the optical densities at times t_1 and t_2 respectively.

Statistical Analysis

The data were subjected to student *t* test for comparison between groups. The values are expressed as mean ± SEM. Significance level was set at $P<0.05$, $P<0.01$, $P<0.001$.

RESULTS

Extracted Lectin from *Morus Nigra* by Sephadex G75

It was found that in elution fraction of *Morus Nigra* presented on pick (Fig.1).

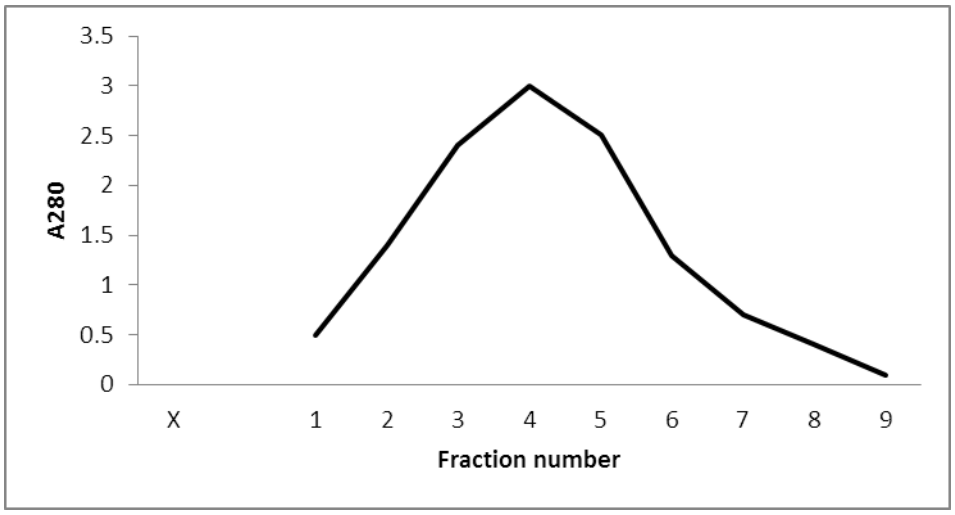

Figure 1: Extracted lectin from the Morus Nigra by sephadex G75.

Hemagglutinin Assay

The extracted lectin from *Morus Nigra* showed a highly agglutination when addition of rabbit erythrocytes suspension (Fig. 2).

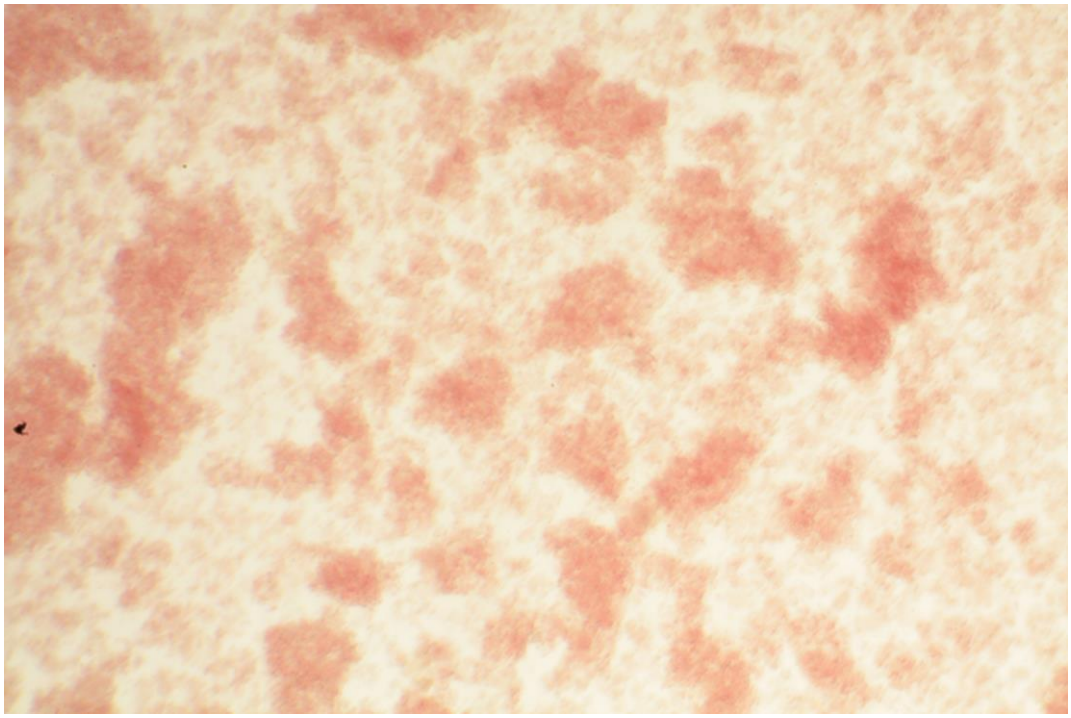

Figure 2: hemagglutinin of lectin extracted from Morus Nigra with suspension of rabbit erythrocytes GX40.

Inhibition Tests

The results of sugar inhibition tests using a large number of simple sugars and glycoproteins for *Morus Nigra* lectin are presented in table1 and 2.

The extracted lectin from *Morus Nigra* did not show any inhibition by all simple sugars tested, only with D-glucosamine, xylose and galacto-pyranose at 200mM concentration. of the glycoproteins tested, only fetuin and casein were inhibitory requiring the same concentration.

Table1: Inhibition of the heamagglutinating activity of the lectin extracted from the Morus Nigra by glycoproteins.

Glycoproteins	Hemagglutinating activity
Fetuin	+
Insuline	-
Casein	+
BSA	-
Ovalbumin	-

+: Inhibition of the heamagglutinating activity.

-: non inhibitory.

Table2: Inhibition of the heamagglutinating activity of the lectin extracted from the Morus Nigra by Sugars.

Sugars	Hemagglutinating activity
Glucose	-
Galactose	-
Lactose	-
Mannose	-
D-glucosamise	+
Xylose	+
Galactopyranose	+
Manitol	-
Maltose	-
Melibiose	-
inositol	-
Fucose	-
Raffnose	-
Arabinose	-
Fructose	-
N-acétyl-glusamine	-
Sorbose	-
Saccharose	-
Methyl-fucopyranoside	-
Methyl-mannopyranoside	-
N-acétyl-galactosamine	-
Sorbitol	-
Methyl-B-L-fucopyranoside	-
Xylitol	-
Cellulose	-
Rhamnose	-

+: Inhibition of the heamagglutinating activity.

-: non inhibitory.

Effect of PH on Heamagglutinating Activity of Extracted Lectin from *Morus Nigra*

The extracted lectin was stable in the PH 3to12 retaining 50% of its hemagglutinating activity at PH 2 (Table 3).

Table 3: effect of PH on heamagglutinating activity of extracted lectin from Morus Nigra.

PH	1	2	3	4	5	6	7	8	9	10	11	12
Heamagglutinating activity	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

+++: highest heamagglutinating activity.

Effect of Heat on Heamagglutinating Activity of Extracted Lectin from *Morus Nigra*

In addition, the hemagglutinating activity of extracted lectin from *Morus Nigra* when submitted to heat treatment was stable until 100°C during 1h (Table 4).

Table 4: effect of Heat on heamagglutinating activity of extracted lectin from Morus Nigra.

Heat	40°C	60°C	80°C	100°C
Heamagglutinating activity	+++	+++	+++	+++

+++: highest heamagglutinating activity.

Blood Human Test (ABO)

Extracted lectin from *Morus Nigra* presented a spécifique highly agglutination to B blood human group (Table 5).

Table 5: effect of suspension erythrocyte human on heamagglutinating activity of extracted lectin from *Morus Nigra*.

Blood human	A	B	O
heamagglutinating activity	+++	---	---

+++: highest heamagglutinating activity

---: non heamagglutinating activity

Effects of Lectins Extracted from *Morus Nigra* on Phagocytic Activity

Significant increase in phagocytic activity was observed in treated group dose -dependent were compared with control (Figure 3).

Effects of Lectins Extracted from *Morus Nigra* on Half-Time $T_{1/2}$ of Carbon in Blood

Figure 4 show a significant decrease in half-time of carbon in blood dose-dependent in treated group were compared with control.

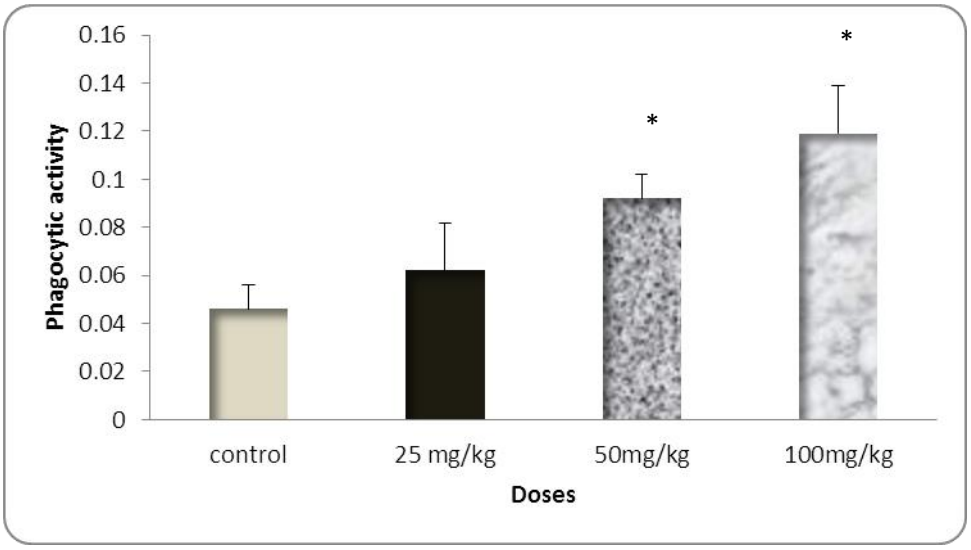

Figure 3: Effect of lectins extracted from *Morus Nigra* on phagocytic activity.

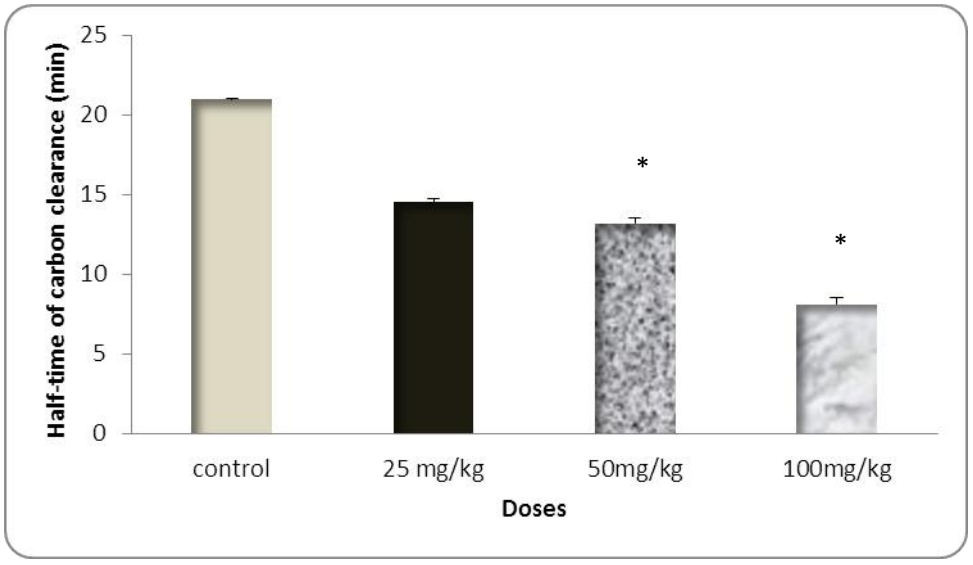

Figure 4: Effect of lectins extracted from *Morus Nigra* on half -life $t_{1/2}$ of carbon in blood.

DISCUSSION

Cross-linked guar-gum, a galactomannan consisting of chains of (1→4) linked β-D-manose with α-D-galactose linked (1→6) as single unit side chains, has been used as an efficient, inexpensive and rapid general affinity medium for the purification of lectins from land plants.[21] The utilization of affinity chromatography is also an important tool in the process of purification of algae lectins. Many lectins from these vegetables were isolated by this technique, such *Ptilota filicina* J. Agardh,[21] *Enantiocladia duperreyi*[22] and *Caulerpa cupressoides.*[23] The hemagglutination inhibition studies carried out with purified *Morus Nigra* lectin, revealed that the lectin is not inhibited by simple sugars but by glycoproteins.

This is in general agreement with those found for the numerous marine algal lectins, such as *Cystoclonium purpureum* (Huds.) Batters, *Solieria chordalis* (C. Agardh) J. Agardh, *Plumaria elegans* and *Ptilota serrata* (24), *Gracilaria bursa-pastoris* (Gmelin) Silva.[25] *Solieria filiformis*,[26] and *Gracilaria verrucosa* (Hudson) Papenfus.[27] *Morus Nigra* lectins are, in general, more specific for complex oligosaccharides often glycoproteins.[13] Therefore, the inhibition of the hemagglutinating activity from the *Morus Nigra* lectins by glycoproteins was also observed in some marine algal, such as *Agardhiella tenera* Schmitz, *Ulva lactuca*,[28] *Bryothamnion seaforthii* (Turner) Kützing and *B. triquetrum*,[29] and *Amansia multifida* Lamouroux.[30] The hemagglutinating activity from the *Morus Nigra* lectin was not affected by exposure to a temperature of100°C for 1h . The absence of carbohydrate in the structure of the lectin differ of the observed to another lectins from marine algae: *Codium tomentosum* (Huds) Stackhouse, *Bryothamnion seaforthii* and *Bryothamnion triquetrum*,[29] *Solieria filiformis* (Kützing) Gabrielson,[26] *Enantiocladia duperreyi*[22] and *Caulerpa cupresssoides*.[23]

The reticulo-endothelial system (R.E.S)consist of the spleen, thymus and other lymphoid tissues, together with cells lining the sinuses of the spleen, bone marrow, and lymph nodes and capillary enthelium of the liver (kuppfers cells), and of the adrenal and pituitary glands, these comprise the sessile or fixed macrophage, are transported by the body fluids or wander through the tissues. The RES is the best defined functionally by its ability to scavenge debris or other foreign matter and forms first line of defense. the rate of removal of carbon particles, by the sessile intravascular phagocytes in the liver and spleen, from the blood stream is a measure of reticulo-endothelial phagocytic activity. In the present study, carbon clearance test, extracted lectin from *Morus Nigra* treated groups, exhibited significantly high phagocytic index.[31] This indicates stimulation of the reticulo-endothelial system by drug treatment. It may be possible that the extracted lectin from *Morus Nigra* influence the mechanism of phagocytosis, largely distributed monocytes macrophages or R.E.S which result in significant increase in the phagocytic index with carbon clearance test.[32]

REFERENCES

1. Goldstein, I.J., Hughes, R.C., Monsigny, M., Ozawa, T. & Sharon, N. What should be called a lectin? Nature, 1980; 285: 60.
2. Peumans, W.J. & Van Damme, W.J.N. Lectin as plant defense proteins. Plant Physiology, 1995; 109: 347-352.

3. Cummings, R.D. 1997. Lectins as tools for glycoconjugate purification and characterization. *In* Glyco-science, status and perspectives. (H.J. Gabius & S. Gabius, eds.) Champman & Hall GmbH, Weinheim, 1981; 191-199.
4. Lis, H. & Sharon, N. Lectins in higher plants. The Biochemistry of Plants 6:371-447.
5. Staswick PE. Storage proteins of vegetative plant tissues. Annu Rev Plant Physiol Mol Biol, 1994; 45: 303–322.
6. Peumans WJ, Van Damme EJM. Lectins as plant defense proteins, 1995.
7. Yeh KW, Chen JC, Lin MI, Chen YM, Lin CY. Functional activity of sporamin from sweet potato (*Ipomoea batatas* Lam.): a tuber storage protein with trypsin inhibitory activity. Plant Mol Biol, 1997; 33: 565–570
8. Staswick PE. Developmental regulation and the influence of plant sinks on vegetative storage protein gene expression in soybean leaves. Plant Physiol, 1989a; 89: 309–315
9. Staswick PE. Preferential loss of an abundant storage protein from soybean pods during seed development. Plant Physiol, 1989b; 90: 1252–1255
10. Mignery GA, Pikaard CS, Hannapel DJ, Park WD. Isolation and sequence analysis of cDNAs for the major potato tuber protein, patatin. Nucleic Acids Res, 1984; 12: 7987–8000
11. Maeshima M, Sasaki T, Asahi Y. Characterization of the major proteins in sweet potato tuber roots. Phytochemistry, 1985; 24: 1899–1902
12. Coleman GD, Chen TH, Ernst SG, Fuchigami L. Photoperiod control of poplar bark storage protein accumulation. Plant Physiol, 1991; 96: 686–692
13. Nsimba-Lubaki M, Peumans WJ. Seasonal fluctuations of lectin in bark of elderberry (*Sambucus nigra*) and black locust (*Robinia pseudoacacia*). Plant Physiol, 1986; 80: 747–751
14. Van Damme EJM, Barre A, Bemer V, Rouge´ P, Van Leuven F, Peumans WJ. A lectin and a lectin-related protein are the two most prominent proteins in the bark of yellow wood (*Cladrastis lutea*). Plant Mol Biol, 1995a; 29: 579–598
15. Peumans WJ, Hause B, Van Damme EJM. The galactose-binding and mannose-binding jacalin-related lectins are located in different subcellular compartments. FEBS Lett, 2000a; 477: 186–192
16. Hamshou M, Smagghe G, Van Damme, EJM. Entomo-toxic effects of fungal lectin rhizoctonia solani towards spondoptera littoralis. Fungal Biol, 2010; 114(1): 34-40.

17. Correia, MTS, Coelho, LCBB. Purification of a glucose/mannose specific lectin, isoform1, formseeds of cratylia mollis mart. (Camaratu bean). Appl. Biochem. Biotechnol, 1995; 55(3): 261-273.
18. Cheng W, Li J, You T, Hu C. Anti-inflammatory and immunomodulatory activities of the extracts from the inflorescence of Chrysanthemum indicum Linné. *Journal of Ethnopharmacology*, 2005; 101(1-3): 334-7.
19. Shah AS, Wakade AS, Juvekar AR. Immunomodulatory activity of methanolic extract of Murraya koenigii (L) Spreng leaves. *Indian Journal of Experimental Biology*, 2008; 46(7): 505-9.
20. Sampaio, A.H., Rogers, D.J. & Barwell, C.J. A galactose-specific lectin from the red marine alga *Ptilota filicina*. Phytochemistry, 1998b; 48: 765-769.
21. Benevides, N.M.B., Holanda, M.L., Melo, F.R., Pereira, M.G., Monteiro, A.C.O. & Freitas, A.L.P. Purification and partial characterization of the lectin from the marine green alga *Caulerpa cupressoides* (Vahl) C. Agardh. Botanica Marina, 2001; 44: 12-22.
22. Benevides, N.M.B., Silva, S.M.S., Oliveira, S.R.M., Melo, F.R., Freitas, A.L.P &Vasconcelos, I.M. Proximate analysis, toxic and antinutritional factors of ten Brazilian marine algae. Revista Brasileira de Fisiologia Vegetal, 1998b; 10: 31-36.
23. Benevides, N.M.B., Holanda, M.L., Melo, F.R., Pereira, M.G., Monteiro, A.C.O. & Freitas, A.L.P. Purification and partial characterization of the lectin from the marine green alga *Caulerpa cupressoides* (Vahl) C. Agardh. Botanica Marina, 2001; 44: 12-22.
24. Rogers, D.J., Fish, B. & Barwell, C.J. Isolation and properties of lectins from two red marine algae: *Plumaria elegans* and *Ptilota serrata*. *In* Lectins: biology, biochemistry, clinical biochemistry. (T.C. Bog-Hansen & D.L.J Freed, eds.). Sigma Chemical Company, St. Louis, 1990; 7: 49-52.
25. Okamoto, R., Hori, K., Miyazawa, K. & Ito, K. Isolation and characterization of a new hemagglutinin from the red alga *Gracilaria bursa-pastoris*. Experientia, 1990; 46: 975-977.
26. Benevides, N.M.B., Leite, A.M. & Freitas, A.L.P. Atividade hemaglutinante na alga vermelha *Solieria filiformis*. Revista Brasileira de Fisiologia Vegetal, 1996; 8: 117-122.
27. Kakita, H., Fukuoka, S., Obika, H., LI, Z.F. & Kamishima, H. Purification and properties of a high molecular weight hemagglutinin from the red alga *Gracilaria verrucosa*. Botanica Marina, 1997; 40: 241-247.
28. Sampaio, A.H., Rogers, D.J. & Barwell, C.J. Isolation and characterization of the lectin from the green marine alga *Ulva lactuca* L. Botanica Marina, 1998a; 41: 427-433.

29. Ainouz, I.L., Sampaio, A.H., Freitas, A.L.P., Benevides, N.M.B. & Mapurunga, S. Comparative study on hemagglutinins from the red algae *Bryothamnion seaforthii* and *Bryothamnion triquetrum*. Revista Brasileira de Fisiologia Vegetal, 1995; 7: 15-19.
30. Rogers, D.J., Fish, B. & Barwell, C.J. Isolation and properties of lectins from two red marine algae: *Plumaria elegans* and *Ptilota serrata*. *In* Lectins: biology, biochemistry, clinical biochemistry. (T.C. Bog-Hansen & D.L.J Freed, eds.). Sigma Chemical Company, St. Louis, 1990; 7: 49-52.
31. Necib Y, Bahi A, Zerizer S, Cherif A, Boulakoud M S. Immunomodulatory Activity of Argan oil (*Argania Spinosa. L*). *International Journal of Pharma Sciences Review and Research*, 2013; 23(1): 11, 57-59
32. Necib Y, Bahi A, Zerizer S. Immunomodulatory Effect of Argan oil (*Argania spinosa. L*) After Exposure to Mercuric Chloride in Mice. *International Journal of Pharma Sciences Review and Research,* 2013; 23(1): 37, 191-193.