INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 20, No. 2, p. 69-81, 2022

RESEARCH PAPER OPEN ACCESS

Anti-oxidant and Immunomodulatory Effects of newlectins extracted from Algerian plants: *Ephedra alata, Zizyphus jujuba and Calycotome villosa*

Ahlem Bahi, Youcef Necib*, Fateh Merouane

Laboratory of Microbiological Engineering and application,Faculty of Natural and Life Sciences, University Mentouri Brothers Constantine1, Algeria

Key words: Extracted lectins, Haemagglutination, Immunomodulatory, Anti-oxidant.

http://dx.doi.org/10.12692/ijb/20.2.69-81 Article published on February 7, 2022

Abstract

The lectins present in plants of *Ephedra alata, Zizyphus jujuba and Calycotome villosa* were extracted by soluble proteins (crude extract) in phosphate buffer (0.1M, pH 7.2). Peptide fraction and purified peptide of *Ephedra alata, Zizyphus jujube and Calycotome villosa* showed the highest anti-oxidant activity in the DPPH, SOD and Ferric reducing assay radical scavenging model. Extracted lectin of *Ephedra alata, Zizyphus jujubaand Calycotome villosa* showed the thermostability is more than 100°C. However, the lectin of *Ephedra alata, Zizyphus jujub aand Calycotome villosa* was stable in the pH ranging between 3-12, 2- 12 and 1- 12respectively and were not inhibited by the sugars. Immunomodulatory activitiesof extracted lectins from *Ephedra alata, Zizyphus jujube and Calycotome villosa* were evaluated on phagocytic activity by carbon clearance test. Adult *Albinos wistar* mice randomly divided into three groups were the first served as a control, while the remaining groups respectively treated with extracted lectins from plants of *Ephedra alata, Zizyphus jujubaand Calycotome villosa* at the dose of 25 and 100 mg/kg by intraperitoneal injection (IP). Change in phagocytic activity was determined after 48 h injection of carbon ink suspension. In carbone clearance test, extracted lectins from *Ephedra alata, Zizyphus jujube and Calycotome villosa* exhibited significantly phagocytic index dose-dependent against the control group, indicating stimulation of the reticulo-endothelial system. The present study thus reveals that extracted lectins from *Ephedra alata, Zizyphus jujuba and Calycotome villosa* holds promise as an immunomodulatory agent, which acts by stimulating dose-dependent phagocytic function.

* **Corresponding Author:** Youcef Necib ✉ bahiahlem@yahoo.fr

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 (Goldstein *et al.,* 1980). The lectins were redefined as proteins possessing at least one non-catalytic domain, which binds reversibly to a specific mono or oligosaccharide (Penman's and Van Damme, 1995).However, antibodies and proteins with enzymatic activity related to carbohydrates cannot be considered as lectins (Cumming, 1997). 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 (Lis & Sharon, 1981). Lectins are heterogeneous proteins of non-immune origin and with at least one non-catalytic domain. Lectins are able to specifically recognise carbohydrates (Carlini and Grossi-de-Sa, 2002). These molecules could reversibly bind to carbohydrates without altering their covalent structure (Pusztai and Bardocz, 2009). Lectins have been extensively distributed in nature. These molecules may have several functions in living organisms, but the entomotoxic properties of plant lectins are important in pest control strategies (Michiels *et al.,* 2010). In fact, the majority of plant lectins bind to O- and N-glycans of animal glycoconjugates. This means that lectins are supposed to play a part in plant defense against plant-eating (phytophagous) invertebrates or higher vertebrates (Peumans and Van Damme, 1995). Certain plant tissues, such as seeds, bark and bulbs, contain high lectin concentrations, which might indicate that lectins play a role as storage proteins (Michiels *et al.,* 2010). Lectins extracted from different plant sources exhibit a considerable degree of structural similarity but also considerable differences in their carbohydrate-binding specificities (Carlini and Grossi-de-Sa, 2002). Decades of research have led to the classification of plant lectins into twelve lectin families (Van Damme *et al.,* 2008). Several phenomena induce the expression of lectin including salt stress, pathogen infection, jasmonic acid treatment and insect herbivory (Michiels *et al.,* 2010). In the present work, we describe the extraction of a newlectin from the roots plants of *Ephedra alata, Zizyphus jujubaChristi and Calycotome villosa*collected from Algeria and their immunomodulatory and anti-oxidants effects.

Materials and methods

The lectins extracted from roots plants *Ephedra alata, Zizyphus jujubaand Calycotome villosa*used in this work originated from Algerian. The *Ephedra alata* was collected from Oued souf *Zizyphus jujuba and Calycotome villosa* were collected from Mila. Human blood groups A, B and O erythrocytes werecollected from healthy donors. The rabbit was obtained by venous puncture ofhealthy animals.

Extraction of lectins

Therootsof each plant of *Ephedra alata, Zizyphus jujuba and Calycotome villosa* were washed briefly, roughly ground and then homogenized in a chiled warning blender with phosphate buffer saline pH7.2. The homogenized was then centrifuged at 6000 rpm for 30min, the remaining debris was removed by passing the supernatant through filter paper (Hamshou *et al.,* 2010). The supernatant was applied to (10 x 1.2 cm) column of pr-swollen sephadex G25 and G75.Active material was eluted from the column with the solution of PBS, fractions of 5ml were collected in each tube and the absorbance was measured at 280 nm in 1 cm path length cell using spectrophotometer UV.

Other fraction of supernatant was applied to a DEAE cellulose column (10× 1.2 cm) previously equilibrated with phosphate buffer (0.1 M, pH 7.2) and eluted with the same buffer by maintaining a flow rate of 5ml/h. The elution profile was monitored at absorbance of 280 nm. Aftereluting the unadsorbed fractions, adsorbed fractions were eluted with linearconcentration gradient of 0–0.3 M NaCl in phosphate buffer (0.1 M, pH 7.2). All fractions were

collected and tested for hemagglutination activity. All fractions collected were lyophilized and used foranti-oxidant assay.

Hemagglutinin assay

The experiment was performed in microtiter plates, according to Correia and Coelho (1995).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.

Metal ions test

To evaluate the effect of metal ions and EDTA on hemagglutinating activity, serial aliquots of two-fold dilutions of lectin solution were previously dialysed against 5 mMEDTA. The material wasused for hemagglutination assays in the absence and presenceof either 5 mM $CaCl_2$, $MgCl_2$, or $MnCl_2$. The hemagglutinating activity was measured by the addition of rabbit erythrocytes.

Inhibition tests

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

pH test

Different buffer solutions with pH ranging from 1 to 12 were prepared to study the stability of extracted lectins of*Ephedra alata, Zizyphus jujuba and Calycotome villosa*.

Heat stability test

The heat stability of the hemagglutinating activity of roots plants *Ephedra alata, Zizyphus jujuba and Calycotome villosa* lectin was determined by incubationof aliquots of lectin solutions at different temperatures (40, 60, 80 or 100°C) for 1h and the remaining hemagglutinating activity was determined.

Limite hemagglutinating activity test

This test determines the agglutinative power and deducts the title lectin. In a first step, 50 µl of buffer were added to each well, a volume of 50µl of the extract was added to the first well and arrange of concentration by double dilution was performed in the subsequent wells. Avolumeof50 µl red blood cells was addedto50µlof extract to each well. Reading hem agglutinating activity was carried out after 1 hour at room temperature ambient.

The hemagglutinating activity is expressed as which is the reciprocal of the greatest dilution ratio for which hemagglutination is observed.

In-Vitro antioxidant assay

Assay for in vitro DPPH- free radical scavenging activity (DPPH assay)

The change in absorbance at 517nm has been used as a measure of anti-oxidant property. The assay was standardized using ascorbic acid as standard. The reaction mixture 4mlcontained, 0.1ml of (5 mg/ml) various concentration of lectin samples in0.1M Phosphate buffer (pH 7.2), 3.9 ml of DPPH (0.025gm/l) solution was added to all theabove test tubes. Incubated at room temperature in the dark for 30 minutes. A blank determination with 0.1ml methanol solution instead of standard treated similarly was maintained. For control 0.1ml of methanol with 3.9ml of DPPH solution was used. The optical density was measured at 517 nm using a spectrophotometer according to the method (Oyaizu *et al*., 1986). The scavenging activity of DPPH free radical in percent was calculated according to the equation.

Percent inhibition % = (A-B) x100/A

Where: A= Absorbance of control reaction.
B= Absorbance of the test sample.

Superoxide anion scavenging activity

Superoxide anion radical scavenging activity was measured (Duan X *et al.*2007) with some modifications. The various fractions of purified lectins were mixed with 3 ml of reaction buffer solution (pH 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM NBT. The reaction solution wasilluminated by exposure to 30W fluorescent lamps for 20 minutes and the absorbance wasmeasured at 560 nm using a spectrophotometer. Ascorbic acid (0.5 mM) was dissolved in methanol and used as a standard to convert the inhibition capability of purified lectins to the Ascorbic acid equivalent.

The reaction mixture without any sample was used asthe negative control. The Superoxide anion radical scavenging activity (%) was calculated as: [(Ao–A1)/Ao] × 100 where Aowas the absorbance of the control and A1was the absorbance of purified lectins

Ferric (Fe^{3+}) reducing power assay

Ferric cyanide (Fe^{+3}) reducing power was determined by the method [11] Fe^{+3} reducing power as an indicator of anti-oxidant activity is widely accepted. In this method anti-oxidant compounds give a colored complex with potassium ferricynaide in the presence of trichloroacetic acid and ferric chloride, which is measured at 700nm. An increase in the absorbance of the reaction mixture indicated the reducing power of the sample. The reaction mixture 9 mlcontained 1ml of 50, 100, 150, 200µg/ml of purified lectins, mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was mixed well and was incubated for 20min at 50°C, cooled rapidly and mixed with 2.5 ml of 10% trichloroacetic acid and 0.5ml of 0.1% ferric chloride. Ablank was prepared with 1ml methanol instead of samples, treated similarly was maintained.

The intensity of iron (II) – ferricynaide complex was determined by measuring the formation of Perls Prussian blue at 700 NM after 10 min. The higher absorbance of the reaction mixture indicates increased reducing power. Ascorbic acid is used as a standard control.

The relative percentage reducing the power of the sample as compared to the maximum absorbance tested which appeared in Ascorbic acid at 10µg/ml was calculated by using the formula:

(A-Amin) / (A max-A min) ×100.

Here, Amax = absorbance of maximum absorbance tested, Amin= absorbance of minimum absorbance tested and A= absorbance of the sample

Protein determination

The protein concentrations of the crude and purified lectins of were determined by the method (Bradford, 1976). Bovine serum albumin was used for standard preparations.

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 access to water. All the experiments were performed in accordance with the institutional animal ethics committee.

The phagocytic activity index was determined as per the method reported by Cheng*et al.* (2005). Phagocytic activity of reticulo-endothelial system was assayed by carbon clearance test. The 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 and III were administrated extractedlectins of plants *Ephedra alata, Zizyphus jujuba and Calycotome villosa* at the dose of 25 and 100mg/kg by interperitoneally injection, respectively. After 48h,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 10 min. Blood sample drops (14) were mixed with 0.1% sodium carbonate solution (4ml) for the lysis of erythrocytes and the absorbance was measured at 675 nm using a spectrophotometer. The phagocytic activity is expressed by the phagocytic index K, which measures all the reticulo-endothelial system functions in 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 (Shah*et al.,* 2008):

$$K = \frac{\ln OD1 - \ln OD2}{t2 - t1}, \quad t_{1/2} = \frac{0.983}{k}, \quad \alpha = \sqrt[3]{K} \frac{\text{Body Weight of animal}}{\text{Liver + Spleen wt}}$$

Where OD_1 andOD_2 are the optical densities attimes t_1 and t_2 respectively.

Statistical analysis

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

Results

The extracted lectin fromEphedra alata, Zizyphus jujuba and Calycotome villosa by sephadex G25 and G75

It was found that in elution fraction ofof*Ephedra alata, Zizyphus jujuba and Calycotome villosa* presented one pick in sephadex G25 and sephadex G75, but the*Calycotome villosa*presented one pick in sephadex G25 and two picks in sephadex G75, which explainthe presence probably two types of lectins in this extract (Fig.1).

Determination of Protein concentration

The purified lectins show a high concentration of protein after purification with values of 0.11 and 0.252 mg/ml in plants of *Ephedra alata and Calycotome villosa,* respectively.

However, a purified lectin from the plant *Zizyphus jujuba*presented a lower concentration of protein (0.085mg/ml) (Table 1).

Table 1. Protein concentration in crude and purified lectins of *Ephedra alata, Zizyphus jujuba and Calycotome villosa.*

	Roots of plants	Protein concentration (mg/ml)
Crude	*Ephedra alata*	0.38±0.01
	Zizyphus jujuba	1.79±0.02
	Calycotome villosa	2.19±0.01
Purified lectins	*Ephedra alata,*	0.11±0.002
	Zizyphus jujuba	0.085±0.002
	Calycotome villosa	0.252±0.001

The value represents in the results are mean ± SEM of three replicates.

In-vitro anti-oxidant activity of fraction peptides and purified lectins of Ephedra alata, Zizyphus jujuba and Calycotome villosa

Peptide fraction A and purified peptide of *Ephedra alata* showed the highest anti-oxidant activity up to 50.54% and 79.97%, 55.5% and 62.3% and 64.1% and 70.11% in the DPPH, SOD and Ferric reducing assay radical scavenging model, respectively. Peptide fraction C and purified peptide of *Zizyphus jujuba* showed the highest anti-oxidant activity up to 55.49% and 76.3%, 68.1% and 70.9% and 68.2% and 68.9.11% in the DPPH, SOD and Ferric reducing assayradical scavenging model, respectively. Peptide fraction A and purified peptide of *Calycotome villosa*showed the highest anti-oxidant activity up to 62.63% and 76.7%, 67.2% and 70.7% and 53.9% and 55.2% in the DPPH, SOD and Ferric reducing assayradical scavenging model, respectively (Table 2).

Hemagglutinin assay

The extracted lectins from the plants of *Ephedra alata, Zizyphus jujuba and Calycotome villosa* showed a high agglutination when the addition of rabbit erythrocytes suspension.

Table 2. Free radical-scavenging assay of fraction peptide and purified lectins of *Ephedra alata, Zizyphus jujuba and Calycotome villosa*.

Plants	Free radical-scavenging assay (%).		
	DPPH	SOD	Ferric reducing assay
Ephedra alata			
A	50.54±0.13	55.3±0.13	64.1±0.15
B	48.34±0.1	43.5±0.1	48.9±0.1
C	25.88±0.12	36.2±0.14	40.1±0.13
D(purified lectin)	79.97±0.15	62.2±0.1	70.1±0.1
Zizyphus jujube			
	17.02±0.09	12.4±0.1	26.2±0.1
A	23.29±0.1	21.3±0.1	35.8±0.12
B	55.49±0.15	68.1±0.15	68.2±0.15
C	53.62±0.15	30.2±0.13	43.1±0.12
D	76.3±0.15	70.9±0.15	68.9±0.12
E(purified lectin)			
Calycotome villosa			
A	62.63±0.15	70.2±0.12	53.9±0.13
B	29.66±0.1	27.3±0.1	35.6±0.1
C	41.15±0.1	33.7±0.1	47.1±0.1
D (purified lectin)	76.7±0.12	70.7±0.12	55.2±0.13
Standard (Ascorbate)	79.19±0.12	76.17±0.17	71.47±0.13

Effect of Metal ions on heamagglutinating activity of extracted lectin from Ephedra alata, Zizyphus jujuba and Calycotome villosa

The hemagglutinating activity of the extracted lectin of *Ephedra alata, Zizyphus jujuba* was not affected by the presence of 5mM EDTA, showing that the lectin is not a metallic protein but extracted lectin of *Calycotome villosa* the hemagglutinating activity was inhibited by th presence of metals showing that the lectin is a metalloproteine (Table 3).

Table 3. Effect of metal ions on heamagglutinating activity of extracted lectin from*Ephedra alata, Zizyphus jujuba and Calycotome villosa*.

Metal ions (5mM) plants	EDTA	$MnCl_2$	$MgCl_2$	$CaCl_2$
Ephedra alata	+++	+++	+++	+++
Zizyphus jujuba	+++	+++	+++	+++
Calycotome villosa	+++	-	-	-

+++: highest heamagglutinating activity.

Table 4. Inhibition of the heamagglutinating activity of the lectin extracted from*Ephedra alata, Zizyphus jujuba and Calycotome villosa*by Sugars.

Sugars	*Ephedra alata*	*Zizyphus jujuba*	*Calycotome villosa*
Glucose	-	-	-
Galactose	-	-	-
Lactose	-	-	-
Mannose	-	-	-
N-acétyl-glusamine	-	-	-

+: Inhibition of the heamagglutinating activity.

-: non inhibitory.

Inhibition assays

The results of sugar inhibition tests using a large number of simple sugars for theplantsof*Ephedra alata, Zizyphus jujuba and Calycotome villosa*lectins are presented in Table 4. The extracted lectin from*Ephedra alata, Zizyphus jujuba and Calycotome villosa*did not show any inhibition by all simple sugars.

Table 5. Effect of pH on heamagglutinating activity of extracted lectin from*Ephedra alata, Zizyphus jujuba and Calycotome villosa.*

pH	1	2	3	4	5	6	7	8	9	10	11	12
Ephedra alata	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Zizyphus jujuba	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Calycotome villosa	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

+++: highest heamagglutinating activity.

: no heamagglutinating activity.

Effect of pH on heamagglutinating activity of extracted lectin fromEphedra alata, Zizyphus jujuba and Calycotome villosa

The extracted lectin from*Ephedra alata, Zizyphus jujuba and Calycotome villosa* was stable in the P^H 2-12 and 1- 12, respectively (Table 5).

Table 6. Effect of heat on heamagglutinating activity of extracted lectin from therootsof*Ephedra alata, Zizyphus jujuba and Calycotome villosa*.

Heat	40°C	60°C	80°C	100°C
Ephedra alata	++	++	++	++
Zizyphus jujuba	++	++	++	++
Calycotome villosa	++	++	++	++

++: highest heamagglutinating activity.

-: nonheamagglutination activity.

Effect of heat on heamagglutinating activity of extracted lectin fromEphedra alata, Zizyphus jujuba and Calycotome villosa

In addition, the hemagglutinating activity of extracted lectin from *Ephedra alata, Zizyphus jujuba and Calycotome villosa*are resisted up to 100°C (Table 6).

Blood human test (ABO)

Extracted lectinsof*Ephedra alata, Zizyphus jujuba and Calycotome villosa*notpresent any specific agglutination to the blood human group (Table 7).

Table 7. Effect of suspension erythrocyte human on heamagglutinating activity of extracted lectin from *Ephedra alata, Zizyphus jujuba and Calycotome villosa*.

Blood human	A	B	O	AB
Ephedra alata	+++	+++	+++	+++
Zizyphus jujuba	-	-	-	-
Calycotome villosa	+++	+++	+++	+++

Limits of hemagglutination test

The hemagglutinating activity of extracted lectins from *Ephedra alata, Zizyphus jujuba and Calycotome villosa*was1:12(4168), 1:9 (512) and 1:10 (1042) *respectively* Table 8.

Effects of lectins extracted from Ephedra alata, Zizyphus jujuba and Calycotome villosaon phagocytic activity

A highly significant increase in phagocytic activity was observed intreatedgroupswith lectins extracted from *Ephedra alata, Zizyphus jujuba and Calycotome villosa,* respectively dose-dependent were compared with control (Fig.2).

Effects of lectins extracted from Ephedra alata Zizyphus jujube and Calycotomevillosaon half-time $t_{1/2}$ of carbon in blood

Fig. 3 show a highly significant decrease in half-time of carbon in blood dose-dependent in treated group with lectins extracted from *Ephedra alata, Zizyphus jujube and Calycotome villosa* respectively were compared with control.

Table 8. The limit haemagglutinating activity of extracted lectins from*Ephedra alata, Zizyphus jujuba and Calycotome villosa.*

Dilution	1 :2	2 :4	3 :8	4 :16	5 :32	6 :64	7 :128	8 :256	9 :512	10 :1042	11 :2084	12 :4168
Ephedra alata	++	++	++	++	++	++	++	++	++	++	++	-
Zizyphus jujuba	++	++	++	++	++	++	++	++	-	-	-	-
Calycotome villosa	++	++	++	++	++	++	++	++	++	-	-	-

+++: highest heamagglutinating activity.

---: non heamagglutinating activity.

Effects of lectins extracted fromEphedra alata, Zizyphus jujuba and Calycotome villosaon Corrected phagocytic index

The results of this study showed in Fig. 4that the corrected α wassignificantly increased intreatedgroupswith lectins extracted from *Ephedra alata, Zizyphus jujuba and Calycotome villosa*respectively dose-dependent were compared with control.

Discussion

The imbalance between the formation of ROS and anti-oxidant defense leads to oxidative stress, resulting in potential cellular damage (Souza *et al*., 2005). Several diseases such as cancer, diabetes, premature aging and degeneration disorders have been linked to oxidative stress caused byROS.

In living cells, ROS are the primary source of oxidative stress conditions. Eukaryotic cells protect themselves by anti-oxidant defense mechanisms such as enzymes, radical scavengers, Hydrogen donors, electron donors, Peroxide decomposers and metal chelating agents. A natural plant contains various anti-oxidants constituent that acts in a different way and is used in traditional medicine (Harmsma *et al*., 2004).Free radicals generated during the course ofthe metabolic process of an organism are known to play a role in several disorders. Anti-oxidant principles present in the plant have been shown to possess free radical scavenging activity.

DPPH assay is one widely used method for screening anti-oxidant activity of natural products. The maximum value of inhibition of DDPH and SOD scavenging activity was observed in standard ascorbic acid, DPPH and SOD scavenging potential of purified lectins of plants*Ephedra alata, Zizyphus jujuba and Calycotome villosa* may be due to hydroxyl grouppresent in the lectins. The Ferric Fe^{+3} reducing power assay mainly depends on the reducing capacity of Fe^{+3} - Fe^{2+} conversion and serves it as a significant indicator of its potential anti-oxidant activity. Among all the purified lectins showed a high ferric reducing power. Similarly, in the glycoprotein extract from *C.borg*, *C.amenda*, *C.casne* has abundant ferric reducing power in a concentration-dependent manner. Scavenging of H_2O_2 by purified lectins may be attributed due to the electron-donating to H_2O_2, thus neutralizing it to water. Purified lectins were capable of scavenging hydrogen peroxide in a concentration-dependent manner. There are some previous researches on the anti-oxidant activity of endophytic fungi from other medicinal plants. For example, two anti-oxidant pestacin, isopestacin from the endophytes fungi *Pestalotiopsis microspora* reported that 22.5% of extracts from endophyticfungi and *Garcinia planta* exhibit remarkable anti-oxidant activity Previously reported that methanolic extract of *Viscum album* has anti-oxidant capacity had been reported that *Viscum album* has a high anti-oxidant activity was (82.23%). Free radicals cause significant alteration in the structure of biological membranes that interferes with cellular integrity and metabolism, leading to cellular toxicity. Many researchers have employed the use of these free radical generators for the determination of anti-oxidant capacity of the given protein. Proteins have the ability to inhibit free radical generation in both in vitro and in vivo systems. Purified lectins isolated in this investigation have the highestanti-oxidant activity against free radicals. Peptides like *G. lucidum* peptide (GLP), cottonseed protein hydrolysate (CPH), fermented marine blue mussel peptide (*Mytilus edulis*), and

giant squid muscle peptide were also reported as potentanti-oxidants against free radicals (Elias *et al.*, 2008).Many natural anti-oxidants were comparably less potent than synthetic anti-oxidants, but if used at higher concentrations, could replace the use of synthetic anti-oxidants owing to their toxicity.

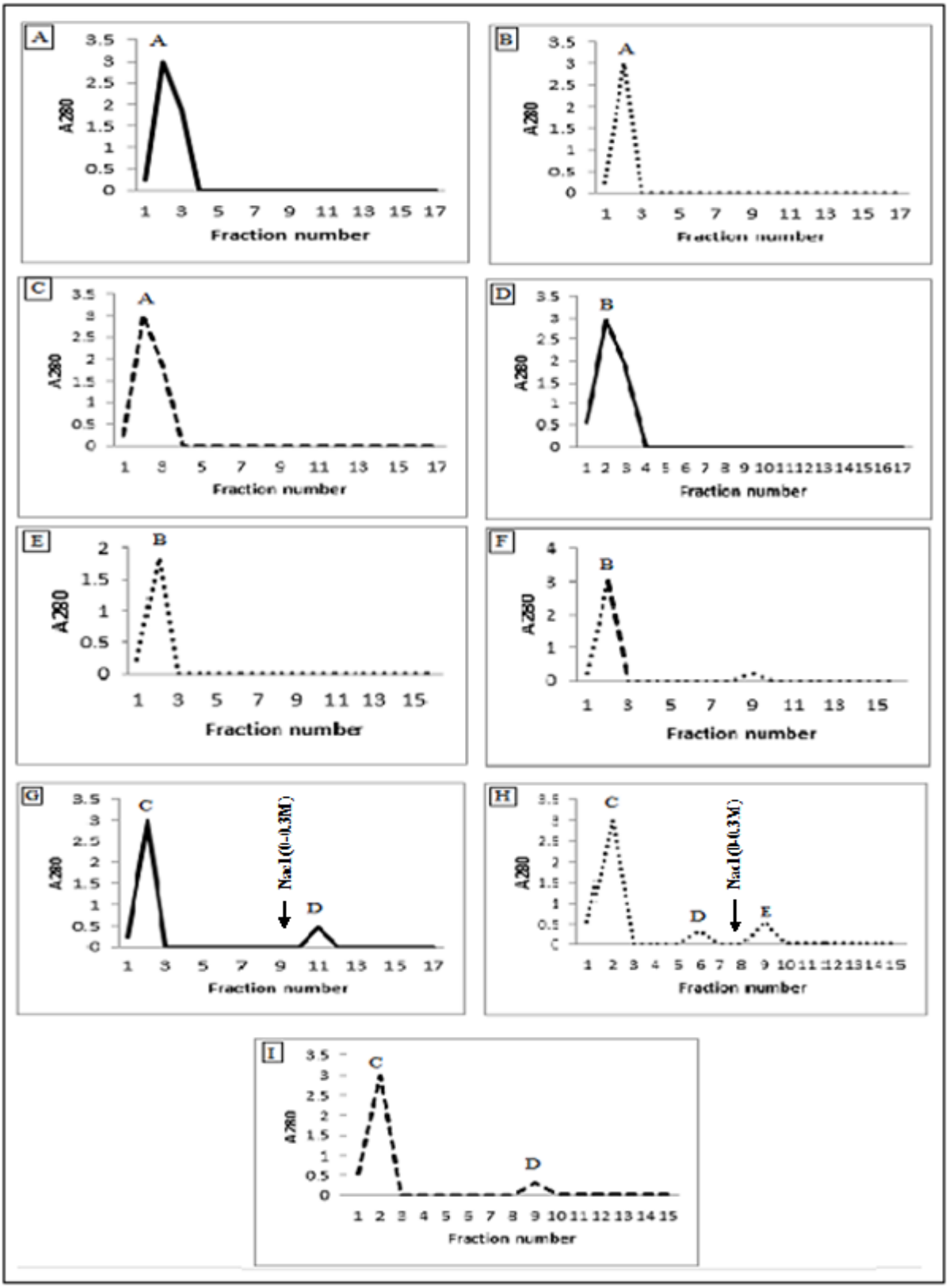

Fig. 1. Extracted lectin from *Ephedra alata, Zizyphus jujuba and Calycotome villosa* by sephadex G25 (A, B, C), G75 (D, E, F) and ion exchange (G, H,I).

Some lectins have been isolated from the roots of plants. The data presented from this study showed that the roots of plants contained a measurable amount of hemagglutinating lectin.

The lectin of*Ephedra alata and Calycotome villosa*doesnot require divalent cations for the maintenance of itsbiological activity since the addition of EDTA to the reaction medium did not affect the haem agglutinating activity, suggesting that this lectin of *Ephedra alataand Calycotome villosa* is not a metallic protein. However, the lectins from *Zizyphus jujuba*exhibited dependence on metalssuch as Ca2+, Mn2+ and Mg2+, as is the case with most plant lectins.

Extracted lectin from *Zizyphus jujuba*did not show positive agglutination with human blood groups while agglutinated red blood cells of rabbit; this result is accorded with Deeksha *et al.* (2015).

However, *Ephedra alataand Calycotome villosa* presented a positive agglutination with all human groups.

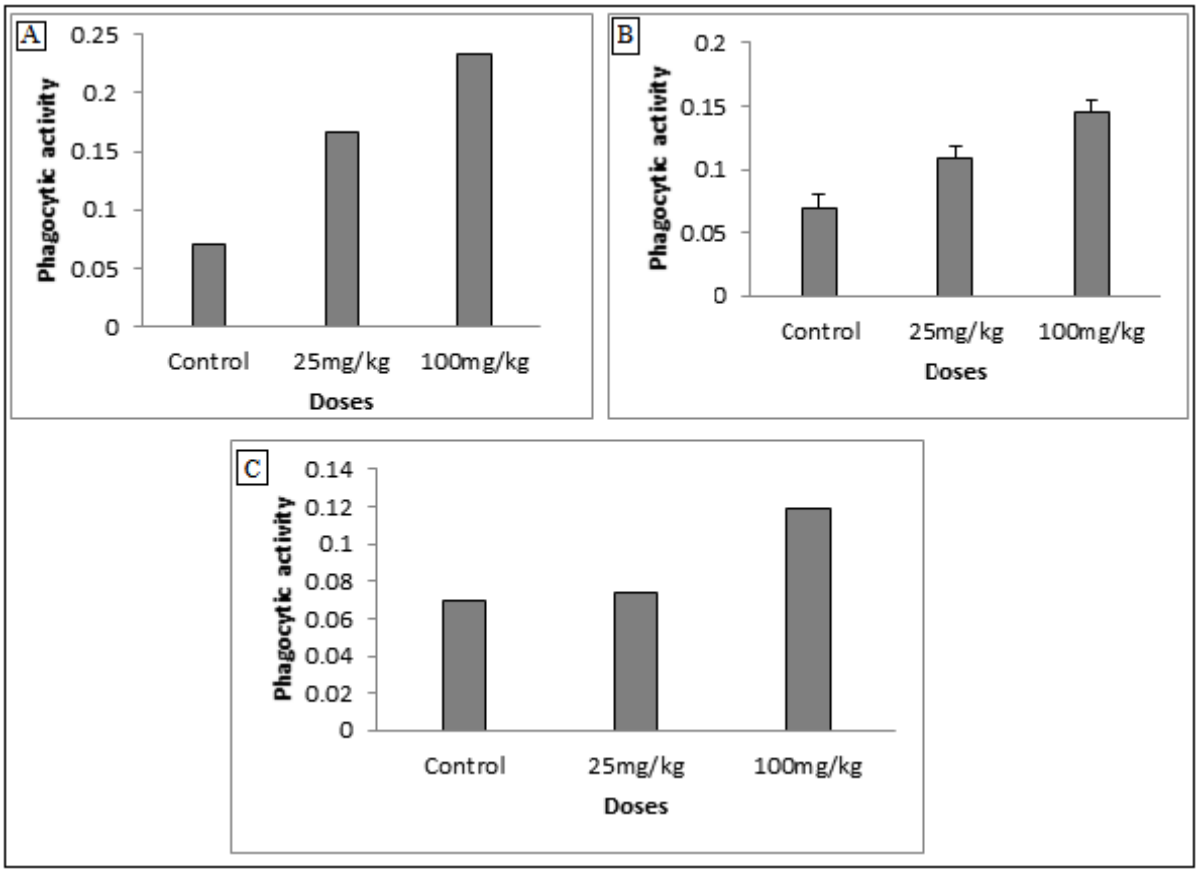

Fig. 2. Effect of lectins extracted from *Ephedra alata (A), Zizyphus jujube (B) and Calycotome villosa* (C)on phagocytic activity.

The work of Necib *et al.* (2014) demonstrated that the extracted lectin from roots of the plant showed a positive agglutination with human erythrocytes ofthe ABO system. The thermo stability and pH stability characteristics of lectins are known to differ from lectin to lectin. The hemagglutinating activity of these extracted lectins was thermo stable and pH-sensitive. Lectins are known to be heat-labile and their activity can be decreased by heat treatment (Cole, 1994). The finding suggests that the hemagglutination activity of extracted lectin from plants: *Ephedra alata, Zizyphus jujuba and Calycotome villosa* were stable at the pH range between 1– 12 and 2-12respectively. An agglutination with relatively high thermo stability up to 80°C and 100°C from extracted lectins of *Ephedra alata, Zizyphus jujuba and Calycotome villosa,*

respectively, is due to that lectin activity may be brought for the denaturation of lectin. These results are accorded by the work of Necib *et al.* (2014).Therefore the diverse specificities of lectins with culicid may be related to the physiological function of these molecules components based on carbohydrate interactions. Sugar specificity of the extracted lectin from *Ephedra alata, Zizyphus jujuba and Calycotome villosa* did not show any specificity from all sugars tested.

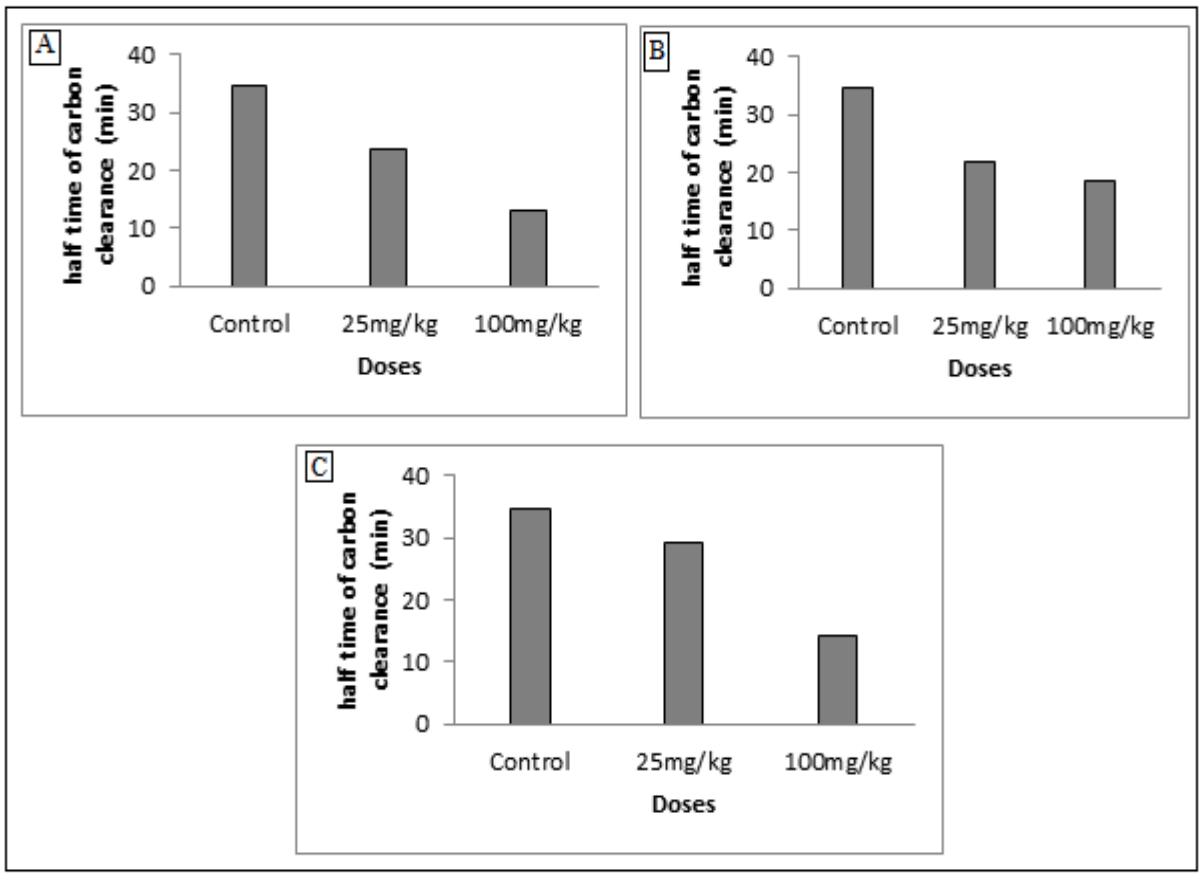

Fig. 3. Effect of lectins extracted from*Ephedra alata (A), Zizyphus jujube (B) and Calycotome villosa(C)*on half - life $t_{1/2}$ of carbon in blood.

The reticulo-endothelial system (R.E.S) consists 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 best defined functionally by its ability to scavenge debris or other foreign matter and form the first line of defense.

The rate of removal of carbon particles by the sessile intravascular phagocytes in the liver and spleen from the bloodstream is a measure of reticulo-endothelial phagocytic activity. In the present study, the carbon clearance test, extracted lectin from*Ephedra alata, Zizyphus jujuba and Calycotome villosa* treated groups, exhibited significantly high phagocytic index (Necib*et al.,* 2013).

This indicates stimulation of the reticulo-endothelial system by drug treatment. It may be possible that the extracted lectin from *Ephedra alata, Zizyphus jujuba and Calycotome villosa* influence the mechanism of phagocytosis, largely distributed monocytes macrophages or R.E.S, which result in a significant increase in the phagocytic index with carbon clearance test ((Necib*et al.,* 2013).

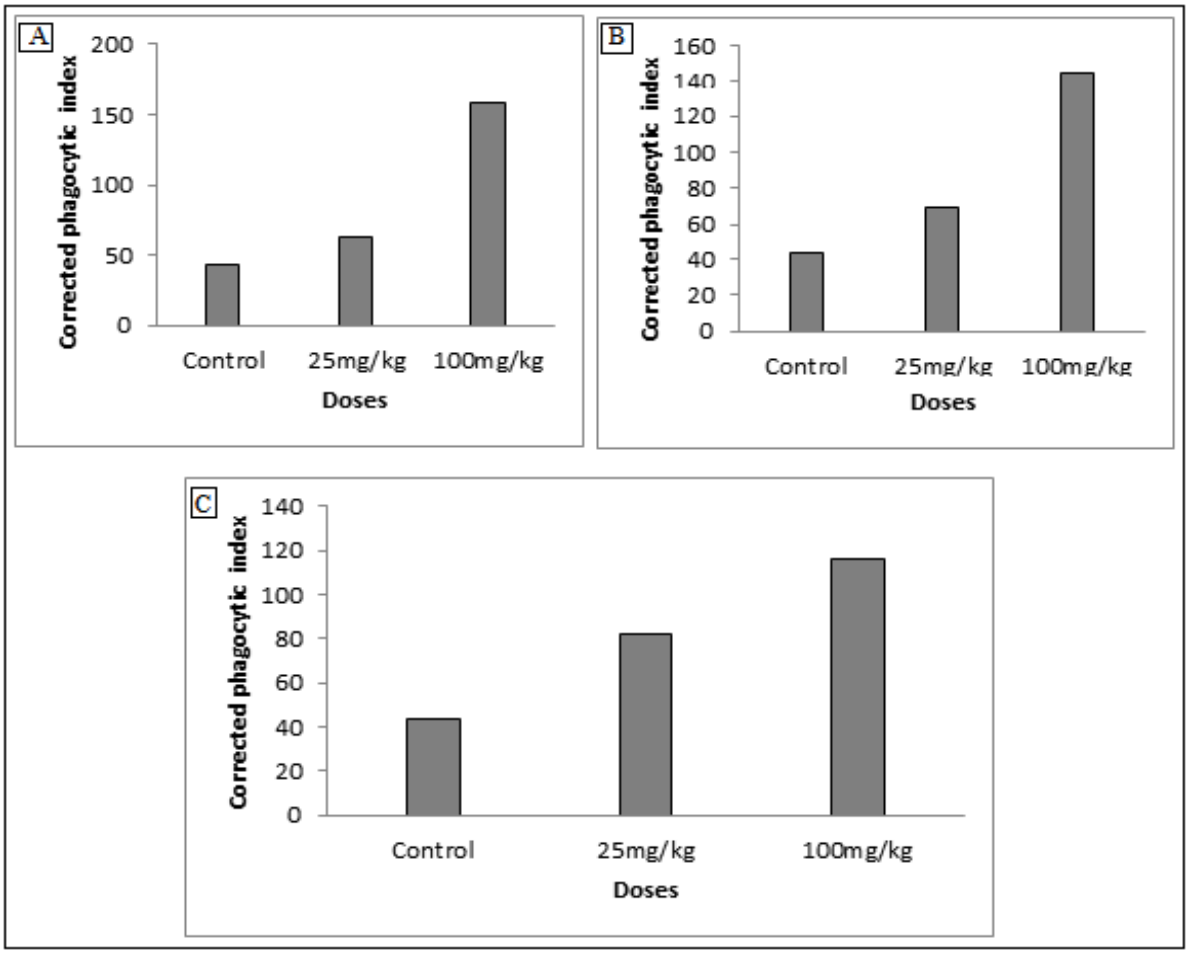

Fig. 4. Effect of lectins extracted from*Ephedra alata (A), Zizyphus jujube (B) and Calycotome villosa*(C) on corrected phagocytic index.

Conclusion

Our findings presented that the extracted lectins from *Ephedra alata, Zizyphus jujuba and Calycotome villosa* showed the highest anti-oxidant activity in the DPPH, SOD and Ferric reducing assay radical scavenging model and immunomodulatory agent, which acts by stimulating dose-dependent phagocytic function.

References

Bradford MA. 1976. Anal Biochemestry **72,** 248-54.

Elias RJ, Kellerby SS, Decker EA. 2008. Critical Review in Food Science and nutrition **48,** 430–441.

Carlini CR, Grossi-de-Sa MF. 2002. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides, Toxicology **40 (11),** 1515–1539.

Cheng W, Li J, You T, Hu C. 2005. Anti-inflammatory and immunomodulatory activities of the extracts from the inflorescence of Chrysanthemum indicum Linné. Journal of Ethnopharmacology **101(1-3),** 334-7.

Cole MD. 1994. Key antifungal, antibacterial and antiinsect assays – a critical review. Biochemical System Ecology **22,** 837–856.

Correia MTS, Coelho LCBB. 1995. Purification of a glucose/mannose specific lectin, isoform1, form seeds of cratylia mollis mart. (Camaratu bean). Applied Biochemical Biotechnology **55(3),** 261-273.

Cumming RD. 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,

p 191-199.

Deeksha MK, Sangha DS, Khurana MB, Binay S. 2015. Screening for lectin quantification in Brassica Spp and vegetable crops. Journal of environmental and Applied Bioresearch 30 (1), 20-24. Duan X *et al.*, Molecules, 2007 **12(4),** 759-771.

Goldstein IJ, Hughes RC, Monsigny M, Ozawa T & Sharon N. 1980. What should be called a lectin? Nature **285,** 60.

Hamshou M, Smagghe G, Van Damme EJM. 2010. Entomo-toxic effects of fungal lectin rhizoctonia solani towards spondoptera littoralis. Fungal Biology **114(1),** 34-40.

Harmsma M, Gromme M, Ummelen M, Dignef W, KJ Tusenius. 2004. International journal of oncology **125,** 1521-1529.

Irobi ON, Moo-Young M, Anderson WA. 1996. Antimicrobial Activity of Annatto (Bixa orellana) Extract. **34(2),** 87-90.

Lis H, Sharon N. 1981. Lectins in higher plants. Biochemistry of Plants **6,** 371-447.

Michiels K, Van Damme EJM, Smagghe G. 2010. Plant-insect interactions: what can we learn from plant lectins? Arch Insect Biochemical Physiology **73(4),** 193–212.

Necib Y, Bahi A, Merouane F, Bouadi H, Boulahrouf K. 2014. Comparative study of a new lectin extracted from roots of plants: Cyperus rotundus, Pistacia lentiscus and Ruta graveolens.World Journal of pharmacology research **4(1),** 1720-1733.

Necib Y, Bahi A, Derri N, Merouane F, Bouadi H and Boulahrouf K. 2014. Immunomodulatory of lectin extracted from roots of blak mulberry (*Morus Nigra*).World Journal of pharmacology research, **4(1),** 1707-1719.

Necib Y, Bahi A, Zerizer S, Cherif A, Boulakoud MS. 2013. Immunomodulatory Activity of Argan oil (*Argania Spinosa. L*). Intertional Journal of Pharmacology Sciences Review and Research **23(1),** 57-59.

Necib Y, Bahi A, Zerizer S. 2013. Immunomodulatory Effect of Argan oil (*Argania spinosa. L*) After Exposure to Mercuric Chloride in Mice. International Journal of Pharmacology Sciences Review and Research **23(1),** 191-193.

Peumans WJ, Van Damme WJN. 1995. Lectin as plant defense proteins. Plant Physiology **109,** 347-352.

Pusztai A, Bardocz S. 2009. Biological effects of plant lectins on the gastrointestinal tract: metabolic consequences and applications. *Trends Glycosciences Glycotechnology* **8(41),** 149–165.

Shah AS, Wakade AS, Juvekar AR. 2008. Immunomodulatory activity of methanolic extract of Murraya koenigii (L) Spreng leaves. Indian Journal of Experimental Biology **46(7),** 505-9.

Shaista R, Sakeena Q, Ishfak HW, Showkat AG, Akbar M, Rabia H. 2014. Purification and partial characterization of a Fructose-binding lectin from the leaves of Euphorbia helioscopia. Pakistan Journal of Pharmacology *Sciences* **27(6),** 1805-1810.

Souza ME, Pinto MM. 2005. Síntesis of xanthones: an overview. Curr Med Chem **12,** 2447–2479.

Van Damme EJM, Lannoo N, Peumans WJ. 2008. Plant lectins. Advance Botany Research **48,** 109–209. http://dx.doi.org/10.1016/50065-2296(08)00403-5.