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Anti-inflammatory, immunomodulatory and antimicrobial effects of newlectins extracted from roots of Algerian plants: *Urtica dioica*.L., *Anacyclu spyrethrum* L., *Brassica napus* L. *and Calycoto mespinosa* L.

Ahlem Bahi[1], Youcef Necib[1*], Imen Torche[1], Sana Boufker[1]

[1]*Laboratory of Microbiological Engineering and application,Faculty of Natural and Life Sciences, UniversityMentouri Brothers Constantine, Algeria*

Key words: Extracted lectins, Haemagglutination, Immunomodulatory, Antimicrobial, Anti-inflammatory.

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Abstract

The lectins are present inthe root of plants *Urtica dioica*.L., *Anacyclus pyrethrum* L., *Brassica napus* L., *and Calycotome spinosa* L.were extracted by soluble proteins (crude extract) in phosphate buffer (0.1M, pH 7.2). The lectin of the plants agglutinated, specifically rabbit erythrocytes and the lectin was extracted from *Anacyclus pyrethrum* L. pecific for blood B human group. Extracted lectin of plants showed the thermostability is more than 100°C. However, the lectin of all plants were stable in the differentes pH ranging and were inhibited by the sugars glucose, galactose and mannose for lectin extracted from plant *Brassica napus. L* and N-acetyl-glucosamine for lectin extracted from *Urtica dioica.L.* Immunomodulatory activities of extracted lectins from*Urtica dioica.L and Brassica napus. L* were evaluated on phagocytic activity by carbon clearance test.. The present study thus reveals that extracted lectins from*Urtica dioica.L and Brassica napus. L* holds promise as an immunomodulatory agent, which act by stimulating dose-dependent phagocytic function. Furthermore, extracted lectins of *Urtica dioica.L, Anacyclus pyrethrum. L, Brassica napus. L and Calycotomespinosa. L* showed highly antimicrobial and anti-inflammatory activities.

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

Introduction

Lectins constitute a group of proteins orglycoproteins of non-immune origin, which bindreversibly to carbohydrates and usually agglutinate cellsor precipitate polysaccharides and glycoconjugates (Goldstein *et al.,* 1980). The lectins were redefined as proteins possessingat least one non-catalytic domain, which bindsreversibly to a specific mono or oligosaccharide (Peumans and Van Damme, 1995).However, antibodiesand proteins with enzymatic activity related tocarbohydrates cannot be considered lectins (Cumming, 1997). As aconsequence of their chemical properties, they havebecome a useful tool in several fields of biologicalresearch (immunology, cell biology, membranestructure, cancer research and genetic engineering).Lectins are present in a wide range of organisms, frombacteria to animals, being present in all classes andfamilies, although not in all the kinds and species (Lis and 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 extractionof a newlectin from the roots plants of *Uriticadioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L*collected from Algeria and their immunomodulatory, antimicrobial and anti-inflammatory effects.

Materials and methods

The lectins were extracted from roots plants *Urtica dioica.L, Anacyclus pyrethrum. L, Brassica napus. L and Calycotomespinosa. L* used in this work originated from Algerian. 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 *Urtica dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L*were washed briefly, roughly ground and then homogenized in a chilled warning blender with phosphate buffer saline pH7.2. The homogenization 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 G200.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 pathlength cell using spectrophotometer UV.

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 dilutionsof 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. 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 inhibitedthe agglutinating activity.

pH test

Different buffer solutions with pH ranging from 1 to 12 were prepared to study the stability of extracted lectins of*Urtica dioica L, Anacycluspyrethrum L, Brassica napus L and Calycotome spinosa L.*

Heat stability test

The heat stability of the hemagglutinating activity ofroots plants *Urtica dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L* lectin was determined by incubationof aliquots of lectin solutions at different temperatures (40, 60, 80, 100 or 120°C) for 1h and the remaininghemagglutinating activity was determined.

Limite hemagglutinating activity test

This testdetermines theagglutinativepowerand deducts thetitlelectin.In the first step, 50 µlof bufferwereadded to each well, avolumeof50µlof the extractwas added to thefirst well andarangeof concentrationbydouble dilutionwas performedinthe subsequent wells. Avolumeof50 µl red blood cellswere addedto50µlof extractto each well.

Readinghemagglutinating activitywas carried outafter 1 hourat room temperature ambient.Thehemagglutinatingactivity is expressedaswhich is the reciprocalof the greatest dilution ratiofor whichhemagglutinationis observed.

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 a 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 a 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 groups: II, III and VI were administrated extractedlectins from roots plants *Urtica dioica.L and Brassica napus. L* at a dose of 25, 50 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 15 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):

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

$$\alpha = \sqrt[3]{K}\frac{-Body\ Weight\ of\ animal}{\text{Liver+Spleen wt}}$$

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

Determination in *vivo* anti-inflammatory activity

LPS-induced edema in rats

7 groups of five animals each were used. Paw swelling was induced by sub-plantar injection of LPS (25µg in 50µl of saline). The lectin is extracted from the root of plants *Urtica dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L*at dose 100mg/kg was administrated by gavage 60 minutes before LPS injection. Diclofénac (3mg/kg) wa used as a reference drug. Control group received NaCl (0.9%). The inflammation was quantified by measuring the volume displaced by the pawat time 1, 2 and 4h after LPS injection. The difference between the left and right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals.

Determination of antibacterial and antifungal activity

Microorganisms used

The test organisms *Escherichia coli ATCC25922, Staphylococcus aureus ATCC43300, Klebsiella pneumonia ATCC 70603, Bacillus cereus, Candida albicans, Acinetobacter sp, Aspergillus niger, Fusarium oxysporum, Aspergillus fumigates and Aspergillus flavus*.

Antifungal activity

Antifungal activity was performed on sterile Petri plates(100x15 mm) containing 10 ml agar sterilized at15 psi and 120°C for 20 min. Sterile paper disks, 1 cm in diameter, were placed at the surface of a heavily seededmedium with the tested organism. A 10 µl aliquot of the lectin was extracted from the root of the plants *Urtica dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L* was added to the disk. Petri dish was incubated at37°C for 48 hrs, at the end of which the diameter of theclear zone of inhibition surrounding the sample wastaken as a measure of the inhibitory power of thesample against the particular test organism (Irobi*et al.,* 1996).

Antibacterial activity

Antibacterial activity of lectin extracted from the root of plants *Urtica dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L*was investigated by the disc diffusion method (Cole, 1994). The microbial strains were obtained fromstock cultures in nutrient agar (0.7%). Onehundredmilliliters of warm nutrient agar (NA) (43°C) and 0.5 mlof bacteria suspension (105-106 CFU /m1) were mixedand 10 ml volumes were distributed in sterile Petriplates (90 X 15 mm) and allowed to solidify. Sterileblank paper discs (6 mm diameter) impregnated with20µl of a sterile solution of lectin extracted from the root of plants *Urtica dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L*(1.0mg/ml, 2.0 mg /ml).Plates were incubated at 37°C for 24 hrs. A transparentring around the paper disc revealed antimicrobialactivity. Zones of growth inhibition around discs weremeasured in millimeters.

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 $P<0.05$, $P<0.01$, and $P<0.001$.

Results

Extracted lectins from roots of *Urtica dioica L, Anacyclus pyrethrum L, Brassica napus L and Calycotome spinosa L* by sephadex G200 It was found that in elution fraction of roots of *Urtica dioica L, Anacyclus pyrethrum L and Calycotome spinosa L* presented one pick but the *Brassica napus L* presented which explains the presence of probably two types of lectins in this extract (Fig.1).

Hemagglutinin assay

The extracted lectins from therootsof*Urtica dioica.L(A), Anacyclus pyrethrum. L (B), Brassica napus. L (C) and Calycotome spinosa. L(D)* showed a high agglutination when the addition of rabbit erythrocytes suspension (Fig.2).

Inhibition assays

The results of sugar inhibition tests used a large number of simple sugars for therootsof*Urtica*

*dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L*lectins are presented in Table1 and Fig. 3. The extracted lectin from the roots of *Anacyclus pyrethrum. L and Calycotome spinosa. L* did not show any inhibition by all simple sugars; *Brassica napus. L presented* inhibition with glucose, galactose and mannose and *Urtica dioica.L* inhibited by N-acetyl-glucosamine at 200 mM concentration.

Table 1. Inhibition of the heamagglutinating activity of the lectin extracted from the roots of *Urtica dioica.L, Anacyclus pyrethrum. L, Brassica napus. L and Calycotome spinosa. L* by Sugars.

Sugars	*Urtica dioica.L*	*Anacyclus pyrethrum. L*	*Brassica napus. L*	*Calycotome spinosa. L*
Glucose	-	-	+	-
Galactose	-	-	+	-
Lactose	-	-	-	-
Mannose	-	-	+	-
N-acétyl-glusamine	+	-	-	-

+: Inhibition of the heamagglutinating activity.

-: non inhibitory.

Effect of pH on heamagglutinating activity of extracted lectin fromthe rootsof Urtica dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L

The extracted lectin from the roots of *Urtica dioica.L, Anacyclus pyrethrum. L, Brassica napus. L and Calycotome spinosa. L* were stable in the P^H 2-12, 4 - 12, 1-12 and 4 - 12, respectively (Table 2).

Table 2. Effect of pH on heamagglutinating activity of extracted lectin from the roots of *Urtica dioica.L, Anacyclus pyrethrum. L, Brassica napus. L and Calycotome spinosa. L.*

pH	1	2	3	4	5	6	7	8	9	10	11	12
Urtica dioica.L	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Anacycluspyrethrum. L	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++
Brassica napus. L	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+++
Calycotome Spinosa. L	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

+++: highest heamagglutinating activity.

-: no heamagglutinating activity.

Effect of heat on heamagglutinating activity of extracted lectin from the roots of Urtica dioica.L, Anacycluspyrethrum.

L, Brassica napus. L and Calycotomespinosa. L In addition, the hem agglutinating activity of extracted lectin from the roots of *Calycotome spinosa. L* is inactivated at 80°C, while *Brassica napus. Land Urtica dioica.L* are resisted up to 100°C. However, the extract of *Anacycluspyrethrum. L*showed a thermostability more than 120°C (Table 3).

Table 3. Effect of heat on heamagglutinating activity of extracted lectin from therootsof*Urtica dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L.*

Heat	40°C	60°C	80°C	100°C	120°C
Urtica dioica.L	++	++	++	++	+
Anacycluspyrethrum. L	++	++	++	++	++
Brassica napus. L	++	++	++	++	+
Calycotomespinosa. L	+++	+++	+++	+	+

++: highest heamagglutinating activity.

-: nonheamagglutination activity.

Blood human test (ABO)

Extracted lectinsof*Urtica dioica. L, Brassica napus. L*and *Calycotomespinosa. L* not presented any specific agglutination to human blood group. However, extractedlectin from the roots plant of *Anacycluspyrethrum. L* presented specific agglutination to B blood human group (Table4, Fig 4).

Limits of hemagglutination Test

The haemagglutinating activityof extracted lectins from therootsof*Urtica dioica. L, Anacycluspyrethrum. L and Calycotomespinosa. L* were1:7(128)and*Brassica napus. L*was 1:6(64) Table 5 and Fig. 4.

Effects of lectins extracted from the roots of Brassica napus. L and Urtica dioica.L on phagocytic activity

No Significant increase in phagocytic activity was observed in treated groups with lectins extracted from the roots plants *Brassica napus. L and Urtica dioica. L* respectively dose-dependent were compared with control (Figure 6).

Table 4. Effect of suspension erythrocyte human on heamagglutinating activity of extracted lectin from theroots plants *Urtica dioica.L, Anacycluspyrethrum. L, Brassica napus. L and Calycotomespinosa. L.*

Blood human	A	B	O	AB
Urtica dioica.L	+++	+++	+++	+++
Anacyclus pyrethrum. L	-	+++	-	+++
Brassica napus. L	-	-	-	-
Calycotome spinosa. L	+++	+++	+++	+++

Table 5. The limite haemagglutinating activity of extracted lectins from theroots plants *Urtica dioica. L, Anacyclus pyrethrum. L,Brassica napus. Land Calycotome spinosa. L.*

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
Urtica dioica.L	++	++	++	++	++	++	++	-	-	-	-	-
Anacyclus pyrethrum. L	++	++	++	++	++	++	++	-	-	-	-	-
Brassica napus. L	++	++	++	++	++	++	-	-	-	-	-	-
Calycotome spinosa. L	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	-

+++: highest heamagglutinating activity

---: non heamagglutinating activity

Effects of lectins extracted from the roots ofBrassica napus. L and Urtica dioica.L on half-time $t_{1/2}$ of carbon in blood

Fig. 7 shows no significant decrease in half-time of carbonin blood dose-dependent in the treated group with lectins extracted from the roots plants *Brassica napus. L and Urtica dioica. L* respectively were compared with control.

Effects of lectins extracted from the roots of Brassica napus. L and Urtica dioica.L on Corrected phagocytic index

The results of this study shown in Fig. 8 that the corrected α wassignificantly increased intreatedgroupswith lectins extracted from the roots plants *Brassica napus. L and Urtica dioica.L*respectively dose-dependent were compared with control.

Anti-inflammatory activity of lectins extracted from the roots plants Brassica napus. L, Urtica dioica.L, Anacyclus Pyrethrum. L and Calycotome spinosa. L.

The anti-inflammatory effects of the extracted lectin from theroots plant *Brassica napus. L,Urtica dioica. L, Anacyclus pyrethrum. L and Calycotome spinosa. L*on LPS induced edema in rat's hind paws are presented in Table 6. There was a gradual increase in edema paw volume of rats in the control group. However, in the test groups, lectin was extracted from

the root of the plants'*Brassica napus. L,Urtica dioica. L, Anacyclus pyrethrum. L and Calycotome spinosa. L*(100mg/kg)showed a significant reduction in the edema paw volume. The results showed that lectin was extracted from the root of plants *Urtica dioica.L and Calycotome spinosa. L*causes a significant reduction in inflammationcompared to standard anti-inflammatorydrugs, but lectinsare extracted from the root of the plant *Brassica napus. Land Anacyclus pyrethrum. L*causes no significant reduction in inflammationcompared to standard anti-inflammatory drugs.

Table 6. Anti-inflammatory activity of lectins extracted from the roots plants *Brassica napus. L, Urtica dioica. L, Anacyclus pyrethrum. L and Calycotome spinosa. L.*

Experiment	Control	LPS	Diclofénac	*Brassica napus. L*	*Urtica dioica.L*	*Anacyclus pyrethrum. L*	*Calycotome spinosa. L*
1h After treatment	0.28±0.001	0.34±0.003	0.25±0.001	0.30±0.002	0.24±0.001	0.29±0.003	0.26±0.002
2h After treatment	0.28±0.001	0.36±0.003	0.24±0.001	0.27±0.002	0.22±0.002	0.26±0.001	0.24±0.001
4h After treatment	0.28±0.001	0.44±0.003	0.20±0.001	0.19±0.001	0.10±0.001***	0.17±0.001	0.14±0.001*

Table 7. Determination of antibacterial and antifungal activity of lectins extracted from root of plants *Brassica napus. L, Urtica dioica. L, Anacyclus pyrethrum. L and Calycotome spinosa. L.*

Microorganisms	Diameter of zone of inhibition (mm)			
	Brassica napus. L	*Urtica dioica.L*	*Anacyclus pyrethrum. L*	*Calycotome spinosa. L*
Escherichia coli ATCC25922	NA	NA	NA	NA
Staphylococcus ayreus ATCC43300	NA	NA	NA	NA
Klebsiella pneumonia ATCC 70603	NA	NA	NA	NA
Bacillus cereus	0.01	NA	0.01	0.1
Candida albicans	NA	NA	NA	NA
Acinetobacter sp	NA	NA	NA	NA
Aspergillus niger	NA	NA	NA	1.5
Fusarium oxysporum	NA	NA	NA	NA
Aspergillus fumigates	NA	NA	NA	NA
Aspergillus flavus	NA	NA	11	NA

NA: No activity.

Antibacterial and antifungal activity of lectins extracted from the roots plants Brassica napus. L, Urtica dioica. L, Anacyclus pyrethrum. L and Calycotome spinosa. L

Extracted lectin from the root of plants *Brassica napus. L, Anacyclus pyrethrum. L and Calycotome spinosa. L*inhibited the growth of *Bacillus ceraus* with a diameter of zone inhibition of 0.01, 0.01 and 0.1 mm respectively, but lectin was extracted from the root of the plant *Urtica dioica.Ldoes* not present any activity of inhibition. However, lectinswere extracted from the root of the plant's *Brassica napus. L, Urtica dioica. L, Anacyclus pyrethrum. L and Calycotome spinosa. L*did not show any zone of inhibition in the test plates after 24h of incubation with *Escherichia coli ATCC25922, Staphylococcus ayreus ATCC43300, Klebsiella pneumonia ATCC 70603, Candida albicansand Acinetobacter sp.* Furthermore, extracted lectins from the root of plants *Anacyclus pyrethrum. L and Calycotome spinosa. L*inhibited the growth of *Aspergillus flavus*(11mm) and *Aspergillus niger* (1.5mm), respectively (Table 7).

Discussion

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.Extracted lectin from roots of *Brassica napus. L* did not show positive agglutination with human blood groups while agglutinated with red blood cells of rabbits; this result is accorded with Deeksha*et al.* (2015). However, *Calycotome spinosa. L and Uritica dioica.L* presented a positive agglutination with all human groups. The work of Necib*et al.* (2014) demonstrated that the extracted lectin from roots of the plant showed a positive agglutination with human erythrocytes of ABO system. However, extracted lectin from root of *Anacycluspyrethrum. L* indicating that lectins are specific toB blood group.

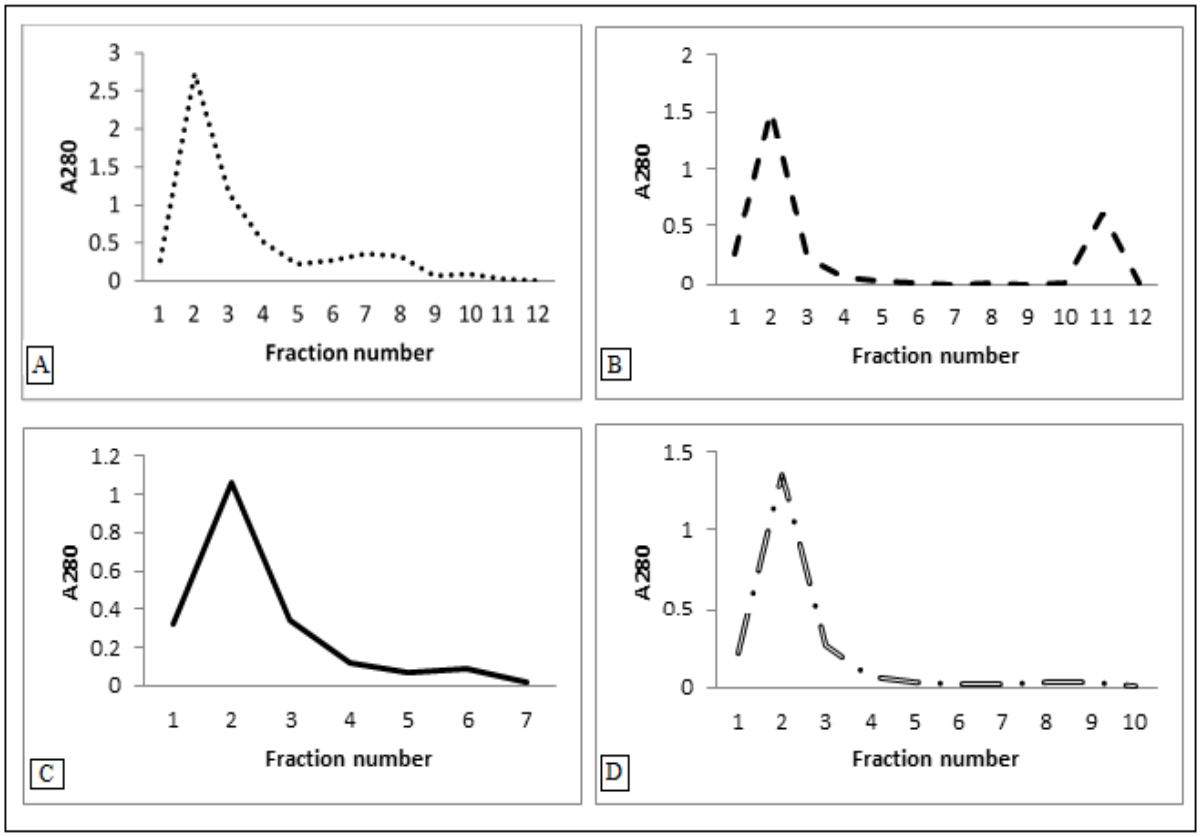

Fig. 1. Extracted lectin from theroots plants *Anacyclus pyrethrum. L (A) , Brassica napus. L (B) , Calycotome spinosa. L (C) and Urtica dioica. L(D)*by sephadex G200.

This result is accorded with the work of Necib*et al.* (2014). The thermostability and pH stability characteristics of lectins are known to differ from lectin to lectin. The hemagglutinating activity of these extracted lectins was thermostable 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 roots plants: *Anacycluspyrethrum. L, Brassica napus. L, Urtica dioica.L* and *Calycotomespinosa. L* were stable at the pH range between 2 - 12, 4 -12; 4-12and 1-12, respectively. An agglutination with relatively high thermostability up to 80°C and 100°C from extracted lectins of roots of *Calycotome spinosa. L* and *Brassica napus. L* respectively, it is that lectin activity may be broughtfor the denaturation of lectin.

These results are accorded to 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 roots of *Brassica napus. L* and *Urtica dioica.L.* were examined by competitive inhibition of various sugars against rabbit erythrocytes, but *Calycotomespinosa. L* did not show any specificity from all sugars tested.

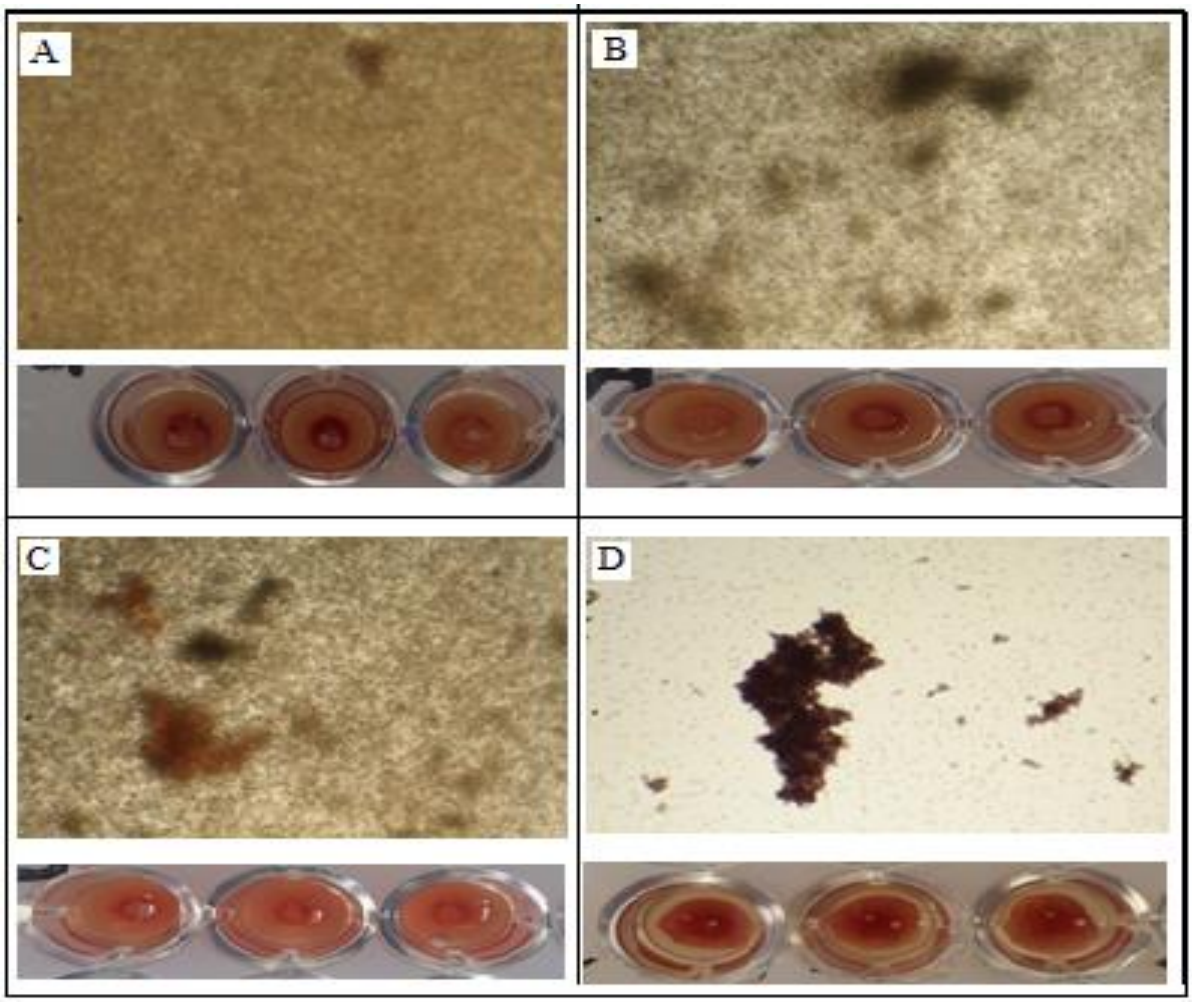

Fig. 2. hemagglutinin of lectin extracted fromtheroots plants *Urtica dioica.L (A), Anacyclus pyrethrum. L (B), Brassica napus. L (C) and Calycotome spinosa. L (D)* with suspension of rabbit erythrocytes GX40.

The activity of the lectin extracted from *Brassica napus. L*was completely inhibited by glucose, galactose and mannose with the minimum inhibitory concentration of 200mMbut extracted lectin from *Urtica dioica.L.* was completely inhibited by N-acetyl-glucosamine at the same concentration. It may be that the lectin reacts with a more extended structure of monosaccharides unit.

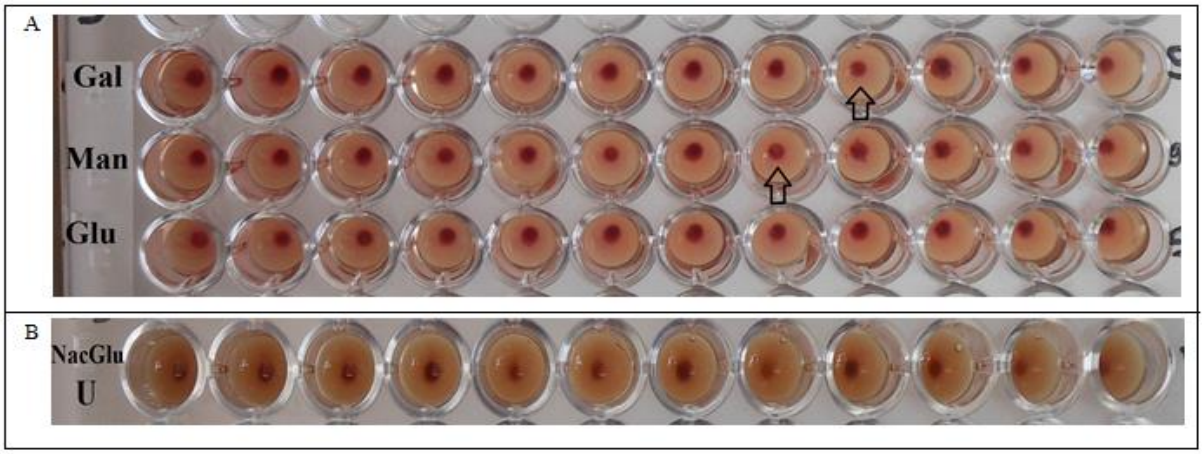

Fig. 3. Inhibition of the heamagglutinating activity of the lectin extracted from the roots plants *Brassica napus. L (A) and Urtica dioica. L (B)* by Sugars.

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.

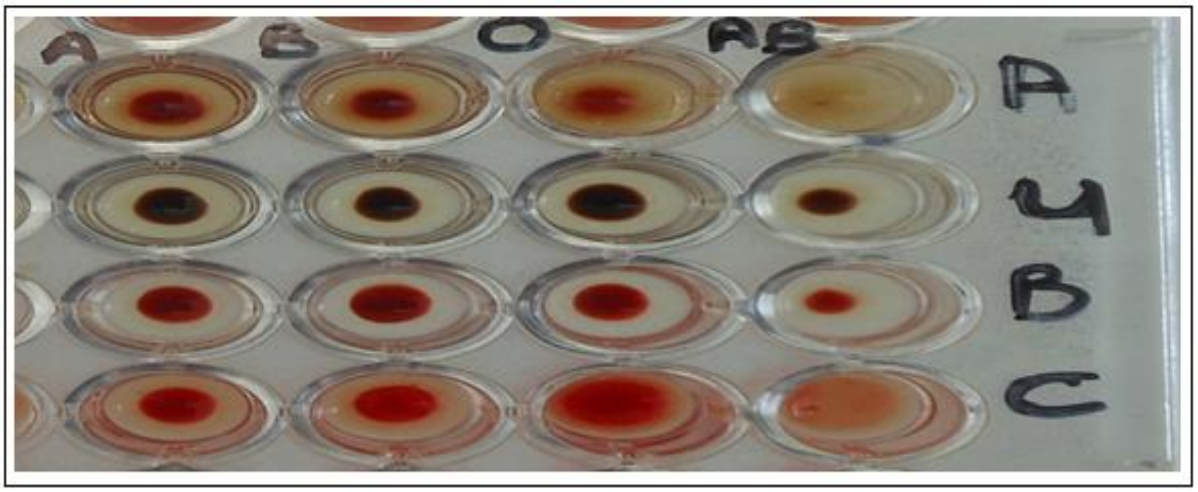

Fig. 4. Effect of suspension erythrocyte human on heamagglutinating activity of extracted lectin from theroots plants *Urtica dioica.L, Anacyclus pyrethrum. L, Brassica napus. L and Calycotomespinosa. L.*

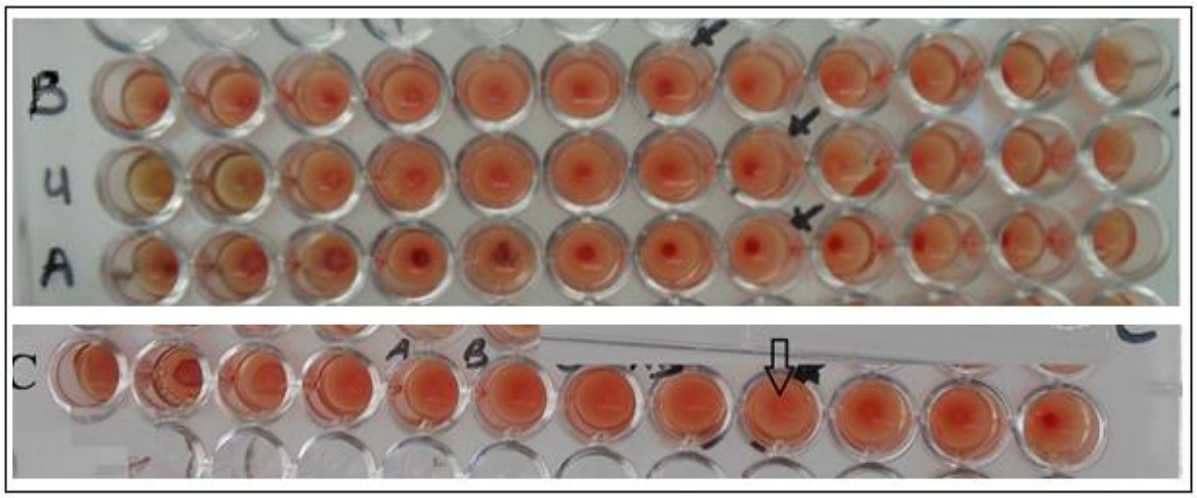

Fig. 5. The limite haemagglutinating activity of extracted lectin from the roots plants *Brassica napus. L,Urtica dioica. L, Anacyclus pyrethrum. L and Calycotome spinosa. L.*

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, carbon clearance test, extracted lectin fromtheroots plants *Anacyclus pyrethrum. L, Brassica napus. L, Urtica dioica.L* and *Calycotome spinosa. L*treated groups, exhibited a 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 theroots plants *Anacyclus pyrethrum. L, Brassica napus. L, Urtica dioica.L* and *Calycotome spinosa. L*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).

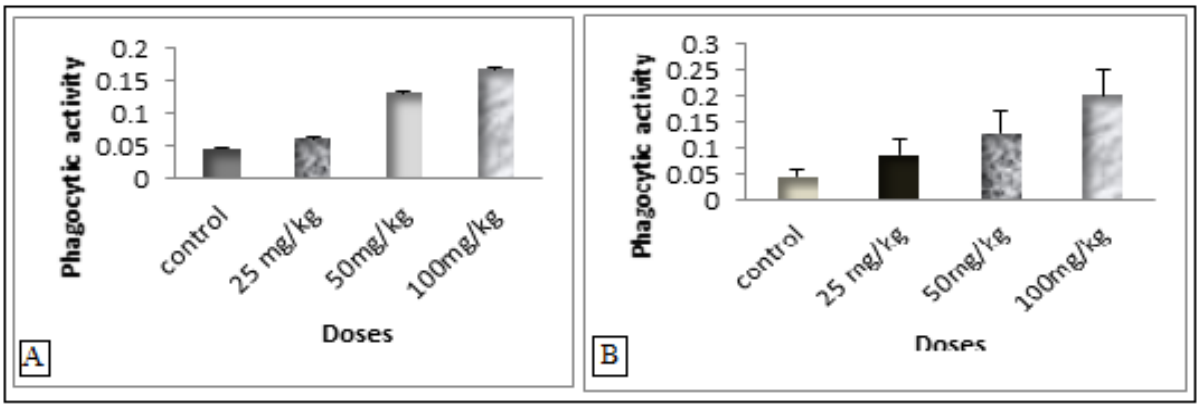

Fig. 6. Effect of lectins extracted from the roots plants *Urtica dioica.L (A) and Brassica napus. L (B)*on phagocytic activity.

In inflammatory reactions induced by exogenous stimulilipopolysaccharide (LPS) which induce neutrophil migration by indirect mechanisms, resident macrophages are believed to be required for the control of neutrophil recruitment.LPS evokes biphasic edema that lasts up to 6 h: the first two hours are sustained byhistamine and serotonin release from mast cells, and the second phase (3–6 h) involves neutrophilinfiltrate, and the release of prostaglandin E2, cytokines (mainly interleukin-1β) and NO (Kulinsky, 2007; Vinegar*et al.,* 1969).Since lectin extracted from root of plants showed an anti-inflammatory effects via inhibition of the paw edema inducedby LPS, such effects seem to be associated with the inhibition of neutrophil migration.Similar effects have also been observed in the plantlectins from *Arum maculatum* (Assreuy*et al.,* 1997) and *Pisum arvense*(Assreuy*et al.,* 1997), although another lectin from *Luetzelburgia*.The present study provides evidence that the lectin extracted from root of plants acts as a potent anti-inflammatory agent in rats in the acute inflammation model.

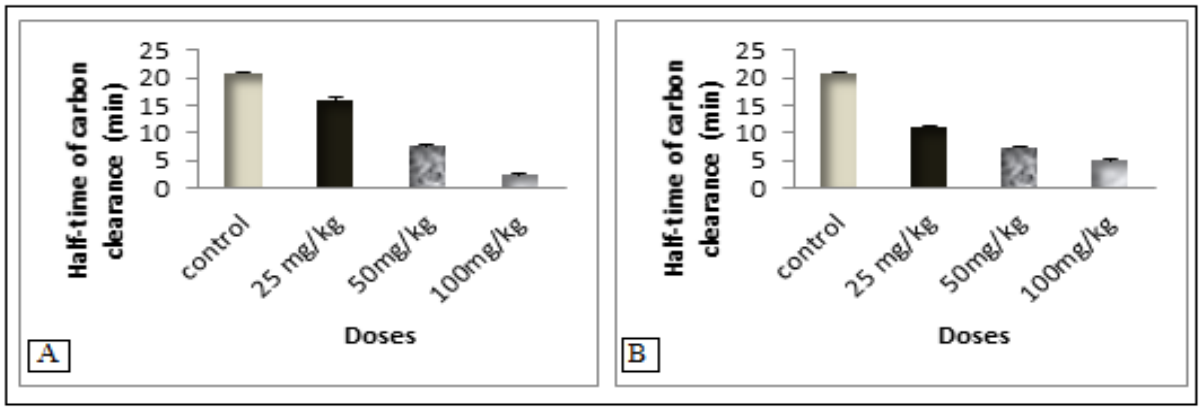

Fig. 7. Effect of lectins extracted fromthe roots plants*Urtica dioica.L (A) and Brassica napus. L*(B) on half -life $t_{1/2}$ of carbon in blood.

Lectin extracted from the root of plants has shown a distinct antimicrobial activity and variable, which is proportional to the diameter of the inhibition zone. The same result was obtained with the lectin of green algae *Bryopsis plumose* against bacterial strains of *Enterococcus faecalis, KCTC 3206; Staphylococcus aureus KCTC 1927 Hirae Enterococcus and Escherichia coli KCTC 3616, KCTC 1116*(Han*et al.,* 2010). Lectin extracted from plants *Calycotome spinosa L, Anacyclus pyrethrum L, Brassica napus L*, showed no antimicrobial activity for the tested strains, except Bacillus *Bacillus cereus* in a partial manner, a similar result was obtained for *Phthirusapyrifolia* (Costa*et al.,* 2010).

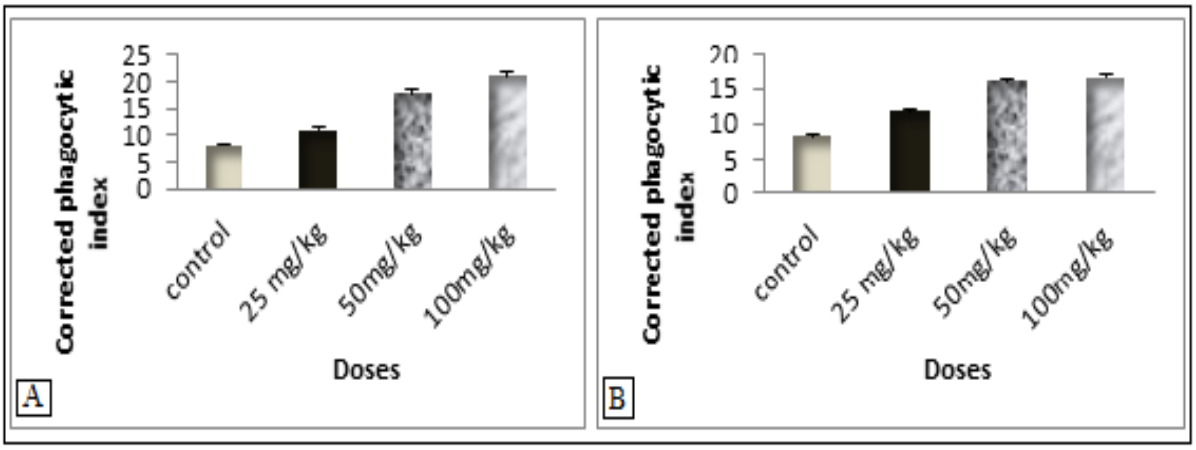

Fig. 8. Effect of lectins extracted from the roots plants *Urtica dioica.L (A) and Brassica napus. L (B)*on corrected phagocytic index.

However, an extracted lectin from plant EHLpresented resistance to three bacterial strains: *Klebsiella pnuemoniae, Escherichia coli and Pseudomonas aeruginosa* (Shaista*et al.,* 2014). In another study carried out on lectin *Tinospora tomentosa* shows their inhibition of *Vibrio mimicus* (17mm), *Staphylococcus aureus and Bacillus cereus* (8mm), *Salmonella typhi* (9mm), *Shigella dysentery* (20mm)(Repon*et al.,* 2014).

Further studies are needed for isolation and purification of this lectin extracted from roots which might be responsible for anti-inflammtory, immunomodulatory and antimicrobial activity.

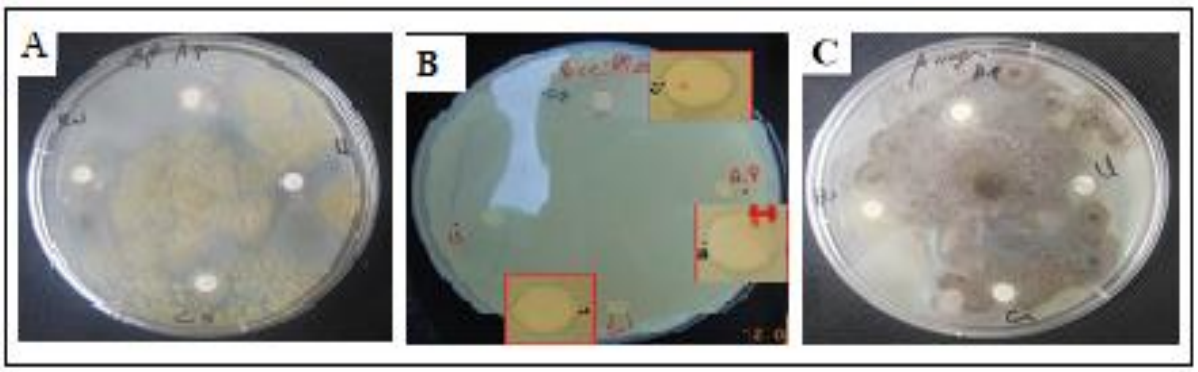

Fig. 9. Extracted lectins against *Aspergillus flafus (A), Bacillus cereus (B) and Aspergillus niger (C) respectively*.

Conclusion

It can be concluded that the extracted lectins from roots of plants holds promise as an immunomodulatory agent, which act by stimulating dose-dependent phagocytic function and showed highly antimicrobial and anti-inflammatory activities.

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