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ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTIMICROBIAL PROPERTIES OF NEW LECTINS PURIFIED FROM ROOTS OF ALGERIAN PLANTS: *MORUS NIGRA, RUTA GRAVEOLENS, CYPERUS ROTUNDUS AND PISTACIA LENTISCUS*.

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ABSTRACT

In this work, the use of desialylated stroma from erythrocytes represented the cheapest method to purify the lectins. To evaluate the antioxidant potential of lectins purified from roots of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus*, in vitro antioxidant activities of lectins using DPPH, SOD, Fe^{+3} reducing assay, H_2O_2 scavenging and lipid peroxydation inhibitions were carried out. DPPH, SOD and Fe^{+3} assays showed that lectins purified from root of *Morus nigra*, Ruta *graveolens, Cyperus rotundus* and *Pistacia lentiscus* significantly reduced DPPH, SOD and Fe^{+3} free radicals with the low IC50 value observed in *Morus nigra (*115.34, 211.11 and 281.25 µg/ml) and in *Ruta graveolens* (208.95, 158.75 and 116.2 µg/ml) respectively compared to standard ascorbic acid. Furthermore, the purified lectins of *Morus nigra* and *Ruta graveolens* showed highest reducing of H_2O_2 and lipid peroxidation inhibition (LPI) with IC50 value 98.76, 27.27 and 163.04, 36.40 respectively compared to standard ascorbic acid. Another hand, purified lectins of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* showed a significant reduction in the edema paw volume and presented a highly activity antimicrobial. The lectins isolated from roots of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* relieved that it was capable of

scavengenging the free radical and exhibit antifungal, antimicrobial and anti-inflammatory activity.

KEYWORDS: purified lectins, antioxidant, anti-microbial, anti-inflammatory.

INTRODUCTION

Natural antioxidants may have free-radical scavengers, reducing agents, complexes of pro-oxidant metals, quenchers of singlet oxygen etc. Recently research has been increased considerably in finding natural occurring antioxidants for use in foods or medical products to replace synthetic antioxidants, which are being restricted due to their adverse reaction such as carcinogenicity. Antioxidant constituents from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance.[1] Free radicals which have one or more unpaired electrons (superoxide, hydroxyl, peroxyl) are produced in normal or pathological cell metabolism; the compounds that can scavenge free radicals have great potential in ameliorating the diseases and pathological cells.[2] Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species.[3] Plants contain bioactive compounds have been reported to possess strong antioxidant properties.[4] Many of the endophytic extract reported the presence of biological activities like antimicrobial, antioxidant, anticancer and anti-HIV properties.[5] Apart from the biological properties, the reports published on endophytic antioxidant properties were very few. Lectin or glycoprotein's are carbohydrate binding proteins that interact with specific sugars and induce several biological activities. Lectin have attracted great research due to their various biological activities like cell agglutination, antitumor, immunomodulatory, antifungal, antiproliferative and antiviral activities These proteins were powerful antioxidants which could inhibit lipid peroxidation and scavenge free radicals.[6] In the present work we describe the purified of a new lectin from the roots of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* collected from Algeria and their Antioxidant, antimicrobial and anti-inflammatory effects.

MATERIALS AND METHODS

The lectins isolation from roots of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* used in this work originated from Algeria.

Lectins Purification by stroma column

The roots of each plant of *Morus nigra*, *Ruta graveolens*, *Cyperus rotundus* and *Pistacia lentiscus* 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 30 min; the remaining debris was removed by passing the supernatant through filter paper.[7] Erythrocyte membranes (stroma) were obtained by lysis of rabbit red blood cells. The membranes were fixed with 1% glutaraldehyde overnight at 4°C, then the stroma were washed with distilled water and freeze dried. Two hundred milligrams of erythrocyte stroma was physically entrapped in a chromatographic column with Sephadex G-25. The crude extract was applied to the stroma column (10 x 1.2 cm) and eluted with buffer at a flow rate of 1 ml, until the A280 of the collected fractions was below 0.01. The bound lectin was eluted with acetic acid (3%) and the pH of each collected fraction was adjusted to 6.

Protein determination

The protein concentrations of the crude and purified lectins of were determined by the method.[8] Bovine serum albumin was used for standard preparations.

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 antioxidant property. The assay was standardized using ascorbic acid as standard. The reaction mixture 4ml contained, 0.1ml of (50, 100, 150, 200 µg/ml) various concentration of lectin samples in 0.1M Phosphate buffer (pH 7.2), 3.9 ml of DPPH (0.025gm/l) solution was added to all the above 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 the method.[9] Scavenging activity of DPPH free radical in percent was calculated according to the equation.

Percent inhibition % = (A-B) x 100/A

Where: A= Absorbance of control reaction.

B= Absorbance of test sample.

The % inhibition of observance was plotted against the sample or the standard concentration to obtain the amount of antioxidant necessary to decrease the initial concentration of DPPH to 50% (IC50). IC50 values were calculated from calibration curve, IC50 values are defined as the concentration of a test compound required to achieve half maximal inhibition and lower IC50 value indicates greater antioxidant activity.

Superoxide anion scavenging activity

Superoxide anion radical scavenging activity was measured[10] 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 was illuminated by exposure to 30W fluorescent lamps for 20 minutes and the absorbance was measured at 560 nm using 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 as negative control. The Superoxide anion radical scavenging activity (%) was calculated as.

[(Ao–A1)/Ao] × 100 where Ao was the absorbance of the control and A1was the absorbance of purified lectins.

Ferric (Fe3+) reducing power assay

Ferric cyanide (Fe^{+3}) reducing power was determined by the method.[11] Fe^{+3} reducing power as an indicator of antioxidant activity is widely accepted. In this method antioxidant compound give a colored complex with potassium ferricynaide in the presence of trichloro acetic 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 ml contained 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 were incubated for 20min at 50ºC, cooled rapidly and mixed with 2.5 ml of 10% trichloroacetic acid and 0.5ml of 0.1% ferric chloride. A blank 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 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, A max = absorbance of maximum absorbance tested, A min= absorbance of minimum absorbance tested and A= absorbance of the sample

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried out.[12] A solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The purified lectins at different concentrations in 3.4 ml phosphate buffer were added to 0.6 ml of H_2O_2 solution (0.6 ml, 43 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Percentage of H_2O_2 radical scavenging activity.

$$= \frac{\text{A control} - \text{Atest} \times 100}{A\ control}$$

Where: A control is the absorbance of the control. A test is the absorbance in the presence of the sample.

Lipid peroxidation inhibition (LPI) assay

Purified lectins were dissolved in 1 ml of 10 mM Tris–HCl buffer (pH 7). The fractions were added with a mixture of ethanol (2.5 ml) and linoleic acid (32.5 µl) and final volume was adjusted to 5 ml with distilled water. The reaction mixture was incubated in a screw capped tube at 60°C in the dark. At 24 h intervals, aliquots of the reaction mixture were withdrawn for measurement of oxidation by the Thiobarbituric acid method. The reaction mixture (100 µl) was added to a mixture of 0.8 ml of trichloroacetic acid (TCA, 100 mg ml−1) and 1.5 ml of TBA (8 mg ml−1) solution in water. The mixture was cooled in an ice bath and centrifuged at 8000 × *g* for 10 min. The absorbance of the supernatant was measured at 532 nm using spectrophotometer. BHT (0.5 mg) was also assayed under the same conditions for comparison purposes.[147]

LPI (%) = [(C–S)/C] × 100 where C was the absorbance of the control and S was the absorbance of lectins.

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 purified lectins of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* at dose 1.5 mg/kg were administrated by intraperitoneal injection 30 minutes before LPS injection. Diclofénac (3mg/kg) wa used as reference drug. Control group received NaCl (0.9%). The inflammation was quantified by measuring the volume displaced by the paw at 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 ayreus ATCC43300, Klebsiella pneumonia ATCC 70603, Bacillus cereus, Candida albicans, Acineto bacter 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 at 15 psi and 120°C for 20 min. Sterile paper disks, 1 cm in diameter, were placed at the surface of heavily seeded medium with the tested organism. A 10 µl aliquot of the purified lectins of Morus *nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* was added to the disk. Petri dish was incubated at 37°C for 48 hrs, at the end of which the diameter of the clear zone of inhibition surrounding the sample was taken as a measure of the inhibitory power of the sample against the particular test organism.[13]

Antibacterial activity

Antibacterial activity of purified lectins of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* was investigated by the disc diffusion method.[14] The microbial strains were obtained from stock cultures in nutrient agar (0.7%). One-hundred millilitres of warm nutrient agar (NA) (43°C) and 0.5 ml of bacteria suspension (105-106 CFU/m1) were mixed and 10 ml volumes were distributed in sterile petri plates (90 X 15 mm) and allowed to solidify. Sterile blank paper discs (6 mm diameter) impregnated with 20µl of sterile solution of purified lectins of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus*

(1.0mg/ml, 2.0 mg/ml). Plates were incubated at 37°C for 24 hrs. A transparent ring around the paper disc revealed antimicrobial activity. Zones of growth inhibition around discs were measured in millimeters.

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

Lectins Purification by stroma column

It was found that in elution fraction after addition of acetic acid 3%, the purified lectins from roots of *Ruta graveolens*, *Cyperus rotundus* and *Pistacia lentiscus* presented a single peak but the purified lectin of *Morus nigra* presented two peak (Fig.1).

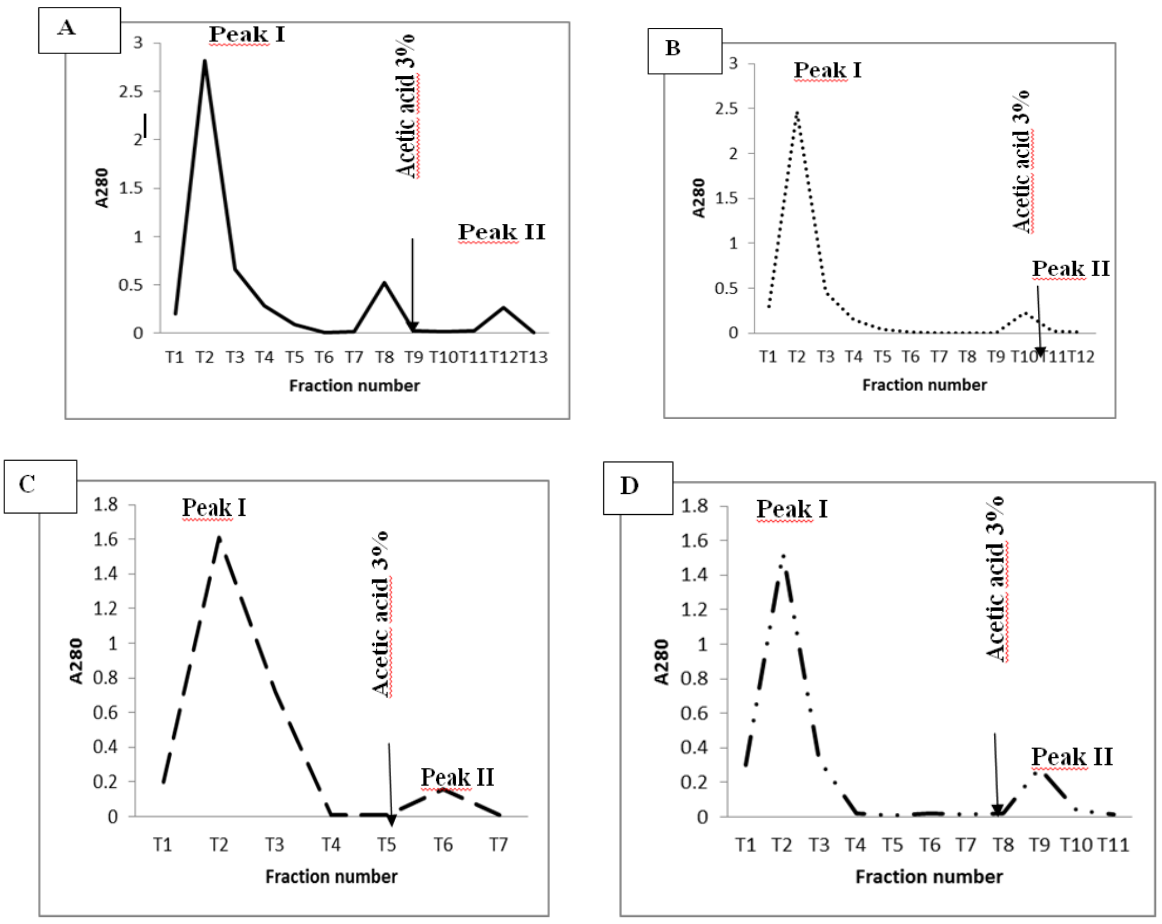

Figure 1: Purification of the lectins from root of *Morus nigra (A)*, *Ruta graveolens(B)*, *Cyperus rotundus (C)* and *Pistacia lentiscus(D)* by affinity chromatography on sephadex G25 column. The column was previously equilibrated with buffer (0.1 M, pH 7.2), the

first peak (I) was eluted with the equilibrium buffer and the second peak (II) eluted with acetic acid 3%.

Determination of Protein concentration

The purified lectins show a highly concentration of protein after purification with values of 0.054, 0.057 and 0.053 mg/ml in roots of *Morus nigra, Ruta graveolens and Cyperus rotundus* respectively. However, purified lectin from root of *Pistacia lentiscus* presented lower concentration of protein (0.017 mg/ml) (Table 1).

Table 1: Protein concentration in crude and purified lectin of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus.*

	Roots of plants	Protein concentration (mg/ml)
Crude	*Morus nigra*	0.42±0.01
	Ruta graveolens	1.93±0.02
	Cyperus rotundus	1.12±0.01
	Pistacia lentiscus	0.10±0.01
Purified lectins	*Morus nigra*	0.054±0.002
	Ruta graveolens	0.57±0.002
	Cyperus rotundus	0.053±0.001
	Pistacia lentiscus	0.017±0.001

Value represent in the results are mean ± SEM of three replicates.

In vitro antioxidant activity of purified lectins of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus*

The four lectins purified from root of Morus *nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* tested for antioxidant activity using DPPH radical scavenging was determined. The isolated lectins from root of plants *Morus nigra and Ruta graveolens* successive showed the maximum antioxidant activity with IC50 values of 115.34 µg/ml and 208.95 µg/ml respectively. The *Cyperus rotundus* and *Pistacia lentiscus* purified lectins also showed antioxidant activity with IC50 values of 215.85 µg/ml and 359.37 µg/ml respectively. The known antioxidant ascorbic acid exhibited IC50 value of 97.1 µg/ml as shown in table 2. Superoxide anion radical scavenging was determined of each of the purified lectins. The values of antioxidant activity determined by superoxide anion radical scavenging method follow the same order as that of DPPH assay. Among the four purified lectins from roots of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* showed the maximum antioxidant activites with IC50 values of 211.11%g/ml, 158.75 µg/ml,

357.14µg/ml and 335.7µg/ml respectively when compared with standard (ascorbic acid). The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. In his assay the ability of purified lectins from roots of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* to reduce Fe^{+3} to Fe^{+2} were determined. Among the four purified lectins from *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* showed the maximum antioxidant activities with IC50 values of 281.25µg/ml, 116.2µg/ml, 334.52µg/ml and 300 µg/ml repectively. The known antioxidant ascorbic acid exhibited IC50 values of 116.2 µg/ml as show in table 2. Hydrogen peroxide and lipid peroxidation inhibition assay determined that: the purified lectins showed the maximum scavenging activities were 98.76 µg/ml and 27.27µg/ml respectively for *Morus nigra*, 163.04µg/ml and 36.40µg/ml respectively for *Ruta graveolens*, 211.53µg/ml and 46.72 µg/ml respectively for *Cyperus rotundus* and 201.75µg/ml and 75.20µg/ml respectively for *Pistacia lentiscus* when compared with standard.

Table 2: Free radical-scavenging assay of purified lectins of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus.*

Plants	Free radical-scavenging assay (IC50 µg/ml).				
	DPPH	SOD	Ferric reducing assay	H_2O_2	Lipid peroxidation inhibition
Morus nigra	115.34	211.11	281.25	98.76	27.27
Ruta graveolens	208.95	158.75	116.2	163.04	36.4
Cyperus rotundus	215.34	357.14	334.52	211.53	46.72
Pistacia lentiscus	359.37	335.7	300	201.75	75.20
Ascorbic acid (standard)	97.1	71.47	106.89	81.32	26.47

Anti-inflammatory activity of purified lectins of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus*

The anti-inflammatory effects of the purified lectins of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* on LPS induced edema in rat's hind paws are presented in table 3.

There was a gradual increase in edema paw volume of rats in the control group. However, in the test groups, purified lectins from root of plants *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* (1.5 mg/kg) showed a significant reduction in the edema paw volume. The results showed that purified lectins of Morus *nigra and Ruta graveolens* causes significant reduction in inflammation compared to standard anti-inflammatory Drug, but

purified lectins of Cyperus *rotundus* and *Pistacia lentiscus* causes no significant reduction in inflammation compared to standard anti-inflammatory Drug.

Table 3: Anti-inflammatory activity of purified lectins of *Morus nigra Ruta graveolens, Cyperus rotundus and Pistacia lentiscus.*

Experiment	Control	LPS	Diclofénac	*Morus nigra*	*Ruta graveolens*	*Cyperus rotundus*	*Pistacia lentiscus*
1h After treatment	0.28±0.001	0.34±0.003	0.25±0.001	0.24±0.002	0.25±0.001	0.23±0.003	0.26±0.002
2h After treatment	0.28±0.001	0.36±0.003	0.24±0.001	0.22±0.002	0.23±0.002	0.20±0.001	0.24±0.001
4h After treatment	0.28±0.001	0.44±0.003	0.20±0.001	0.14±0.001*	0.11±0.001***	0.17±0.001	0.19±0.001

Values are given as mean ± SEM for group of 5 animals each. *P≤0.05, compared to standard drug. **P≤0.01, compared to standard drug. ***P≤0.001, compared to standard drug.

Antibacterial and antifungal activity of purified lectins of Morus *nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus*

purified lectins of *Morus nigra, Cyperus rotundus* and *Ruta graveolens* inhibited the grouth of Bacillus ceraus with diameter of zone inhibition of 0.01, 0.01 and 0.1 mm respectively, but purified lectins of *Pistacia lentiscus* not presented any activity of inhibition. However, purified lectins of Morus *nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* 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 albicans and Acinetobacter sp*. Furthermore, purified lectins of *Morus nigra, Cyperus rotundus* and *Ruta graveolens* inhibited the grouth of *Aspergillus flavus* 9, 4 and 3mm respectively however, the purified lectin of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* inhibited the grouth of *Aspergillus niger* 5, 6, 3 and 4mm respectively (Table 4).

Table 4: Determination of antibacterial and antifungal activity of purified lectins of *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus.*

Microorganisms	Diameter of zone of inhibition (mm)			
	Morus nigra	*Ruta graveolens*	*Cyperus rotundus*	*Pistacia lentiscus*
Escherichia coli ATCC25922	-	-	-	-
Staphylococcus ayreus ATCC43300	-	-	-	-

Klebsiella pneumonia ATCC 70603	-	-	-	-
Bacillus cereus	0.01	0.1	0.01	-
Candida albicans	-	-	-	-
Acinetobacter sp	-	-	-	-
Aspergillus niger	5	6	3	4
Fusarium oxysporum	-	-	-	-
Aspergillus fumigates	-	-	-	-
Aspergillus flavus	9	3	4	-

-: No activity.

DISCUSSION

Imbalance between formation of ROS and antioxidant defense leads to oxidative stress, resulting in potential cellular damage.[15] Several diseases such as cancer, diabetes, premature aging and degeneration disorders have been linked to oxidative stress caused by ROS.[16,17] In living cells ROS are the primary source of oxidative stress condition.[18] Eukaryotic cells protect themselves by antioxidant defense mechanisms such as enzyme, radical scavengers, Hydrogen donors, electron donors, Peroxide decomposers and metal chelating agents.[19] A natural plant contains various antioxidants constituent that acts in different way and are used in the traditional medicine.[20] Free radicals generated during the course of metabolic process of an organism are known to play role in several disorders. Antioxidant principles present in the plant have been shown to posses' free radical scavenging activity.

DPPH assay is one widely used method for screening antioxidant activity of natural products.[21] Maximum value of inhibition of DDPH and SOD scavenging activity was observed in standard ascorbic acid, DPPH and SOD scavenging potential of purified lectins from root of plats *Morus nigra, Ruta graveolens, Cyperus rotundus* and *Pistacia lentiscus* may be due to hydroxyl group present in the lectins.

The Ferric Fe^{+3} reducing power assay mainly depends on the reducing capacity of Fe^{+3} - Fe^{2+} conversion and serve it has an significant indicator of its potential antioxidant activity.[22] Among the 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 the concentration dependent manner.[23,24] Scavenging of H_2O_2 by purified lectinse may be attributed due to the electron donating to H_2O_2, thus neutralizing it to water.[25] Puried lectins were capable of scavenging hydrogen peroxide in a concentration dependent manner. There are some previous researches on the antioxidant activity of endophytic fungi from other

medicinal plants. For example[26,27] two antioxidant pestacin, isopestacin from the endophytes fungi *Pestalotiopsis microspora* reported that 22.5% of extracts from endophytic fungi and *Garcinia planta* exhibit remarkable antioxidant activity.[28] Previously reported that methanolic extract of *Viscum album* has antioxidant capacity has been reported that *Viscum album* has a high antioxidant activity was (82.23%).[29,30]

Free radicals cause significant alteration in the structure of biological membranes that interferes with cellular integrity and metabolism leading to cellular toxicity.[31] Many researchers have employed the use of these free radical generators for determination of antioxidant capacity of the given protein. Proteins have the ability to inhibit the free radical generation in both in vitro and in vivo systems.[32] Purified lectins isolated in this investigation have the highest antioxidant 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 potent antioxidants against free radicals.[31,33,34] Lipid peroxidation leads to rapid development of rancid and stale flavors and is considered as a primary mechanism of quality deterioration in lipid foods and oils. Addition of the antioxidants to foods could stabilize the products over extended shelf life periods.[35] Peptides found in daily consuming food products can also act as antioxidants. Peptides from egg yolk and casein have inhibited lipid oxidation in various muscle foods like beef, tuna.[36,37] Purified peptide was also a potent antioxidant, with 80.46% inhibiting lipid oxidation. Many natural antioxidants were comparably less potent than synthetic antioxidants, but if used at higher concentrations could replace the use of synthetic antioxidants owing to their toxicity.

In inflammatory reactions induced by exogenous stimuli lipopolysaccharide (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 by histamine and serotonin release from mast cells, and the second phase (3–6 h) involves neutrophil infiltrate, and the release of prostaglandin E2, cytokines (mainly interleukin-1β) and NO.[38,39] Since lectin extracted from root of plants showed an anti-inflammatory effects via inhibition of the paw edema induced by LPS, such effects seem to be associated with the inhibition of neutrophil migration. Similar effects have also been observed in the plant lectins from *Arum maculatum*[40] and *Pisum arvense*[41], although another lectin from *Luetzelburgia*. The present study provides evidence that the

lectin extracted from root of plants acts as potent anti-inflammatory agent in rats in acute inflammation model.

Lectin extacted from root of plants have 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.[42] Lectins purified from root of *Morus nigra*, *Cyperus rotundus* and *Ruta graveolens*, showed no antimicrobial activity for the tested strains, except *Bacillus cereu*s in a partial manner, a similar result was obtained for *Phthirusa pyrifolia*.[43] However, purified lectins from plant EHL presented resistance to three bacterial strains: *Klebsiella pnuemoniae, Escherichia coli and Pseudomonas aeruginosa*.[44] 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).[45]

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