IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
**Research Article**
September 2016 Vol.:7, Issue:2


# Physicochemical Characterization of Phytolectin Isolated from Legume Plants and Antibiogram Study against Soil Borne Pathogens

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

**Andrewslin Jani[1], V. Jeba Priya[2], R. Jeyalakshmi and Sreedevi, S[2]**

*1. Assis. Professor, Depmt of Microbiology, MCC, Mariagiri-629753.*

*2. 2nd M.sc Students, Depmt of Microbiology, MCC, Mariagiri-629753, India.*

**Submission:** 3 September 2016
**Accepted:** 7 September 2016
**Published:** 25 September 2016



**Keywords:** Rhizobium, Lectin, Legume plants and Carrier molecules

**ABSTRACT**

In the present study, the legume plant (*Pisum sativum* L.) was cultivated using rhizobium as bio fertilizer using carrier material as compost. Phytolectin was isolated from the seeds of legume by ammonium sulphate precipitation at 70% saturation which was dialyzed and purified. The phytolectin was characterized by haemagglutination and biochemical test. It showed maximum stability at pH 8.0 and 25°C. The isolated lectin was subjected to SDS- PAGE and its molecular weight was found to be 27.5kDa. The antibacterial activity of isolated lectin was tested against soil borne pathogens and compared with commercial antibiotics revealed highly effective. The overall results of present study showed that the antibacterial activity of the lectin could be enhanced if the component was completely purified. Hence, the study expressed that the isolated plant lectin extract served as a potential source of novel herbal drug for treating infectious diseases caused by some of the soil borne pathogens.

## INTRODUCTION

*Rhizobium* is the most well-known species in the group of bacteria that act as a primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of lumps or nodules where nitrogen fixation takes place (Adenike *et al*., 2003). They are regular in structure, appearing as straight rods; in the root nodules, the nitrogen-fixing form exists are irregular cells called bacteroids which are often club and y shaped (Sharma *et al*., 2009). The word Lectin has been derived from Latin word which means choose because lectins are very specific to the site to which it binds. Lectins are carbohydrate-binding proteins which bind to glycoproteins, glycolipids and also polysaccharides (Goldstein and Hayes, 1978) which mediate various kind of biological processes by binding to a different sugar moiety (Sharon and Lis, 1998). Lectins were characterized for their agglutination properties with erythrocytes of human and other animals, which is the easiest and most convenient method of detection of lectin activity (Laija *et al*., 2010; Lam and Bun, (2011). The screening of a number of breast cancers against lectins shows different specificities (Kilpatrick, 1991).

Lectins are found commonly in most legumes and their toxic effects have been seen (Liener, 1986). It has been suggested, for instance, that the potato lectin (which is considered as a cell wall protein) immobilized avirulent strains of *Pseudomonas solanacearum* in the cell wall (Sequeira and Greham, 1977). The bioactive properties of lectin obtained from red kidney bean (*Phaseolus vulgaris*) possess the ability to agglutinate red blood cells and had a molecular weight of 32kDa. (Shia *et al*., 2007). The haemagglutination activity of BveL was specific for any human blood group (Silva *et al*., 2007). There is amble of works studied of this kind, hence the present work was planned by the following objectives

- To isolate and characterize the nitrogen-fixing bacteria *Rhizobium* from root nodules of Legume plants
- To prepare biofertilizer using compost as a carrier material
- To check the growth rate of the legume plant
- To separated the Lectin from Legume seeds and confirmed by the Haemagglutination Assay
- To detect the molecular weight SDS-PAGE was performed

- To perform the Antibiogram study of selected plant pathogens like *E. coli, Klebsiella, Bacillus, Proteus, Shigella, Staphylococcus, and Pseudomonas*

## MATERIALS AND METHODS

### Sample collection

The samples were collected from different fields of Thiruvattar. Root nodules were isolated from the legume plants. The samples collected in sterile polyethene bags and immediately transported to the laboratory.

### Isolation of Rhizobium species from Root Nodules

The surfaces of the nodules were removed by surface sterilization with 1% mercuric chloride for 30 seconds. Excess mercuric chloride bis removed by washing with sterile water. The nodules were transferred to a Petri plate containing 70% ethanol for 1 minute and then washed with sterile water. The nodules were crushed with a clean pestle and mortar. The suspension containing a large number of rhizobial cells serially diluted up to $10^{-5}$ using sterile water. YEMA medium was prepared was prepared and poured in Petri plate. After cooling 0.1ml of the sample was inoculated and incubated at room temperature for 3 days.

### Identification of Rhizobium

The Rhizobium was identified by the staining technique carried out were simple and gram staining. The biochemical test done were Motility Indole, Methyl red, Voges-Proskauer, Catalase, Oxidase, Urea hydrolysis, and Hydrogen sulfide production.

### Mass Multiplication of Rhizobium

250 ml of nutrient broth of YEMA broth was prepared and loopful of isolated white colonies from YEMA plate was used for mass multiplication of Rhizobium. The flask was incubated at room temperature on a rotary shaker for 2-6 days the count of viable cells in final broth culture should not be less than 1X $10^6$ per ml.

**Preparation of Carrier Material and Carrier Based Culture**

Compost was selected as a suitable carrier. The desirable character for a good carrier material was

- Water holding capacity
- Particle size
- Organic matter

Carrier materials were allowed to sun dried up to a moisture content of 5%. Heat drying of carrier material leads to a deleterious effect on the organism. Carrier material was grinded using pestle mortar. The preheated carrier was filled up to two third of the capacity of steel sterilized at 121$^{o}$C for 3 hours continuously on autoclave. A carrier material was mixed with 10% calcium carbonate to neutralize the pH (6.5 to 7.0). The Rhizobial microbial broth was mixed with finely powdered and sterilized carrier material up to one-third of the water holding capacity of the carrier.

**Pot Experiment**

The pots used in the experiment were sterilized. Seeds were sown in sterilized pot containing autoclaved soil and biofertilizer. Pots were labeled properly. The pots were observed and watered regularly till 21 days. Between 15 days, interval growth parameters of the plant were analyzed.

**Isolation and Biochemical Characterization of Lectin from Legume Plants**

**1. Isolation of Lectins**

The uncoated seeds were soaked in PBS for overnight. Then the seeds are grinded with minimum volume of PBS and the plates were collected in 50 ml centrifuge tubes and were centrifuged by Eppendorf centrifuge 7500 rpm, at 4$^{o}$C for 20 mins. The supernatant was taken by salting out process. The supernatant was then 70% saturated with ammonium sulfate and allowed to stand for 24 hours. The precipitated phytolectins were collected by centrifugation at 2500 rpm for 30 minutes. The samples were dialyzed with PBS, dried and kept at 4$^{o}$C.

## 2. Haemagglutination Assays

Healthy human venous blood (4-5) was collected in a 15 ml tube to which the anticoagulant EDTA was previously added. A 1ml blood sample was centrifuged in 2 ml microtube at 1000 rpm for 5minutes at room temperature by Eppendorf mini spin. The pellet was collected and 10 ml of phosphate buffered saline (PBS) was added. The mixture of blood and PBS was centrifuged at 100o rpm for 5min at room temperature. After centrifuge, the pellets were collected and 100 µl of pellet was added to 10 ml of PBS solution (pH 7.2). The haemagglutination activity of legume lectin was detected when blood erythrocytes were added to it. The assay was carried out in a 96 well round bottom microtiter plate. The first well of each row was served as a positive control to which 100 µl of normalized sample and 100 µl of blood was added and last well served as a negative control since it contains 100 µl of blood and 100 µl of PBS solution. Between the positive and negative control, each well contains blood, PBS, and lectins. First of all 100 µl PBS was added to all the wells. Then 10 µl of normalized crude was poured to the first well and it was serially diluted till the negative control. Similar procedure was followed for the other samples. Finally, 100 µl of processes blood samples was poured to each well after the plate was placed on a plane surface without disturbing it. After two hours the haemagglutination assay result was observed.

## Protein Purification and Determination

The electrophoretic fractionation of phytolectin was done by Polyacrylamide Gel Electrophoresis and the total protein in the seed extracts of legume was estimated by using the method was modified by Lowry et al., 1951.

## Solubility Test

The solubility tests were performed using water and NaOH solution.

## Colour Reaction

The colour reactions were performed with the isolated samples of legume lectin with 2% biuret reagent.

**pH Stability**

The legume lectin was adjusted to different pH values ranging 3 to 12, adding 40 µl of the universal buffer. After being left for 1 hour at room temperature, the samples were adjusted back to pH 7.4 with 30 µl of 0.5 M Tris HCL buffer, pH 7.7 and assayed for haemagglutinating activity.

**Thermal Stability**

The thermal stability of legume lectin was determined by incubating the lectin (1mg/ml 0.05M sodium acetate buffer, pH 6.0 containing 0.15 NaCl) at an elevated temperature ranging between 20$^{o}$C to100$^{o}$C for a period up to 60 minutes. The samples were color and done the hemagglutinating activity.

**Antimicrobial activity against soil pathogen**

**Microbial strains**

Soil pathogens are isolated from soil by serial dilution agar plating method. Both Gram-positive and Gram-negative strains were used in this study.

**Testing of Antimicrobial Activity**

Muller-Hinton medium powder was dissolved in distilled water and autoclaved for 20 minutes for sterilization, poured into sterile Petri dishes and the allowed to cool. The entire surface of Muller-Hinton medium was lawned with nutrient broth culture using cotton swabs. Well were cut and the well as control was applied to the inoculated media. The plates were then kept at 37$^{o}$C in an incubator. The plates were examined after overnight incubation. The zones showing complete inhibition, clear none, was measured and the diameters of the clear zones.

**RESULT**

**Isolation of Rhizobium from Root Nodules**

The Rhizobium was isolated from root nodules of a legume plant. The microscopical, cultural and biochemical test showed as Gram-negative, motile and grown in Yeast Extract Mannitol

Agar medium. The colonies with mucoid, glistening, and elevated morphology were enumerated from the agar surface. The colonies varied in size between 1.5 to 4.5mm. The growth of Rhizobium on YEMA medium is shown in plate 1. The biochemical test shows positive in indole, Voges-Proskauer, Catalase, Gelatin hydrolysis, Oxidase, Starch hydrolysis, Urea hydrolysis and also it shows negative in Methyl red.

**Preparation of Biofertilizer**

Carrier material like compost is sterilized, dried and crushed. The Rhizobium was grown on YEMA broth for 48 hours incubation at 37$^{o}$C on a rotary shaker. The bacterial suspensions are mixed with carrier materials uniformly. Legume seeds are grown prepared biofertilizer in different length and height was recorded. After 30 days of germination, changes occur in the test and control plants. Rhizobial biofertilizer using compost as a carrier material showed high growth rate.

**Isolation of Lectin**

Better yielded plant seed (compost with Rhizobium as a biofertilizer) was collected and the extract of the seed was treated with solid ammonium sulphate and the fraction was precipitated at 70% saturation. The pellet was collected and dialysed using PBS solution.

**Determination of Lectin**

The lectin agglutinate the human erythrocyte, this confirms that the fraction is phytolectin. The protein yield and the molecular weight also studied. The molecular weight of the lectin by SDS-PAGE revealed 27.5 Da. The lectin was non-soluble in Water and soluble in NaOH. The color was also give positive in biuret reagent. The lectin was stable at pH 8 and temperature is 25$^{o}$C

**Antibiogram Study of Lectin**

Antibiogram study of Lectin was done by selected soil pathogens such as *E. coli*, Bacillus, Proteus, *Shigella, Klebsiella,* and *Pseudomonas*. Then it was compared with commercial antibiotics such as Gentamycin ($G^{10}$), Chloramphenicol, Kanamycin, and Erythromycin. Lectin showed high resistant to *E. coli*, *Bacillus*, *Proteus*, *Shigella* and *Pseudomonas* when compared with commercial antibiotics.

**Table- 1: Biochemical Characterization of Rhizobium**

| Sr. No | Character of Rhizobium | Result |
|---|---|---|
| **1** | Gram staining | Gram-negative |
| **2** | Motility | Positive |
| **3** | Indole | Positive |
| **4** | Methyl red | Positive |
| **5** | Voges-Prokauer | Positive |
| **6** | Catalase | Positive |
| **7** | Gelatin hydrolysis | Positive |
| **8** | Oxidase | Positive |
| **9** | Starch hydrolysis | Positive |
| **10** | Urea hydrolysis | Positive |

**Table-2: Biochemical Characterization of Lectin**

| Sl No | Biochemical Test | Result |
|---|---|---|
| **1.** | Haemagglutination assay | Agglutinate 'A' positive blood |
| **2.** | Solubility | Nonsoluble in water<br>Soluble in NaOH |
| **3.** | Colour reaction | Positive in biuret |
| **4** | Lowry's method | 1.48 mg/ml |
| **5** | Molecular weight | 27.5 KDa |
| **6** | pH stability | 8 |
| **7** | Thermal stability | 25 |

**Table-3:** Anti-microbial Activity of Lectin against Soil Pathogens

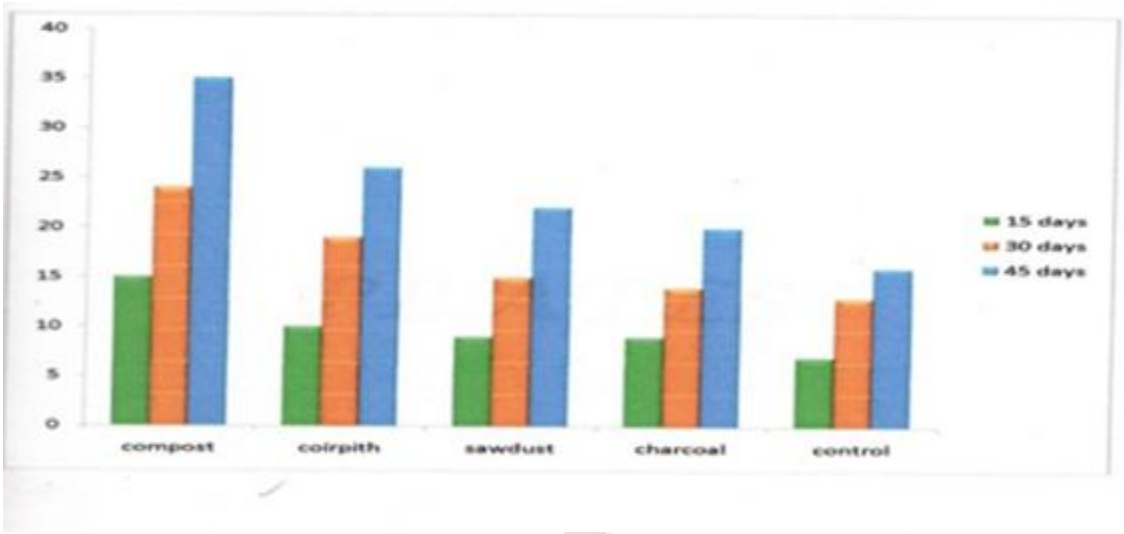
| Sr. No | Organism | 50 µl | 100 µl |
|---|---|---|---|
| **1** | *E .coli* | 12 | 16 |
| **2** | *Bacillus* | 13 | 19 |
| **3** | *Proteus* | 13 | 16 |
| **4** | *Shigella* | 14 | 19 |
| **5** | *Klebsiella* | 11 | 13 |
| **6** | *Pseudomonas* | 13 | 17 |

**Table-4: Antibiogram of Commercial Antibiotics against the Selected Soil Pathogens**

| S. No | Antibiotic Disc | *E. coli* | | *Bacillus* | | *Proteus* | | *Shigella* | | *Klebsiella* | | *Pseudomonas* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Z** | **S** | **Z** | **S** | **Z** | **S** | **Z** | **S** | **Z** | **S** | **Z** | **S** |
| **1** | Gentamycin ($G^{10}$) | 9 | R | 11 | R | 8 | R | 7 | R | 9 | R | 7 | R |
| **2** | Chloramphenicol | 13 | I | 9 | R | 9 | R | 13 | R | 12 | R | 4 | R |
| **3** | Kanamycin | 7 | R | 2 | R | 7 | R | 7 | R | 7 | R | 2 | R |
| **4** | Erythromycin | 5 | R | 7 | R | 1 | R | 3 | R | 10 | R | 4 | R |

**Z-zone of inhibition in mm, R- Resistant, S-Sensitive, I-intermediate**

**Fig: 1 Growth of Legume.Plant Using Various Carrier Materials**



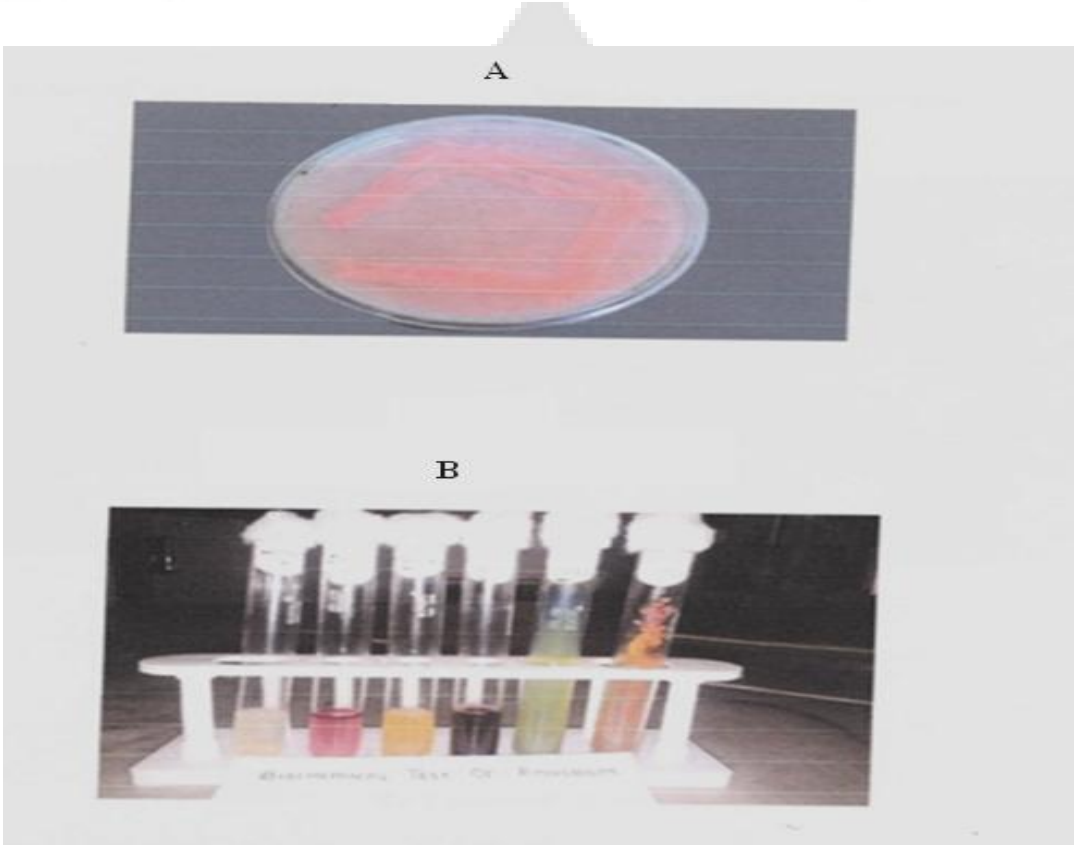




**Fig: 2 and 3- Confirmation of Rhizobium colony through A) YEMA plate B) Biochemical characterization**

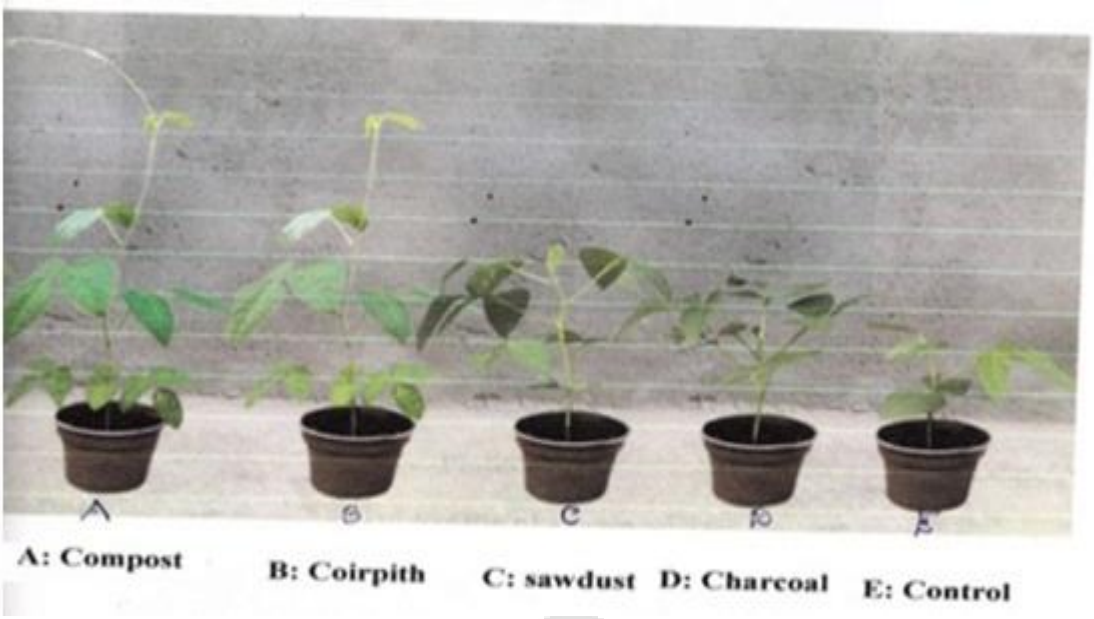



**Fig: 4- Influence of four various experimental carrier molecules and its growth efficiency of Legume plant**

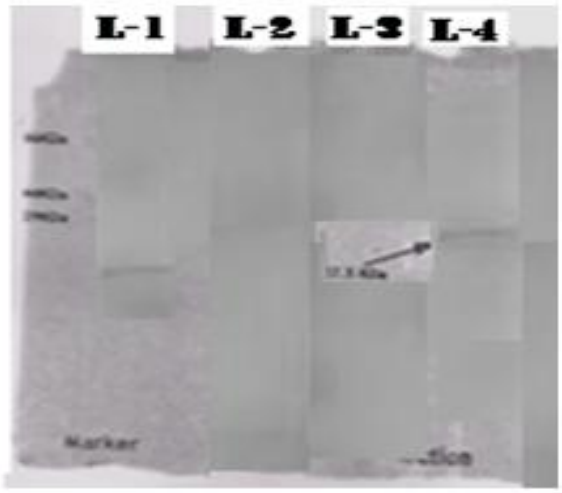



**Fig: 5- SDS Profile for confirmation of Lectin**

## DISCUSSION

In the present work biochemical characterization was performed and pure culture was maintained at culture slant. Rhizobiums are temperature and pH sensitive. They were unable to grow in the presence of Methylene blue and lactose. It utilizes glucose and search as sole carbon source (Deora and Seth V.K, (2010)) and it was confirmed the purity of Rhizobium isolates by physiological and biochemical characterization (Decak *et al*., (2006). Carrier material like compost is used to prepare the biofertilizer, the Rhizobium was mass multiplicated and mixed with the carrier material. Nandi *et al*., (2000) was performed. Mass Scale production of Rhizobium in the fermentation medium. Molasses could serve as a superior carbon source for Rhizobial growth (Silva *et al*., 2001). Then it was applied for the growth of Legume plant. Similarly, Patil and Verma, (2013) isolated Rhizobium and produced Biofertilizer using different carriers (lignite, bagasse, rice husk, groundnut shell). The growth was studied on different plants. The viability of Rhizobium was studied in four different bioinoculant carriers at various temperatures (Saha *et al*., 2001; Feng *et al*., (2012). purified the *Pinellia ternate* lectin from 95% saturated ammonium sulfate precipitation by applying mannose-sepharose 4B affinity chromatography (Moreira *et al*., 1991; Sekhar, 2012) was purified the ammonium sulfate precipitated lectin dialyzed against PBS (Kocourek. and Horejisi,1981; Chao and Burton, (1984). In the present study the molecular weight of lectin by SDs PAGE was revealed as 27.5 kDa., Feng *et al*., (2012) suggested that the lectins a glycoprotein with the molecular weight of 12.165 kDa had proved the lectins molecular weight is 22 kDa. New lectin from seeds *Moringa oleifera* have the molecular weight is 26.5 kDa (Andrea *et al*., 2009). The bioactive properties of lectin obtained from red kidney bean had a molecular weight of 32 kDa (Balaji *et al*., 2012) and Shia *et al*., 2007.

After the growth of legume plant, high yield giving plant seed was selected for further investigation. The seeds of legume plant were collected and the extract was prepared by using PBS. The seed extract was treated with solid ammonium sulfate and the fraction was precipitated at 70% saturation. The pellet was collected and dialyzed using PBS solution. A hemagglutination test was performed to check the confirmation of the phytolectin. Then the concentration of the protein was detected by Lowry's method and the molecular weight was checked by using SDS-PAGE method. The solubility, color reaction, pH stability, temperature stability was also

performed. The lectin was found to have high-affinity inhibitory effect against the bacterial pathogens tested while compared with commercial antibiotics when compared with commercial antibiotics. Nair *et al* (2013) had studied antimicrobial activity using agar well diffusion method. The results showed that the pea lectin had a potent antibacterial activity against Gram-negative and Gram-positive bacteria towards human and plant pathogens.

In this work the Antibiogram study against soil bacteria such as *E. coli, Bacillus, Proteus, Shigella, Klebsiella* and *Pseudomonas* when compared with commercial antibiotics. Nair *et al* (2013) had studied antimicrobial activity using agar well diffusion method. The results showed that the pea lectin had a potent antibacterial activity against Gram-negative and Gram-positive bacteria towards human and plant pathogens. The lectin A was purified from *Crotalaria paulina* seeds has the ability to act against the bacterial infections this kind of opinion had been suggested by following authors Pando *et al*., (2004); Chandrika and Shalia, (1987); Hsu *et al*., (2006) Nitrogen fixing bacteria Rhizobium was isolated from root nodules of leguminous plant and characterized Rhizobium by macroscopic, microscopic and cultural and biochemical test (Adenike and Geretan, 2003; Andrea *et al*., 2009). The Rhizobium was grown on YEMA broth for 48 hours incubation at 37$^{o}$C on a rotary shaker. The biofertilizer is prepared by using sterilized different carrier materials such as compost, coir pith, charcoal powder, this kind of similar result was already reported by Ayouba, 1994; Charungchitrak *et al*. (2011)

## CONCLUSION

*Rhizobium* is the most well-known species of the group of bacteria that act as a primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of lumps or nodules where nitrogen fixation. Plant lectins are a heterogeneous group of proteins or glycoproteins that share in common their ability to bind specific sugar residues and to agglutinate cells. Because lectins are found in many different species and in many different organs and tissues of plants, it is assumed that they play fundamental biological roles, including part of their defense mechanisms. Apart from this result clearly showed when the plant seed (compost with Rhizobium as a biofertilizer) extract was treated with solid ammonium sulfate and the fraction was precipitated at 70% saturation as a result better yield was present. Furthermore from the PAGE analysis expressed the result was confirmed the lectin molecular

weight 27.5 kDa. The lectin was non-soluble in water and soluble in NaOH. The color was also given positive results in biuret reagent. The lectin was stable at pH 8 and temperature is 25$^{o}$C. In this work the Antibiogram study against soil bacteria such as *E. coli*, *Bacillus*, *Proteus, S. Klebsiella* and *Pseudomonas* suspensions are mixed with carrier materials uniformly. Legume seeds are inoculated and the growth rate was checked at different intervals.

## REFERENCES


1. Adenike. H. K. and Geretan. O. B (2003). Purification and partial characterization of a lectin from the fresh leaves of *Kalanchoe crenata* (Andra.). *Journal of Biochemistry and Molecular Biology*, 37: 229-233.
2. Andrea.F.S. Luciana. A. L, Adriana.C. C. Argolo.J/A Teixeira.P. Pavia.M.G. Luana.C.B.B. (2009). Isolation of a seed coagulant *Moringa oleifera* lectin. *Process Biochemistry*
3. Ayouba. G., Bohlool. B.B and Schmidt. E.L. (1994). Lectins: a possible basis for specificity in the Rhizobium-legume root nodule symbiosis. *Science.* 185: 269-271.
4. Balaji. P., arun.K.P., Kumar.S. R., Sundaram.M and Brindha. P. (2012). Isolation and identification of Adaptogenic protein from *Cicer arietinum* Linn. *Int J pharm Pharm Sci*. 4, 79-82
5. Chandrika R, and Shalia. M. S (1987). Isolation and properties of a lectin from the seeds of *Mimosa invisa*. *L. J. Biosci*.12. 383-391.
6. Chao.W. L, Burton. J. C. (1984). Rhizobium inoculants preparation. *Trop. Agric*. Pp 8: 291-295.
7. Charungchitrak. S, Petsom. A, Sangvanich. P, Karnchanat. A. (2011). Antifungal and antibacterial activites of lectin from the seeds of *Archidendron jiringa*. *Nielsen Food Chemistry*. 126. 1025-1032.
8. Deora.G.S., Seth.V.K., (2010). An improvised Portable fermentor for small scale production Of Rhizobium Culture. *Curr. Sci*. 42: 394-395.
9. Deca. A. K, Liener. I.E. Sharon.N and Goldstein. I. J. Ed. (2006). The lectins: properties, functions and applications in Biology and Medicine. *Orlando, Florida, Academic Press*. 33-247.
10. Feng.R, Zhang.W. (2012). Characterization and biological activities of lectin isolated from Pinelia ternate. *Advances in Biological Chemistry*. 2. 115-122.
11. Goldstein. I. J and Hayes. C. E (1978). The lectins: Carbohydrates-binding proteins of plants and animals. Adv. Carbohydr. Chem. Biochem.35: 127-340.
12. Hsu. K. L, Pilobello. K.T and Mahal, L.K. (2006). Analysing the dynamic bacterial glycome with a lectin microarray approach. *Nat. Chem. Biol*., 2: 127=157.
13. Kilpatrick. D.C. (1991). Lectin interactions with human leucocytes: mitogencity, cell separation, clinical applications, in D.C Kilpatrick, E. Van Driessche, T.C Bog Hansen (Eds). Sigma Chemical Company, St Louis, USA. *Lectin Reviews*, 1: 69-80.
14. Kocourek. J and Horejisi.V (1981). Nature (London), 290: 188.
15. Laija.S.N., Mahesh.S., Smith.L.S and Remani. P (2010). Isolation and Partial Characterization of two plant lectins. *Current Research Journal of Biological Sciences*.2 (4): 232-237.
16. Lam. S. K, and Bun.T (2011) lectins: production and practical applications. *Appl microbial Biotechnol*. 89. 45-55.
17. Liener. I. E (1976). Phytohaemagglutinin (phytolectin). *A Review Plant Physiology*. 27 291-391.
18. Liener. I. E (1986). Nutritional significance of lectins in the diet. San diego. Academic Press. Inc): 527-552.
19. Sharon, N and Lis. H. (1998): Lectins: carbohydrates- specific proteins that mediate cellular recognition. *Chem. Arev*., 98: 637-674.
20. Moreira. R. A, A. Inouz. I. L, Oliveira. J. T. A and Cavada. B.S (1991). Plant lectins: Chemical and biological aspects. *M. Emoriasdo Instituto Osvaldo Cruz* , 86: 212-218.

21. Nair. S.S., Madembil. N.C., Nair.P Raman. S, Veerabadrappa.S.B. 2013 comparative analysis of the antibacterial activity of some phytolectins. *International Current Pharmaceutical Journal*. 18-22.
22. Nandi. P, Bhattacharya.P, Singh.T (2000). Simplified methods of mass production and application of biofertilizers. *Proc.Natl.Sem.Organic farming*, 166-178.
23. Gomes, P.M.G., Napoleao. F. S., Correial. T.H. and Coelho. L.C. (2010) Antimicrobial activity of secondary metabolites and lectins from plants. *Applied microbiology and microbial biotechnology*. 396-406.
24. Pando. L.A, Carvalho. D.D., Toyama. M.H., Ciero. L., Novello. J. C., Pascholatti. S. F., Maragoni.S. (2004). Purification and characterization of a lectin from *crotalaria paulina* seeds. *Protein*. J. 7.437-444.
25. Patil. N.N., Verma.O.P (2013). Preparation of biofertilizers on the yield of Chickpea (*Cicer arietinum L.). Ind. J. Microbiol*. 39: 249-251.
26. Saha. A. K., Jauhari. K.S. (2001). Dilution potima of broth cultures for maximizing production of carrier based inoculants. *Curr.Sci*, 57: 507-509.
27. Sekhar. C. 2012. Isolation and Characterization of Soybean (*Glycine max*). Lectin *National Institute of technology*, 2-24
28. Sharma.A. Bun.T., Howong. J., Lin.P. 2009. Purification and characterization of a lectin from *Phaseolus vulgaris* cv. (Anaszari Beans). *Journal of Biomedicine and Biotechnology* 5-6-10.
29. Sharon. N. and H. Lis, (2004). History of lectins from hemagglutinins to biological recognition molecules. *Glycobiology*. 14: R 53-R 62.
30. Shia.J, Xuea.S. J., Kakudab.Y., Ilicb.S., Kimb.D (2007). Isolation and characterization of lectins from kidney beans (*Phaseolus vulgaris* ). *J. Procbio*. 7 - 15.
31. Silva. J. A., Damico.D.C.S., Baldasso. P. A., Mattoli. M.A., Winck. F. K., Fraceto. l. F., Novello. J. C. (2007). Isolation and Biochemical characterization of Galactoside-binding lectin from *Bauhina variegate* Candida (BvcL) seeda. 26: 193-201.
32. Silva. A. L. C., Horta.A.C.G., Moreeria.R.D. (2001). Isolation and partial characterization of a lectin from *Bauhinia pentandra* (Bong) Vog. *Ex.Steua.R.Bras.Fisol*, l 13(3): 262-269.