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ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF A LECTIN ISOLATED AND PURIFIED FROM *ADHATODA VASICA* L.

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ABSTRACT

Adhatoda vasica belongs to a member of the family Acanthaceae. It is a well-known plant drug in Ayurvedic and Unani medicine and which is found in worldwide distribution. A lectin has been isolated from the leaves of Adhatoda vasica by the purification procedure of 80 % ammonium sulphate precipitation, followed by dialysis against 50 mM Tris HCl pH (7.6) and sephadex G -100 column chromatography. The Flow rate of 1.5 mL 12 min^{-1} fractions showed haemagglutinating activity against on rabbit 4% erythrocytes. The purified lectin named as Adhatoda vasica Lectin (AVL) results for molecular mass determinations produced a single band on SDS PAGE (20 KDa), revealing that tetrameric nature. It agglutinated against human A, B, AB, and O system, cow, sheep, Goat, mice, rabbit, and big erythrocytes and result that rabbit erythrocyte specificity. Haemagglutination was inhibited by D-galactose sugar specificity. The antimicrobial activity of the purified lectin was carried out by Agar disc diffusion using appropriate standards. The antimicrobial effect of AVL was evaluated by determining the minimal inhibitory, bactericide and fungicide concentrations. Highest bacteriostatic and bactericide effects were detected for S. paratyphi 13 mm µg ml^{-1} and antifungal activity 13.25 mm µg ml^{-1} S. aureus and S. epidermis MIC value (3.59, 2.5 and 1.72, 1.6)µg ml^{-1} E. coli and S. paratyphi respectively. The thermo stability of AVL is 30°C to 60°C. pH stability of the lectin in the range of 5 - 7. AVL confirmed a remarkable antibacterial activity against the pathogenic bacteria and inhibited the growth of fungi.

KEY WORDS: Adhatoda vasica Lectin; Haemagglutination; Lectin Isolation; Antimicrobial; Protein purification.

INTRODUCTION

Lectins are proteins of non-immune origin that bind to carbohydrates and sugar containing substances in a specific and reversible way or precipitate glycoconjugates [12]. The main properties of lectins are based on their ability to interact with carbohydrates and thus combine with glycocomponents, leading to their biological properties [13]. The interaction of lectins with particular carbohydrates can be as specific as the interaction between those of antigen and antibody or substrate and enzyme [24]. Lectins bind not only to oligosaccharides on cells but also to free floating glycans including monosaccharides. Lectins are able to tightly bind to and cause the precipitation of specific polysaccharides and glycoproteins because they are polyvalent. Lectins can recognize mono, oligo or polysaccharides, as well as glycoconjugates and thus recognize glycoproteins or glycolipids on the surface of cells [34].

The lectins are arranged as dimers or tetramers, and existing as multiple isoforms sharing similar biochemical properties [11]. In addition to their carbohydrate binding activity, some of the lectins such as those of hemagglutinin, galactosidase, poly nucleotide adenosine glucosidase, or ribosome inactivating proteins, also show other biological activities [4]. Lectins in higher plants defend against pathogenic bacteria and fungi by recognizing and immobilizing the infecting microorganisms via binding, thereby preventing their subsequent growth and multiplication. They act as a sort of immune system for plants by “sticking” themselves to the structural carbohydrates (sugars) of invaders. Recently, lectin biology has applied these tools in the biomedical field and also in the treatment of diseases, including cancer [21]. Another application reported in the literature involving lectins is their antimicrobial activity where lectins may act against microorganisms by interfering with their growth and playing a role in defense systems [28].

Antibacterial activity on Gram-positive and Gram-negative bacteria occurs through the interaction of lectin with components of the bacterial cell wall including techoic and teichuronic acids, peptidoglycans and lipopolysaccharides; The polysaccharide chitin is constituent of fungi cell wall and chitin-binding lectins showing antifungal activity; impairment of synthesis and/or deposition of chitin in cell wall may be the reasons of antifungal action [20]. Probably the carbohydrate binding property of lectin is involved in the antifungal mechanisms and lectins of different specificities can promote distinct effects.

Hence, in this present study we made an attempt to isolate lectins with potential medicinal application. Thus in this present study we report, the isolation, purification and characterization of a lectin from the leaves of Adhatoa vasica (AVL). We have also investigated antibacterial and antifungal activity and their growth.

MATERIALS AND METHODS

2.1. Isolation of the Lectin from Adhatoda vasica Leaf

Leaf samples were homogenized with PBS at 4˚C. The extract was centrifuged at12, 000 rpm for 15 min to remove debris and the crude extract was stored at 4˚C. The extracted protein was under the process of ammonium sulphate precipitation in following concentrations (30%, 40%, 50%, 60%, 70% and 80%) and dialyzed against 50 mM Tris- HCl (pH 7.6) previously described [29].

2.2. Column chromatography (Syed et al., 2001[37])

The partially purified proteins were applied to a Sephadex G-100 column (16 mm diam x 22 cm). Pre washed by 50 mM Tris–HCl, (pH 7.6). Partially purified proteins were subjected in to Sephadex G-100 column. The column was eluted with 50 mM Tris–HCl, (pH 7.6) (the method was done at 18 °C of under this condition). The every fraction were collected and monitored by UV assay (at 280 nm). Lectin active proteins were collected for protein estimation, Haemagglutination assay by titer plate method and stored at 4 °C.

2.3 Quantification of protein (Lowry et al., 1951[22])

The protein concentration of the fractions was measured using bovine serum albumin (BSA) as the standard. The absorbance was read at 660 nm.

2.4. Haemagglutination inhibition assay

Haemagglutination activity was measured as previously described [14] with little modification. Briefly, serial two-fold dilutions of the lectin solution in microtiter v-plates (25 µL) was mixed with 25 µL 4% suspension of human red blood cell in phosphate buffered saline, pH 7.2 at room temperature (the erythrocytes of human blood group A, B and O). Readings were recorded after about 30 minutes at room temperature, when the blank had fully sediment. The haemagglutination titer, defined as the reciprocal of the highest dilution exhibiting haemagglutination, was treated as one haemagglutination unit. Specific activity was expressed as the number of haemagglutination units per mg protein (wang et al., 2000[40]).

2.5. Blood group specificity (Arne Forsgren et al., 2003[5])

Haemagglutination activity was assayed for purified lectin in a micro titer plate by serial dilution on 50µl of PBS (pH 7.4). A 50 µl of 4% human RBC (A, B and O) and animal (sheep, rabbit, cow, goat, chicken and mice) was added respectively and the specificity of lectin was observed.

2.6. Effect of temperature on haemagglutinating activity

The effect of temperature on the agglutinating activity of the lectin from A.vasica was determined by carrying out assay at different temperatures according to the method described by Kuku et al., 2009[18]. The purified lectin was incubated in a water bath for 2 hr at various temperatutre: 10°C-100°C, and then cooled to 20°C. Haemagglutination assay was carried out as previously described.

2.7. Effect of pH on Haemagglutinating Activity (Arne Forsgren et al., 2003[3])

The effect of pH on the activity of the lectin from A.vasica was determined by incubating the lectin in the following buffers at different pH values: Glycine buffer (pH 2.0 to2.8), Citrate buffer, (pH 3.0 to 5.8), Phosphate buffer (pH 6.0 to 8.0), Tris - Hcl buffer (pH 8.2 to 9.0), Glycine-NaoH buffer (pH 9.2 to 10.4).After incubation for 2 hrs at room temperature. The samples were readjusted to pH 7.4 by dialysis and assayed for the haemagglutination activity. The control values were the agglutination titre of the lectin in PBS, pH 7.2.

2.8. Inhibition of Lectin-Induced Haemagglutination by Carbohydrates (Wang et al., 2000[40])

The haemagglutination inhibition tests to investigate inhibition of lectin induced haemagglutination by various carbohydrates were performed in a manner analogous to the haemagglutination test. Serial twofold dilutions of sugar samples were prepared in phosphate-buffered saline. All of the dilutions were mixed with an equal volume (25 µl /25: l) of a solution of the lectin with haemagglutination units. The mixture was allowed to stand for 30 min at room temperature and then mixed with 50 µl (50: l) of a 4% rabbit erythrocyte suspension. The minimum concentration of the sugar in the final reaction mixture, which completely inhibited haemagglutination units of the lectin preparation, was calculated.

2.9. Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed under denaturing conditions in 7.5% (w/v) polyacrylamide slab gel at pH 8.9by Trudel and Asselin, 1990[38].

2.9.1. Protein staining after electrophoresis

Protein staining was done using an aqueous silver nitrate Blum et al., 1987 and modified by Claimer et al., 2012[8].

2.10. Antimicrobial Activity Screening (Romanenko et al., 2005 [32])

The purified lectins were screened for their antibacterial activities by the agar well-diffusion method by measuring the diameter of the inhibitory zones in mm using a concentration of AVL 50, 25, 12.5 (µg/15µl) of lectins. Mueller Hinton Agar, Sabouraud Dextrose Agar medium was used for determining antibacterial, antifungal activity respectively. The diameters of the zones of inhibitions of the samples were then compared with the diameter of the zone of inhibition produced by the standard antibiotic disc such as ampicillin and tetracycline. The plates were incubated for 24 hours at 37°C. One well containing extractant serves as control in each plate. The plates were examined for zones of inhibition, which indicate the degree of susceptibility of the test organism.

2.10.1. Determination of Minimum Inhibitory Concentration (MIC) by broth dilution method (Amsterdam 1996[2])

The purified A. vasica Lectin (AVL) was added using with sterile pipette in various concentration like 10 µg to 100 µg in to sterile test tube and final volume 10 ml make up with nutrient broth medium. The tubes were then inoculated with 0.05 ml of the standardized microbial culture and further incubated at 37°C for 24 hours in orbital Shaker and observed for any microbial growth in form of turbidity. The test procedure was repeated to check the reproducibility of the results. Minimal Inhibitory Concentration (MIC) was determined as the lowest lectin protein concentration at which there was ≥ 50% reduction in optical density relative to the control well OD at 490 nm. Ampicillin was used as standard. To determine minimal bactericide concentration (MBC), inoculations (10µl) from wells treated with protein or AVL that was found to inhibit bacterial growth were transferred to NA (Nutrient Agar) plates and incubated at 37 C for 24 h. The lowest protein concentration showing no bacterial growth was recorded as the MBC. The assay was performed in triplicate.

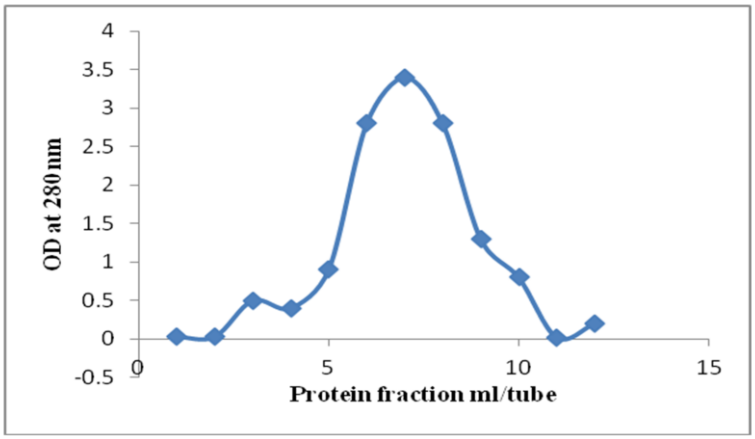
RESULTS AND DISCUSSION

Most plants contain one or more carbohydrate-binding proteins termed plant lectins. Although the functions of these proteins in plants are just beginning to be understood, lectins have been enormously important to the development of the field of glycobiology. Most lectins are multivalent and capable of agglutinating cells. In addition, since lectins differ in

the types of carbohydrate structures they recognize with high affinity, they are also useful in the characterization of glycoconjugates [10]. He describes the diversity of the plant lectins, how they are isolated and characterized and what is known about their structures and the carbohydrates they recognize. In addition, the many ways in which plant lectins can be useful in studying glycoconjugates.

A.vasica leaves were homogenized using 1X PBS (Phosphate Buffer Saline), filtered and centrifuged at 12,000 rpm for 30 min. Then the crude proteins in the supernatant were collected and stored at -20°C for future use [19; 23; 35; 39 and 42], a 2-step extraction procedure was developed. The crude proteins from the leaves of Adhatoda vasica, were subjected to analysis of agglutination activity against Rabbit, Sheep, Cow, Mice, Chicken, Human A,B and O blood group samples of erythrocytes (RBC) using with 96 well V titer plate for screening the presence of lectin. Agglutination of the red blood cells by the crude extract and the various fractions that were obtained during purification was estimated as described by Bing et al., 1967[6].

Figure.1. Column chromatography of *A. vasica* Lectin on Sephadex G-100.



Gel filtration chromatography (Sephadex G- 100 column chromatography in system) Flow rate of 1.5 mL 12min−1 and detection at 280nm U.V Spectrophotometer reciprocal of Haemagglutination unit.

Adhatoda vasica lectin has a specific for the rabbit blood group and it has 4 and 1.8 mg protein. The Purification fold at11.59 % and 74.26 % in the 80 % of ammonium sulphate saturation and followed that sephadex G - 100 column chromatography respectively. The specific activity was calculated by Haemagglutination assay, which is conform that maximum activity was observed at above mentioned saturation 1/16, 1/128 HU (Haemagglutination Unit). Lectins may present in leaves or different parts of the plant. Similarly in leaf crude extract agglutination activity was found which has the ability to agglutinate native RBC. A. vasica leaf lectins had an ability to agglutinate only the rabbit blood so it's a blood group specific lectin and both leaf lectins are multivalent lectins because only the multivalent

lectins can agglutinate blood groups tested for haemagglutination. In the purification of lectin from A. vasica the column chromatography on Sephadex G-100 (Fig.-1) removed proteins with low molecular weight. Seven fractions were collected and identified high absorbance at 280 nm UV spectrometer (Shimadzu - 1800). Based on the Haemagglutinating activity used as a main fraction of among the seven fractions [38], Subsequent purification resulted in 74.26 -fold purification and an overall yield of 155% (Table.1). The effect of Haemagglutination also proved that the purified protein was lectin. Moreover, gel filtration chromatography on Sephadex G-100 confirmed the apparent molecular weight of the A. vasica lectin as 20kDa and named as AVL. The purification of AVL folds at 74.26 indicating that the purity of the product was high. However, the lectin from A. vasica agglutinated Rabbit red blood cells specific which is typical of many lectins [18 and 41].

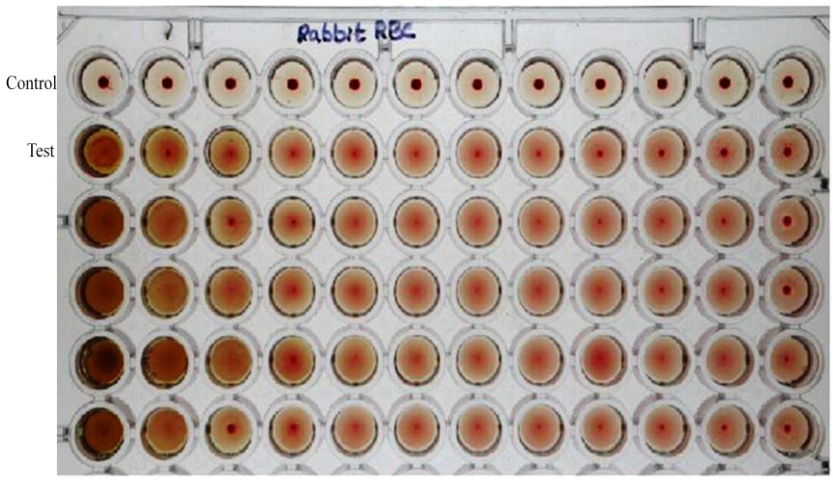
Table.1. Purification status of Adhatoda vasica leaf lectin

Fractions	Volume (mL)	Protein ($mg.mL^{-1}$)	Total rotein (mg)	Total activity[a] $(H.U.)mL^{-1}$	Specific activity[b] HAU	Purification Fold	Yield (%)
Crude extract	50	12.3	615	1287	2.092	1	100
$(NH_4)_2SO_4$ fraction (60%)	12	4	48	1164	24.25	11.59	90.44
Sephadex G-100	3	1.8	5.4	839	155.37	74.26	65.2

[a] - The total activity was obtained by multiplication between the HAU total mass for each step. [b-] The haemagglutinating activity unit (HAU) was calculated using the reciprocal of the highest titter with visible haemagglutination on Rabbit erythrocytes The high agglutination activity was observed A. vasica leaf protein in all the blood samples. Especially much more activity was observed against on Rabbit erythrocytes (Figure.2). In contrast to the lectin identified previously in the endosperm of maize seeds, which recognizes preferentially human erythrocytes group B [30], the capacity of AVL to agglutinate Rabbit erythrocytes is significantly different from that of other erythrocytes, indicating that the specificity of AVL is addressed to blood group determinants. Similarly in leaf crude extract agglutination activity was found which has ability to agglutinate native RBC. Adathoda vasica leaf crude extract had an ability to agglutinate only the rabbit blood so it's a blood group specific lectin and multivalent lectins because only the multivalent lectins can agglutinate blood groups tested for haemagglutination [44 and 45]. The precence of lectin was confirmed by this agglutination activity and the specific activity of crude protein result was 2.09 HU µg /ml

respectively. Adhatoda vasica has a high specific agglutination activity against rabbit blood group lectin.

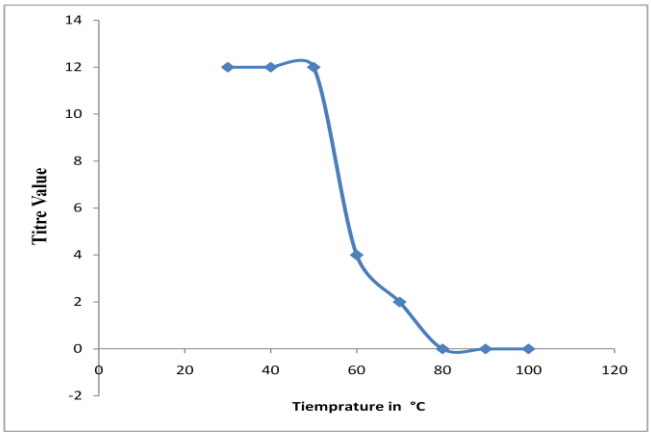
Figure.2. Lectin Specific activity for *Adhatoda vasica*



The hemagglutinating activity unit (HAU) was calculated using the reciprocal of the highest title with visible haemagglutination on Rabbit erythrocytes

The effect of temperature stability leaf lectin were subjected to moist heat treatment for 2 hrs at 100˚C and found stable but the Adhatoda vasica and lectins are denatured at 100˚C in this result ambient temperature at 50°C. Lectin in this study result was found to be heat stable from 20ºC to 50°C (Figure. 2 and Table 2) thus they are heat labile and not thermophilic and in 80 ºC no haemagglutination was observed. In comparison lectin extracted from C. annuum was found to be stable between 20ºC to 30°C [1 and 18]. However, the haemagglutinating activity from the Pterocladiella capillacea lectin was affected only by exposure to a temperature of 70°C this occurs in some plant lectin and marine algae [5]. Well, lectin from Ganaderma caperise is not affected after exposure to temperature at and above 70°C for 60 minutes [25]. Haemagglutinating activity of lectins from Pterocladiella ostreatus is reduced at or above 40°C [40]. However, some lectins exhibit a remarkable degree of thermo resistance. Under these circumstances, substantial purification of a target lectin can be accomplished by heating a crude extract at a temperature where the target lectin is stable, but extraneous protein are denatured and precipitate from solution. The former reported that heat stability of this protein differs from lectin to lectins while the latter, reported that lectins are known to be heat labile and their activity can be decreased by heat treatment. Hence, a decrease haemagglutinating activity of lectins as temperature increase shows that its activity depends on the native conformation of the protein.

Figure.3.Effect of Temperature in *A. vasica* lectin Haemagglutinon on Rabbit erythrocyte



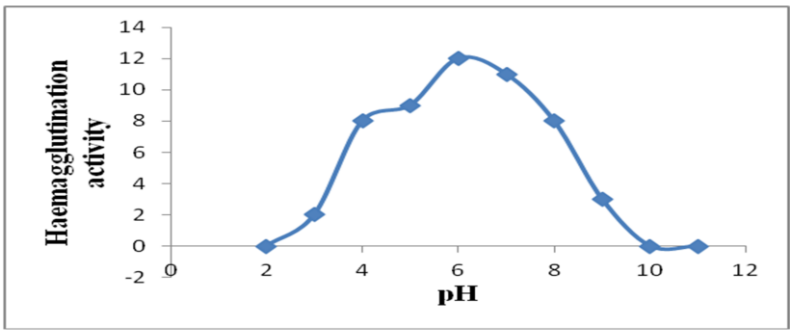
Thermal stability of *A. vasica* lectin at different temperatures (from 30 to 100 ◦C) as a function incubation time. The haemaglutination activity (HA) was expressed as a percentage function of the control HA (not thermally treated).

Table.2. Effect of temperature on haemagglutinating activity against A. vasica Lectin.

Temprature (°C)	30	40	50	60	70	80	90	100
Haemagglutinating activity (number of units)	12	12	12	4	2	0	0	0

The effect of pH range A. vasica leaf lectin agglutinate native RBC using with following four different buffers namely, Glycine-HCl (pH 2 to 2.8), Citrate Buffer (pH 3 to 5.8), Phosphate Buffer (pH 6 to 8.0), Tris Buffer (pH 8.2 to 9.0), Glycine Buffer (pH 9.2 to 10.4) over this experiment observed the effect of pH on A. vasica leaf lectin in which complete agglutination was found at pH 5-7 and partial agglutination at pH 4. There was some agglutination occur above pH 8, pH 10-11 with less optimum activity(Figure.3) and this experiment shows agglutination of trypsinized RBC took place over the range pH 4-10. This result suggests that the protein has two binding sites, one site more active at range of pH 6 – 8 and the other at basic range of pH 6 – 8. This result is however, in contrast with that of [1] in which Manila clam lectin activity was stable between pH 6 and pH 9 and was temperature-dependent.

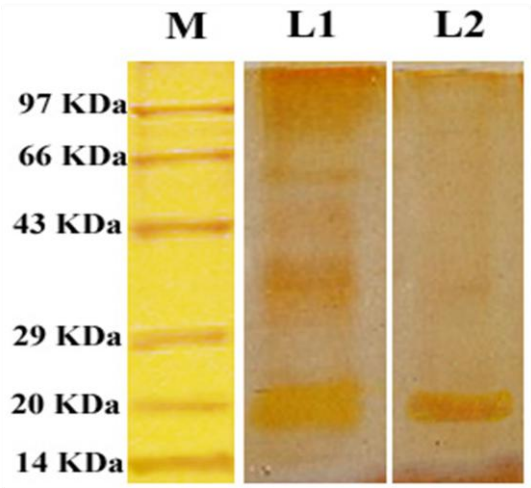
Figure.4. Effect of pH on Haemagglutinating activity of *A. vasica* Lectin



A. vasica stability as a function of pH. The data are represented by average standard deviation (n = 3). Haemaglutination assay was conducted at pH 7.4.
1.GLYCINE-HCL : (pH 2 to 2.8)
2.CITRATE BUFFER : (pH 3 to 5.8)
3. PHOSPHATE BUFFER : (pH 6 to 8.0)
4. TRIS BUFFER : (pH 8.2 to 9.0)
5. GLYCINE BUFFER : (pH 9.2 to 10.4)

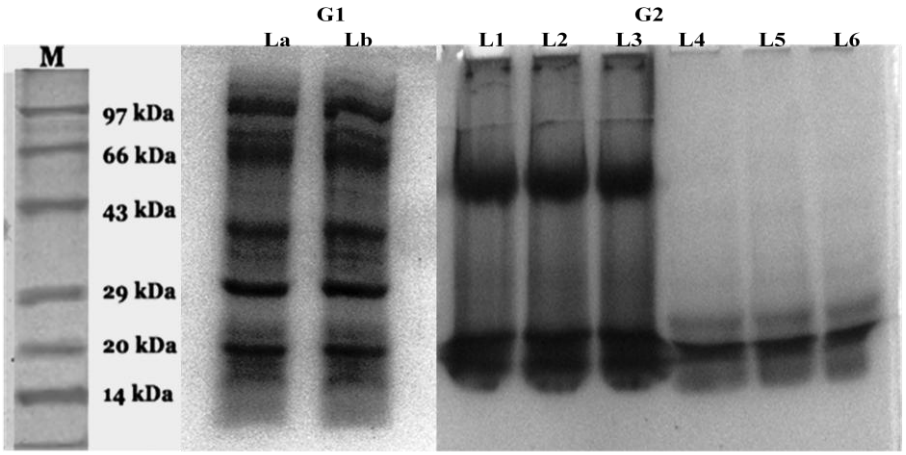
The lectin's sugar specificity was determined by haemagglutinating inhibition assays (Table 4). The hapten inhibition studies to define the sugar specificities of the purified lectin of A. vasica showed that The activity of the lectin was completely inhibited by galactose with minimum inhibitory concentration of 50 mM and 25 mM respectively. The above Sugar inhibitory was observed, and general agreement with those found for the numerous marine algal lectins, such as Cystoclonium purpureum Batters [15], Solieria chordalis [31], Gracilaria bursa-pastoris (Gmelin) Silva [28], Solieria filiformis [7]. Maltose, fructose and sucrose had no effect on haemagglutination activity. This is in slight variance with the result gotten by kuku et al., 2009 [18] where the lectin extracted from C. annum was slightly inhibited by sucrose at higher concentration of 200 mM. Galactose enhanced the haemagglutination activity of the lectin as the concentration increases as shown in 50 mM and 25 mM, while galactose inhibit activity of lectin extracted from C.annuum at concentration above 200 mM as reported by Kuku et al., 2009 [18]. Simlarly A.vasica lectin Galatose inhibiton was observed. This shows that leaf lectin is not specific for any monosccaharides lectin activity was inhibited by D-galactose, D-galactosamine and N- acetyl – galactosamine which is monosaccharides. Leaf lectin may be specific for methylated sugars or oligosaccharide. The sugar specificity of lectins is usually established by the hapten inhibition technique in which different carbohydrates are tested for their ability to inhibit either haemagglutination or polysaccharide (glycoprotein) precipitation by the lectin. The specificity of lectin is defined in terms of the best monosaccaharide inhibitor. The results from such studies allow the classification of lectins.

Figure.5. Silver Nitrate staining of *A. vasica* Lectin



Silver stained M- Marker protein, Lane 1 – Partial purified *A. vasica* Lectin, Lane 2- purified *A. vasica* lectin

Figure.6. Protein profile of *A. vasica* Lectin on SDS PAGE



CBB stained M- Marker protein, G1 –Crude protein CBB stained gel, G 2–partially and purified protein CBB stained gel
Lane a,b – Crude protein of *A. vasica* Lectin; Lane 1,2,3 – Partial purified *A. vasica* Lectin; Lane 4,5,6- purified *A. vasica* lectin

Purified lectin proteins from A.vasica were electrophoresis on SDS Polyacrylamide gel and which was stained by aqueous silver stain (Figure.5, 6). Molecular weight determination for the haemagglutinin of leaf lectin was performed. Lectin from Japanese jack bean (Canavalia gladiata agglutinin, CGA) was purified by affinity chromatography on a maltamyl-sepharose column [17] and Sephadex G-50 by Yamauchi and Minamikawa 1990[43]. In the present study Sephadex G-75 column was used for the isolation of Canavalia gladiata. Upon SDS PAGE, under both reducing and nonreducing conditions, CGL yielded two polypeptide bands of molecular weight 26 and 17 KDa indicating that they are not linked by disulphide bonds. A lectin from Japanese jack bean (Canavalia gladiate agglutinin) was shown to have a protein subunit with a molecular weight of 30,000 [17], whereas AVL isolated in the present study exhibited a low molecular weight of 20 kDa in Figure 5 and 6 protein profile of A. vasica lectin stained by CBB as well as Silver nitrate stain. Similarly Concanavalin isolated from Canavalia ensiformis by Sheldon et al., 1998[35] yielded five bands of molecular weights 78, 74, 54, 32 and 30 kDa on SDS PAGE. Carbohydrate binding, particularly mono and disaccharides, is the defining feature of the lectins and is the basis of many methods of classification of plant lectins. The saccharide binding specificities of lectins can be utilised in the purification, characterization and sequencing of polysaccharides, polypeptides and glycoproteins.

Table.3. Antibacterial activity of A. vasica lectin

Bacterial Cultures	AVL (µg/ml)				Standard
	C	50	25	12.5	
Gram Positive					
Staphylococcus aureus	-	12	10	10	45 (M)
Staphylococcus epidermis	-	12	10	09	40 (T)
Gram Negative					
Escherichia coli	-	08	08	08	30 (K)
Salmonella paratyphi	-	13	10	10	25 (E)

C - DMSO (Dimethyl sulphoxide). Measurements are given in millimeter (mm). Erythromycin (E); Kanamycin (K); Methicillin (M); Tetracycline (T).

AVL was more efficient in killing the gram-positive bacteria Figure 7 shows S. aureus than the gram-negative bacteria such as E. coli, S. Paratyphi Similarly, the N-acetyl-D-glucosamine-binding lectin from Araucaria angustifolia seeds was also more active against gram-positive (Clavibacter michiganensis) than gram-negative (Xanthomonas axonopodis) bacteria, promoting the formation of pores and severe disruption of the C. michiganensis membrane and bubbling on the X. axonopodis cell wall [7]. The increment of antibacterial activity was likely due to the concentration of AVL, indicating that it is one of the main active components present in the leaf lectin. A thermo-resistant lectin isolated from Eugenia uniflora seeds inhibited the growth of S. aureus and P. aeruginosa with an MIC (1.5 µg ml $^{-1}$) similar to those determined for A. vasica lectin, but it was less effective in inhibiting (MIC of 3.5, 1.7 µg ml $^{-1}$) the growth (Table 3) of S. aureus and E. coli [27 and 28].

Figure.7. Antimicrobial activity of *Adhatoda vasica* lectin

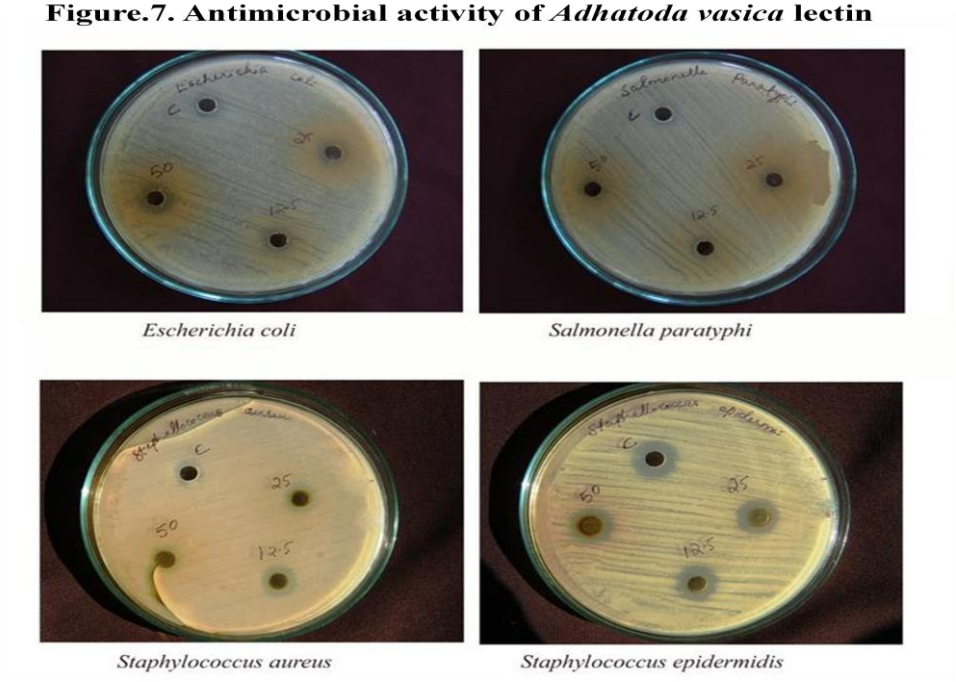
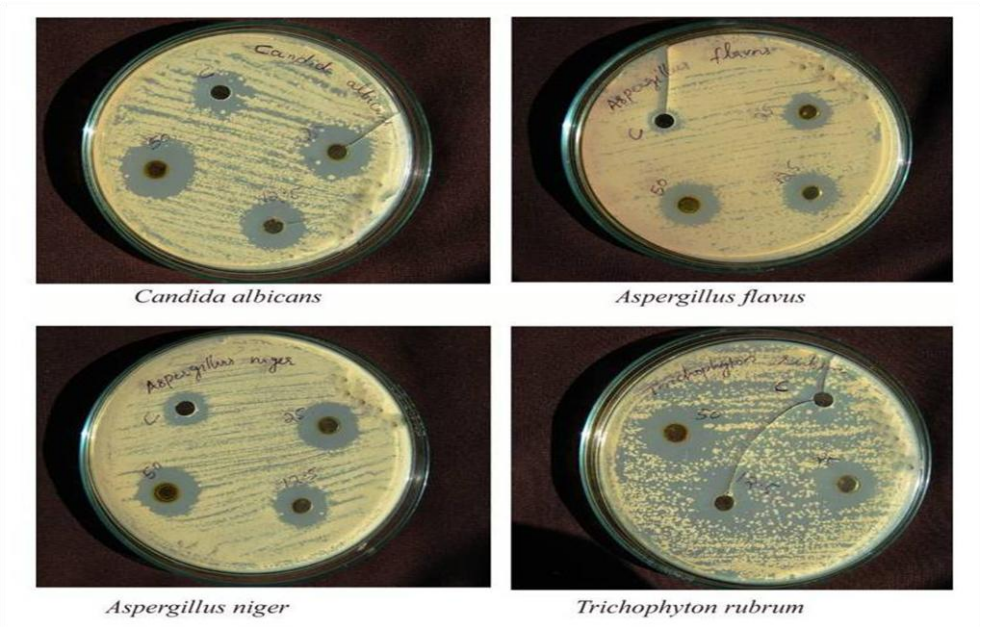


Figure.8. Antifungal activity of *Adhatoda vasica* lectin



The difference in the susceptibility of gram-positive and gram-negative bacteria may be linked to the difficulty of lectins to cross the gram-negative bacteria outer cell wall to reach the periplasmic space (Nunez et al. 2011). Additionally, the high level of peptidoglycan (which contains GlcNac) in the cell wall of gram-positive bacteria may Provide more interaction sites for chitin-binding lectins.

Table.4. Antifungal activity of A. vasica lectin

Fungal Strains	Adhatoda vasica lectin (µg/ml)			
	50	25	12.5	Control
Aspergillus flavus	18	13	16	-
Trichophyton rubrum	14	12	12	-
Aspergillus niger	16	13	13	-
Candida albicans	16	13	12	-

Table.5. Minimal Inhibitory concentration and Minimal Bacterial growth against of AVL

S.No.	Microorganism	MIC µg/ml of AVL	MBC
1	Staphylococcus aureus (+)	3.59	ND
2	Staphylococcus epidermis (+)	2.50	ND
3	Escherichia coli (-)	1.72	ND
4	Salmonella paratyphi (-)	1.61	ND

MIC and MBC values are expressed in µg ml^{-1} of protein. (+) Gram-positive and (-) Gram-negative bacteria. ND – Not Detecting.

Antifungal activity against AVL got higher zone of inhibition in Figure 8 and table 4 shows the value of inhibition effect 50 µg/ml concentration got average highest antifungal activity of AVL lectin. The lowest 12.5 µg/ml concentration of AVL got average of 13.25 mm inhibition zone were calculated. AVL was more efficient of inhibiting C. albicans growth. Plant lectins are active against fungi by recognizing and immobilizing the micro-organisms via binding to carbohydrate components, thereby preventing their subsequent growth and multiplication [9]. The chitin-binding property of AVL may be involved in its antifungal mechanism. A chitin binding lectin, showed inhibitory activity against in Table 5. C. albicans with a MIC value of 95 µg ml $^{-1}$ [16] similarly AVL antifungal activity on agar plate zone of inhibition value 13, 12, 13, 12 mm of A. flavus, T. rubrum, A. niger, C. albicans respectively.

CONCLUSION

In conclusion, Adhatoda vasica plant may be considered as a medicinal plant having antimicrobial potentiality. Its leaves contain a specific types of lectins like molecules those may have many pharmacological potentialities. AVL represents the first isolated proteinaceous constituent of the plant and being active against several pathogenic microorganisms could be used as an antimicrobial agent for human and animal infections.

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