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Bacteriostatic activity of con a lectin from *Canavalia ensiformis*

S.R. Kulkarni * **and V.J. Tayade**

Bombay College of Pharmacy, Kalina, Mumbai, India.

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ABSTRACT

The aim of this work was to explore the therapeutic applications of Con A lectin from *Canavalia ensiformis* and to explore its antibacterial activity. Activity of lectin was quantified by their ability to agglutinate erythrocytes using Hemagglutination assay. Characterization and purity of Con A lectin was evaluated by using SDS-PAGE analysis. The reversal of hemagglutination activity of lectin was evaluated by using the sugars namely; mannose, galactose, lactose, fructose, glucose. The antibacterial activity of lectins was tested against *Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis, Escherichia coli* using pour plate method. Amoxycillin was used as standard. At 250mg/ml concentration Con A lectin showed good bacteriostatic activity.

1. Introduction

Lectins constitute a group of proteins or glycoproteins of non-immune origin, which bind reversibly to carbohydrates and usually agglutinate cells or precipitates glycoconjugates[1]. Majority of leguminous lectins can agglutinate variety of plants and animal cells such as erythrocytes, lymphocytes and malignant cells [2].The first plant lectin to be purified and crystallized was Con A and its primary and three-dimensional structure was determined[3]. Lectin extraction is usually carried out by different methods such as maceration using water/buffer solution with controlled pH and ammonium sulphate precipitation [4]. Molecular structure of Con A lectin was reported to be homotetramer with each subunit 26.5kDa, 58kDa,80kDa [4]. The drugs from natural sources with low cost of production, low side effects and good efficacy have become a focus now-a-days [5]. Lectins are used to analyze the carbohydrates present on the cell walls of gram-positive and gram-negative bacteria. Bacteria contains the cell wall composed of peptidoglycan, teichoic acids (Gram-positive organisms), and Lipolysaccharides (Gram-negativeorganisms).Peptidoglycan is a polymer of alternating N-acetlglucosamine (GlcNAc or NAG) and N-acetylmuramic acid (NAM) units connected by short pentapeptides. Amongst the gram-positive and gram-negative bacterias, gram-positive bacteria were more susceptible for lectin as compared to gram-negative bacteria [6]. The microorganisms develop resistance to the synthetic antibiotics and hence make them lesser effective. In such cases, natural products which are a part of our daily diet serve as the best alternative for new antibacterial drug discovery [7-8].

2. Materials and Methods

2.1 *Source of Material*

Jack Bean meal was procured from local market and used for the extraction of lectins.

2.2 *Extraction of Con A lectin from Canavalia ensiformis*

Con A meal was defatted in hexane, it was kept for maceration in 0.5M NaCl at 4ºC for 4 hrs followed by filtration. The homogenate obtained was centrifuged at 6000 rpm for 15

Corresponding Author: Dr. Savitak R Kulkarni, Bombay College of Pharmacy, Kalina, Mumbai, India. **E-Mail:** svt_kulkarni@yahoo.co.in ; vaishali.tayade276@gmail.com

minutes. The precipitate obtained was collected by 80% saturation of ammonium sulphate, which was collected after 12hrs and dialyzed against distilled water for 24 hrs and the supernatant was lyophilized [9].

2.3 *Hemagglutination assay*

Hemagglutination activity of Con A was detected by using rat erythrocytes. The lectin solution was serially diluted in microtiter V plate that was further mixed with 50µl of 4% suspension of rat erythrocytes. Hemagglutination was observed after 1 h at room temperature. The titre of tested lectin was expressed as the reciprocal of the highest dilution showing agglutination of rat erythrocytes [9].

2.4 *SDS-Page*

Gel electrophoresis was carried out using SDS-PAGE, using Biolit Midrange molecular weight markers (14-95KDa) [10].

2.5 *Sugar inhibition tests*

Sugar inhibition of hemagglutination was further performed on V bottom microtiter plate. Glucose, Lactose, Mannose, Fructose and Galactose was dissolved in 0.1M PBS were added to serial dilutions of extracts and then incubated for 1h at 37ºC. To serve as control, serial dilutions of extracts in 0.1M PBS was also prepared. Sugar inhibition assay was then performed using 2% rat erythrocytes [11].

2.6 *Test organisms*

Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis, Escherichia coli. The organisms were cultured in the microbiology lab. After suitable growth was seen, the culture solution (*Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis,* and *Escherichia coli*) was diluted to 10^6 in the test tube was taken along with sterile water.

2.7 *Antimicrobial Activity Screening*

The partially purified lectin was screened for the antibacterial activity by the pour plate method. Different concentrations of Con A 100mg/ml and 250mg/ml were added. Nutrient agar medium was used for determining the antibacterial activity against *Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis and Escherichia coli.* In this method, 10^8dilutions of microbes were used. 1ml of this dilution were taken in the test tubes aseptically, 1ml of different concentration of Con A lectin 100mg/ml, 250mg/ml, were added to the above test tubes, finally they were mixed with nutrient agar medium and poured into plates and allowed to solidify and incubated at 37ºC for 24hrs. Standard Amoxycillin 100µg/ml, 250µg/ml was used [12-13].

2.8 *Viable Count Method*

4µl of microbial culture *Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis, and Escherichia coli* were added in each test tube containing nutrient broth (5ml). 1ml of nutrient broth containing culture was transferred into the petriplate containing molten agar. It was allowed to solidify and the plates were incubated for 24hrs at 37ºC ± 2ºC. Number of the colonies of bacteria was counted and results of different extract concentrations were compared to that of the standard drug.

Table 1: Antimicrobial Activity of Con A lectin using pour plate method

Sr. No.	Amount to f lectin solution/plate Microbes(10^8 dilution)	100 (mg/ml)	250 (mg/ml)	Control (+)	Sterlity Control (-)	Control (Extract)	Standard (Amoxycillin) 100µg/ml	Standard (Amoxycillin) 250µg/ml
1.	*Streptococcus mutans*	++	+	+++	-	-	+	-
2.	*Staphylococcus aureus*	++	+	+++	-	-	+	-
3.	*Bacilus subtilis*	++	+	+++	-	-	+	-
4.	*Escherichia coli*	+++	+++	+++	-	-	+	-

Maximum growth: +++, Slight Decrease in Growth: ++, Decrease in Growth: +

Table 2: Antimicrobial Activity of Con A lectin using Viable Count Method

Concentration of extracts Con A /standard	No. of colonies *S.aureus*	No. of colonies *S.mutans*	No. of colonies *B. subtillis*
100mg/ml	82×4	76×4	70×4
250mg/ml	72×4	66×4	56×4
Control(Microbial suspension+N.Agar)	306×4	215×4	227×4
100µg/ml (Standard)	67×4	68 ×4	50×4
250µg/ml (Standard)	43×4	55×4	47×4

Table 3: Results of Sugar Inhibition Assay

Sugars	Dilution (Sample: PBS)						
	2	**4**	**8**	**16**	**32**	**64**	**Control**
Mannose	+	+	-	-	-	-	-
Galactose	+	+	+	-	-	-	-
Lactose	+	+	+	-	-	-	-
Glucose	+	+	+	-	-	-	-
Fructose	+	+	+	-	-	-	-

Agglutination: +; No Agglutination: -

Table 4: Description of Crude and Lyophilised extract and its Yield

Description	*Canavalia ensiformis*
Crude extract description	Dark brown semisolid extract
Lyophilized extract description	Buff colored powder
Lyophilized yield (%w/v)	2.8

Table 5: Viable Count of Con A lectin containing different concentration of extracts

Concentration of extracts Con A extract	No. of colonies *S.aureus*	No. of colonies *S.mutans*	No. of colonies *B. subtillis*
100mg/ml	82×4	76×4	70×4
250mg/ml	72×4	66×4	56×4
Control(Microbial suspension+N.Agar)	306×4	215×4	227×4
100µg/ml(Standard Amoxicillin)	67×4	68 ×4	50×4
250µg/ml	43×4	55×4	47×4

Table 6: % Inhibition of Con A Lectin against microorganisms

Sr. No.	Microorganisms	Control	Con A extract (Viable Count)		% Inhibition	
			100mg/ml	250mg/ml	100mg/ml	250mg/ml
1	*S.aureus*	306	82×4	72×4	73.20	76
2	*S.mutans*	227	76×4	56×4	76	56
3	*B.subtilis*	215	76×4	66×4	70	66

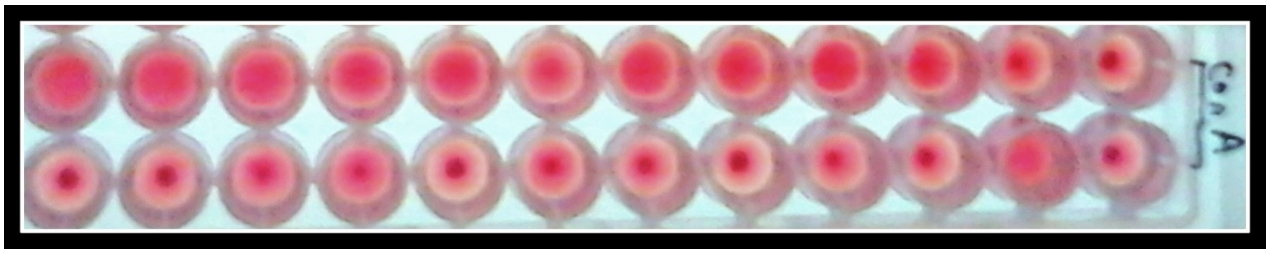

Figure 1: Hemagglutination Assay of Con A lectin. The HA titre of Con A lectin is 512 or 2^{10}

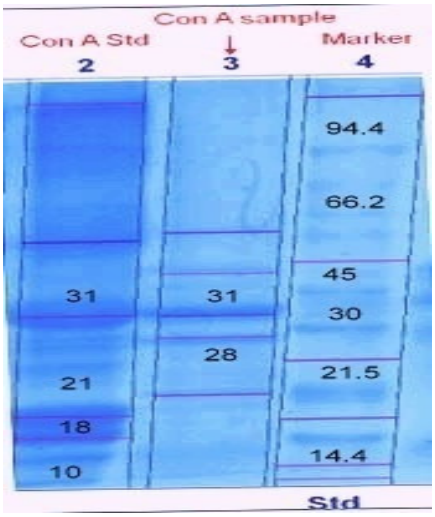

Figure 2: Gel Electrophoresis

3. Results and Discussion

Extraction of Con A lectin from jack bean meal was carried out, the crude extract obtained was dark brown semisolid in colour, after lyophilization the extract was buff colored powder, and the yield of lyophilized extract was found to be 2.8% w/v as given in Table no:3. The Hemagglutination Assay titre of Con A lectin was found to be 2^{10} or 512shown in Figure no: 2. Molecular weight determination of Con A lectin was carried out using Gel Electrophoresis studies. Con A standard showed bands at31, 21, 18 10kDa, and Con A sample showed bands at 31, 28 kDa given in Figure no: 3 which was compared to the Standard Protein Mid-range molecular weight markers. Con A is a mannose binding lectin which can bind specifically to the mannose sugar; this sugar may inhibit the activity of lectins which was studied by Sugar inhibition assay method, results are given in Table No: 2.

The antibacterial activity of Con A lectin extract was studied against *Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis, Escherichia coli.* Results are shown in Table no.1.As per the obtained results Con A lectin showed good bacteriostatic activity at 100mg/ml and 250mg/ml concentration against *Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis.* But at a same concentration *Escherichia coli* was found to be multiplied by thriving on Con A lectin. In case with the viable count method, Con A lectin extract was studied against *Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis,*

results are shown in Table no: 2, the viable counts of the bacterias were compared to that of Standard Amoxycillin. Con A lectin helps in triggering the growth of *Escherichia coli* this probably may be attributed to some differences in composition of cell wall of gram negative and gram positive bacteria. Con A lectin at a concentration of 250 mg/ml showed good bacteriostatic activity similar to that of Standard Amoxycillin at (100µg/ml, 250µg/ml). Con lectin at 250mg/ml concentration showed 76% inhibition against *S.aureus*, 66% inhibition against *S.mutans* and 56% inhibition against *B.subtilis* microorganisms.

4. Conclusion

A Con A lectin was extracted from jack bean meal and was partially purified by ammonium sulphate precipitation method. Con A lectin acts as an effective bacteriostatic agent against *Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis* which helps to decrease the growth of these bacteria. Standard Amoxycillin showed inhibitory activity at 250µg/ml whereas, Con A extract showed static activity at 250mg/ml against gram positive organisms namely; *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus mutans*. The Con A lectin showed a feature of growth promoter activity for *Escherichia coli* so this probably may be attributed to the differences in the cell walls of gram positive and gram negative bacterias.

Conflict of interest statement

We declare that we have no conflict ofinterest.

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