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Studies on characterization of *Maytenus senegalensis* lectins with RBC surface markers of cancer, diabetes and sickle cell anemia

Ragini Chahande*[1], Snehalparate[2],Rajani G Tumane[3], Shubhangi K Pingle[4]
1. Seth KesarimalPorwal College, Kamptee,Nagpur, Maharashtra , INDIA
2. Dr.Ambedkar College Nagpur, Deekshabhoomi, Nagpur, Maharashtra,INDIA.
3.ICMR- National Institute of Occupational Health, Ahmedabad, Gujarat INDIA
4. Regional Occupational Health Centre (Southern),ICMR Complex, Kannamangala PO, Poojanahalli Road, Devanahalli Taluk, Bengaluru-562110, Karnataka, INDIA
*E mail: ragini.chahande@gmail.com

ABSTRACT
The lectins from Maytenus senegalensis Leaves (MSL) have the ability to bind with carbohydrate-binding proteins and agglutinate erythrocytes. Studies reported the crude extract of MSL was used for the treatment of inflammatory diseases. However, little is known about the active components of conferring therapeutic properties. The present study aimed at the characterization of MSL lectins and its interactions with surface markers of cancer, diabetes, and sickle cell anemia RBCs. The molecular weight determination, sugar inhibition, physical and chemical characterization was performed in purified lectins of MSL. Results indicated that partially purified lectins from MSL do not exhibit specificity towards human blood groups. The horse and dog erythrocytes showed high agglutination as compared to calf, hen, goat, and rat. Further, no significant difference in hemagglutination Unit (HAU) was observed between diabetic and normal erythrocytes of humans. MSL lectins show the difference in HAU with erythrocytes of breast, cervix, and endometrium cancer when compared with normal RBC's. Further, a comparison between HAU of sickle cell RBC's and normal RBC's suggest significant difference in AS and SS type. MSL lectin is thermostable and worked in all temperature ranges 30–100 ºC. The study provides a molecular basis for understanding the interaction of MSL lectins recognition sites on cell surfaces of RBC's helps to elucidate the mechanisms for the diagnostic approach.
Keywords: *agglutination, anaemia, carbohydrate binding, cancer, hemagglutinin*

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INTRODUCTION

"Lectin" emerges from Latin word "legere" which means "to select" by William Boyd in 1954. Lectins are the proteins present in variety of organisms responsible for showing agglutination property. Lectins and hemagglutinins are proteins/glycoproteins, which have at least one non-catalytic domain that exhibits reversible binding to specific monosaccharide or oligosaccharides [1]. They bind with the carbohydrate present on the surface of erythrocytes without hampering the carbohydrate properties. Many lectins are differentiated on the basis of carbohydrate specificity and others on basis of lectin families[2-7].

Applications of the lectins depend on their properties. Within the plant, lectins play an active role in transport of carbohydrates and in the formation of symbiotic associations with rhizobia to facilitate the formation of root nodules [8-11]. The antifungal and anti-insect activities of lectins can be made use of in the control of pathogens [12-16].

Maytenus senegalensis, family Celastraceae is a tropical shrub or tree found in regions of African continent, in Madagascar and east to Bangladesh with a wide altitude range from sea level to highlands [17-20]. Usually a shrub, but can be a tree of 8 m high, the trunk straight with drooping branches and many sucker shoots, either with or without spines. Bark is grey, rough and thick, often bunched together, on smooth red or grey green branches which have no hairs or dots. Leaves are alternate or in clusters, smooth, often fleshy, variable in shape, oval, the tip often wider than the base [4, 21-25]. Flowers appears as white-cream-green in dense stalked clusters, often covering the tree. The wide spectrum of uses of the *Maytenus senegalensis* plant in African traditional medicine for treatment of multiple diseases is attributed to the phytochemical contents in it such as tannins, saponins, alkaloids and terpenoids. Scientific studies have proven that *Maytenus senegalensis* is a unique anti-inflammatory plant due to the presence in it of maytenoic acid [26-29]. Consequently, the roots could potentially be employed in

therapeutic preparations for the treatment of inflammatory-based diseases (rheumatoid arthritis, chronic peptic ulcer.

MATERIAL AND METHODS

Materials

Plant material

Plant leaves of *Maytenus senegalensis* (wild shrub) is a perennial weed used for isolation of the lectin, plants were collected locally during the period October to December. Leaves of plants are collected from Nagpur region of Central India. After thorough washing of the plant leaves with tap water in order to remove soil particles and organic material, chopped in pieces (about 1 cm long; the diameter varies between 1.5 and 3 mm).

Blood samples

Around 5mL of blood sample of A, B, AB and O was collected from healthy individuals (18 to 50 years of age) in a sterile syringe, with the help of volunteers. The collected blood was mixed with 0.05 g of EDTA and stored at 4ºC. These blood samples were used to carry out further experiments. Blood samples of cancer and sickle patients were collected from nearby hospitals. Animal erythrocytes samples such as dog, horse, calf, goat were collected from the nearby veterinary hospitals.

Chemicals

All the procedures were done with the use HiMedia and Serva chemicals including, Bovine serum albumin (BSA), Sodium hydroxide purchased from HiMedia, India Ltd, Ethanol [Jiangsu Huoxi international Trade co. Ltd], Sodium Dihydrogen phosphate [Merck specialities Pvt. Ltd], Disodium hydrogen phosphate from Thomas baker Pvt. Ltd, Sodium potassium tartarate, HCl, FolinCiocalteau (FC) reagent from Merck. Ethylenediaminetetra acetic acid (EDTA) was purchased from Sigma (St Louis, MO). (Dextrose, Fructose, Lactose, Maltose, Arabinose, Glucose, Sucrose, Sorbitol, Galactose, Xylose, Mannose) were purchased from Bio system.Other chemicals includes Glycerol, Bromophenol blue, β- mercaptoethanol, TrisHCl, Glycine, APS (Ammonium per sulphate), Coomassie brilliant blue (CBB), Methanol, Glacial acetic acid, TEMED.

Methods

Preparation of plant extract

Leaves of *Maytenus senegalensis* are collected from the wild and washed 2-3 times with tap water followed by distil water. Leaves are cut into small pieces. The leaves (approx. 50 gm.) with 10 ml saline water (9% NaCl) are crushed into mortar and pestle. The extract was filtered by a filter paper.The filtrate was collected and preserved at 4°C for further identification, characterisation and application studies of lectins.

Isolation and Purification of crude extract

The crude extract was purified and dialyzed to remove impurities and salts from extract with the help of dialysis membrane- 50 (Av flat width- 24.26 mm, Av diameter- 14.3 mm, Capacity approx.- 1.61 m/cm) . The soluble fractions were dialyzed for 24 to 48 h at 4°C against several changes of saline.

Partial purification of *Maytenus Senegalensis* by ammonium sulphate precipitation

Ammonium sulphate precipitation is one of the most widely used techniques for the purification of proteins by altering their solubility in the presence of high salt concentration. It is used as precipitant as it is highly soluble in nature and stabilizes protein structure. The solubility behaviour of the ammonium sulphate is expressed as a function of the percentage of the protein saturation. The protein precipitate is assayed to determine the total protein content. The ammonium sulphate concentration is added to the point that maximum protein is precipitated while leaving the contaminants in the solution. The precipitated protein is subsequently recovered by centrifugation.

The extract was brought to 70-90 % saturation with 6.21 gm./10 ml. Extract was slowly stirred with ammonium sulphate at 4°C. Hence, all the proteins will be separated through centrifugation at 6,000 rpm for 5 min. the supernatant is discarded and precipitate dissolved in in normal saline dialysed against saline for one day and stored at 4°C[30].

Preparation of 2% erythrocytes

The collected blood of A, B, AB, and O was washed 2-3 times with saline by centrifuging at 2000×g for 10 minutes at room temperature. 200 micro litre of the pellet was suspended in 10 ml of 1N NaCl (to yield a 2% RBC suspension). This suspension was then used for the quantitative haemagglutination assay[31].

Determination of the Hemagglutinating Activity

The determination of the hemagglutinating activity (HA) was performed in micro titre plates. 100 µl of *Maytenus senegalensis* leaves (MSL) lectin was twofold serially diluted with physiological saline, and 100 µl of 2% human red blood cell (RBC) suspension were added. The results were read after 30 min at room temperature, when the control was fully sedimented. The lectin activity was expressed as H.U

(hemagglutinating unit). One HU is defined as the minimum amount (in microgram) of lectin per ml that could cause agglutination of 1 ml of a 2% suspension of unmodified human erythrocytes [32].

Agglutination inhibition assay

The sugar specificity of MSL lectin was analysed in the hemagglutination test. The sugar base to be tested was formulated into a 100 to 500 mM concentration of aqueous solutions. The sugars which are used are of varied nature as D-glucose, D-fructose, D-sucrose, D-lactose, D- sorbitol, D- maltose, D- arabinose, D-galactose, D-xylose, D-Mannose, and D- ribose. 100 µl of sugar solution was twofold serially diluted with stroke-physiological saline, 10 µl of lectin solution were mixed and incubated at room temperature for 1 h.100 µl of 2% human red blood cell suspension were added to detect hemagglutinating activity [33].

Protein estimation

Estimation of protein concentration was done in purified MSL by Folin Lowry method. Mixed 500µl of purified MSL solution with 500µl of distilled water and make up the volume up to 1ml. To this, add 4ml of alkaline copper reagent to each tube and incubate for 10mins at room temperature. After incubation add 0.5ml of Folinciocalteau reagent and kept in dark for 30 minutes. Read absorbance on semi autoanalyzer (Bio system Pvt Limited) at 670nm [34].

pH stability

pH stability experiment is done to observe the optimum pH for maximum activity of lectins present in the MSL. Different pH buffers ranging from pH 2- 12 were used. Glycine–HCl buffer solution at pH 2.0, citrate buffer solution at pH 3.0 to 5.0, phosphate buffer at pH 6.0 to 8.0, and glycine–sodium hydroxide buffer solution at pH 9.0 to 12.0 were used to maintain the pH.

In this experiment, the acid–base stability of the lectin was studied by a hemagglutinating test. 50 µl of purified plant extract is serially double diluted against normal saline, and treated with 50 µl of different pH buffers. Incubation was done for approx.30 min. freshly prepared 2% RBCs suspension were added [35].

Temperature stability

The lectin solution in aliquots of 200 µl was heated to the desired temperature using a constant temperature water bath. All the tubes were completely submerged in the water bath throughout the investigation and ensured to prevent the evaporation of water or loss of solution. A series of thermal treatments were carried out at 30 °C, 40 °C, 50 °C, 60 °C 70 °C, 80 °C, 90 °C, and 100 °C for 30 min. At the end of the thermal treatment, it was cooled in room temperature and stored at 4 °C until further analysis. The effect of temperature on lectin stability was determined by measuring hemagglutinating activity of the incubated lectin extract.

Molecular determination by SDS-Polyacylamide Gel Electrophoresis

SDS PAGE is an analytical method used to separate proteins based on molecular weight and evaluate the digestion of lectin by bis-acrylamide homogenous gel. The gel was run for 2 hours using TRIS- glycine-SDS running buffer at constant voltage of 150 V for stacking and separating gel. After the electrophoresis, the gel was washed with distilled water for several times, and then stained for 30 min with Coomassie brilliant blue R-250.Finally distained in 10% acetic acid solution and analysed in densitometer (Bio Rad. PD Quest Software-8.0.1) [36].

RESULTS

In the present study, an enriched lectin with the agglutinating activity was purified and was designated as *Maytenus senegalensis* leaves (MSL).

Purification of *Maytenus senegalensis* plant extracts lectin:

MSL extract was purified by dialysis and ammonium sulphate precipitation method. 70-90% and 100% saturation was used to examine characteristic properties of MSL lectin. It showed strong agglutination activity with healthy human erythrocytes.

Estimation of Protein concentration by Follin Lowry's method:

Total Protein content present in dialyzed extract of MSL lectins (100µl) was found to be 0.148652 mg/ml by standard graph.

Determination ofHemagglutination activity:

The partially purified extract of MSL lectin showed hemagglutination activity by formation of carpet till eleven times dilution in case of represented blood groups (figure 1(a)). The formation of carpet and button pattern of MSL lectin extract in titer plate showed different concentrations of titer requirement. The minimum concentration of lectin required for agglutination activity was observed as 0.000145168 mg/ml shown in Table 1. The experiment showed that the MSL agglutinates human erythrocytes, having no specificity towards any of the blood group. The partially purified MSL lectin has also observed hemagglutination activity with diabetic human erythrocytes. The similar results were observed as

compared to normal erythrocytes (Figure 2). The MSL lectin showed hemagglutination activity by formation of carpet till eleven times dilution in case of all diabetic blood groups (Figure 1(b)). Therefore, the minimum concentration of lectin required for agglutination activity with the diabetic erythrocytes was observed as 0.000145168 mg/ml.

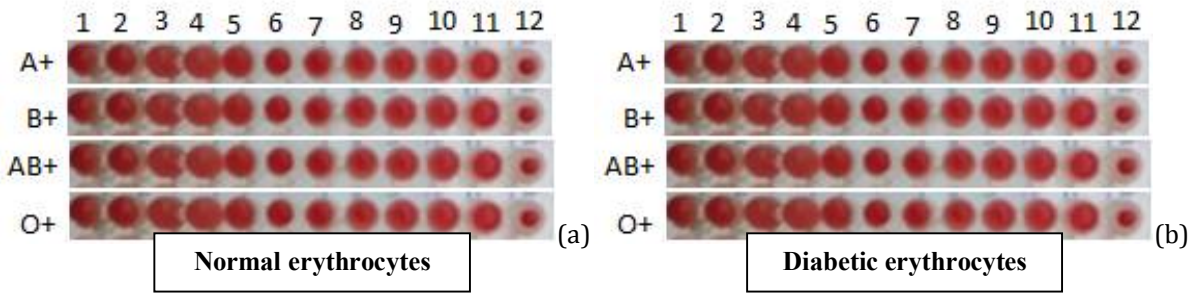

Figure 1. (a) Hemagglutination Titer of MSL lectin with Normal Human Erythrocytes of A Rh +ve, B Rh +ve, AB Rh +ve , O Rh +veblood group (b) Hemagglutination Titer with Diabetic Human Erythrocytes of A Rh +ve, B Rh +ve, AB Rh +ve , O Rh +ve blood group**.**

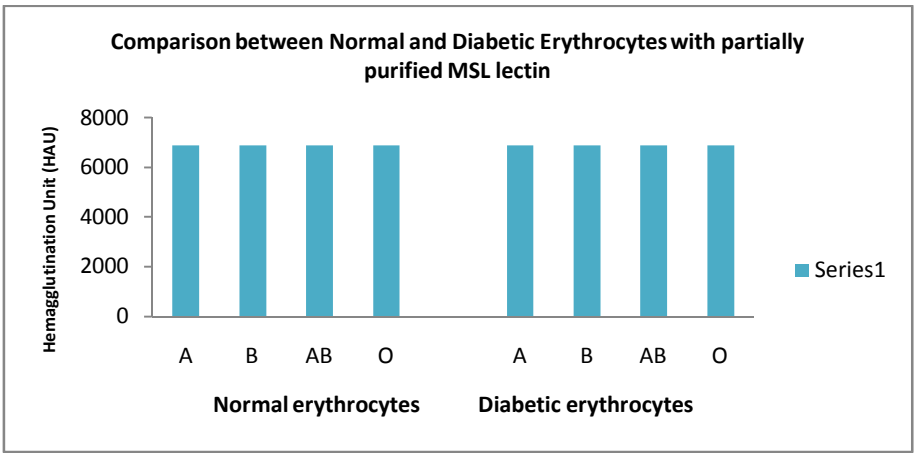

Figure 2. Graphical representation of comparison between normal (A Rh +ve, B Rh +ve, AB Rh +ve, O Rh +ve) and diabetic erythrocytes(A Rh +ve, B Rh +ve, AB Rh +ve, O Rh +ve) with partially purified MSL lectin- Hemagglutination Unit (HAU) analyzed.

Table 1 Hemagglutination activity using MSL lectins

Human erythrocytes	Hemagglutination Unit (HAU)	Total protein (mg/ml)	Specific Activity (HAU/mg/ml)
A,B,AB,O Rh^+	6888.572	0.000145168	47452413.8

HAU- The reciprocal of the highest dilution of the lectin solution exhibiting hemagglutination, referred as one hemagglutination unit.

The MSL purified lectins were also used to study the agglutination patterns of erythrocytes of cancer and sickle cell patients. The partially purified MSL lectin showed hemagglutination activity by formation of carpet till eleven times dilution in normal erythrocytes and erythrocytes from bladder cancer patient. Up to twelve times dilution was observed in case of erythrocytes of cervix and endometrium cancer patients and in case of breast cancer erythrocytes hemagglutination activity by formation of carpet till fifteen times dilution as compared to normal erythrocytes was observed (Figure 3). The activity shown by breast cancer was extreme as compared to normal. The activity of endometrium and cervix cancer is slightly more than normal whereas bladder cancer shows similar activity compared to normal erythrocytes (Figure 1). The minimum concentration of lectin required for agglutination activity with the normal erythrocytes and bladder cancer erythrocytes was found to be 0.000145168 mg/ml, for cervix and endometrium cancer RBCs, it was observed as $7.2584e^{-05}$ mg/ml. For breast cancer erythrocytes, the minimum concentration was found to be $9.073e^{-06}$ mg/ml as shown in Table 3.

The partially purified extract of MSL lectin showed hemagglutination activity by formation of carpet till ten times dilution in case of erythrocytes of SS-Type of sickle cell patient as compared to eleven times dilution in AS Type of sickle cell erythrocytes and normal erythrocytes (Figure 4). The agglutination activity shown by sickle cell (SS type) erythrocytes was lower as compared to AS Type and normal healthy erythrocytes (Figure 2). The minimum concentration of lectin required for agglutination activity with the normal erythrocytes and AS Type sickle cell erythrocytes was found to be 0.000145168 mg/ml whereas for SS Type sickle cell erythrocytes, it was observed to be 0.000290336 mg/ml as shown in Table 4.

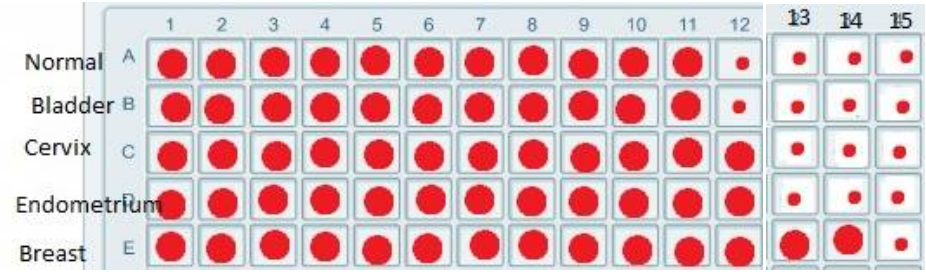

Figure 3 Hemagglutination titer representing comparative study between normal and different cancer (Bladder, Cervix, Endometrium, Breast) erythrocytes with partially purified MSL lectins

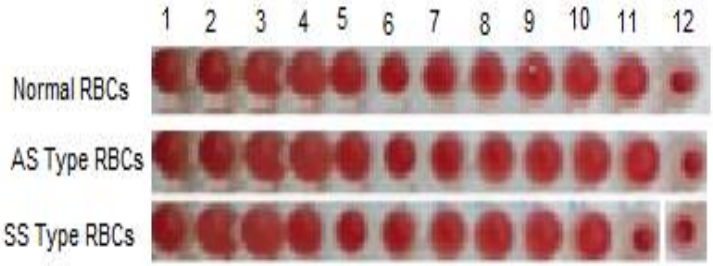

Figure 4.Hemagglutination titer representing comparative study between normal and sickle cell (AS Type and SS Type) erythrocytes with partially purified MSL lectins

Table 2 Comparison of hemagglutination activity of cancer types with normal erythrocytes using MSL lectins

Types of cancer analysed	Hemagglutination Unit (HAU)(ug/ml)	Total protein (mg/ml)	Specific Activity (HAU/mg)
Normal (control)	6888.572	0.000145168	47452413.8
Bladder	6888.572	0.000145168	47452413.8
Cervix	13777.14	$7.2584e^{-05}$	-
Endometrium	13777.14	$7.2584e^{-05}$	-
Breast	55108.58	$1.8146e^{-05}$	-

Table 3 Comparison of hemagglutination activity of sickle cell erythrocytes with normal erythrocytes using MSL lectins

Types of samples analysed	Hemagglutination Unit(HAU)(ug/ml)	Total protein (mg/ml)	Specific Activity (HAU/mg)
Normal (control)	6888.572	0.000145168	47452413.8
AS Type	6888.572	0.000145168	47452413.8
SS Type	3444.286	0.000290336	11863103.4

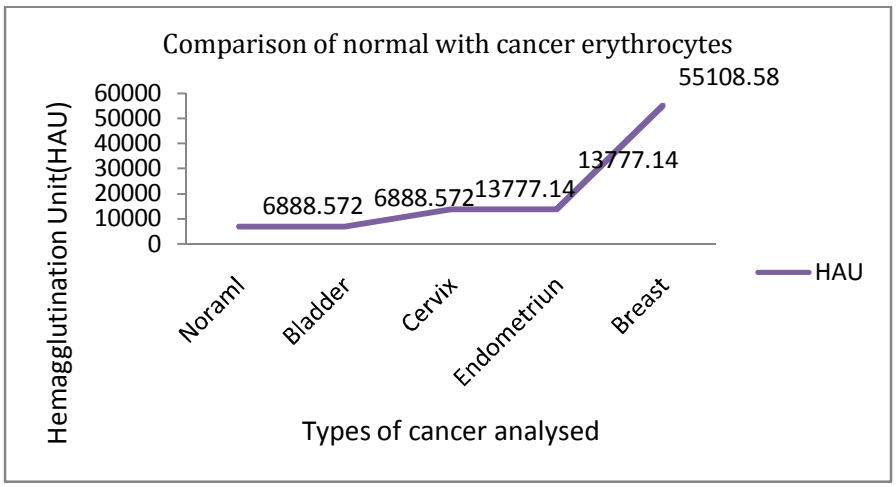

Figure 5 Graphical representation of HAU(Hemagglutination Unit)ofnormal and cancerous (Bladder, Cervix, Endometrium, Breast) erythrocytes

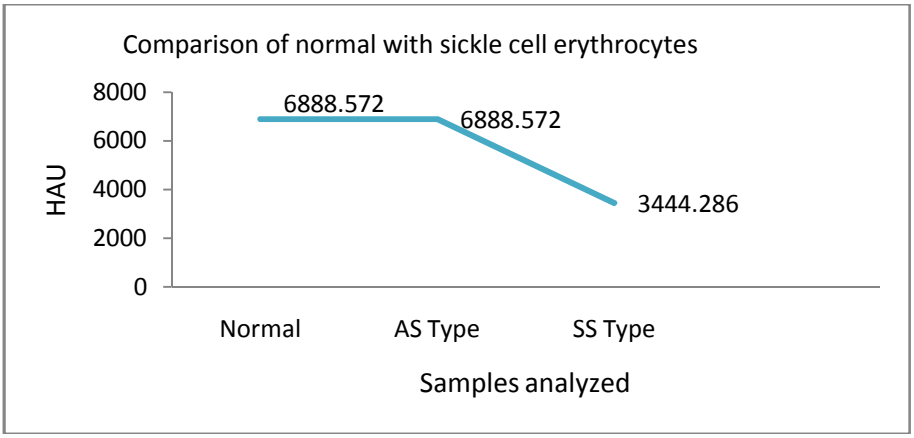

Figure 6 Graphical representation of HAU of normal and sickle cell (AS Type, SS Type) erythrocytes

Agglutination activity determination on animal erythrocytes:

MSL plant lectins also showed agglutination against animal erythrocytes like calf, horse, dog, hen, goat and rat (Table 2). High agglutination activity was observed in horse and dogs erythrocytes whereas moderate agglutination activity was observed in calf, hen, rat and goat erythrocytes.

Table 5 Agglutination study of MSL lectins with animal erythrocytes

S.No.	Animals	Agglutination
1	Calf	++
2	Horse	+++
3	Dog	+++
4	Hen	++
5	Rat	++
6	Goat	++

+++ shows high agglutination of erythrocytes; ++ shows moderate agglutination of erythrocytes
+ shows low agglutination of erythrocytes; - shows negative agglutination of erythrocytes

Effect of pH and Temperature on MSL lectins:

We examined the effect of pH on human erythrocytes by the partially purified *Maytenus senegalensis* leaves lectin. It showed heamagglutination activity in all pH ranges. HA activity was observed between pH 3 and pH 11. The HA activity of the crude homogenates remained stable and high in pH 3-6, but it decreases at pH above 7 or below 3 and become totally inactive at pH above 12 or below 2. At extreme acidic and basic pH, no agglutination was found and cells gets haemolysed during incubation (Figure 9).

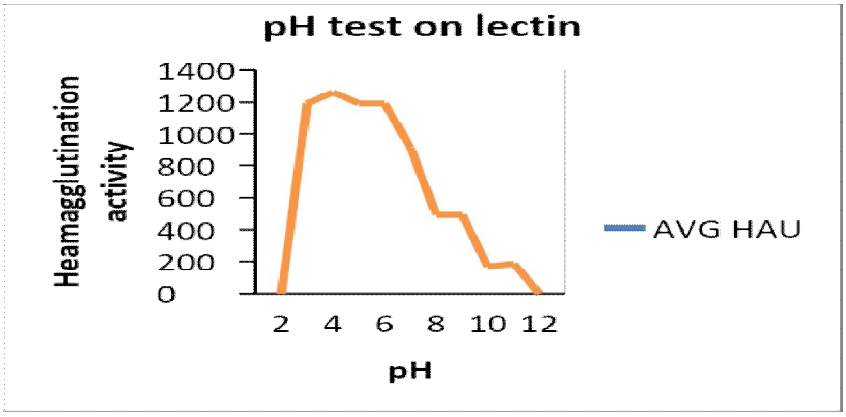

Figure 7 Graphical representation of effect of pH on heamaggluting activity of purified MSL lectin

The graph of temperature range in degree Celsius (°C) against agglutination activity in percentage (%) was plotted to determine optimum temperature for maximum stability. Results showed that the lectin present in this plant is thermo stable in nature and sustain a wide range of temperature as shown in (figure 2).

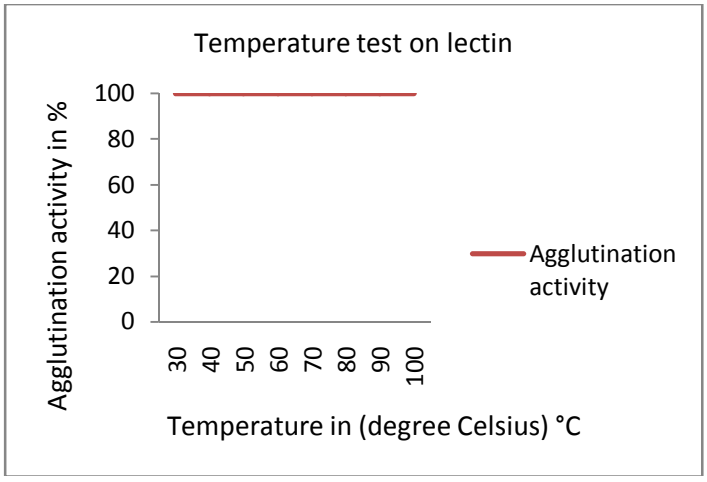

Figure 1 Graphical representation of effects of temperature on heamagglution activity of purified MSL lectin

Carbohydrate inhibition assay:

In this experiment, it was observed that lectins of *Maytenus senegalensis* are non- specific to the sugars tested at various concentrations from 100 mM to 500 mM as observed with agglutination (carpet pattern) as shown in Figure. These lectins showed no inhibition to the following simple sugars. This indicates the possibilities of existence of carbohydrate binding domain specific for sugar derivatives but not for simple sugar units. There are many plant lectins which show specificity towards certain sugar derivatives, such as wheat germ agglutination (WGA) is specific for GlcNac, MorusRubra lectin has also shown the specificity for N-acetyl –D- galactosamine in a literature.

Table 6 Carbohydrate inhibition assay by purified MSL lectins, concentration of sugars varies from 100 mM to 500 mM.

Types of Sugar	Inhibition
Glucose	NI
Sucrose	NI
Lactose	NI
Sorbitol	NI
Fructose	NI
Maltose	NI
Arabinose	NI
Galactose	NI
Xylose	NI
Mannose	NI
Ribose	NI

NI = No Inhibition

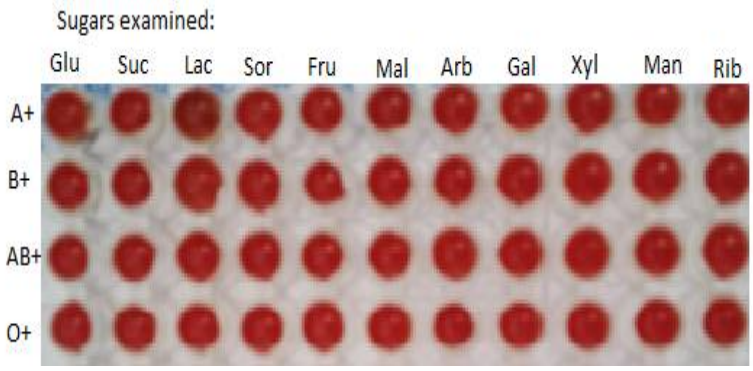

Figure 2Hemagglutination inhibition assay in presence of different sugars

Note: Glu- glucose, Suc- Sucrose, Lac- Lactose, Sor- Sorbitol, Fru- Fructose, Mal- Maltose, Arb- Arabinose, Gal- Galactose, Xyl- Xylose, Man- Mannose and Rib- Ribose.

Results of SDS-PAGE analysis

Analysis of SDS-PAGE gels was performed with the aim to identify protein band expression which was observed in partially purified MSL lectins. Molecular marker and sample were loaded in lane 1 and lanes 2 respectively and stained with coommasie brilliant blue which non-specifically bind to protein present in partially purified MSL lectin. No bands were observed after analysis of gel for protein band by Quantity one software. The partially purified MSL lectin required further purification for band expression will be carried out in further studies.

DISCUSSION

In recent years, reports have been suggested about many structural and biochemical uses of plant lectins and their increasing use as biotechnological tools. Many lectins from various plant parts were identified and isolated. The study mainly focused on characterization of lectins with RBC surface markers of cancer, diabetes and sickle cell anemia.

The present study, useda conventional ammonium sulphate precipitation method to purify the MSL lectins. Results observed 100% ammonium sulphate saturation followed by dialysis for purification of MSL lectins. In the same line Ramteke & Patil *et al* (2005) and Mothong (2009) were observed the similar saturation for initial purification of lectins from Tridaxprocumben leaves [37-38].

In this study, partially purified MaytenusSenegalensis (MSL) plant lectins does not exhibit specificity towards strong agglutination activity with erythrocytes of human blood group (A, B, AB and O Rh+) revealing its non- specificity.Other blood group nonspecific reported lectins are wheat germ agglutinin, soybean lectin, blackgram lectin, *A. integra*lectin, rice lectin [39-42].

Further, horse and dog erythrocytes showed high agglutination with lectins as compared to calf, hen, goat and rat erythrocytes. Different isolated and purified lectins were found to be agglutinate with erythrocytes from different animals. Ramteke & Patil (2005) noted that lectin from Tridaxprocumbans agglutinates dog erythrocyte. Because lectins have the ability to bind sugars and precipitated in various glycoconjugates which can identify cell surface sugars and separate glycoproteins. The same virtue attributes them with a biological activity of agglutinating human and animal erythrocytes[37].

On the other hand, no difference in hemagglutination titre was observed between diabetic and normal erythrocytes of human blood. It does not show any difference in titer towards specific markers on erythrocytes of diabetic and normal erythrocytes The inhibition of HA by MSL lectins with simple sugars were analyzed, suggested that it cannot be inhibited by any of the sugars. Reported studies on plant lectins showed specificity towards certain sugar derivatives of wheat germ agglutination (WGA) (GlcNac), and Morus Rubra lectin (N-acetyl –D- galactosamine [43-46].

This study were also observed MSL lectins agglutinates most of erythrocytes of breast cancer cells and moderately agglutinates with cervix and endometrium cancer cells when compared with normal erythrocytes. Similar observation was not found with erythrocytes of bladder cancer cell. Results indicated that specific cell surface markers were highly active on the erythrocytes of breast, cervix and endometrium cancer cells. Lectins have depicted effects on glycan motifs on the surface of cancer erythrocytes reflecting their cytotoxity. In the same lines, Brooks and coworkers (1991) found that lectins were extremely specific for identifying cells with the improperly assembled membrane glycoproteins of the breast cancer erythrocytes as compared with the erythrocytes of normal person [47].David *et al*., (1978), also reported variations in lectin agglutination between erythrocytes of normal persons and cancer patients. It can be assumed that the sugar specific epitopes may be increased in the erythrocytes of

the breast cancer cases as compared to the normal individuals[48].Hemagglutination studies were also carried out by Mitchell *et al.,* (1985) using *H. pomatia* agglutinin and *P. vulgaris* leucoagglutinin with erythrocytes of breast and colon cancer and reported the difference between the binding pattern in metastatizing human breast and colon cancer with HPA, and PHA – L [49]. Hasija (1991) reported that the binding pattern of cancer cells differ with different lectins[50]. Durgawale *et al.,* (2001) reported results with Synadenium root (Hook F) lectin and stated that more receptor sites were available on the surface of cancerous cells [51].

Further, hemagglutination titre comparison between sickle cell (AS Type and SS Type) erythrocytes and normal erythrocytes suggest that SS type erythrocytes have less markers present on their surface as compared to AS Type and normal erythrocytes which can binds to the MSL lectin.

Plant lectins showed optimum activity at different pH and temperature range. In this study, optimum pH for the purified lectin activity of *Maytenus senegalensis* was from pH 3 to 11. Extreme acidic and basic pH i.e. above pH 12 or below pH 3 inhibits the lectin activity of MSL plant whereas at neutral pH gives best agglutination activity. Thus more acidic and more basic medium are not favourable for haemagglutination activity of MSL lectin, still works in between 3-11 pH. Studies on, *Euphorbia tirucalli* lectin showing highest activity between pH 7-8 range, *Amaranthus cruentus* seed lectin was having stability between pH range of 3-8. *Vicia Graminea and V.mungo* lectin activity was reported to be unaffected in pH range of 6-9 and 4-7 respectively [35].

Thermal stability of lectins was studied in MSL shows maximum activity at all temperature range from 30–100 °C. The hemagglutination activity of the purified lectin is heat-labile in nature. Julio G. Palharini *et al* (2017) has described the stability of Euphorbia tirucalli lectin between range 25-75 ºC which shows similarity in our findings [52].

It can be stated that cellular adhesion and interactions are largely dependent on properties of the erythrocytes surface membrane. The alteration in the cell membrane depends on molecular basis. The role of lectins in research has been increased steadily. Plant lectins showed optimum activity at different pH and temperature range. Thus more acidic and more basic medium are not favourable for haemagglutination activity of MSL lectin. MSL lectin worked in all temperatures range 30–100 ºC. It was found to be heat labile and has good thermal resistance power. It indicated that this lectin was thermostable in nature.

CONCLUSION

Result explained differences in glycosylation of the surface protein in erythrocytes of different cancer cases. MSL lectin manifest significant changes in heamagglutination pattern in all blood groups showing the alteration in the erythrocytes membrane. Moreover, the structure of MSL lectin was partially characterized, the agglutination activity of MSL lectin was not inhibited by any specific simple sugars. Thus more acidic and more basic medium are not favourable for heamagglutination activity of MSL lectin. It indicated that this lectin was thermo-stable in nature. Further, heamagglutination titre of SS type erythrocytes have less markers present on their surface as compared to AS Type and normal erythrocytes which can binds to the MSL lectin. The present study has provided a molecular basis for understanding the interaction of lectins with their receptors on cell surfaces and helps to elucidate the mechanisms by which agglutination occurs. Quantitation of the lectin molecules bound on the cell surface provides useful information for analysing the energies involved in lectin-induced aggregation.

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