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Characterization of *Cocus nucifera* (fresh) Lectin

Dr. M. Krishnaveni
Assistant Professor, Department of Biochemistry, periyar University, Salem - 636 011
Email - Krishnavenim2011@gmail.com

Abstract: Lectin isolated from *Cocos nucifera* (Fresh) was assessed for protein, carbohydrate by qualitative (thin layer chromatography) and quantitative analysis (spectrophotometry). The lectin obtained showed its ability to bind with glucose, sucrose, fructose, ribose and showed thermostability at 50 C as well as pH stability towards acidic pH. Hemeagglutination assay showed its human blood group specificity to O+, A+, B+, AB+ and among the animal blood tested, *Cocos nucifera* (Fresh) lectin showed specificity to pig blood. The protein content assessed by lowry's and dye method. The protein content was found to be high in dye method (17.10±1.98 mg/g). Whereas, the specific activity was found to be high in lowry's method 648.10 HAU/mg protein. The erythrocyte membrane stability was found to be moderate and possess good antioxidant potential which will relieve pain, inflammation. Also showed stability to denaturing agent urea and also to salts such as Na_2So_4, $CaCl_2$ at 25Mm concentration while for $FeCl_3$ the stability was noticed with both 25mM, 100Mm. SDS PAGE result showed band at 45, 55, 116KDa.

Key Words: *Anti-inflammatory, Antioxidant, Specific activity, Thermostable lectin.*

1. INTRODUCTION :

Lectin, a carbohydrate binding protein of non immune origin, have the ability to agglutinate, glycoconjucates(1), this property attracts researchers to use in molecular biology as a tool for the study of glycoconjugates in targeted drug delivery system(2-3). Physiological functions for lectins depend upon their general properties, locations in tissues. The binding site for specific carbohydrate is the major characteristic of lectins and help in the determination of their physiological role. Lectin binding exclusively to carbohydrate on the cell surface is reversible, non covalent, and exhibit more than two binding site, for agglutination reaction with carbohydrates of complex nature allowing attraction for different di, oligosaccharides, in order to exhibit anti-bacterial, anti-fungal, anti-tumor, anti-nematode, anti-viral activity. Plant lectin act as a reserve of nitrogen and as protein specific recognition factors and are classified by its monosaccharide binding specificity. Most of the plant lectins do not interact with endogenous cellular glycan but shows strong affinity for sugars from other organisms such as bacteria, fungi, herbivorous invertebrates, vertebrates thereby representing a defense molecule. Hence, it was decided to isolate lectin from *Cococus nucifera* (Fresh) in an attempt to view the distinctive properties and their characteristics.

2. MATERIALS AND METHODS:

2.1. SAMPLE COLLECTION AND ISOLATION OF LECTIN

Cocos nucifera was purchased from the local market at Salem, Tamil Nadu, India. 100gm of *Cocos nucifera* (Fresh) was used as a sample for the analysis. The lectin was isolated by soaking the sample overnight in 50mM phosphate buffered saline (pH 7.4), homogenized, filtered, centrifuged for 30min at 16,000 rpm.(4) The supernatant was used for the analysis. The sample was labeled as V1 for SDS PAGE.

2.2. PROTEIN ASSAY

The lectin was assessed for its protein content by Lowry and Dye method (5-6). To 10µl sample, added 1ml alkaline copper reagent, incubated for 10 minutes, added 0.1ml Folinciocalteu reagent, incubated for 30-60min at room

temperature and read at 660nm. For dye method, to 10µl sample added 5ml Coomassie brilliant blue dye, mix well and after 5 minutes read at 595nm using spectrophotometer. BSA was used as a standard. Specific activity was calculated from the obtained protein.

2.3. CARBOHYDRATE ANALYSIS

The total sugar content was performed by anthrone method (7). The crude lectin was converted into its monomers on boiling (3hr) with 5ml 2.5N HCl, neutralized with alkali, centrifuged. To 10µl lectin added 3ml 0.2% Anthrone reagent, heated at 100°C for 7min., cooled, read at 630nm. Glucose was used as a standard.

2.4. THIN LAYER CHROMATOGAPHY

Thin layer chromatography was adopted to study the protein, carbohydrate. (8-9) Silica gel prepared was activated by heating at 110°C, then the isolated lectin was loaded on the gel leaving 1cm from the bottom and allowed to run in a solvent system for protein [Butanol (8): Glacial acetic acid(2): water(2)] and in [Butanol (4): Acetone(5): Phosphate buffer (1)] for carbohydrate. After 30 minutes, the plates were taken out, sprayed with ethanolic ninhydin for protein and with anisaldehyde for carbohydrate. Dried, observed the spots obtained and calculated its Rf value.

2.5. RED BLOOD CELL (RBC) SUSPENSION PREPARATION

Human blood samples (2ml) from volunteer was collected, washed repeatedly with normal saline (0.9%), prepared 2-5% erythrocyte suspension a fresh. And animal blood sample was collected from the slaughter house early in the morning freshly on the day of experiment and processed similarly. Only hemeagglutination assay was conducted with all human and animal blood samples and other parameters with human blood group O^+ RBC suspension alone.

2.6. HEMEAGGLUTINATION ASSAY

The hemeaggultination assay (10) was performed by serially diluting lectin samples, agglutination was observed visually with carpet and button pattern after 1 hr incubation with 2-5% RBC suspension. Hemagglutination unit is defined as the last dilution that mediated agglutination. Specific activity is HAU per mg protein.

2.7. HEMEAGGLUTINATION INHIBITION ASSAY

Agglutination inhibition assay (11) was performed with sugars like glucose, sucrose, ribose, fructose, xylose, lactose, maltose, mannose. To equal volume lectin sample, added various sugar solutions at different concentrations, 2-5% RBC suspension was added, incubated for 1hr and examined for agglutination, agglutination inhibition.

2.8. EFFECT OF PH , TEMPERATURE , UREA, SALT

The effect of pH was assessed by selecting pH range from 1 to 10 (12) using buffer solutions such as 0.1N HCL(pH 1), 0.2M Glycine HCL, sodium acetate, sodium phosphate, Tris HCL, glycine sodium hydroxide, carbonate - bicarbonate buffer (pH 2-10). To equal volume lectin sample added buffer solutions of varying pH range, tested for agglutination. The effect of temperature was assessed at different temperature ranging from 10° to 100° for 3hrs. After 3hrs centrifuged, supernatant was used for the agglutination assay using 2-5% RBC suspension. The effect of denaturing agent urea on lectin hemeagglutination was assessed in 2, 5, 8, 10M solution. Lectin extracted was assessed both with and without heat. Equal volume of samples were treated with respective concentration of urea, assessed for its hemeagglutination activity.

Effect of salt was assessed using $FeCl_3$, Na_2So_4, $CaCl_2$ 25 and 100mM concentration. The test was performed similar to hemeagglutination test. Minimum concentration for hemeagglutination was noticed.

2.9.ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity (13) was studied with O^+ RBC suspension. To 10µl sample, 1ml phosphate buffer, 2ml hyposaline, 0.5ml RBC suspension was added, incubated for 15-30minutes, centrifuged for 5min.The supernatant was measured at 560nm. Standard drug was diclofenac sodium. Control was also developed. The RBC membrane stabilization in percentage was calculated as follows:Membrane stabilization (%)=100–[(O.D of drug treated sample/O.D of control) *100].

2.10.ANTIOXIDANT ACTIVITY

Antioxidant activity was studied using DPPH (14).To various concentrations of lectin ranging from 5 to 500 µg/ml, added 100µl of 0.01mM methanolic DPPH solution, incubated in dark for 30 min. at ambient temperature, recorded the absorbance at 517nm using spectrophotometer. Standard was developed with ascorbic acid. Control was also maintained. The percentage of free radical scavenging activity was calculated by the formulae:[(Ac–As)/Ac]× 100.

2.11. SDS PAGE

SDS-PAGE was done according to Laemmli (15). 50µl protein sample was mixed with sample buffer (Tris–HCl, pH 6.8 containing 2-mercaptoethanol, glycerol, bromophenol blue, 5% SDS) in 2:1, heated at 90°C for 5min., after that 25µl sample was loaded along with high molecular weight protein markers. Perform electrophoresis at 50mA in the beginning, later at 100mA. Stain the bands with Coomassie brilliant blue R250. The molecular weights were determined by comparison with standard protein markers.

2.12. STATISTICAL TOOL

All Quantitative experiments were done thrice, measurements were taken using Schimadzu UV1800 spectrophotometer. Standard deviation was calculated.

3. RESULTS AND DISCUSSION

3.1Nutrient assay

Table.1 Protein, Carbohydrate by TLC, Quantitative analysis of carbohydrate in *Cocos nucifera* (Fresh)

Lectin Source	Nutrient	Results
Cocos nucifera (Fresh)	Protein (TLC) Rf Value	0.19±0.30
	Carbohydrate(TLC) Rf Value	0.81±0.14
	Carbohydrate(Anthrone method) mg/g	21.98±10.01

Values are Mean ± SD for Three experiments

Protein and carbohydrate content in *Cocos nucifera* (Fresh) lectin by thin layer chromatography (Table.1). Rf value reported was 0.19±0.30 for protein, for carbohydrate 0.81±0.14. The quantitative estimation of carbohydrate by anthrone method was found to be 21.98±10.01.

Table.2 Protein profile of *Cocos nucifera* (Fresh)

Lectin Source	Total protein (mg/g)		Total HAU	Specific activity (HAU/mg protein)		Purification fold/ Yield(%)
	Lowry method	Dye method		Lowry method	Dye method	
Cocosnucifera (Fresh)	7. 85±1.82	17.10±1.98	512	648.10	296.81	1/100

Values are Mean ± SD for Three experiments

Table.2 shows the protein profile in *Cocos nucifera* (Fresh) lectin. The protein content and specific activity by lowry method was found to be 7.85±1.82mg/g, 648.10 HAU/mg. While by dye method it was 17.10±1.98, 296.81HAU/mg. The purification fold is1/100.

3.2.In vitro Erythrocyte membrane stabilization test

Table.3 Anti-inflammatory activity of *Cocos nucifera* (Fresh)

Lectin Source /Standard	Antiinflammatory activity (%)
Cocos nucifera (Fresh)	46.00±0.00
Diclofenac sodium (Standard)	100.00±0.00

Values are Mean ± SD for Three experiments

Anti-inflammatory drugs act both by inhibiting lysosomal enzymes as this enzymes are released on inflammation and also it act by stabilizing the lysosomal membrane. human red blood cell membrane and lysosomal membrane are comparable, the human red blood cell membrane stability by *Cocos nucifera* (Fresh) was assessed. The anti – inflammatory activity observed was 46.00±0.00% (Table.3).

3.3.Hemeaagglutination assay

Table.4 Hemeagglutination assay of *Cocos nucifera* (Fresh) in human, animal erythrocytes

Lectin Source	Blood group	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	2^{11}	2^{12}	2^{13}	2^{14}	2^{15}	2^{16}
Fresh coconut lectin	O^+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
	A+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	B+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
	AB+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	Cow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	Goat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Pig	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	-

+ Hemeagglutination - No Hemeagglutination

Table 4 shows the results of blood group specificity of *Cocos nucifera* (Fresh) lectin by hemagglutination assay, which was assessed using human O^+, A^+, B^+, AB^+as well as using cow, goat, pig red blood cell suspension. *Cocos nucifera* (Fresh) lectin was specific to all human blood groups studied as well as to pig blood and showed non-specificity to cow and goat blood .

3.4.Hemeagglutination Inhibition assay

Table.5 Hemeagglutination inhibition assay of *Cocos nucifera* (Fresh)

Lectin Source	Sugars	10mM	20mM	50mM	100mM	200mM
Fresh coconut lectin	Glucose	+	-	+	+	-
	Sucrose	+	+	-	+	+
	Ribose	-	+	-	-	-
	Fructose	-	-	-	-	+
	Xylose	-	-	-	-	-
	Lactose	-	-	-	-	-
	Maltose	-	-	-	-	-
	Mannose	-	-	-	-	-

+Hemeagglutination, - Hemeagglutination Inhibition

Table. 5 shows the results of sugar binding specificity of *Cocos nucifera* (Fresh) lectin through hemeagglutination inhibition assay with sugars. Hemeagglutination was observed with glucose at 10, 50, 100mM concentration, for sucrose at 10,20,100, 200mM concentration, for ribose at 20mM concentration and for fructose at 200mM concentration. All the remaining sugars were showing hemeagglutination inhibition at all concentration tested.

3.5.Effect of pH

Table.6 pH stability of *Cocos nucifera* (Fresh)

Lectin Source	pH									
Cocos nucifera (Fresh)	1	2	3	4	5	6	7	8	9	10
	+	-	-	-	-	+	-	-	-	-

- Hemeagglutination Inhibition, + Hemeagglutination

Table.6 shows the pH stability of *Cocos nucifera* (Fresh) lectin. The pH studied was pH 1 to 10 (pH 1- 0.1N HCL and 0.2M for the remaining pH 2 to 10). pH 1 and pH 6 only showed hemeagglutination.

3.6.Effect of Temperature

Table.7 Effect of Temperature on *Cocos nucifera* (Fresh)

Lectin Source	Temperature in °								
Cocos nucifera (Fresh)	10	20	30	40	50	60	70	80	100
	+	-	-	-	+	-	-	-	+

- Hemeagglutination Inhibition, + Hemeagglutination

Table.7 shows the effect of temperature on *Cocos nucifera* (Fresh) lectin. It was studied by heating at various temperature for three hours and subjected to hemeagglutination assay. The results depicts that *Cocos nucifera* (Fresh) lectin was stable at 10, 50, 100°C confirming it to be a thermostable lectin.

3.7.Effect of urea

Table. 8 Effect of Denaturing agent Urea on *Cocos nucifera* (Fresh)

Lectin Source	Crude lectin heated				Crude lectin Not Heated			
	2M	5M	8M	10M	2M	5M	8M	10M
Cocos nucifera (Fresh)	-	-	-	-	+	+	+	+

- Hemeagglutination Inhibition, +Hemeagglutination

Table.8 shows the results of effect of denaturing agent urea on *Cocos nucifera* (Fresh) lectin by heating as well as by not heating for hemeagglutination. *Cocos nucifera* (Fresh) lectin subjected to heat showed hemeagglutination inhibition. Whereas, lectins not subjected to heat showed hemeagglutination at all the concentration studied.

3.8.Effect of salt

Table.9 Effect of various salts on *Cocos nucifera* (Fresh)

Lectin Source	Salt	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	2^{11}	2^{12}	Lectinactivity HAU
Cocos nucifera (Fresh)	$FeCl_3$ (25mM)	-	-	-	-	-	-	-	-	-	-	-	+	4096
	$FeCl_3$ 100mM	-	-	-	-	-	+	-	-	-	-	+	+	64/2048/4096
	Na_2SO_4 25mM	-	-	-	-	-	-	-	-	-	-	+	+	2048/4096
	Na_2SO_4 100mM	-	-	-	-	-	-	-	-	-	-	-	-	-
	$CaCl_2$ 25mM	-	-	-	-	-	-	+	-	-	-	-	-	128
	$CaCl_2$ 100mM	-	-	-	-	-	-	-	-	-	-	-	-	-

- Hemeagglutination Inhibition, + Hemeagglutination

Table. 9 shows the result of $FeCl_3$, Na_2SO_4, $CaCl_2$ on *Cocos nucifera* (Fresh) lectin at two different concentration 25, 100mM. $FeCl_3$ at 25mM showed 4096 HAU. While for 100mM it was 64/2048/4096 HAU. While, 25mM Na_2SO_4 showed agglutination at 2048, 4096 HAU, while calcium chloride at 25mM showed agglutination at 128HAU. No agglutination was observed with other salts tested at two different concentration.

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3.9.Antioxidant activity

Fig. 1 DPPH antioxidant activity of *Cocos nucifera* (Fresh)

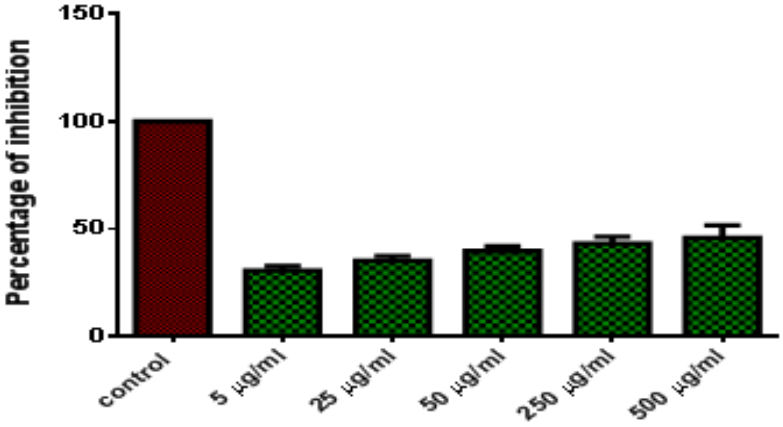

Fig.1 shows the free radical scavenging ability of *Cocos nucifera* (Fresh) lectin at 500µg/ml, 250 µg/ml, 50µg/ml, 25µg/ml, 5µg/ml against methanolic DPPH. The percentage of inhibition observed for ascorbic acid was 100% . the inhibition in percentage was found to be increased as the concentration increases and IC50 value reported was 82.61µg/ml. The percentage of inhibition observed was 45.78, 43.15, 39.73, 35.21, 30.59%.

3.10.SDS PAGE

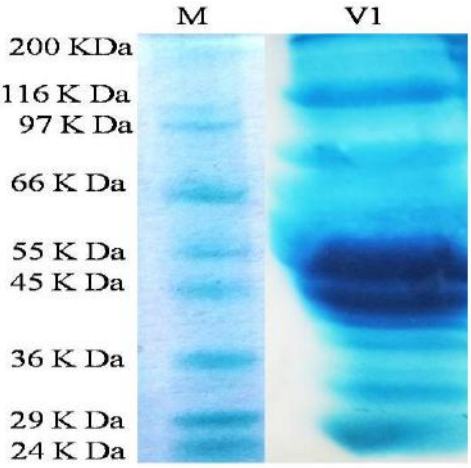

Fig.2 shows the SDS PAGE results of *Cocos nucifera* (Fresh) lectin. The band was observed at 45, 55, 116KDa.

4. CONCLUSION

The results of present study showed, that *Cocos nucifera* (Fresh) lectin could agglutinate O+, A+, B+, AB+ of human and pig blood of animal. *Cocos nucifera* (Fresh) lectin was specific to glucose, sucrose, ribose, fructose. The protein content was found to be high in dye method. While, the specific activity was found to be high in lowry's method. The erythrocyte membrane stability was found to be moderate with good antioxidant potential which aid to relieve pain, inflammation. Stability to urea and salts was observed. SDS PAGE showed band at 45, 55, 116KDa. The obtained results show, that *Cocos nucifera* (Fresh) lectin might be very much useful in the aspects of pharmaceutical purpose.

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