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Partial Purification and Characterization of Lectin from the Seeds of *Cissus poplunea*

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Research Article

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ABSTRACT

Lectin is a glycoprotein substance, usually of plant origin, of non-immunoglobulin nature and capable of binding to carbohydrate moieties of complex glycoconjugates. This underlies its clinical significance. Hence, purification steps comprising of centrifugation, salting-out, ultra filtration, dialysis and anion exchange affinity chromatography were used to purify the protein from the seed of *Cissus populnea*. The purified lectin agglutinated non-specifically red blood cells of human type A, B and O. The hemagglutinating activity of the lectin towards human erythrocytes was inhibited by D-fructose, D-glucose and $CuSO_4$. However the lectin activity was enhanced by D-galactose and $MgCl_2$. Stability studies showed the purified protein to be stable at a spectrum of 20-40°C and at pH range of 6-8 and 10-11. The kinetic study on the purified protein indicated 26271HU and 278.2 L for V*max* and K*m,* respectively. However, result from paper chromatography on the carbohydrate isolate during purification indicated presence of a ketone sugar having same appearance with fructose standard, a sugar clinically established as the major source of energy during spermatogenesis. It was observed that heamagglutinating activity of the lectin from *Cissus populnea* towards human erythrocytes was non-selective to type of blood groups. It could be stated that *Cissus populnea* consumption may pose no threat to patient with challenge in metabolising glucose, since its main carbohydrate content is fructose.

Keywords: Lectin; Cissus populnea; glucose; fructose; erythrocytes;

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1. INTRODUCTION

Interest in medicinal plants as a re-emerging health-aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being, and the bio-prospecting of new plant-derived drugs (Hoareau and Dasilva, 1999). While medicinal plants and traditional medicine are integral parts of the health delivery system in developing societies like Nigeria where there is a heavy reliance on such. In addition to purified plant derived drugs, there is an enormous market for crude herbal medicines as dietary supplements, and for therapeutic purposes in both the developed and developing countries of the world (De Smet, 1997). It is incontrovertible that clinical plant-based research has made particularly rewarding progress in various significant fields of medicine, example the use of taxoids and camptothecins in anticancer and artemisinin compounds in anti-malarial therapies.

Against this backdrop, this study focuses on *Cissus populnea*, an otherwise promising herbal therapy associated with a myriad of uses in different parts of the world. The seed gum is used for soup and as soup thickener. It is also widely used as medicine for the treatment of venereal diseases and indigestion and as drug binder (Iwe and Atta, 1993). Its extracts have been credited with antibacterial properties (Kone et al., 2004), as an anti-trypanosomal plant and a source of gum powder (Atawodi et al., 2002) and as a component of an herbal anti-sickling Nigerian formula (Moody et al., 2003). In Benin Republic, it is used for its diuretic properties; may render the plant as an anti-hypertensive agent and the relief of hypertension may result in improved fertility. The aqueous extract of the stem bark is used as a fertility enhancer in males in South Western Nigeria (Ojekale et al., 2006). Same efficacy was also reported by Awoyinka & Smith (2003) in experiment carry out on albino rats.

The use of *Cissus populnea,* as an aphrodisiac and fertility enhancer amongst the males has been attributed to the declining fertility trend that has been established in this population over the years coupled with the attendant increasing levels of erectile dysfunction (Joint Report, 2004). In addition to this, there have been reports in the scientific literature on the possible toxic effects of *C. Populnea* (Smith et al., 2002; Geidam et al., 2004). Despite all these reports, little is known on the possible clinical significance of its lectin which if well documented it would greatly increased the awareness and exploration of *C. populnea* in traditional plant remedies. Hence this, study aims at purifying and characterizing a lectin from the seeds of *Cissus poplunea.*

2. MATERIAL AND METHODS

2.1 Materials

2.1.1 Collection of plant material

Seeds of *Cissus populnea* were purchased from open market in Ilisan-Remo, Ogun State, Nigeria.

2.1.2 Collection of blood samples

Ten blood samples of 5ml each of different blood groups were collected from Babcock University Medical Laboratory, Ilisan-Remo. Ogun State, Nigeria. The blood samples were

collected in heparinised bottles (ethylene diamine tetra acetate (EDTA) bottle) to prevent the blood from coagulating and kept in fridge to preserve them till the time of use.

2.2 Methods

2.2.1 Treatment of blood samples

Blood samples of known blood groups of A, B, AB & O were collected from individuals at Babcock University. 5ml of each sample was centrifuged at 1500 × g for 5 minutes at room temperature. The red blood cells obtained were then washed by centrifugation at1500 × g for 5minutes at room temperature with 0.01M phosphate- buffered saline (pH 7.2). This was repeated twice, after which the cells were mixed with 3% formaldehyde in EDTA bottle and allowed to stir gently overnight, before it was centrifuged at 1500× g for 5 minutes, the following day. The centrifuged red cells were then washed again as before, three times with 0.01M phosphate- buffered saline (pH 7.2) after which the cells were collected into a stopped bottle and 76.8ml of 0.01M phosphate- buffered saline was added to make the cell 4% thereafter, it was stored in the fridge.

2.2.2 Isolation and purification of lectin from *Cissus populnea*

The defatted samples were dissolved in water in ratio 1:20 An aliquot of it were separated and kept in refrigerator for carbohydrate analysis while the other part was centrifuged at 1500 revolution per minutes (rpm) for 30minutes. The pellets were discarded and supernatant collected for ammonium sulphate precipitation as described by Trowbridge (1974). The precipitated protein were pulled together and dissolved in 240ml of distil water, the resulting mixture was concentrated by ultra filtration (Millipore, India) at 1500g for 30 minutes before dialyses against 0.15M NaCl – 0.01M NaPO4 buffer for 24hours. The dialysed sample was further purified by anion exchange affinity chromatography method using 2g DEAE- Sephadex A-50 previously dissolved in 50ml of 0.15M NaCl – 0.01M NaPO4 buffer before equilibration in a column (8.5 × 1.5cm). Thereafter, the column was washed with the same buffer and loaded with dialyzed precipitated protein and eluted with 0.2M glucose solution. The eluents were assay for lectin using hemagglutination activity as marker.

2.2.3 Assay for lectin activity

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). A serial two-fold dilution of the lectin solution was mixed with 50 µl of a 4% suspension of human erythrocytes in phosphate buffered saline, pH 7.2 at room temperature (the erythrocytes of human blood group A, B and O were fixed with 3% formaldehyde). The plate was left undisturbed for 1h at room temperature in order to allow for agglutination of the erythrocytes to take place. The hemagglutination titre of the lectin expressed as the reciprocal of the highest dilution exhibiting visible agglutination of erythrocytes was reckoned as one hemagglutination unit. Specific activity is the number of hemagglutination units per mg protein (Wang et al., 2000).

2.2.4 Test of hemagglutination inhibition by various carbohydrates

The hemagglutination inhibition tests to investigate inhibition of lectin-induced hemagglutination by various carbohydrates were performed in a manner analogous to the

hemagglutination test as described by Kuku et al. (2009). Serial two-fold dilutions of sugar samples were prepared in phosphate buffer saline. All the dilutions were mixed with an equal volume (50 µl) of the lectin solution of known hemagglutination units. The mixture was allowed to stand for 1 hr at room temperature and then mixed with 50 µl of a 4% human erythrocyte suspension. The hemagglutination titres obtained were compared with a non-sugar containing blank. In this study, the sugars used were: glucose, galactose, maltose, fructose, sucrose and lactose. The minimum concentration of the sugar in the final reaction mixture which completely inhibited hemagglutination units of the lectin sample were obtained (Wang et al., 2000).

2.2.5 Effect of temperature on hemagglutinating activity

The effect of temperature on the agglutinating activity of the lectin from *C. Populnea* was determined by carrying out assay at different temperatures according to the method described by Cavada et al. (1996). The purified lectin was incubated in a water bath for 30 min at various temperatures: -10, -4, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100°C, and then cooled to 20°C. Hemagglutination assay was carried out as previously described.

2.2.6 Effect of pH on hemagglutinating activity

The effect of pH on the activity of the lectin from *C. populnea* was determined by incubating the lectin in the following buffers at different pH values of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. This was done by altering the pH of 0.15M NaCl – 0.01M NaPO4 buffer using concentrated HCl and 1M NaOH and assaying for hem agglutinating activity. The control values were the agglutination titre of the lectin in PBS, pH 7.2.

2.2.7 Effect of salts on hemagglutinating activity

The hemagglutination inhibition tests to investigate inhibition of lectin- induced hem agglutinations by various salts were performed in a manner analogous to the hemagglutination test. Serial two-fold dilutions of salt samples were prepared in phosphate buffer saline. All the dilutions were mixed with an equal volume (50 µl) of the lectin solution of known hemagglutination units. The mixture was allowed to stand for 1 hr at room temperature and then mixed with 50 µl of a 4% human erythrocyte suspension. The hemagglutination titres obtained were compared with a non-salt containing blank. In this study, the salts used were: Calcium Chloride, iron (iii) sulphate, sodium sulphate, copper sulphate and Magnesium chloride. The minimum concentration of the sugar in the final reaction mixture which completely inhibited hemagglutination units of the lectin sample were obtained (Wang et al., 2000).

2.2.8 Determination of sugar content

Into the aliquot of the defatted sample, distilled water was added slowly and cooled before the froth was removed. The undissolved materials were removed by centrifugation at 1500×g for 30minutes at room temperature. Then absolute ethanol was added slowly with a pasteur pipette to the supernatant with constant stirring until the solution became cloudy. The polysaccharide precipitate was collected by centrifugation at 1500×g for 30minutes at room temperature and spread in a petri-dish to dry at room temperature. It was then re-suspended in distil water at 50mg/ml and centrifuged at 1500×g for 5minutes. The supernatant was retained while the precipitate was discarded. The polysaccharide extract was then hydrolysed with 2MHCl followed by neutralization with 4M NaOH. Paper

chromatography of the hydrolysis was carried out as described by Rendina (1972) using Sodium acetate: N, N-Dimethylaniline: water as solvent mixture in ratio 110:50:40, with 1% solutions of glucose, mannose, galactose and fructose as sugar standards. Tollen's reagent was used as the staining solution.

2.2.9 Determination of total protein

Biuret test as described by Plummer (1987) was used in determination of total protein in *Cissus populnea*.

2.3 Statistical Analysis

All analysis was carried out at least duplicate so as to get a very good result and graph plotted with the latest version of graph pad prism 5.0.

3. RESULTS AND DISCUSSION

It was observed that throughout the process of purification, the specific activity generally increased and the time for blood agglutination got shorter as purification of lectin increases (Table 1). Although, there was a little deviation from these trends, with ammonium sulphate having a lower specific activity and a longer hemagglutination time to that of crude. However, as soon as ammonium sulphate was removed via dialysis the specific activity increased hence, it can be inferred that ammonium sulphate inhibits hemagglutination activity of lectin. With this result, the lectin gotten from affinity chromatography is still the best having highest hemagglutination activity of 206HU/mg at 800µl volume. The data presented from this study showed that the extract of the seeds of *C. populnea* contained adequate amount of hemagglutinating protein (lectin). However, the lectin from *C. populnea* agglutinated red blood cells non-specifically which is typical of many lectins (Kuku et al., 2009).

Lectin in this study was found to be heat stable from 20ºC to 40°C (Fig.1) thus they are heat labile and not thermophilic. In comparison lectin extracted from *C. annuum* was found to be stable between 20ºC to 30°C (Alexander et al., 2004; Kuku et al., 2009). However, the hemagglutinating activity from the *Pterocladiella capillacea* lectin was affected only by exposure to a temperature of 70°C this occurs in some plant lectin (Cavada et al., 1996) and marine algae (Benevides et al., 1998). Well, lectin from Ganaderma caperise is not affected after exposure to temperature at and above 70°C for 60minutes (Ngai and Ngi, 2004). Hemagglutinating activity of lectins from *Pterocladiella ostreatus* is reduced at or above 40°C (Wang et al., 2000). The difference in all these values may be supported by the submissions of Okamato et al. *(*1990) and Leiner (1994). 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 hemagglutinating activity of lectins as temperature increase shows that its activity depends on the native conformation of the protein. More so, Lectin activity was found to be stable at two optimum ranges of pH 6 – 8 and pH 10-11 but with high optimum activity at range of pH 6 – 8 (Fig. 2). 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 Alexander et al. (2004) in which *Manila clam* lectin activity was stable between pH 6 and pH 9 and was temperature-dependent.

Table 1. Purification profile of lectin

Volume (µl)	25.0	50.0	100.0	200.0	400.0	800.0	Time taken for hemagglutination
Crude: Specific activity (HU/mg)	3.00	13.50	9.00	27.00	54.00	13.50	50 minutes
(NH4)2SO4: Specific activity (HU/mg)	0.38	0.75	1.50	3.00	9.00	27.00	60-80 minutes
Dialysed: Specific activity (HU/mg)	3.25	9.50	19.00	54.00	54.00	103.00	25-30 minutes
Affinity chromatography: Specific activity (HU/mg)	25.75	51.50	77.25	103.00	154.50	206.00	5- 12 minutes

Table 2. Effect of salts on hemagglutinating activity of *Cissus populnea'* lectin

Salt (mmol/L)	800	400	200	100	50	25	PBS
$CaCl_2$	+	++	+++	+++	+++	+++	+
$Fe2(SO_4)_3$	+	+	+	+	+	+	+
Na_2SO_4	+	+	+	+	+	+	+
$CuSO_4$	_	_	+	+	+	+	+
$MgCl_2$	++++	+++	++	+	+	+	+

Key: +, hem agglutinating activity; -, no hemagglutinating activity; PBS, phosphate-buffered saline.

Lectin activity of *Cissus populnea* was slightly inhibited by $CaCl_2$ (Table 2) at higher concentration of 400mM and 800mM but enhanced the activity of lectin at concentrations of 200, 100, 50 and 25mM in contrast to lectin extracted from *Manila clam* which is Ca^{2+}-dependent *(*Alexander et al., 2004). $Fe_2(SO_4)_3$ and Na_2SO_4 has no effect on hemagglution activity of lectin extracted from *Cissus populnea.*

Similar observation has been reported by Okamoto et al. (1990), where Fe^{3+} salts were devoid of any effect on hemagglution activity of lectin extracted from *Gracilariabursa-pastori*. $CuSO_4$ inhibited lectin activity of at higher concentration of 400 and 800 mM while $MgCl_2$ enhanced the lectin activity of *Cissus populnea* as the concentrations were increased. However, Ca^{2+}, $Mg2^{+}$, Mn^{2+}, and Zn^{2+} ions do not affect the hemagglutinating activity of lectins isolated *G. frondosa* (Kawagishi et al., 1990).

Sugar inhibitory studies carried out in this study (Table 3), is in general agreement with those found for the numerous marine algal lectins, such as *Cystoclonium purpureum* (Huds.) Batters (Kamiya et al., 1980), *Solieria chordalis* (C. Agardh) J. Agardh (Rogers and Topliss, 1983), *Gracilaria bursa-pastoris* (Gmelin) Silva (Okamoto et al., 1990), *Solieria filiformis* (Benevides et al., 1996). Maltose, fructose and sucrose had no effect on hemagglutination activity. This is in slight variance with the result gotten by kuku *et al*., 2009 where the lectin extracted from *C. annum* was slightly inhibited by sucrose at higher concentration of 200 mM. Galactose enhanced the hemagglutination activity of the lectin as the concentration increases as shown in the Table 3, while galactose inhibit activity of lectin extracted from *C. annuum* at concentration above 200 mM as reported by Kuku et al. (2009).

Table 3. Sugar inhibition study of hemagglutinating activity of *Cissus populnea*

Sugar (mmol/L)	800	400	200	100	50	25	PBS
Maltose	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Glucose	_	_	+	++	+++	+++	+
Galactose	++++	+++	++	+	+	+	+
Fructose	_	+	++	+++	++	+	+

Note: +, hem agglutinating activity; -, no hemagglutinating activity; PBS, phosphate-buffered saline.

However, fructose slightly inhibited the activity of lectin as concentration increases. This is in contrast to that observed by Alexander et al. (2004). A cursory critical insights should be emphasised on these observations since in the presence of a low concentration of 100mM fructose lectin from *C. populnea*-lectin is found to agglutinate red blood cells though at a higher concentration of 800mM the blood-clot is reversed. This behaviour profoundly has clinical implication to the use of *Cissus populnea.*

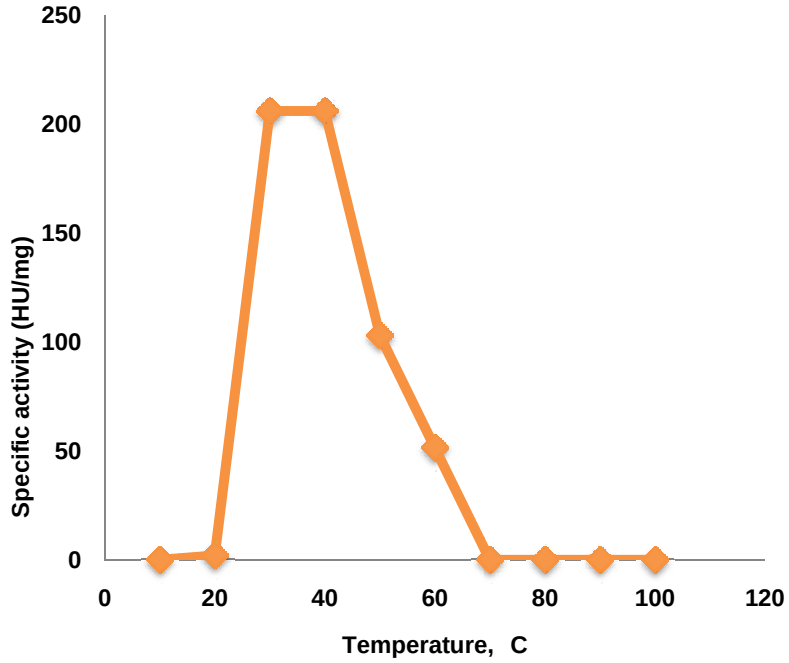

Fig. 1. Graph of specific activity against temperature

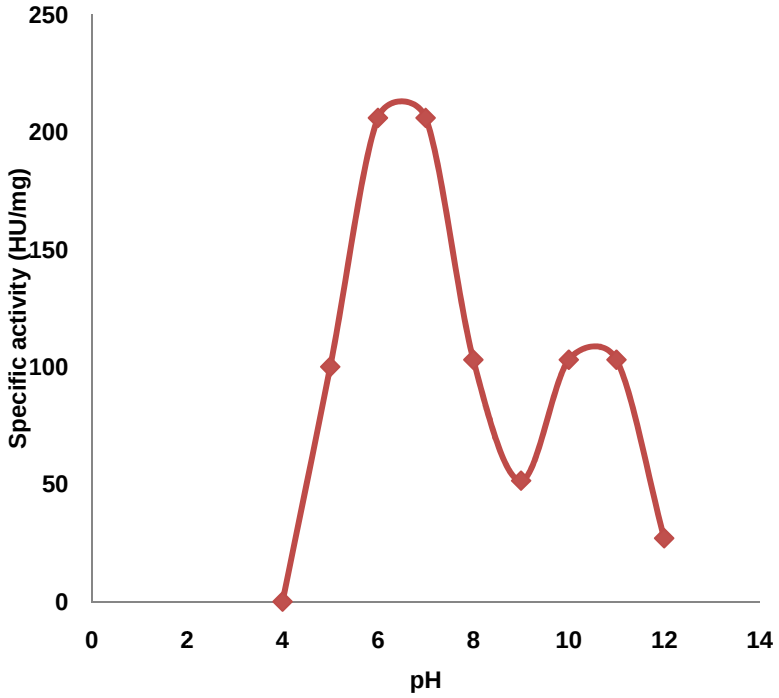

Fig. 2. Graph of specific activity against pH

4. CONCLUSION

In this study, heamagglutinating activity of the lectin from *Cissus populnea* towards human erythrocytes was found to be non-selective to type of blood groups. *Cissus populnea* consumption also may pose no threat to patient with challenge in metabolising glucose, since its main carbohydrate content is fructose.

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