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Chemical Modification and Denaturation Effects on the Hemagglutinating Activity of Two Pterocarpus Species Seeds Lectins

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Authors' contributions

The research was carried out in collaboration among all the authors. Authors OOO and AOA conceptualized the study. Authors MTF, SPO, ERO and AIA performed the experiment with supervision by author OOO and AOA. Author OOO did the data analysis. Authors OOO and AOA jointly wrote the draft manuscript. All authors revised the manuscript and approved the final manuscript for publication.

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ABSTRACT

Aims: *Pterocarpus osun* and *Pterocarpus soyauxii* seeds lectins were subjected to various chemical modifications in order to detect the amino acid residues involved in their hemagglutinating and sugar-binding activities.

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Methodology: The lectins were purified using salt precipitation and size exclusion chromatography. Hemagglutinating activity and sugar specificity of the lectins were also established. Chemical modification of arginine was done using phenylglyoxal hydrate, and 5,5- dithiobis-(2-nitrobenzoic acid) (DTNB) was used to modify cysteine. Phenylmethylsulfonyl fluoride (PMSF) was employed for serine modification and tryptophan residue was modified with N-bromosuccinimide (NBS). Denaturants effects on the hemagglutinating activity were carried out with chaotropic agents, acid, disulphide bridge reducer and cross-linker agent.
Results: *Pterocarpus osun* seeds lectin is mannose specific while *Pterocarpus soyauxii* seeds lectin is galactose/lactose-binding lectin. Hemagglutinating activities of the two lectins were completely lost when tryptophan residue was modified with NBS and the loss was reversed by dialysis. Modifications of Cysteine, Arginine and Serine have no effect on the hemagglutinating activity of *P. osun* lectin. Nevertheless, the modifications of same amino acids slightly reduced the activity of *P. soyauxii* lectin, which dialysis and prolonged incubation were able to overturn. Mannose was found to bind and inhibit hemagglutinating activity of *P. osun* lectin in the presence of various modifiers but galactose and lactose could not inhibit the hemagglutinating activity of *P. soyauxii* lectin in the presence of modifiers. All denaturants employed significantly affected the hemagglutinating activity of the two lectins. However, the effects were reversible except for *P. osun* lectin denatured with 8M urea.
Conclusion: The results revealed that tryptophan residue is essential for hemagglutinating activity of the *Pterocarpus* species seeds lectins studied in this research. Cysteine, Arginine and Serine are also needed for sugar binding by *P. soyauxii* lectin but not so important in *P. osun* sugar binding ability.

Keywords: Lectin; amino acid; residue; denaturing agents; sugar; tryptophan; carbohydrate structure; arginine; glycoconjugate.

1. INTRODUCTION

Proteins with the ability to bind glycoconjugate on the surface of cells and possibly agglutinate the cells are referred to as lectins [1]. Lectins are naturally ubiquitous biomolecules, with presence in animal, plants and microorganism [2]. Lectin is of non-immune origin and possesses no catalytic functions. They are multivalent [3] because they contain per molecule more than one binding site for carbohydrate. This property confers on them the ability to form network with many cells and agglutinate the cells out of solution.

Focus on lectin research has been tremendously increased recently because they exhibit some important biological properties like precipitating of glycan and glycoprotein, mitogenic simulation of lymphocytes, blood group specificity and preferential agglutination of cancerous cells [4]. With these properties, lectins have become indispensable tools in analytical and preparative biochemistry and in many area of biomedical research. Mostly, lectins are used in the purification and characterization of glycoconjugates. Aforementioned properties of lectins are linked to their interaction with carbohydrate-moiety [5].

Present in the binding sites of lectin are amino acid residues that possess ionizable side-chain that can contribute to interaction between functional group present on sugar and the lectin. Though this binding is similar to enzyme-substrate association where catalytic conversion of substrate occur, but lectin does not chemically alter the carbohydrate structure neither do new entity is produced. Identification of unique amino acid residue present in the binding site of lectin is crucial for the proper understanding of the relationship that exists between the lectin structure, their biological functions and applications in several biochemical fields [6].

Chemical modification of proteins is a process performed to prepare derivatives of protein for the purposes of amino acid sequence determination and to identify amino acids residues involved in binding and catalysis [7]. In the early 60, chemical modification of protein started with a procedure like affinity labelling, which gained ground for identification of enzyme active site amino acid residues. Specific examples is the modification of histidine residue of chymotrypsin with substrate-like compound N-p-Tosyl-L-phenylalanine chloromethyl ketone (TPCK), which eventually led to the complete elimination of its catalytic activity [8].

However, a simpler approach was later introduced. This involved the use of the side chain selective reagents that can react with one or more side chain groups in a predictable manner [9]. This method is still widely used today to identify amino acid side chains required for binding and biological activity. The effects of these group specific modifying reagents on specific amino acid residues present in the active/binding site of some lectin have been reported [10-12]. The method was applied to determine the involvement of amino acid residues in the haemagglutinating activity and biological functions of such lectin.

Lectins from Pterocarpus genus that is a member of Dalbergieae tribe and leguminosae family have been reported [13]. *Pterocarpus osun* and *P. soyauxii* are species of Pterocarpus. Both are native to Sub-African countries especially Nigeria, Congo, Equatorial Guinea and Cameroun. Their leaves are edible and occasionally used as vegetable because of the high nutrient content. Several ethnomedicinal uses have been reported generally for Pterocarpus species [14-17]. Recently, lectins of different blood and sugar specificity were reported from these species. The lectins were purified into homogeneity from the seeds and elicited antioxidant potential [18-19]. Based on comparison, our research group sought to deduce the rationale behind the differences in their sugar specificity.

Therefore, chemical modification of *P. osun* and *P. soyauxii* seed lectin binding site amino acid residues side-chain, using specific group modifying agents, were carried out in order to establish the amino acids involved in both the blood and sugar binding of these lectins and compare with other lectin in the same Sub-family that have the same specificities.

2. MATERIALS AND METHODS

2.1 Materials

Sodium Dihydrogen Phosphate (NaH_2PO_4), Sodium Chloride (NaCl), Sodium Azide are products of Qualikem Finechem Pvt Ltd. Vadodara, India. Sodium hydroxide (NaOH), 5, 5-dithiobis-(2-nitrobenzoic acid), N-bromosuccinimide, phenylglyoxal hydrate, phenylmethylsulfonyl fluoride, urea, glutaraldehyde, Trichloroacetic acid, Sephadex G-75, guanidine chloride, and guanidine chloride were all purchased from Sigma Aldrich chemical Ltd, Saint Louis, United States. Bio-Gel P-100 and 2-mercaptoethanol are bought from Bio-Rad chemical company, California, USA. All other chemicals used were of analytical grade.

2.2 Methods

2.2.1 Preparation of *Pterocarpus osun* and *Pterocarpus soyauxii* seeds crude extract

The dried matured seeds of *P. osun* and *P. soyauxii* were removed from the winged fruits and pulverized into powder using a blender, after which 50g of the powder was defatted using petroleum ether. 20g of the defatted powder was extracted in ten volumes of 10 mM Phosphate Buffered Saline (PBS) solution (pH 7.2). After stirring with a magnetic stirrer for about 10 hours, the mixture was centrifuged at 10,000 rpm for 20 minutes using refrigerated centrifuge (Thermoscientific TGL 16A) and the supernatant (termed the crude protein extract) was collected and subjected to salt precipitation.

2.2.2 Purification of *Pterocarpus osun* and *P. soyauxii* seeds lectin

2.2.2.1 Ammonium sulphate precipitation

Pterocarpus *osun* and *P. soyauxii* seeds crude protein extract was subjected to salt precipitation (70% ammonium sulphate saturation) and left for about 18 hours at room temperature. The mixture was centrifuged to collect the precipitate, which was redissolved in minimal volume of PBS (pH 7.2). The precipitate was then dialyzed against PBS extensively. The dialysate was centrifuged to remove debris. The supernatant obtained was lyophilized.

2.2.2.2 Gel filtration on Sephadex G-75 and Biogel P-100

Sephadex G-75 was packed into a chromatographic column (2.5 x 40 cm) and equilibrated with PBS pH 7.2. Seven milliliters of the dialyzed and lyophilized ammonium sulphate precipitate of *P. osun* seeds crude extract (5 mg/ml) that contain about 8 mg proteins was layered on the column. The elution was carried out using the same buffer and 5 ml fractions were collected at the flow rate of 20 ml/hr. The fractions were monitored for protein by measuring the absorbance at 280 nm, after which hemagglutinating assay was carried out on every other test tubes. The test tubes with agglutinating activity were immediately pooled and freezed.

Biogel P-100 was separately packed into another chromatographic column of the same size for purification of lyophilized sample of *P. soyauxii* seeds. The same volume of 5 mg/ml protein solution containing 12.5 mg protein was layered on the column. The sequence of protocols above was followed.

2.2.3 Red blood cells fixation, hemagglutinating activity assay and sugar specificity test

Human and rabbit red blood cells (RBC) were fixed with glutaraldehyde using method of Kuku et al. [20]. The whole blood sample was collected into heparinized bottle. The blood was washed several time with PBS to obtain RBC. Two percent packed RBC was prepared in 1% glutaraldehyde-PBS (v/v) solution and incubated for 1 hr. The fixed RBC was collected using centrifugation and washed with PBS to remove glutaraldehyde and 2 % v/v of the fixed RBC was prepared in PBS that contains 0.02 % sodium azide.

Hemagglutination assay to detect the presence of lectin was carried out in 96-wells U-shaped microtitre plate. The crude extract and other fractions were serially diluted up to 24 wells already filled with 100 µl PBS. Fixed RBC of rabbit and human ABO blood group (50 µl) was separately added into each well and allowed to incubate for at least two hours before taking the hemagglutinating titre. Hemagglutinating titre was read as the reciprocal of the last dilution showing visible agglutination of RBC.

Sugar specificity of the lectins was determined following the hemagglutination assay procedure except for the addition of solution of 0.2M sugars after the serial dilution of the lectin. This was followed by 30 minutes incubation before adding the fixed RBC. The hemagglutinating titre obtained was compared with original lectin.

2.2.4 Effect of chemical modification of amino acid residues on hemagglutinating activity

This was carried out by treating the lectin samples with specific modifying agents; the residual activity of the modified lectin was determined by hemagglutination assay. Lectin samples incubated in the absence of modifying reagents served as the control.

2.2.4.1 Modification of tryptophan residue

Spande and Wiktop [21] method of modifying tryptophan using N-bromosuccinimide (NBS) was adopted with slight amendment. In brief, aliquot of 1 mg/ml lectin sample prepared in 100 mM sodium acetate buffer, (pH 5.0) was mixed with same volume of 10 mM NBS, which was added in five installments within 60 min at 20ºC. The residual hemagglutinating activity of the lectin was evaluated after dialyzing the solution against distilled water to remove excess modifying reagent.

2.2.4.2 Modification of Serine residue

This was carried out by incubating the lectin (100 µg) in 100 µl of 50 mM Tris-HCl buffer (pH 7.4) with 10 mM phenylmethylsulfonyl fluoride (PMSF) at 27ºC for 1 hour [22]. Excess modifying reagent was removed by dialyzing against distilled water after which the residual hemagglutinating activity was determined. The lectin sample in the absence of modifying agent was used as positive control.

2.2.4.3 Arginine residues modification

Arginine residues in the lectin sample were modified with phenylglyoxal hydrate using the procedure of Riordan [23]. One hundred microlitres of 1 mg/ml of the lectin sample in PBS, pH 7.2 was incubated with 10 µl of 10 mM phenylglyoxal hydrate (in 0.1 M sodium carbonate, pH 8.0) at room temperature for 1 hour (with the addition of 10 µl of reagent every 15 minutes). Excess modifying agent was removed by dialyzing against distilled water after which the residual hemagglutinating activity was determined. The lectin sample in the absence of modifying agent was used as positive control.

2.2.4.4 Cysteine modification

The cysteine residues in the sample were modified according to the method of Habeeb [24]. One hundred microlitres lectin sample in 50 mM phosphate buffer (pH 8.0) was incubated with 10 µl of 10 mM 5,5- dithiobis-(2-nitrobenzoic acid) (DTNB) at 27ºC for 1 hour (with the addition of 10 µl of reagent every 15 minutes). Excess modifying agent was removed by dialyzing against distilled water after which the residual hemagglutinating activity was determined. The lectin sample in the absence of modifying agent was used as positive control.

2.2.5 Effect of denaturants on hemagglutinating activity

The effect of denaturing agents such as chaotropic (urea, guanidine HCl), cross-linker (glutaraldehyde), acid (trichloroacetic acid), and disulphide reducer (2-mercarptoethanol) on lectin activity was carried out by incubating an aliquot of the purified lectin with each of the denaturant for 6 hours. The hemagglutinating activity was determined before and after dialysis against distilled water. The lectin sample in the absence of denaturant was used as positive control.

3. RESULTS

3.1 Hemagglutinating Activity

Phosphate Buffer Saline-extract of the two *Pterocarpus spp.* agglutinates erythrocyte of rabbit. *Pterocarpus soyauxii* extract further agglutinated human blood groups A, B and O, which *P. osun* did not agglutinated. The *P. soyauxii* showed slight preference for erythrocytes of rabbit. These indicate that *P. osun* is specific for rabbit erythrocytes while *P. soyauxii* is non-specific for all the blood groups. The results are shown in Table 1.

Table 1. Hemagglutinating activity of *Pterocarpus osun* and *Pterocarpus soyauxii*

	Hemagglutinating Titre	
Erythrocytes Source	***P. osun***	***P. soyauxii***
Rabbit	2^{11}	2^{12}
Human Blood Group A	2^{0}	2^{8}
Human Blood Group B	2^{0}	2^{7}
Human Blood Group O	2^{0}	2^{8}

3.2 Purification of *P. osun* and *P. soyauxii* Seeds Lectins

The gel filtration chromatogram of *P. osun* seeds lectin on Sephadex G-75 column is depicted in Fig. 1. One peak of hemagglutinating activity and two protein peaks were recorded. The hemagglutinating activity is resided in the first peak of protein. The fractions with hemagglutinating activity were pooled, concentrated and used for further studies.

Gel filtration elution profile of *P. soyauxii* seeds lectin on Biogel P-100 is shown in Fig. 2. Two

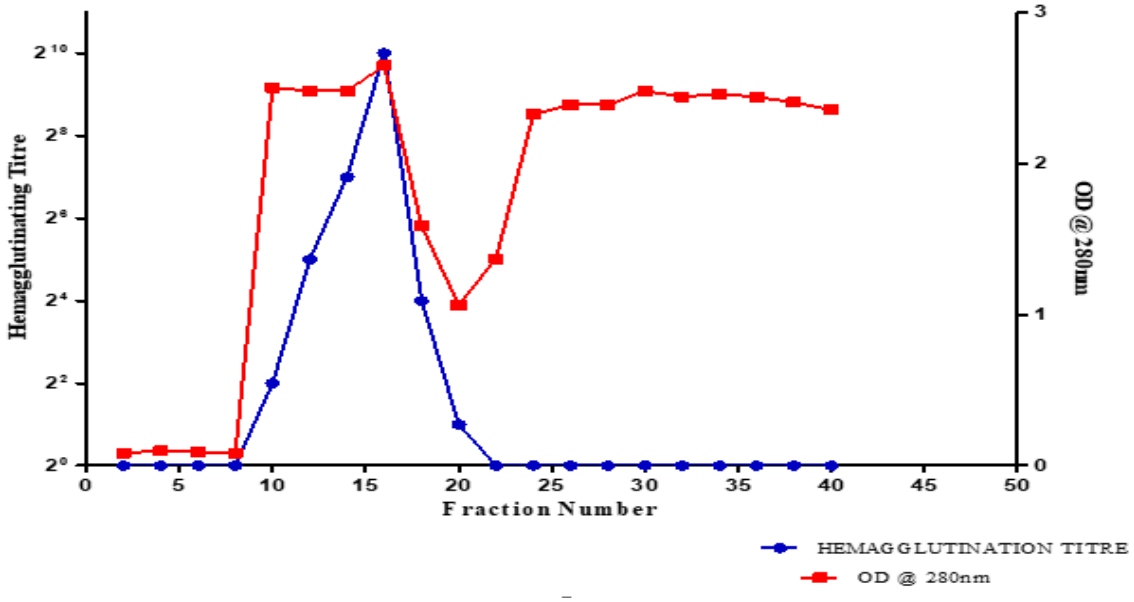

Fig. 1. Chromatogram of Gel Filtration Chromatography of the Ammonium Sulphate Precipitate Dialysate of *Pterocarpus osun* Seed Crude Extract on Sephadex G-75

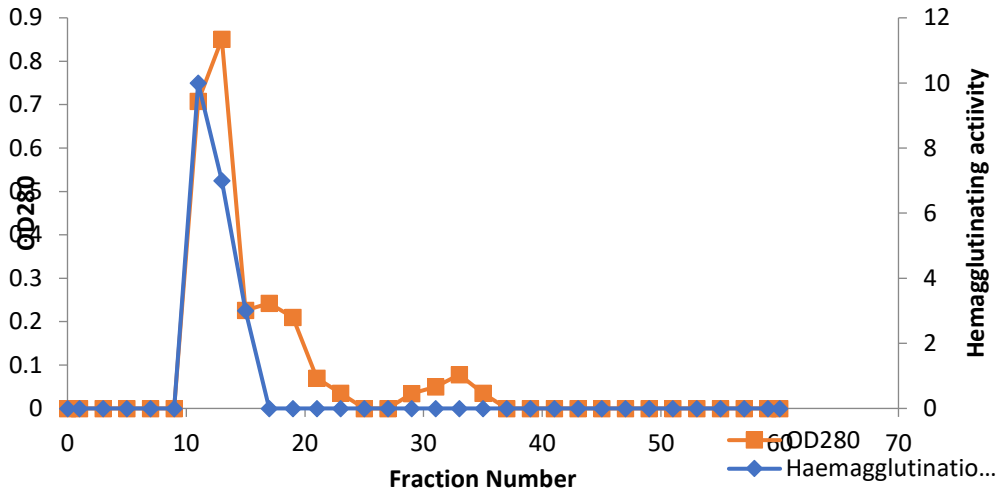

Fig. 2. Chromatogram of Gel Filtration Chromatography of the Ammonium Sulphate Precipitate Dialysate of *Pterocarpus soyauxii* Seed Crude Extract on Biogel P-100

protein peak elicited hemagglutinating activity. The fractions that show hemagglutinating activity were pooled, concentrated and used for further studies.

3.3 Chemical Modification of *P. osun* and *P. soyauxii* Seeds Lectins

The effects of chemical modification of amino acid residues on hemagglutinating activity of the purified lectins are summarized in Table 2 and Table 3. Generally, only the NBS has significant effects on the hemagglutinating activity of the lectins from the two species. Dialysis for about 24 hours was able to reverse the effect. DTNB, PMSF and Phenylglyoxal did not affect the hemagglutinating activity of *P. osun* even after 6 hours of incubation. However, there was reduction in the activity when the three modifiers were separately incubated with *P. soyauxii* lectin for 1hr. Incubation for longer time and dialysis against PBS was able to reverse the effect of the chemicals on the lectin activity. Further studies were carried out to determine the effect of the chemical modification of amino acid residues on the sugar binding ability of the lectin. Table 4 and 5 show the detail results of the experiments. The binding of sugar (mannose) to the *P. osun* lectin in the presence of DTNB, PMSF and phenylglyoxal resulted in complete loss of hemagglutinating activity, which the modifier could not achieve. This shows that the modified amino acid residues (arginine, cysteine and serine) are located in the binding site of the sugar and have no significant roles in binding of sugar by the *P. osun* lectin. The results presented in Table 4 revealed that DTNB, PMSF and phenylglyoxal did not alter any amino acid residue at the sugar-binding site of *P. osun* lectin. The sugar (mannose) was able to bind to the lectin and caused complete inhibition of hemagglutinating activity of the lectin despite the presence of the modifiers. NBS modification of tryptophan could not be overturned by the sugar, indicating that the amino acid residue is probably located at the sugar-binding site or close to it thereby affecting the sugar binding. In case of *P. soyauxii* lectin, contrary results were obtained. DTNB, PMSF and phenylglyoxal prevented the binding of galactose and lactose to the sugar-binding site of the lectin. Consequently, galactose and lactose were unable to inhibit the hemagglutinating activity of the lectin. NBS showed the same effect as was obtained for *P. osun*.

3.4 Denaturation Studies

The effects of denaturing agents on hemagglutinating activity of the purified lectin from the two *Pterocarpus* species are summarized in Table 6. The denaturants used are 2% Glutaraldehyde, 6M Urea, 8M Urea, 6M Guanidine HCl, 8M Guanidine HCl, 2% Trichloroacetic acid and 2-mercarptoethanol. All the denaturants affected the hemagglutinating activity in the lectin from the two *Pterocarpus* species. It was also observed that hemagglutinating activity was restored after the denaturants were removed by dialysis against PBS for 24 hours except for effect of the 8M urea on *P. osun* lectins.

Table 2. Effect of chemical modification on hemagglutinating activity of *P. osun*

	1 hour incubation		6 hours incubation	
Chemical Modifiers	**Before Dialysis**	**After Dialysis**	**Before Dialysis**	**After Dialysis**
Control	2^6		2^6	
NBS	2^0	2^5	2^0	2^7
DTNB	2^6	2^5	2^7	2^7
PMSF	2^6	2^6	2^7	2^7
Phenylglyoxal	2^6	2^6	2^7	2^7

Table 3. Effect of chemical modification on hemagglutinating activity of *P. soyauxii*

	1 hour incubation		6 hours incubation	
Chemical Modifiers	**Before Dialysis**	**After Dialysis**	**Before Dialysis**	**After Dialysis**
Control	2^{10}		2^{10}	
NBS	2^0	2^9	2^0	2^{11}
DTNB	2^4	2^{10}	2^{11}	2^{11}
PMSF	2^5	2^{10}	2^{11}	2^{11}
Phenylglyoxal	2^4	2^{10}	2^{11}	2^{11}

Table 4. The effects of chemical modifiers on the sugar binding sites of *P. osun* lectins

		Activity with sugar Mannose	
Chemical Modifiers	**Activity with Modifiers**	**Before Dialysis**	**After Dialysis**
Control (Without Modifiers)	2^{10}	2^0	
NBS	2^0	2^0	2^0
DTNB	2^{10}	2^0	2^0
PMSF	2^{10}	2^0	2^0
Phenylglyoxal	2^{10}	2^0	2^0

Table 5. The effects of chemical modifiers on the sugar binding sites of *P. soyauxii* lectins adding modifiers first

		Activity with Sugars			
		Galactose		Lactose	
Chemical Modifiers	**Activity with Modifiers**	**Before Dialysis**	**After Dialysis**	**Before Dialysis**	**After Dialysis**
Control (Without Modifiers)	2^{10}	2^0		2^0	
NBS	2^0	2^0	2^0	2^0	2^0
DTNB	2^{10}	2^9	2^9	2^8	2^9
PMSF	2^{11}	2^9	2^8	2^8	2^8
Phenylglyoxal	2^{10}	2^9	2^{10}	2^8	2^{10}

Table 6. Effects of denaturants on the hemagglutinating activity of *P. osun* and *P. soyauxii*

	P. osun		*P. soyauxii*	
Denaturants	**Before Dialysis**	**After Dialysis**	**Before Dialysis**	**After Dialysis**
Control	2^7		2^{11}	
2% Glutaraldehyde	2^1	2^6	2^0	2^{11}
6M Urea	2^0	2^7	2^0	2^{11}
6M Urea	2^0	2^0	2^0	2^{10}
6M Guanidine HCl	2^1	2^7	2^0	2^{11}
6M Guanidine HCl	2^0	2^6	2^0	2^{10}
2% Trichloroacetic acid	2^1	2^6	2^0	2^{11}
2-mercarptoethanol	2^1	2^6	2^3	2^{10}

4. DISCUSSION

Amino acids play an enormous role in determining the structure and ultimately the biological activity of proteins. In order to establish the role played by certain amino acids in the structure and function of the lectin purified from *P. osun* and *P. soyauxii*, modifying chemical agents and denaturants were employed. To understand the phenomenon of interaction between lectins and carbohydrates, knowledge of the reactive groups involved in the interaction is essential [25]. Identification of specific amino acids involved in the biological activity of proteins, elucidates the relationship between its structure and the role played by amino acid side chains in its activity. The involvement of some amino acids residues in the hemagglutinating activity of *P. osun* and *P. soyauxii* lectins was investigated using specific modifying reagents such as phenylglyoxal for arginine, DTNB for cysteine, PMSF for serine, and NBS for tryptophan. The modifiers phenylglyoxal, DTNB and PMSF did not produce any alteration in the hemagglutinating activity of the *P. osun* lectin but significantly reduced the *P. soyauxii* lectin hemagglutinating activity, which was overturned by dialysis and long period of incubation. These results indicated that arginine, cysteine, and serine are not playing any important role in the activity of the *P. osun* lectin but might be involved in the maintenance of the active conformation and possibly binding at the active site of *P. soyauxii* lectin. This is in line with the result published by Thakur et al. [26]. *Ganoderma lucidum* lectin hemagglutinating activity remains unchanged after treatment with phenylglyoxal, PMSF and DTNB, though more residue of cysteine was modified after denaturation of the lectin with urea (8M) [26]. Modification of arginine residue in *Erythrina indica* lectin with phenylglyoxal did not show any effect on the activity of the lectin. This is suggesting that arginine may not be involved in either direct interaction with the sugar or may not have a role in maintaining conformation of the sugar-binding pocket and hence not contributing to the hemagglutinating activity of the lectin [27]. Earlier, arginine modification of lectins from *Artocarpus lakoocha* [10], *Phaseolus vulgaris* [11], *Crotalaria striata* [28], and *Araucaria brasiliensis* [29] did not affect the interaction of the lectins.

However, total loss of hemagglutinating activity was observed when both *P. osun* and *P. soyauxii* lectin were treated with N-bromosuccinimide (NBS). This result evidently suggests that tryptophan is either located at the sugar-binding site or is involved in the maintenance of the lectin active conformation. Previous studies have shown that tryptophan is indispensable for the hemagglutinating activity of some lectins, especially galectins [30,31]. Tryptophan residue was implicated in sugar binding activity of both congerin I and II because its modification by 2-nitrophenyl sulfenyl chloride (NPS-CL) led to about 87% loss of the hemagglutinating activity of the congerins [30]. A complete loss of activity of the *Artocarpus lakoocha* lectin was observed when tryptophan, tyrosine and histidine were modified, indicating the involvement of these amino acids in the carbohydrate-binding ability of the lectin [10]. Contrary to these reports and our findings, tryptophan residues were found to be non-essential both in agglutination and in sugar-binding potential of *Trichosanthes dioica* seed lectin [32]. It was speculated that tryptophan residues of the lectin appears to be buried in the protein interior, as they could not be modified under native conditions [32]. However, their report supported our finding on insignificant of cysteine residue in hemagglutinating activity of both Pterocarpus species lectin studied. In addition, tryptophan plays an important role in protein by enriching protein surfaces involved in protein docking and are often found in membrane proteins close to lipid head groups [33]. Tryptophan has a nitrogen atom in its aromatic ring structure; this N-atom can play a role in the binding to non-protein atoms, although such instances are rare [34] but may not be strange for lectins being unique in a world of protein.

Three-dimensional structure of proteins is the functional state that depends on the amino acid sequence of the protein and various bonds, which include hydrogen bonding, hydrophobic bond, ionic interaction, disulphide bridges among others, are responsible for the globular shape of the proteins. Disruption of these bonds by external agents like pH, temperature, heavy metals, detergent and chemical agents affect the secondary and tertiary structure of the protein. In the recent past, addition of various denaturants has been found to affect the tertiary structure of lectins [35-36]. In protein structural studies, introducing these denaturing agents could give some salient information about the amino acid composition and possibly the nature of secondary motifs present in the protein. In our studies, *P. osun* and *P. soyauxii* lectins subjected to different chemicals treatment completely lost

their hemagglutinating activities. However the reaction was reversible when the dialysed against double distilled water. The decrease in activity observed because of the addition of β-Mercaptoethanol, might be an indication that the lectins contains a disulphide bond crucial to its hemagglutinating activity, which β-Mercaptoethanol reduces [37]. By breaking the disulphide bonds, both the tertiary and quaternary structure of some proteins can be disrupted. While the reduction in the hemagglutinating activity of the lectins due to the chaotropic agents (Guanidine-HCl and Urea) might infer that, the lectins are globular protein whose hydrogen bonds and hydrophobic interactions were disrupted by the chaotropic agents. It might also imply that chaotropic agent causes denaturation of the seeds lectin due to disruption in hydrophobic interaction in the lectin interior followed by solvation via alteration in solvent environment, which support its native conformations [38-39]. Chaotropic agents usually have denaturing effects on hydrophobic amino acids [40]. This suggests that one or more of glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine and tryptophan is present in the two *Pterocarpus spp*.

Glutaraldehyde is a bifunctional compound mainly used in chemical modifications of proteins and polymers. This bifunctional compound links covalently to the amine groups of lysine or hydrolysine in the protein molecules induced by the addition of salts, organic solvents, or non-ionic polymers. These solid aggregates are held together by non-covalent bonding and readily collapse and dissolve when dispersed in an aqueous medium. Thus, the chemical modification of proteins with crosslinking agents can be used for the reinforcement of the compact tertiary structures resulting in protein stabilization against pH inactivation [41]. Trichloroacetic acid acts through two mechanisms. The first one is dehydration of the hydration shells around water. These structured regions of water form around hydrophobic patches on the surface of the folded protein. The waters in the shell are stolen into the hydration shell around the precipitant, effectively increasing the hydrophobic effect. This mechanism is in common with other precipitation agents, such as ammonium sulphate. Second, the anionic TCA may trigger partial protein unfolding through disruption of the electrostatic interactions, which determines the native tertiary structure of the protein. As a result, the well-hidden hydrophobic interior of the protein becomes exposed to the solvent.

5. CONCLUSION

In conclusion, this study has shown that N-bromosuccinimide (NBS) has significant effects on the hemagglutinating activity of purified lectins from two *Pterocarpus* species namely *P. osun* and *P*. *soyauxii*. Other modifiers employed, phenylglyoxal, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and Phenylmethylsulfonyl fluoride (PMSF) do not have significant effects on their hemagglutinating activity. In addition, the denaturants employed in this study, glutaraldehyde, urea, guanidine HCl, trichloroacetic acid and 2-mercarptoethanol, affected the hemagglutinating activity of the lectin. It is also noteworthy that the hemagglutinating activity was restored to the lectin samples upon dialysis except with 8M urea.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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