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Original Article

Purification, Characterization and Antioxidant Potential of a Novel Lectin from *Pterocarpus soyauxii* Taub Seeds

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Abstract

The study investigated the presence of lectin in the seeds of *Pterocarpus soyauxii*, purified the lectin and determined its physicochemical properties as well as its antioxidant potential. Purification of *P. soyauxii* seeds lectin (PSSL) was accomplished by ammonium sulphate precipitation and gel filtration on Sephadex G-100, hemagglutination assay was by serial dilution and DPPH radical scavenging, metal chelating and lipid peroxidation inhibition assays were employed to evaluate its antioxidant potential. PSSL agglutinated erythrocytes from human blood group ABO, rabbit and rat. Trypsinization enhanced agglutination which was inhibited by galactose, lactose and arabinose. The lectin activity was independent of metal ion, thermal stable up to 60 °C and at pH range of 6 to 8. Gel filtration estimated molecular weight was 65 kDa and subunit molecular weight was 35 kDa. PSSL showed significant antioxidant activity. The lectin was able to scavenge DPPH radical, chelate iron metal and inhibit lipid peroxidation with IC_{50} of 0.14 ± 1.33 mg/ml, 3.40 ± 0.37 mg/ml and 0.50 ± 0.08 mg/ml respectively. The results indicate that PSSL might be applied as natural antioxidant for the development of drug and food products for the benefit of human health.

Keywords: antioxidant; hemagglutination; lectin; *Pterocarpus soyauxii*; seed

Introduction

Globally research towards the development of drug from natural sources for various ailments that is ravaging the world is increasing. Many plants have been examined for their pharmacological, nutraceutical and therapeutic potentials and different bioactive agents have also been isolated from them. Among biologically active agents is lectin, which is a protein or glycoprotein. It has the ability to bind reversibly and specifically to carbohydrate on the surface of the cells thereby precipitating or agglutinating them (Lam and Ng, 2011). Lectins are well distributed in nature, found in animals, plants and microorganism (Sharon and Lis, 2004; Hamid *et al*., 2013). Lectins have been found to be valuable tools in scientific research especially in the area of biocontroling, bone marrow transplantation, cancer diagnosis, HIV/AIDS and others (Inbar and Chet, 1997; Bouwman *et al*., 2006; Lam and Ng, 2011; Huskens and Schols, 2012; Yau *et al*., 2015). In addition, lectins have been shown to possess various biological activities like anticoagulant, antitumor, antibacterial, antiproliferative, anti-inflammatory, analgesic, insecticidal and antiviral (Singh *et al*., 1999; Lam and Ng, 2011; Kumar *et al*., 2012; Hamid *et al*., 2013; Santos *et al*., 2014; Dan *et al*., 2016; Lagarda-Diaz *et al*., 2017; Hendrickson and Zherdev, 2018). They are considered strong candidates for therapeutic usage because they are macromolecules with noticeable resistance to adverse conditions like pH, temperature variations and isotonicity, with no major changes to their biological functions (Coffey *et al*., 1993). Also, the characteristic property of lectins to recognise other molecules in a distinct way makes it relevant in various research and applications (Santos *et al*., 2014).

Recently, lectin research received a boost when it was reported to have the ability to scavenge free radicals (Pan and Ng, 2015; e Lacerda *et al*., 2017; Olodude *et al*, 2017) which provided them the potential to protect the human body against damage caused by free radicals and reactive oxygen species released during oxidative stress. The development of several chronic diseases such as cancer, aging, arthritis, cardiovascular diseases, diabetes and neurodegenerative disorder is linked to oxidative stress (Anitha and Sathisha, 2017). Treatment of these disorders needs supply of external antioxidant to improve the internal

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defence system which may be overwhelmed by the excessive exposure to oxidative stress (Anitha and Sathisha, 2017). Therefore, there is need for development of more natural antioxidant from potential food sources which can reduce or thwart oxidative stress and its harmful effects. This possibly may replace the synthetic antioxidants which are high-priced and have adverse side effects.

Among the plant family which had their lectins well investigated for various biological properties is Leguminosae. Legume seeds have been the major focus of lectin-related research. Leguminous lectins are the most studied group of lectins and hundreds of these proteins have been isolated and extensively investigated in relation to their chemical, physicochemical, structural and biological properties (Sun *et al*., 2011). The studies of leguminous lectins are focused mainly on the subfamily *Papilionoideae* but yet some tribes of this family are rarely investigated. Few lectins from *Dalbergieae* tribe have been isolated, characterized and includes *Lonchocarpus capassa* (Joubert *et al*., 1986), *Vatairea macrocarpa* (Calvate *et al*., 1998), *Pterocarpus angolensis* (Echemendia-Blanco *et al*., 2009), *Platypodium elegans* (Benevides *et al*., 2012), *Platymiscium floribundum* (Pereira-Junior *et al*., 2012), *Vatairea guianensis* (Silva *et al*., 2012a), *Centrolobium tomentosum* (Almeida *et al*., 2016), and *Centrolobium microchate* (de Vasconcelos *et al*., 2015). *Pterocarpus soyauxii* is a species of *Pterocarpus* genus belonging to the *Dalbergieae* tribe and family Leguminosae. It is native to central and tropical West Africa countries like Nigeria, Congo-Kinshasa and Angola. The common English name is African padauk/coralwood while it is called Osun pupa by the Yoruba tribe of Western Nigeria. The leaves are edible and contain large amounts of vitamin C; the fresh leaves are used as vegetables and as livestock feed for goats, they are also recommended for consistent use for diabetics (Uzodimma, 2013). They are used in herbal medicine to treat skin parasites and fungal infections (Gill, 1992). The leaves have also been reported to be capable of normalising the hematological abnormalities associated with pathophysiology of diabetes mellitus (Saliu *et al*., 2012). The ethanolic leaf extract of *Pterocarpus soyauxii* has been reported to have antimicrobial activity. At high concentration, it significantly inhibited the growth of *Escherichia coli, Salmonella typhi, Shigella flexneri, Staphylococcus aureus, Klebsiella pneumoniae* and *Candida albicans* (Osuagwu and Akomas, 2013). Tchamadeu *et al*. (2011) reported that the aqueous stem bark extract of *Pterocarpus soyauxii* had very low toxicity in oral acute high dose administration and no toxicity in oral sub-chronic low dose administration in mice and thus concluded that the plant could be considered safe for oral medication. With enormous information available on the usage of this plant for the possible treatment of various disorders, no information is available at the time of complying this report on the health benefit of the seeds of *Pterocarpus soyauxii*.

The present study was therefore designed to isolate protein with hemagglutinating activity from *Pterocarpus soyauxii* seeds, purify the lectin, determine its physicochemical properties and evaluate the antioxidant potential of the purified lectin.

Materials and Methods

Collection and identification of seeds

The fruits of *Pterocarpus soyauxii* were collected from the Botanical Garden, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The plant and fruit were identified at IFE Herbarium in the same department.

Preparation of the crude extracts of P. soyauxii seeds

Pterocarpus soyauxii seeds were removed from the fruits and air-dried. The seeds were dehulled and milled. The milled samples were defatted with petroleum ether and gentle stirring. The defatting process was further repeated for complete removal of the oily constituents. The defatted samples were homogenized in 25 mM phosphate buffered saline (PBS, pH 7.2) containing 100 mM NaCl. The mixtures were stirred overnight using a magnetic stirrer. The homogenates were centrifuged at 10000 rpm for 20 minutes using cold centrifuge and the collected supernatant termed crude lectin extract was frozen and stored.

Glutaraldehyde fixation of erythrocytes

Human blood groups A, B, O was obtained from healthy donors after informed consent. Rabbit and rat erythrocytes were obtained from healthy rabbit and rat purchased from Teaching and Research Farm, Obafemi Awolowo University, Ile Ife.

The animal and human red blood cells (RBC) were fixed with glutaraldehyde according to the method of Pattanapanyasat *et al*. (2010). Blood samples were collected into heparinized bottles and centrifuged at 3000 rpm for 15 min. The erythrocytes were collected and washed five times with PBS, pH 7.2. A 50% glutaraldehyde was diluted to 1% (v/v) with PBS and chilled at 4 °C. The chilled glutaraldehyde-PBS solution was used to dilute the red blood cells to 2% (v/v). The suspension of cells and glutaraldehyde was incubated for 1 h at 4 °C with occasional mixing. The fixed cells were collected by centrifugation at 3000 rpm for 5 min and washed five times with PBS. The cells were then suspended in PBS, containing 0.02% (w/v) sodium azide, to a final concentration of 2% (v/v) and stored at 4 °C until further use.

Trypsinization of red blood cells

Trypsinization was carried out using the method of Occena *et al*. (2007). Blood samples of blood groups A, B and O were collected in heparinized bottles and centrifuged at 3000 rpm for 15 min. The erythrocytes were collected and washed three times with PBS and 2% suspension was prepared in PBS for each of the blood groups. For trypsinization process, 1% trypsin was mixed with 2% red blood cells suspension in PBS in a ratio of 1:1 (v/v) and was incubated at 37 °C for 1 h. After incubation, the trypsinized cells were washed with PBS three times, diluted and resuspended in PBS (2% v/v), and stored until further use.

Hemagglutination assay

The crude lectin extract of *P. soyauxii* seeds was tested for the presence of lectin by hemagglutination assay performed by the method of Odekanyin and Kuku (2014).

Hemagglutination assay was carried out in 96-well U-shaped microtiter plates. PBS (100 µl) was delivered sequentially into wells arranged in rows of 12 wells. The crude extract (100 µl) was added into the first well to obtain a 1:2 dilutions. A serial dilution was then performed by transferring 100 µl of the diluted sample in a particular well into the next well containing 100 µl PBS. This process was carried out for two complete rows of 12 wells each until the last 24th well. Aliquots (50 µl) of the 2% glutaraldehyde-treated erythrocytes suspension of the animal and human blood samples were added separately to each well and the wells were left for 2 h for agglutination to take place. The hemagglutination titre of the lectin is defined as reciprocal of the highest dilution of the extract exhibiting visible hemagglutination - this will be equivalent of one hemagglutination unit (HU). Blood group specificities of the lectins were determined as described above with different blood groups.

Sugar specificity test

The sugar specificities of the lectins were investigated by comparing sugars on the basis of minimum concentration required to inhibit the agglutination of erythrocyte by lectins as described by Kuku and Eretan, (2004). This was done in two steps. In the first step, lectin was successively diluted until the end-point dilution causing hemagglutination was obtained. 0.2 M of sugar solution was added to each well at 50 µl per well, while the control well contained PBS instead of sugar solution. Fifty microlitres of erythrocyte suspension was added to each well, and the hemagglutination titre of the lectin was determined. Inhibitory sugars caused a reduction in the titre of the lectin activity shown by PBS-control experiment. The sugars tested are: maltose, D-(+)-mannose, lactose, L-(+)-arabinose, sorbose, D-(+)-glucose, galactose, mannitol, N-acetyl-D-glucosamine, mannosamine, 2-deoxy-D-glucose, dulcitol, xylose, methyl α-D--glucopyranoside and D-(+)-glucosamine HCl. In the second step, the minimum concentration of each inhibitory sugar required to inhibit lectin-specific hemagglutination of blood cells by 50% was determined (Kuku and Eretan, 2004). Two-fold successive dilutions of sugar samples were prepared in PBS (0.2 M initial concentration). Lectins were successively diluted in the microtiter plates until the 12th well on each row. Fifty microlitres of the successively-diluted sugar solutions were added to each well containing the successively-diluted lectins while the control well contained PBS instead of sugar solution. Fifty microlitres of erythrocyte suspension was added to each well, and the hemagglutination titre of the lectin was determined.

Purification of P. soyauxii lectin

Ammonium sulphate precipitation

The crude lectin extract of the *P. soyauxii* seeds was subjected to 70% ammonium sulphate precipitation. The ammonium sulphate equivalent to 70% precipitation was slowly added to the crude extracts. The mixture was gently stirred to dissolve completely the salt and centrifuged after 24 h at 3500 rpm for 15 min to obtain the precipitate. The precipitate was dialysed thoroughly against several changes of PBS to remove the salt and undissolved materials from the dialysates.

Gel-filtration on Sephadex G-100

The dialysate of ammonium sulphate precipitate of *P. soyauxii* crude lectin extract was applied on Sephadex G-100 column (2.5 x 40 cm) previously equilibrated with PBS, pH 7.2. The protein was eluted with the same buffer at a flow rate of 15 ml/hr and 5 ml fractions were collected. The fractions were monitored for protein by measuring the absorbance at 280 nm and assayed for hemagglutinating activity.

Determination of protein concentration

Protein concentration of the crude extract, dialysate and other fractions were determined by the method of Lowry *et al.* (1951) using Bovine Serum Albumin (BSA) as standard protein. The absorbance at 280 nm was also used to monitor protein elution in the chromatographic fractions.

Physicochemical characterization of purified lectin

Molecular weight determination

Native molecular weight of the purified lectin was determined by carried out gel filtration on Sephadex G-100 column (1.5 x 100 cm) which had been calibrated with molecular weight markers. Standard protein (5 ml) was applied separately and ran using 10 mM phosphate buffer, pH 7.2 as eluants at a flow rate of 12 ml/h. Fraction of 5 ml were collected and the elution of the protein was monitored at 280 nm. The void volume of the column was determined using Blue dextran which elution's was monitored at 620 nm. The molecular weight markers used were Bovine serum albumin (Mwt 66,000), Ovalbumin (Mwt 45,000), Pepsin (Mwt 35,000), Chymotrypsin (Mwt 25,000); and Lysozyme (Mwt 14,000). 5 mg/ml of each of the standard protein marker were prepared. The purified *P. soyauxii* seed lectin was also subjected to Sodium Dodecyl Sulphate-Polyacylamide gel electrophoresis (SDS-PAGE) for subunit molecular weight determination. The procedure described by Weber and Osborn (1975) was used along with lysozyme (Mwt 14,000), trypsin inhibitor (Mwt 20,000), carbonic anhydrase (Mwt 29,000), ovalbumin (Mwt 45,000), bovine serum albumin (Mwt 66,000), galactosidase (Mwt 118,000) and myosin (Mwt 212,000) as standard protein markers.

Effect of temperature on hemagglutinating activity

The effect of temperature on hemagglutinating activity of *P. soyauxii* seeds lectin and thermal stability of the lectin were done as described by Sampaio *et al.* (1998). The purified lectin was incubated in a water bath for 1 h at different temperatures (30 °C - 100 °C). At each temperature, aliquots of the lectin were taken at 15 min interval for 60 min, rapidly cooled on ice and assayed for hemagglutinating activity. The hemagglutinating activity of the untreated samples at room temperature represented 100% hemagglutinating activity.

Effect of pH on hemagglutinating activity

The effect of pH on the hemagglutinating activity of the lectin was performed by incubating aliquots of the lectin solutions for 1 h with buffers of different pH values ranging from pH 3-13. The buffers used were 0.2 M citrate buffer (pH 3-5), 0.2 M Tris-HCl buffer (pH 6-8), and 0.2 M glycine-NaOH buffer (pH 9-13). The hemagglutination

assays were carried out. Lectin incubated in PBS pH 7.2 was used as the control.

Effect of EDTA and divalent cations on hemagglutinating activity

The effect of ethylenediaminetetraacetic acid and divalent cations on *P. soyauxii* seeds lectin were carried out as described by Wang *et al*. (1996). The purified lectins were dialysed against 10 mM EDTA for 24 h and the hemagglutinating activity of the demetallized lectins was determined. The treated lectins were incubated with 50 µl of each of the following cations: $CaCl_2$, $BaCl_2$, $MnCl_2$, $SnCl_2$, and $HgCl_2$ at 10 mM for 2 hrs and the hemagglutinating activity of each of the samples were determined.

Antioxidant assays

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The hydrogen or radical scavenging properties of *P. soyauxii* seeds lectin was determined by the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) method as described by Brand-Williams *et al*. (1995). DPPH (0.3 mM, 1 ml) was added in methanol to 1 ml of varying concentrations of the lectin/standard (ascorbic acid). The mixture was vortexed and incubated in the dark for 30 min and the absorbance was measured at 517 nm against a DPPH negative control containing only 1 ml of the methanol instead of the lectin.

The percentage inhibition of the DPPH scavenging activity was calculated using the equation below:

DPPH % inhibition = $[1- (Abs_{sample}/ Abs_{control})] \times 100$

Where:

Abs_{sample} = Absorbance of the lectins

$Abs_{control}$ = Absorbance of the control at 517 nm

Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph by plotting inhibition percentage against sample concentration.

Lipid peroxidation inhibition assay

The assay of lipid peroxidation activity was carried out according to the thiobarbituric acid reaction method of Kimura *et al*. (1981) and Hattori *et al*. (1993) with BHT as standard. Ten percent (10%) liver homogenate (0.25 ml) was added to 150 mM Tris-HCl buffer (pH 7.2) (0.1 ml), 1% (w/v) ascorbic acid (0.05 ml), 0.07 M $FeSO_4$ (0.05 ml) and varying concentrations of the purified lectin of *P. soyauxii* seeds. The reaction mixtures were incubated at 37 °C for 1 h. After incubation, 0.5 ml of 0.1 N HCl, 0.2 ml of 9.8% SDS, 0.9 ml of distilled water and 2.0 ml of 0.67% TBA were added sequentially. The reaction mixtures were heated in boiling water at 100 °C for 30 min., cooled and 2.0 ml of butan-1-ol was added and later centrifuged at 3000 rpm for 10 min. The supernatant was collected and measured at 532 nm against reagent blank.

The percentage inhibition was estimated using the expression:

% Inhibition = $(Abs_{control}-Abs_{test} /Abs_{test}) \times 100$

where $Abs_{control}$ = MDA produced by fenton reaction in the absence of extract (control);

Abs_{test} = MDA produced by fenton reaction in the presence of extract.

Metal chelating activity assay

The metal chelating activity assay was carried out according to the method of Singh and Rajini (2004) with some modifications, using EDTA as standard. Solutions of 2 mM $FeCl_2{\cdot}4H_2O$ and 5 mM ferrozine were diluted 20 times. Briefly, an aliquot (1 ml) of varying concentrations of purified lectin of *P. soyauxii* seeds was mixed with 1 ml $FeCl_2{\cdot}4H_2O$. After 5 min incubation, the reaction was initiated by the addition of ferrozine (1 ml). The mixture was shaken vigorously and after a further 10 min incubation period, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated by using the formula:

Chelating effect % = $(Abs_{control} - Abs_{sample}/ Abs_{control}) \times 100$; where $A_{control}$ = absorbance of control sample (the control contains $FeCl_2$ and ferrozine, complex formation molecules);

A_{sample} = absorbance of a tested samples.

Results and Discussion

Pterocarpus soyauxii seeds lectin (PSSL) was purified to homogeneity by combination of ammonium sulphate precipitation and gel filtration on Sephadex G-100. The crude protein extract of petroleum ether-defatted *P. soyauxii* seed powder was subjected to 70% ammonium sulphate precipitation and the precipitate obtained was thoroughly dialyzed. Dialysate was then layered on the Sephadex G-100 column. Three distinct protein peaks (GF-I, GF-II, GF-III) were obtained after the column chromatography. Hemagglutination assay carried out on the pooled fractions of each peak revealed that only the first peak agglutinated erythrocyte (Fig. 1). The same procedure was employed by Galbraith and Goldstein (1970) and e Lacerda *et al*. (2017). Galbraith and Goldstein (1970) used Sephadex G-200 while e Lacerda *et al*. (2017) used Sephadex G-100. E Lacerda *et al*. (2017) obtained three distinct protein peaks and only the first peak exhibited hemagglutinating activity. This is similar to the result of the present study. The methodology established for PSSL purification is efficient because a homogenous molecule was purified by 31 folds with about 13% recovery. The soluble protein concentration and specific activity of the crude lectin extract were 11.6 mg/ml and 176.6 HU/mg proteins respectively and for the purified lectin the value was 1.51 mg/ml and 5427.8 HU/mg proteins respectively (Table 1).

Phosphate Buffer Saline (pH 7.2) extract of petroleum ether-defatted *P. soyauxii* seeds powder showed relatively high hemagglutinating activity against trypsin-treated and untreated erythrocyte of all human blood group and rabbit (Table 2). Only untreated rat erythrocyte was agglutinated. No blood group specificity was detected because all human blood erythrocyte was agglutinated to a similar degree. There was only slight preference for rabbit erythrocyte. Treatment of erythrocyte with trypsin improved the hemagglutination. Non-blood group specificity of *P. soyauxii* seeds lectin may be due to the presence of multiple binding sites where it can recognize all the determinants of each blood type (Saha *et al*., 2014). Agglutination of native

Table 1. Summary of purification of lectin from the seeds of *Pterocarpus soyauxii*

Fraction	[a]Total protein (mg)	[b]Total activity (HU)	[c]Specific activity (HU/mg)	Purification fold
Crude Extract	11.6	2048	176.6	1
Ammonium Sulphate Dialysate	4.9	2048	418.0	2.4
Gel Filtration (Sephadex G-100)	1.51	8196	5427.8	30.7

[a] Protein concentration (mg),
[b] Hemagglutinating activity expressed in hemagglutination units which is reciprocal of the highest dilution of the extract exhibiting visible hemagglutination,
[c] Specific activity is calculated as the ratio of Total activity and total protein.

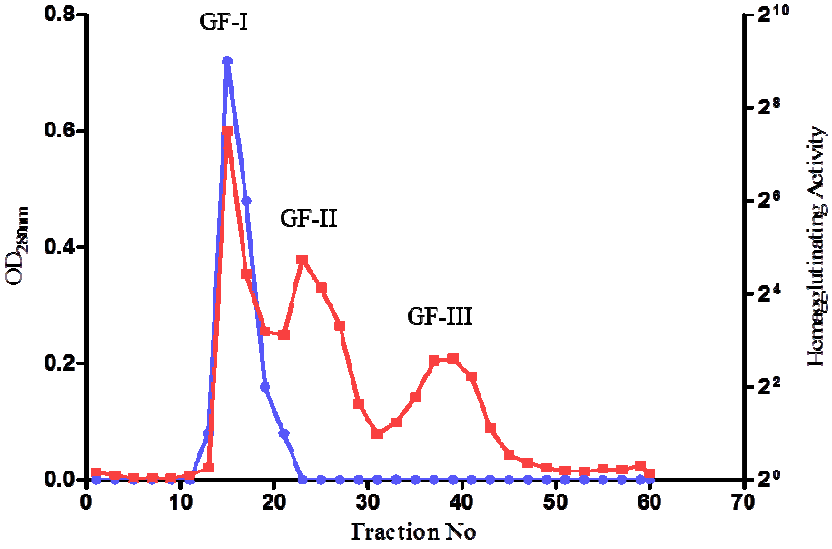

Fig. 1. Purification of PSSL on Sephadex G-100 Column. Approximately 7 ml of dialysate of 70% ammonium sulphate precipitate was layered on the column (2.5 x 40 cm) packed with Sephadex G-100 that was equilibrated with 25 mM Phosphate buffer pH 7.2 containing 100 mM NaCl. The PSSL was eluted with the same buffer at a flow rate of 12 ml/hr and fractions of 5 ml were collected, monitor for protein by measuring the absorbance at 280 nm and hemagglutinating activity of all the fractions was also determined. (Pooled fractions, ●●●●●Hemagglutinating Activity, ▪▪▪▪ -$OD_{280,}$ GF - Protein Peaks)

and enzyme-treated rabbit erythrocyte was observed with *Dioclea lasiocarpa* seed lectin (do Nascimento *et al*., 2012). The same observation was reported by Silva *et al*. (2012a), Santiago *et al*. (2014) and e Lacerda *et al*. (2015) for lectins isolated from *Vatairea guianensis, Canavalia oxyphylla* and *Mucuna pruriens* respectively. Lectin present in the seed of *Mucuna sloanei* agglutinated both bromelain-treated and untreated human and rabbit erythrocyte (Teixeira-Sá *et al*., 2009). Bashir *et al*. (2010) also reported that extent of hemagglutination was the same when erythrocytes of all human blood group were incubated with purified soy-bean (*Glycine max*) seed lectin. *Apuleia leiocarpa* seeds lectin also showed hemagglutinating activity toward all blood tested with preference for rabbit erythrocyte (Carvalho *et al*., 2015). Opposing our findings on hemagglutinating activity, Pereira-Junior *et al*. (2012) and de Vasconcelos *et al*. (2015) reported that no hemagglutinating activity was observed for human erythrocytes when incubated with *Platymiscium floribundum* and *Centrolobium microchaete* seeds lectin respectively.

Lectin specificity with certain molecules or cells has been described by Gupta *et al*. (2012) as dependent on both lectin structure and free sugars or sugar residues of polysaccharides, glycoprotein or glycolipids ligands. Difference in lectin structures and functions may be linked to many different binding site molecular compositions that bind to all kinds of glycosylated ligands. This makes each type of lectin to have high specificity with particular sugars. The sugar specificity of PSSL was investigated by hemagglutination-inhibition assay using different carbohydrates. PSSL hemagglutinating activity was completely inhibited by galactose and galactose-containing sugars. Also, a pentose sugar, arabinose, completely inhibited the lectin activity. Lactose showed better inhibition of PSSL hemagglutinating activity for all human erythrocyte. The minimum inhibitory concentration of sugars that completely inhibited the lectin activity is shown in Table 3. This inhibitory effect on lectin activity gave support to grouping of PSSL as a member of galactose-binding lectin. This is in accordance with sugar specificity reports for majority of leguminous lectins (Konozy *et al*., 2002 and 2003; Bashir *et al*., 2010; Sun *et al*., 2011; Silva *et al*., 2012a; Kuku et al., 2012; Mishra *et al*., 2016; Khurtsidze *et al*., 2017). Some other leguminous lectins that displayed different specificity especially for glucose and mannose or their derivatives have also been reported (do Nascimento *et al*., 2012; Silva *et al*., 2013; Osterne *et al*., 2014; Santiago *et al*., 2014; Ng *et al*., 2015; Pompeu *et al*., 2015). It was observed that none of the sugars that inhibited PSSL

hemagglutinating activity using human erythrocyte was able to inhibit the activity against rabbit erythrocyte completely. The presence of carbohydrate moiety on the rabbit erythrocyte surface that have higher affinity for the carbohydrate binding site of the lectin may possibly be preventing the sugars from binding to the lectin thereby stopping their inhibitory effects. There is little or no arabinose-specific lectin in the leguminous lectin family. Arabinose binding lectins are commonly isolated from microorganisms, mushrooms and also marine organisms (Engel *et al*., 1992; Wang and Ng, 2005). Recently, arabinose was reported to cause partial inhibition of hemagglutinating activity of *Apuleia leiocarpa* seeds lectin (Carvalho *et al*., 2015). Purified lectins from the *Dalbergiae* tribe have been reported to be composed of diverse isoforms (Calvete *et al*., 1998; Silva *et al*., 2012a; Pereira-Junior *et al*., 2012; de Vasconcelos *et al*., 2015). Two isolectins were identified in *C. microchaete* seeds lectin and diverge in lectin chain lengths (de Vasconcelos *et al*., 2015). There is high possibility of isolectins presence in *P. soyauxii* seeds lectin that diverge in their sugar specificity and likely other properties.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of purified PSSL in the presence and absence of reducing agent displayed an electrophoretic profile having a single band corresponding

Table 2. Hemagglutinating activity of PBS extract of *P. Soyauxii* seeds against human and animal erythrocytes

Erythrocyte		Hemagglutinating activity of *P. soyauxii*	
		Non-trypsinized	Trypsinized
Human	A	512	8196
	B	256	16392
	O	1024	8196
Rabbit		16392	65568
Rat		256	ND

ND: Not Determined

Table 3. Inhibition of hemagglutinating activity of PSSL by various carbohydrates in the presence of different erythrocytes

Blood	*Sugar Lactose (mM)	Galactose (mM)	Arabinose (mM)
A	0.138 ± 0.057	2.605 ± 0.520	4.167 ± 1.042
B	0.913 ± 0.345	6.250 ± 3.125	8.333 ± 2.083
O	1.824 ± 0.689	2.085 ± 0.520	4.167 ± 1.042

Minimum Inhibition Concentration required giving a 50% inhibition of the agglutination of different erythrocytes. Data are expressed as mean ± SEM of triplicate determination.
*Other sugars used did not completely inhibit the PSSL hemagglutinating activity.

to apparent molecular mass of 35 kDa. The native molecular weight of PSSL as determined by gel filtration chromatography was 64.6 kDa. The result revealed that PSSL is an homodimeric protein. The result was similar to what was obtained for *P. coccineus* seeds lectin which has 66 kDa as its native molecular weight and 33 kDa as the subunit mass (Pan and Ng, 2015). So also, it is comparable to two species of *Mucuna* lectins. *Mucuna pruriens* and *M. sloanei* gave a native molecular weight of 60 kDa and 65.6 kDa respectively.

Pterocarpus soyauxii seeds lectin (PSSL) was observed to possess high thermal stability, maintaining its full hemagglutinating activity even after incubation up to 60 °C for 1 h. However, activity was drastically reduced at 70 °C and completely lost at 80 °C and higher temperature (Fig. 2a). This implies that the lectins undergo conformational changes under extreme temperatures resulting in the loss of activity. The loss of activity of the lectins with increased temperature is due to destabilisation of sporadic weak interactions of tertiary structure responsible for native conformation of lectin (Singh and Saxena, 2013). These data suggest that PSSL is comparable to other leguminous lectins that exhibited high thermostability. Lectin from *E. speciosa* was also discovered to be stable up until 70 °C, but totally lost its activity at 80 °C (Konozy *et al*., 2003). Lectin isolated from *P. floribundum* seeds by Pereira-Junior *et al.* (2012) maintained its full lectin activity at 60 °C for 1 h, but the activity decreases as the temperature increases. *Canavalia oxyphylla* seed lectin was likewise reported to retain full activity until 60 °C, lost considerable activity at 70 °C and no activity was observed at 90 °C (Santiago *et al.,* 2014). Lectin purified from *Phaseolus coccineus* (Pan and Ng, 2015), *Chenopodium quinoa* (Pompeu *et al*., 2015), *Apuleia leiocarpa* (Carvalho *et al*., 2015) and *Canavalia virosa* (Shanmugavel *et al*., 2016) are all thermostable up to 60 °C for at least 30 min. High thermostability possessed by these lectins may be advantageous, as stable bioactive substance is more efficient during all phases of their processing and on the other hand this is considered as antinutritional factors that cause many adverse phenomena in animals if ingested (e Lacerda *et al*., 2017).

Several studies have revealed that stability under highly acidic and basic conditions is a common property of lectins (Pompeu *et al*., 2015). Lectins are resistant to harsh condition like drastic change in pH. Examination of the effect of pH on *P. soyauxii* seed lectin showed that the lectin was pH sensitive. Hemagglutinating activity was optimum at pH 6-8, relatively insensitive to acidic conditions where it maintained 50% maximal activity between pH 3-5 but was distinctly affected by basic pH with the hemagglutinating activity decreasing gradually by 50% for every unit rise in pH until pH 13 (Fig. 2b). This result is in agreement with

the reported pH stability for lectin from *Erythrina indica* leaves lectin that exhibited pH stability at pH 6.0 - pH 8.0 (Konozy *et al*., 2002), *A. hypogea* seed which maintained its maximum activity at pH 5.0 to pH 8.0 (Sun *et al*., 2011), *Dioclea lasiocarpa* seeds lectin optimum pH was between 6.0 and 8.0 (do Nascimento *et al*., 2012) and *C. virosa* seeds lectin have highest activity at pH 4.0 - pH 9.0 (Shanmugavel *et al*., 2016).

The hemagglutinating activity of PSSL was not affected after prolong dialysis against EDTA. Also, addition of various divalent ions like Ca^{2+}, Mn^{2+} or Mg^{2+} to the dialyzed lectin did not alter the activity. Though, it has been reported that leguminous lectins require divalent cations like Ca^{2+}, Mn^{2+} or Mg^{2+} for their full hemagglutination (Echemendia-Blanco *et al*., 2009, Sun *et al*., 2011, do Nascimento *et al*., 2012, de Vasconcelos *et al*., 2015, e Lacerda *et al*, 2017) there are some exception. The current data proposes that, in contrast to other leguminous lectins, PSSL does not need metal ions for it to be fully active or that the metal ions are most likely bound tightly to the inner part of the molecule, shielding it from the EDTA effect. This is comparable to the report of Pereira-Junior *et al*. (2012), Silva *et al*. (2012a, b), Santiago *et al*. (2014), Pompeu *et al*. (2015) and Khurtsidze *et al*. (2017).

In determining the antioxidant capability of PSSL, three different *in vitro* methods for evaluating antioxidant activity were employed. This includes DPPH radical scavenging, metal chelating and lipid peroxidation assays. All methods showed that PSSL possessed antioxidant activity in a dose-dependent manner and exhibited an IC_{50} of 0.14 ± 1.33 mg/ml, 3.40 ± 0.37 mg/ml and 0.50 ± 0.08 mg/ml for these methods respectively (Figs. 3 A, B and C). Apart from peptides, obtainable by enzymatic hydrolysis, chemical hydrolysis or bacterial fermentation, that have been established to possess strong antioxidant ability, some proteins from various sources have also been reported to have significant ability to ameliorate the harmful effect of free radicals and reactive oxygen species produced during oxidative stress. Previous investigations, especially on the leguminous seeds, have confirmed that protein from these seeds have antioxidant activity (Carrasco-Castilla *et al*., 2012; Petchiammal and Hopper, 2014; Pan and Ng, 2015; e Lacerda *et al*., 2017). Pan and Ng (2015) reported for the first time the ability of leguminous seed lectin to exert antioxidant activity though the antioxidant activity of lectins from other sources such as *Moringa oleifera* seeds (Santos *et al*., 2005), *Pleurotus florida* (Bera *et al*., 2011), and endophytic fungi (Sadananda *et al*., 2014) have been published. Our findings support other studies that reported antioxidant activity for lectins from the *Leguminaceae* family seeds.

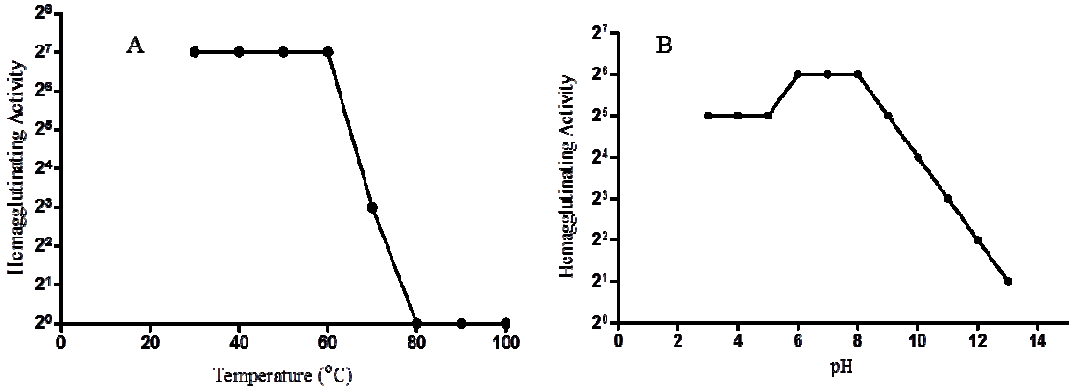

Fig. 2. Effect of temperature and pH on the hemagglutinating activity of PSSL

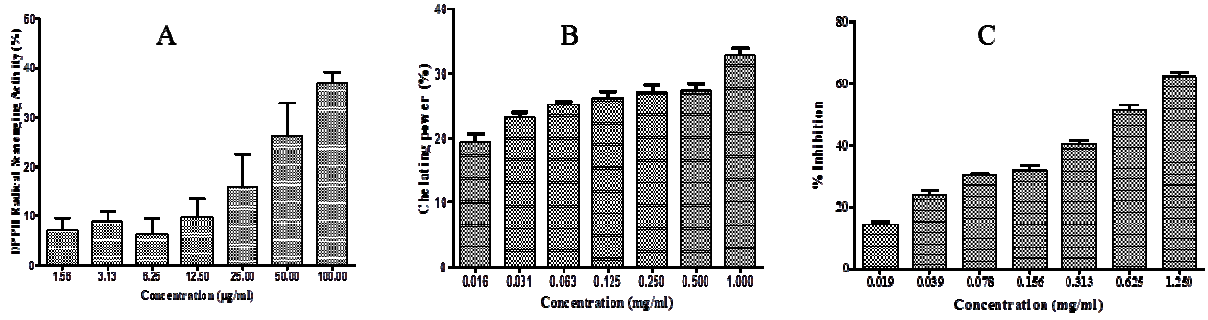

Fig. 3. Antioxidant activity of *P. soyauxii* seeds lectin. [A] DPPH radical scavenging activity [B] Metal chelating activity and [C] Lipid peroxidation inhibition activity. The results are shown as means values ± SD of three separate experiments

Conclusions

The present investigation purified lectin from the seeds of one of the under-utilised plants (*Pterocarpus soyauxii*) in Nigeria. PSSL lectin agglutinate rabbit and all human blood group erythrocytes, and the hemagglutination were inhibited by galactose, lactose and arabinose. PSSL lectin is not a metal dependent lectin, maximum hemagglutinating activity was observed up to 60 °C and around pH values of 6-8. It possessed antioxidant activity which was detected using three different methods. Future studies are required to determine other properties and also biological activities of this lectin. The mechanism of action of its antioxidative prowess will also need future studies.

References

Almeida AC, Osterne VJS, Santiago MQ, Pinto-Junior VR, Silva-Filho JS … Cavada BS (2016). Structural analysis of *Centrolobium tomentosum* seed lectin with inflammatory activity. Archives of Biochemistry and Biophysics 596:73-83.

Anitha N, Sathisha GJ (2017). Evaluation of antibacterial and antioxidant activities of partially purified lectin from tubers of *Xanthosoma violaceum*. European Journal of Biomedical and Pharmaceutical Sciences 4(11):420-425.

Bashir H, Khan T, Masood A, Hamid (2010). Isolation, purification and characterization of a lectin from a local Kashmiri variety of soybean (*Glycine max*). Asian Journal of Biochemistry 5(3):145-153.

Benevides RG, Ganne G, Simoes RC, Schubert V, Niemietz M, … Imberty A (2012). A lectin from *Platypodium elegans* with unusual specificity and affinity for asymmetric complex N-glycans. Journal of Biological Chemistry 287(31):26352-26363.

Bera AK, Rana T, Bhattacharya D, Das S, Pan D, Das SK (2011). Sodium arsenite-induced alteration in hepatocyte function of rat with special emphasis on superoxide dismutase expression pathway and its prevention by mushroom lectin. Basic and Clinical Pharmacology &Toxicology 109(4):240-244.

Bouwman LH, Roep BO, Roos A (2006). Mannose-binding lectin: clinical implications for infection, transplantation and autoimmunity. Human Immunology 67(4-5):247-256.

Brand-Williams W, Cuvelier ME, Besert CLWT (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology 28(1):25-30.

Calvete JJ, Santos CF, Mann K, Grangerio TB, Nimtz M, … Cavada BS (1998). Amino acid sequence, glycan structure and proteolytic processing of the lectin of *Vatairea macrocarpa* seeds. FEBS Letter 425(2):286-292.

Carrasco-Castilla J, Hernandez-Álvarez AJ, Jiménez-Martínez C, Jacinto-Hernández C, Alaiz M, … Dávilla-Ortiz G (2012). Antioxidant and metal chelating activities of *Phaseolus vulgaris* L. var. Jamapa protein isolates, phaseolin and lectin hydrolysates. Food Chemistry 131(4):1157-1164.

Carvalho AS, da Silva MV, Gomes FS, Paiva PM, Malafaia CB, … Correia MT (2015). Purification, characterization and antibacterial potential of a lectin isolated from *Apuleia leiocarpa* seeds. International Journal of Biological Macromolecules 75:402-408.

Coffey DG, Uebersax MA, Hosfield GL, Bennick MR (1993). Thermal extrusion and alkali processing of dry beans (*Phaseolus vulgaris*). Journal of Food Processing and Preservatives 16(6):421-431.

Dan X, Liu W, Ng TB (2016). Development and applications of lectins as biological tools in biomedical research. Medicinal Research Reviews 36(2):221-247.

de Vasconcelos MA, Alves AC, Carneiro RF, Dias AHS, Martins FWV, …. Cavada BS (2015). Purification and primary structure of a novel mannose-specific lectin from *Centrolobium microchaete* Mart seeds. International Journal of Biological Macromolecules 81:600-607.

do Nascimento ASF, Gondim ACS, Cajazeiras JB, Correia JLA, Pires AF, …. Cavada BS (2012). Purification and partial characterization of a novel lectin from *Dioclea lasiocarpa* Mart seeds with vasodilator effects. Journal of Molecular Recognition 25(12):657-664.

Echemendia-Blanco D, Driessche EV, Ncube I, Read JS, Beeckmans S (2009). Stability, subunit interactions and carbohydrate-binding of the seed lectin from *Pterocarpus angolensis*. Protein and Peptide Letters 16(9):1120-1134.

e Lacerda RR, Moreira IC, de-Lacerda ACS, Cabral NL, Lucetti, DL, … Santi-Gadelha T (2015). Lectin isolated from Brazilian seeds of velvet beans (*Mucuna pruriens* (L) DC.) presents analgesic, anti-inflamatory and antihemolytic action. Journal of Medicinal Plant Research 9(8):231-242.

e Lacerda RR, do Nascimento ES, de Lacerda JTJG, da Silva Pinto L, Rizzi C, … De Almeida Gadelha TS (2017). Lectin from seeds of a Brazilian lima bean variety (*Phaseolus lunatus* L. Var. *cascavel*) presents antioxidant, antitumor and gastroprotective activities. International Journal of Biological Macromolecules 95:1072-1081.

Engel M, Bachmann M, Schröder HC, Rinkevich B, Kljajic Z, … Muller WEG (1992). A novel galactose-and arabinose-specific lectin from the sponge *Pellina semitubulosa*: isolation, characterization and immunological properties. Biochimie 74(6):527-537.

Galbraith W, Goldstein IJ (1970). Phytohemagglutinins: a new class of metalloprotein: Isolation, purification and some properties of the lectin from *Phaseolus lunatus*. FEBS Letter 9(4):197-201.

Gill LS (1992). Ethnomedical uses of plants in Nigeria. Uniben Press, University of Benin pp 200-201.

Gupta GS (2012). Animal lectins: forms, function and clinical application, Springer, Vienna pp 1108.

Hamid R, Masood A, Wani IH, Rafiq S (2013). Lectins: Proteins with diverse applications. Journal of Applied Pharmaceutical Science 3(4):S93-S103.

Hattori M, Yang XW, Miyashiro H, Namba T (1993). Inhibitory effects of monomeric and dimeric phenylpropanoids from mace on lipid peroxidation *in vivo* and *in vitro*. Phytotherapy Research 7(6):395-401.

Hendrickson OD, Zherdev AV (2018). Analytical application of lectin. Critical Reviews in Analytical Chemistry 48(4):279-292.

Huskens D, Schols D (2012). Algal lectins as potential HIV microbicide candidates. Marine Drugs 10(7):1476-1497.

Inbar J, Chet I (1997). Lectins and biocontrol. Critical Reviews in Biotechnology 17(1):1-20.

Joubert FJ, Sharon N, Merrifield EH (1986). Purification and properties of a lectin from *Lonchocarpus capassa* (apple leaf) seed. Phytochemistry 25(2):323-327.

Khurtsidze E, Kutchava T, Pomikalova N, Gaidamashvili M (2017). Galactose-binding lectin from mulberry (*Morus alba* L) seeds with growth hormone-like activity. Annals of Agrarian Science 15(1):26-30.

Kimura Y, Kubo M, Tani T, Arichi S, Okuda H (1981). Studies on *Scutellariae radix*. IV. Effects on lipid peroxidation in rat liver. Chemical and Pharmaceutical Bulletin 29(9):2610-2617.

Konozy EH, Mulay R, Faca V, Ward RJ, Greene LJ, … Bhide SV (2002). Purification, some properties of a D-galactose-binding leaf lectin from *Erythrina indica* and further characterization of seed lectin. Biochimie 84(10):1035-1043.

Konozy EH, Bernardes ES, Rosa C, Faca V, Greene LJ, Ward RJ (2003). Isolation, purification, and physicochemical characterization of a D-galactose-binding lectin from seeds of *Erythrina speciosa*. Archives of Biochemistry and Biophysics 410(2):222-229.

Kuku A, Eretan OB (2004). Purification and partial characterization of a lectin from the fresh leaves of *Kalanchoe crenata* (Andr.) Haw. Journal of Biochemistry and Molecular Biology 37(2):229-233.

Kuku A, Odekanyin OO, Okonji RE (2012). Physicochemical properties of a lactose specific lectin from the seeds of *Erythrina senegalensis* DC. Ife Journal of Science 14(1):143-153.

Kumar KK, Chandra KLP, Sumanthi J, Reddy GS, Shekar PC, Reddy B (2012). Biological roles of lectins: A review. Journal of Orofacial Sciences 4(1):20-25.

Lagarda-Diaz I, Guzman-Partida AM, Vazquez-Moreno L (2017). Legume lectins: protein with diverse applications. International Journal of Molecular Sciences 18(6):1242.

Lam SK, Ng TB (2011). Lectins: production and practical applications. Applied Microbiology and Biotechnology 89(1):45-55.

Lowry OH, Rosebrough NJ, Farr AL, Randall R (1951). Protein measurement using Folin phenol reagent. Journal of Biological Chemistry 193(1):265-275.

Mishra RP, Ganaie AA, Allaie AH (2016). Isolation and purification of a Galactose specific lectin from seeds of *Bauhinia variegata* and evaluation of its antimicrobial potential. International Journal of Pharmaceutical Science and Research 7(2):804-815.

Ng TB, Chan YS, Ng CCW, Wong JH (2015). Purification and characterization of a lectin from green split peas (*Pisum sativum*). Applied Biochemistry and Biotechnology 177(6):1374-1385.

Occena IV, Mojica EE, Merca FE (2007). Isolation and partial characterization of a lectin from the seeds of *Artocarpus camansi* Blanco. Asian Journal of Plant Sciences 6(5):757-764.

Odekanyin OO, Kuku A (2014). Characterization of galactose-specific lectin from the skin mucus of African catfish *Clarias gariepinus*. Scientific Research and Essays 9(20):869-879.

Olodude SO, Odekanyin OO, Fatokun AB (2017). Evaluation of antioxidant and carbohydrate hydrolysing enzymes inhibitory activities of *Trilepisium madagascariense* Ficalho seeds hemagglutinin. IOSR Journal of Pharmacy and Biological Sciences 12(6):29-41.

Osterne VJ, Santiago MQ, Pinto-Junior VR, Cajazeiras JB, Correia JL …. Cavada BS (2014). Purification, partial characterization and CNBr-Sepharose immobilization of a vasorelaxant glucose/mannose lectin from *Canavalia virosa* seeds. Applied Biochemistry and Biotechnology 172(7):3342-3353.

Osuagwu GGE, Akomas CB (2013). Antimicrobial activity of the leaves of three species of Nigerian *Pterocarpus* (Jacq.). International Journal of Medicinal and Aromatic Plants 3(2):178-183.

Pan WL, Ng TB (2015). A dimeric *Phaseolus coccineus* lectin with anti-oxidative, anti-proliferative and cytokine-inducing activities. International Journal of Biological Macromolecules 81:960-966.

Pattanapanyasat K, Noulsri E, Lerdwana S, Sukapirom K, Onlamoon N, Tassaneetrithep B (2010). The use of glutaraldehyde-fixed chicken red blood cells as counting beads for performing affordable single-platform CD^{4+} T-lymphocyte count in HIV-1-infected patients. Journal of Acquired Immune Deficiency Syndromes 53(1):47-54.

Pereira-Junior FN, Silva HC, Freitas BT, Rocha BAM, Nascimento KS, … Cavada BS (2012). Purification and characterization of a mannose/N-acetyl-D-glucosamine-specific lectin from the seeds of *Platymiscium floribundum* Vogel. Journal of Molecular Recognition 25(8):443-449.

Petchiammal C, Hopper WAHEETA (2014). Antioxidant activity of proteins from fifteen varieties of legume seeds commonly consumed in India. International Journal of Pharmacy and Pharmaceutical Sciences 6(1-2):476-479.

Pompeu DG, Mattioli MA, Ribeiro RIMA, Goncalves DB, de Magalhaes JT … Granjeiro PA (2015). Purification, partial characterization and antimicrobial activity of lectin from *Chenopodium quinoa* seeds. Food Science and Technology (Campinas) 35(4):696-703.

Sadananda TS, Govindappa M, Ramachandra YL (2014). *In vitro* antioxidant activity of lectin from different endophytic fungi of *Viscum album* L. British Journal of Pharmaceutical Research 4(5):626.

Saha RK, Acharya S, Jamiruddin M, Roy P, Islam MMS, … Shovon, SSH (2014). Antimicrobial effects of a crude plant lectin isolated from the stem of *Tinospora tomentosa*. Journal of Phytopharmacology 3:44-51.

Saliu JA, Elekofehinti OO, Komolafe K, Oboh G (2012). Effects of some green leafy vegetables on the haematological parameters of diabetic rats. Journal of Natural Product and Plant Resources 2(4):482-485.

Sampaio AH, Rogers DJ, Barwell CJ (1998). A galactose-specific lectin from the red marine alga *Ptilota filicina*. Phytochemistry 48(5):765-769.

Santiago MQ, Leitão CC, Pereira-Junior FN, Pinto-Junior VR, Osterne JS, … Cavada BS (2014). Purification, characterization and partial sequence of a pro-inflammatory lectin from seeds of *Canavalia oxyphylla* Standl. & LO Williams. Journal of Molecular Recognition 27(3):117-123.

Santos AFS, Argolo ACC, Coelho LCBB, Paiva PM (2005). Detection of water-soluble lectin and antioxidant component from *Moringa oleifera* seeds. Water Research 39(6):975-980.

Santos AFS, da Silva MDC, Napoleão TH, Paiva PMG, Correia MTS, Coelho LCBB (2014). Lectins: function, structure, biological properties and potential application. Current Topics in Peptide and Protein Research 15:41-62.

Shanmugavel S, Velayutham V, Kamalanathan T, Periasamy M, Munusamy A, Sundaram J (2016). Isolation and analysis of mannose/trehalose/maltose specific lectin from jack bean with

antibruchid activity. International Journal of Biological Macromolecules 91:1-14.

Sharon N, Lis H (2004). History of Lectins: From hemagglutinins to biological recognition molecules. Glycobiology 14(1):53R-62R.

Silva HC, Nagano CS, Souza LA, Nascimento KS, Isídro R, … Damasceno LEA (2012a). Purification and primary structure determination of a galactose-specific lectin from *Vatairea guianensis* Aublet seeds that exhibits vasorelaxant effect. Process Biochemistry 47(12):2347-2355.

Silva MC, Santana LA, Mentele R, Ferreira RS,de Miranda A, … Oliva MLV (2012b). Purification, primary structure and potential functions of a novel lectin from *Bauhinia forficata* seeds. Process Biochemistry 47(7):1049-1059.

Silva HC, Bari AU, Rocha BAM, Nascimento KS, Ponte EL, Pires AF, … Nagano CS (2013). Purification and primary structure of a mannose/glucose-binding lectin from *Parkia biglobosa* Jacq. Seeds with antinociceptive and anti-inflammatory properties. Journal of Molecular Recognition 26(10):470-478.

Singh RS, Tiwary AK, Kennedy JF (1999). Lectins: Sources, activities and applications. Critical Reviews in Biotechnology 19(2):145-178.

Singh N, Rajini PS (2004). Free radical scavenging activity of an aqueous extract of potato peel. Food Chemistry 85(4):611-616.

Singh AP, Saxena KD (2013). Biological Activity of Purified *Momardica Charantia* Lectin. Chemical Science Transactions 2:258-262.

Sun J, Yang QL, Bi J, Zhang CS, Yu LN, Zhu F (2011). Purification and identification of a natural lectin from the seed of peanut *Arachis hypogea*. The Open Materials Science Journal 5:78-82.

Tchamadeu MC, Dzeufiet PDD, Nana P, Nouga CK, Tsofack FN, … Tack I (2011). Acute and sub-chronic oral toxicity studies of an aqueous stem bark extract of *Pterocarpus soyauxii* Taub (Papilionaceae) in rodents. Journal of Ethnopharmacology 133(2):329-335.

Teixeira-Sá DM, Reicher F, Braga RC, Beltramini LM, deAzavedo MR (2009). Isolation of a lectin and a galactoxyloglucan from *Mucuna sloanei* seeds. Phytochemistry 70(17-18):1965-1972.

Uzodimma D (2013). Medico-ethnobotanical inventory of Ogii, Okigwe Imo State, South Eastern Nigeria-I. Global Advanced Research Journal of Medicinal Plants (GARJMP) 2:030-044.

Wang HX, Liu WK, Ng TB, Ooi VEC, Chang ST (1996). The immunomodulatory and antitumour activities of lectins from the mushroom *Tricholoma mongolicum*. Immunopharmacology 31(2-3):205-211.

Wang H, Ng TB (2005). First report of an arabinose specific fungal lectin. Biochemical Biophysical Research Communication 337(2):621-625.

Weber K, Osborn M (1975). Protein and sodium dodecyl sulphate: molecular weight determination on polyacrylamide gels and related procedures. Chapter 3 In "The Protein". Edited by Neurath H and Hill RL. Academic press, New York, Vol 1, pp 179-223.

Yau T, Dan X, Ng CC, Ng TB (2015). Lectins with potential for anticancer therapy. Molecules 20(3):3791-3810.