IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 6 Ver. IV (Nov. – Dec. 2017), PP 29-41
www.iosrjournals.org

Evaluation Of Antioxidant And Carbohydrate Hydrolyzing Enzyme Inhibitory Activities Of *Trilepisium Madagascariense*ficalho Seeds Hemagglutinin

Samuel O.OLODUDE,Oludele O.ODEKANYIN*,Adebayo B. FATOKUN
Biochemistry and Molecular Biology Department, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
**Corresponding Author: O.ODEKANYIN*

Abstract*: A lectin was partially purified and characterized from the seed of T. madagascariense by ammonium sulphate precipitation and gel filtration on Sephadex G-100 and its antioxidant capacities and ability to inhibit two carbohydrate hydrolyzing enzymes implicated in Diabetes mellitus were investigated. T. madagascariense seed lectin (TMSLec) agglutinated non-specifically red blood cell from the human ABO system with preference for enzyme-treated red blood cell and also rat and rabbit erythrocytes were agglutinated. It exhibited sugar binding specificity towards galactose. Its molecular mass as determined by gel filtration was estimated to be 45kDa. The lectin had optima pH between 3.0 and 7.0 and was active up to 40^{O}C and EDTA had no effect on the hemagglutinating activity. Tryptophan was observed to play an important role in the lectin activity. A drastic reduction in lectin activity was recorded when treated with glutaraldehyde and urea. It significantly scavenged DPPH free radicals in a concentration dependent manner and contain 215.7mg of ascorbic acid equivalent per gram of the TMSLec when antioxidant was assessed by FRAP assay.The lectin significantly inhibited the activity of alpha amylase and alpha glucosidase in-vitro with IC_{50} of 0.13 ± 0.01 mg/ml and 0.836 ± 0.01 mg/ml respectively. The study revealed the presence of lectin in the seeds of T. madagascariense and that the lectin possess not only remarkable antioxidants activities but also exhibited inhibitory potential against alpha amylase and alpha glucosidase. It could therefore be useful in the management of diabetes.*

Keywords: *Amylase, Antioxidants, Glucosidase, Lectin, Trilepisiummadagascariense, Seeds*

Date of Submission: 05-08-2017 Date of acceptance: 07-12-2017

I. Introduction

Trilepisiummadagascariense, the urnfig or false-fig, is a species of plant in the family Moraceae. It was formally known as *Bosqueiaangolensis*. It grows to a medium-sized or large tree in primary or secondary forest, or in forest patches. It is native to tropical and subtropical West and Central Africa, and occurs southwards to Zimbabwe, Mozambique, and South Africa. The ripe fruit have the appearance of blue plums or elongated figs. Roasted seeds are eaten and the trees are sometimes cultivated. It has many traditional uses. The stem bark is traditionally used to treat venereal diseases, arthritis, rheumatism, diarrhea, and dysentery while the roots are used against cutaneous and subcutaneous parasitic infections [1]. The methanol extract from the leaves of *T. madagascariense* was reported to inhibit the growth of *Staphylococcus aureus*[2]. Likewise the methanolic extract from the stem bark has been shown to possess antidiarrhoeal activities [3], and previously unknown trilepisflavan and trilepisuimic acid compounds were isolated from it [4].

Diabetes mellitus (DM) is still not completely curable by current antidiabetic drugs. Insulin therapy is the only satisfactory approach in *Diabetes mellitus* management, though it has several drawbacks like insulin resistance, anorexia, brain atrophy and fatty liver in chronic treatment [5]. Treatment of type 2 *Diabetes mellitus* (T2DM) patients with synthetic drugs like sulfonylureas and biguanides are also always associated with various side effects [6]. Recently, herbal drugs from plant source are gaining popularity in the treatment of *Diabetes mellitus,* this is as a result of their efficacy, low incidence of side effects, and low cost [7]. T2DM is common because of increasing obesity and reduced activity levels, environmental and psychosocial stress factors, as well as malnutrition with increased xenobiotic intakes. T2DM is mainly characterized by the increased morbidity and mortality from cardiovascular diseases, also associated with a high risk of atherosclerosis and renal, nervous system and ocular complications [8]. Inhibitors of carbohydrate metabolizing enzymes have been reported to reduce postprandial hyperglycemia by delaying carbohydrate digestion and decreasing intestinal glucose absorption. Clinically used antidiabetic agents are known to inhibit starch degrading enzymes competitively in the brush border of the small intestine. It is therefore necessary to search for novel carbohydrate hydrolyzing enzyme inhibitors from natural sources. The study aims are to extract and purify the lectin from the seeds of *T. madagascariense*;determine some of the physicochemical properties of the purified lectin;evaluate the

antioxidant activity of the lectin; andinvestigate the inhibitory activity of the lectin on carbohydrate hydrolyzing enzymes, to determine the possibilities of its efficacy in the treatment/management of T2DM.

II. Materials and Methods

Collection of sample

Matured ripefruits of False-fig (*Trilepisium madagascariense*)were collected from the Botanical Garden between May and June, 2015 and were identified at the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State.

Methods

Preparation *T. madagascariense* seeds extract

The fruits were macerated to remove the seeds. The seeds were de-hulled, air dried and ground into powder. The crude extract was prepared by homogenizing the powdered sample in phosphate buffered saline (PBS), pH 7.2 (1:10 w/v). The mixture was stirred overnight at 4^{o}C. This was followed by centrifugation at 10,000 rpm for 20 minutes, using cold centrifuge (Centurion Scientific LTD. Model 8880. R-Series). The supernatant which constitute the crude lectin was collected and freezed.

Glutaraldehyde fixation and Trypsinization of red blood cells

The red blood cells from human and animals were fixed with glutaraldehyde according to the method of Pattanapanyasat*et al* [9]. Blood samples were collected in heparinized bottles and centrifuged at 3,000 rpm for 15 minutes. The erythrocytes were collected and washed three times with PBS. 50% glutaraldehyde was diluted to 1% (v/v) with PBS and chilled to 4^{o}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 an hour at 4^{o}C with occasional mixing. The fixed cells were collected by centrifugation at 3,000 rpm, and washed five times with PBS. The cells were re-suspended in PBS, containing 0.02% (w/v) sodium azide, to a final concentration of 2% (v/v), and stored at 4^{o}C until required. For trypsinization process, 1% trypsin was mixed with 2% red blood cells suspension in PBS at a ratio of 1:1 (v/v) and was incubated at 37^{o}C for 1 hourfollowing the method of ocenna*et al*[10]. After incubation, the trypsinized cells were washed with PBS three times, diluted and re-suspended in PBS (2% v/v), and stored until further use.

Hemagglutination assay

The crude extracts of *T. madagascariense* seeds was screened for the presence of lectin by hemagglutinating assay and was carried out by the method of Odekanyin and Kuku [11]. Hemagglutination experiments were performed in a 96-well U-shaped microtitre plate. PBS (100 µl) was delivered sequentially into wells arranged in rows (each row contained 12 wells). Extract (100 µl) was added into the first well and a serial dilution was done by transferring 100 µl of the diluted sample in a particular well into the next well containing 100 µl PBS until the last 24th well. Aliquots (50 µl) of the 2% red blood cells suspension were added to each well and the microtitre plates were left undisturbed for 2 hrs. The titre value was taken as the reciprocal of the highest dilution of the extract causing visible hemagglutination. Specific activity is the number of hemagglutination units per mg protein expressed as hemagglutinating units (HU)/mg.

Sugar specificity by hapten inhibition 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 Odekanyin and Kuku[11]. In the first step, lectin was serially diluted until the end-point dilution causing hemagglutination was obtained. 0.2M of sugar solution was added to each well at 50µl per well while the control well contained PBS instead of sugar solution. 50µl of erythrocyte suspension was added to each well, and the hemagglutinating 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, manitol, N-acetyl-D-glucosamine, mannosamine, 2-deoxy-D-glucose, dulcitol, xylose, α-D-methyl glucopyranoside and D(+)-glucosamine HCl. The minimum inhibitory concentration of sugar required to inhibit the lectin by 50% was carried out as reported by Odekanyin and Kuku[11]. Two-fold serial dilutions of 0.2 M sugar (50 µl) solutions in PBS were diluted with the lectin (l0 µl) solution and allowed to react for 30 min at room temperature. 2% Erythrocyte suspension ((50 µl) was then added and the mixture left for one hour undisturbed. The hemagglutinating titre that was obtained was compared with a non-sugar containing blank.

Protein concentration determination

The total protein concentration of the crude extract and other fractions were determined using Lowry's method [12]. Bovine serum albumin (BSA) was used as standard.

Purification of lectin

Ammonium sulphate precipitation

162 ml of the crude extract of *T. madagascariense* seed was measured. Ammonium sulphate crystals were added under controlled temperature condition on a magnetic stirrer to give a saturation of 0-70% and this was kept at 4^{o}C overnight. The precipitate was collected by centrifugation and re-dissolved in PBS followed by exhaustively against PBS and slightly with distilled water. Then, the resulting solution was lyophilized into powder.

Gel filtration on Sephadex G-100

Sephadex G-100 (10 g) was swollen in 200ml of distilled water for 72 hours at room temperature. The slurry gel was packed into a column (2.5 x 40 cm) according to instructions contained in the GE-Healthcare laboratory technique manual. The packed column was equilibrated with 500ml of 0.025M PBS, pH 7.2. Dialysate of ammonium sulphate precipitate (5 ml) was applied on the column, proteins were eluted with the same buffer and fractions of 5 ml were collected at a flow rate of 20 ml/hr. The fractions were assayed for hemagglutinating activity and monitored for protein by measuring the absorbance at 280 nm. The fractions in the active peak were pooled, dialyzed exhaustively against distilled water and lyophilized for further use.

Determination of apparent molecular weight

The apparent molecular weight of the partially purified lectin was estimated under non-denaturing conditions by gel filtration on Sephadex G-100 column (1.0 x 70 cm) using the following protein markers; bovine serum albumin (Mr 66,000; 5 mg/ml), ovalbumin (Mr 45,000; 5 mg/ml), pepsin (Mr 35,000; 5 mg/ml), trypsin (Mr 24,000; 5 mg/ml) and lysozyme (Mr 14,600; 5 mg/ml). Each standard protein (5ml) was applied on the column and run separately using 25mM phosphate buffer pH 7.2 as eluant at a flow rate of 12 ml/hr. Fractions (5ml) were collected and elution was monitored at 280 nm for each protein. The void volume of the column was determined with blue dextran, the elution of which was monitored at 620 nm. Partition coefficient (K_{av}) was plotted against logarithm of the molecular weight of the protein markers on a semi-logarithm graph.

Effect of temperature, pH, denaturants and EDTA on hemagglutinating activity

The effect of temperature onTMSLec hemagglutinating activity and thermal stability of the lectin were done as described by Cavada*et al*[13]. The partially purified lectin was incubated at various temperatures (30 - 100^{o}C). At each temperature, aliquots of the lectin were taken at 15 minutes interval for 60 minutes and was rapidly cooled on ice and assayed for hemagglutinating activity. The residual hemagglutination activity was tested for at room temperature (25^{o}C). The hemagglutination activity of the untreated sample at room temperature represented 100% activity.

The effect of pH on the activity of the lectin from TMSLec was determined by incubating aliquot of the lectin for 1 hour with 0.2 M (Citrate buffer pH 3 - 6, Tris-buffer pH 6 - 8, Glycine-NaOH pH 8 - 13) and assaying for its hemagglutinating activity. Lectin incubated in PBS (pH 7.2) was used as the control.

The effect of ethylenediaminetetraacetic acid (EDTA) and divalent cations on TMSLec was carried out as described by Wang *et al* [14]. The partially purified lectin was dialyzed against varying concentrations of EDTA (5 - 50 mM) at 4°C for 24 hours and then subjected to hemagglutinating assay. Effect of metal ion on hemagglutinating activity was studied by submitting 50 µl of the EDTA-treated lectin to two-fold serial dilutions in PBS with or without 10, and 20 mM $CaCl_2$, $SnCl_2$, $HgCl_2$, $BaCl_2$ and $MnCl_2$ respectively and its hemagglutinating activity was determined.

Chemical modification studies

This was carried out by treating the lectin sample with specific modifying reagents. The residual activity of the modified lectin was determined by hemagglutinating assay. Modification of tryptophan, and cysteine residues was carried out according to the method of Spande and Witkop[15], arginine residues was also modified using the method of Riodan [16] as modified byKuku *et al* [17].

Antioxidants assays

DPPH free radical scavenging assay

The hydrogen or radical scavenging properties of TMSLec were determined by the stable radical DPPH method as described by Brand-Williams *et al*[18]. The reaction of DPPH with an antioxidant compound which can donate hydrogen atom led to its reduction and there is a colour change from deep violent to light yellow, this change in colour is measured spectrophotometrically at a wavelength of 517 nm. To varying concentration of the sample extracts/standard (Ascorbic acid) (1 ml) was added to 0.3 mM DPPH (1 ml) in methanol and allowed to react in the dark. The mixture was vortexed and incubated in the dark for 30 minutes and the absorbance was measured at 517 nm against a DPPH negative control containing only methanol (1 ml) in place of sample extract.

Ferric reducing antioxidant power (FRAP)

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method with absorbance measured with a spectrophotometer [19]. The principle of this method is based on the reduction of a colorless ferric-tripyridyltriazine complex to its blue ferrous coloured form owing to the action of electron donating in the presence of antioxidants. The stock solutions consist acetate buffer, (300 mM, pH 3.6), 10 mM 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ) solution in 40 mMHCl and 20 mmol/L $FeCl_3.6H_2O$ were mixed together in the ratio of 10:1:1 respectively, to give the working FRAP reagent. The temperature of the solution was raised to 37ºC before using. Aliquot (50 µl) of the lectin at 1mg/ml and standard solution of ascorbic acid ((50 µl), (20, 40, 60, 80, 100 mg/ml)) was added to 1ml of FRAP reagent. Absorbance measurement was taken at 593nm exactly 10 minutes after mixing against reagent blank containing 50 µl of distilled water.

All measurements were taken at room temperature with samples protected from direct sunlight. The reducing power was expressed as equivalent concentration (EC) which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard. Results are expressed in µM Fe.

Metal chelating activity

The metal chelating properties of TMSLec was determined by iron chelating method described by Xie*et al* [20] with some modifications. To 100 µl of varying concentrations of the samples, 100 µl of 2 mM $FeCl_2$ and 100 µl of 5 mM (1 in 20) ferrozine was added 96 well microtitre plate. The mixture was left in the dark for 20 minutes a room temperature. The absorbance was measured at 560 nm. The control mixture contains all the reaction components except the sample which was replaced with distilled water. EDTA was used as positive control.

Carbohydrate inhibitory enzymes assays

Alpha-glucosidase inhibitory assay

The alpha-glucosidase enzyme inhibition assay was performed using standard assay methods as described by Thirumurugan*et al*[21]. Alpha-glucosidase (*Saccharomyces cerevisiae*) (1 mg) was dissolved in phosphate buffer (100 ml, pH 6.8) containing bovine serum albumin (BSA) (200mg). The reaction mixture consist of the partially purified TMSLec at varying concentrations (10 - 1000 µg/ml) with phosphate buffer (490 µl, pH 6.8) and 5mM *p*-nitrophenyl α-D-glucopyranoside (*p*-NPG) (250 *µ*l). After incubating at 37^{o}C for 5 min, alpha-glucosidase (250 µl, 0.15unit/ml) was added and incubated at 37^{o}C for 15 min. The reaction was terminated by the addition of 0.2 M Na_2CO_3 (2ml). Alpha-glucosidase activity was determined spectrophotometrically at 400 nm on UV-Visible spectrophotometer by measuring the quantity of *p*-nitrophenol released from *p*-NPG. Acarbose was used as positive control of alpha-glucosidase inhibitor. The concentration of the partially purified TMSLec required to inhibit 50% of alpha-glucosidase activity under the assay conditions was defined as the IC_{50} value.

The results were expressed as % inhibition calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \text{ X } 100$$

Alpha-amylase inhibitory assay

The alpha-amylase enzyme inhibition assay was carried out as described by Apostolidis*et al*[22]. A total of 250 µl of the partially purified TMSLec (0.01-1.0 mg/ml) was pipetted in a test tube and 250 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing alpha-amylase (0.5 mg/ml) was added. The reaction was started by the addition of 50µl of soluble starch (0.5%) dissolved in 20mM phosphate buffer at pH 6.9. The content of the tubes were incubated at 25°C for 10 minutes. The reaction was terminated by adding 500 µl of dinitrosalicylic acid (DNS) reagent and further incubated in boiling water for 5 minutes after which, it was cooled to room temperature. The content of each test tube was diluted with distilled water (5 ml) and the absorbance was measured at 540 nm using a spectrophotometer. A control was prepared using the same procedure except that the lectin was replaced with distilled water. The alpha-amylase inhibitory activity was calculated as shown below.

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \text{ X } 100$$

III Result And Discussion

Isolation and purification

A lectin from the seed of *T. madagascariense* having antioxidant properties and α-glucosidase and α-amylase inhibitory activities was purified by combination of ammonium sulphate precipitation and gel filtration chromatography on Sephadex G-100. The crude extract contained detectable amount of lectin which was precipitated by 70% $(NH_4)_2SO_4$ saturation and the precipitate dialysate was further purified on the Sephadex G-

100 column (40 x 2.5 cm). Three protein peaks were obtained and the lectin activity resides in the first peak as shown in figure. 1. The TMSLec fraction recovered from the gel filtration column contained 82mg protein with a specific activity of 178.8HU/mg representing 25% yield of the TMSLec activity and 12-fold purification (Table. 3). Apparent molecular mass of the purified TMSLec was 45KDa (Figure. 2). Analysis of purified lectin on SDS-PAGE in the presence of reducing agents gave a single band whose molecular weight was 22 kda. This shows that the purified lectin is an homodimer protein. Anhomodimeric lectins have also been reported in *Dioclealehmanii*[23].

TMSLecstrongly agglutinated human blood (ABO system) erythrocytes and also showed an evident hemagglutinating activity towards rabbit erythrocytes with higher specificity (Table 1). This may be probably due to the presence of carbohydrate component on the cellular surface of human red blood erythrocytes that are relatively less recognized by the TMSLec binding site. The sensitivity of the lectin to agglutinate erythrocytes was greatly enhanced following treatment with trypsin except for rabbit erythrocytes.It has been speculated that trypsin can redistribute the receptor sites on the erythrocytes surfaces which may induce clustering that is more favorable for agglutination [24]. Non-specific agglutination of ABO human erythrocytes was observed for the *Mucunasloanei* seed lectin [25] and the enzyme treated and non-treated rabbit erythrocytes were agglutinated by lectin from *M.pruriens* seed [26]. The hemagglutination of red blood cell of human blood system was completely inhibited by galactose (Table. 2). Similar results have been observed with the seeds lectin from *Cucurbitaceae*sp [27] and *Bauhinia variegata* [28].It was also observed that the other galactose containing sugar like lactose was unable to inhibit the hemagglutinating activity of TMSLec which was unusual because majority of the galactoside lectin always show specificity for the galactose containing sugar [17, 29]. This may be due to the size of the binding site on this lectin, which may not be as extended as other lectins that have been reported to be galactoside[30].

TMSLec was found to retain its native hemagglutinating activity up to 40^{o}C. There was gradual loss of activity as the temperature increased towards 80^{o}C and no activity was recorded above 90^{o}C (Figure. 3). Similar result was reported byAwoyinka and Dada[31]on *Cissuspoplunea* seed lectin which was found to be heat stable from 20^{o}C up to 40^{o}C. Thermostability of TMSLec varies in hemagglutinating activity with temperatures as well with incubation time. The lectin retained about 75% of its maximum activity at 50^{o}C for 60 min of incubation. Progressive inactivation of the hemagglutinating activity was observed as the temperature increase to 90^{o}C when there was total loss of activity. The loss of hemagglutinating activity of TMSLec with increased temperature is probably due to destabilization of weak interaction of tertiary structures responsible for its native conformation.

Examining TMSLec activity at different pH values (pH 3.0 - 13.0) showed that the lectin exhibited maximum activity at a pH range of 3.0 to 7.0; and above this pH, there was a decline in lectin hemagglutinating activity. The maximum activity evidently fell within acidic and neutral pH (Figure. 4). Many lectins have been reported to be active at acidic pH range including the lectin isolated from *Bauhinia pentandra* seeds which showed a maximum activity at pH 3 and a minimum at pH 10 [32]. These characteristics have also been noticed in lectin from *Annona muricata* seeds [33], *Hericiumerinaceum* seed [34] and *D. opposita* tuber [35].

The presence or absence of certain metal ions can lead to conformational changes in lectin which may either increase or decrease its activity. The chelation studies performed with TMSLec showed no effect on the hemagglutinating activity. This could suggest that either the lectin activity was not dependent on metal ions to be fully active or the metal ion are tightly bound to the lectin molecule and could not be removed by dialysis with chelating agent. The removal of metal ions by EDTA has been reported to disrupt the carbohydrate binding site and reduce lectin activity of many plant lectins [36]. Lectins isolated from *Arisaemahelleborifolium* [37] and *D. mangenotiana* [38] also did not show any reduction in hemagglutinating activity when dialyzed exhaustively against EDTA.

Identification of specific amino acids involved in the biological activity of proteins elucidates the relationship between the structure and the role played by amino acid side chains in their activities. Commonly used strategy for identifying the amino acid residues essential for biological activity of any protein is to treat the protein with specific modifying agents [29]. This investigationprovided clues about the amino acids involved in the hemagglutinating activity of TMSLec. Table. 4 shows that Treatment with 5.5'Dithiobis-2-nitrobenzoic Acid (DTNB) did not produce any alteration in the hemagglutinating activity of the TMSLec. The result indicated that cysteine did not play any important role in the activity of the lectin but possibly involve in the formation of di-sulphidebridge that stabilizes the protein conformation. Sharma *et al.*[39]gave a similar remark from the result obtained from Anasazi bean lectin treated with DTNB which did not alter the hemagglutinating activity of the lectin. However the total loss of lectin activity when N-Bromosuccinimide (NBS) was used suggests the involvement of tryptophan (Trp) residues in lectin activity and likely its stability. Involvement of Trp in the hemagglutinating activity of other lectin has been reported[17, 29]. Studies have also reported that Trp is indispensable for the hemagglutinating activity of some legume lectin [40]. Partial inactivation was also observed when the protein was modified with Phenylglyoxal. This was not synonymous to the reports of Thakur *et al.* [41]for the lectin from *Ganodermalucidum*. Also, Konozy*et al*[29] reported that modification of arginine

residue in *E. indica* lectin with phenylglyoxal had no significant effect on the activity of the lectin. The result from the current study is suggesting that arginyl group of TMSLec is probably involved in the binding of sugar moiety on the surface of the cell. It could therefore be proposed that arginine and tryptophan residues in TMSLec may be involved in either direct interaction with the sugar or may have a role in maintaining conformation of the sugar binding pocket and hence contributed to the hemagglutinating activity of the lectins.
Oxidative stress has been linked to imbalance between formation of ROS and antioxidant defense system [42], while reports have it that plants contain various antioxidant constituents and bioactive compound that are useful to health. Free radicals generated during metabolic process of an organism are known to play role in several disorders. [43, 44].DPPH is one of the most widely used methods in evaluating free radical scavenging properties of natural products. TMSLec revealed a mild free radical scavenging potential comparing the result with ascorbic acid (positive control) (Figure. 5). These findings compared well with the study conducted by Sadanand*a et al*[43], the result depicts that lectin extract from *Viscum album* showed a promising free radical scavenging potential in a concentration dependent manner. Valdez-Drtiz *et al*[45] studied Azufrado bean (*Phaseolus vulgaris*) protein concentrate and reported that the protein hydrolysate presented 44% antioxidant activity by DPPH. Bahi*et al*[44] also reported a positive scavenging potential of lectin found in some plant roots. The extent of the reaction may depend on the hydrogen donating ability of TMSLec hydroxyl group present. The FRAP assay is often used to evaluate the ability of an antioxidants to donate an electron [44], which mainly depends on the reducing capacity of Fe^{3+}- Fe^{2+} and serve as a significant indicator of its potential antioxidant activity[46]. The ferric reducing antioxidant properties showed by TMSLec possibly cause the reduction by conversion of ferricyanide complex to the ferrous form and this could suggest that the TMSLec may possibly react with free radicals and convert them to a more stable compound. Similarly, lectin from *V. album*[43] has abundant ferric reducing power though in a concentration dependent manner.Transition metal ions, such as Fe^{2+} and Cu^{2+}can catalyze the generation of reactive oxygen specieswhich accelerates lipid oxidation. Fe^{2+} can also catalyze the Haber-Weiss reaction and inducesuperoxide anions to form more hazardous hydroxyl radicals. These hydroxyl radicals react withadjacent biomolecules to cause severe tissue damage [20], especially lipid oxidation. Therefore, thechelation of transition metal ions by anti-oxidative protein could retard the oxidation reaction. Figure 6shows the ability of TMSLec to chelate the transition metal ion, Fe in dose dependent manner (0.1 – 0.3 mg/ml). Presumably, peptide cleavages lead to enhanced Fe^{2+}binding due to increased concentration ofcarboxylic (COO^{-}) and amine groups in acidic and basic amino acids, thus removing the pro-oxidativefree metal ions from the hydroxyl radical system [47].

The primary goal of the management of type 2 *Diabetes mellitus* is to reduce the blood glucose concentration to a normal or near normal level[48]. Inhibitors of carbohydrate hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase have been helpful as oral hypoglycemic medicines for the control of hyperglycemia exclusively in patient with type 2 *Diabetes mellitus*[49, 50]. Inhibition of these enzymes delays carbohydrate digestion and extends the total carbohydrate digestion time, leading to a decrease in the rate of glucose absorption and therefore reducing the postprandial plasma glucose rise[51].

In this study, the inhibitory potential of TMSLec was investigated *in-vitro* on alpha-amylase and alpha-glucosidase activities. From figure 7, TMSLec showed a great potential of inhibition on alpha-amylase activity when compared with the reference drug (Acarbose). This is similar to the result reported by Russo *et al*[52] for antidiabetic activities of *Smallanthussonchifolius* leaf extract. Related assay was performed by Bansode and Salalkar[53], but the result reported was observed to have a lower inhibitory activity of alpha-amylase when compared with the standard (Acarbose). Meanwhile, TMSLec inhibitory potential of the alpha-glucosidase activity is not as significant as acarbose (positive control). Pseudosaccharides (Acarbose), by virtue of being a substrate analog, inhibit alpha-glucosidase in a concentration dependent manner and the same response was observed with TMSLec against alpha-glucosidase (Figure. 8). This imply that the active component of the TMSLec competed with the substrate for binding to the active site on the enzyme, thereby slowing down or preventing the breakdown of disaccharides to single glucose unit. The results from our study is parallel to the result reported by Shai *et al*[54], though the research work compared two types of alpha-glucosidases (Mammalian and Microbial) which led to the presentation of the claim that a plant extract may inhibit digestion of complex carbohydrates to monosaccharaides in the human gastrointestinal tract and should be validated by inhibition of mammalian enzyme and not only the microbial version of the enzyme.

V. Conclusion

Present study indicated that lectin from *T. madagascariense* seed does not only possess remarkable scavenging activity on DPPH and FRAP activities, but also exhibited inhibitory potential against alpha glucosidase and alpha amylase. The results of this investigation should probably be a basis for further research to develop antidiabetic therapy.

Reference

[1]. Burkill, H. M. (1985). The useful plants of West Tropical Africa, vol 1.Families AD Royal Botanic Gardens, Kew.Library MARC record.

[2]. Sabrina, K., Huffman, M., Se'Venet, T., Hladik, C., Grellier, P., Loiseau, P. and Wrangham, R. (2006). Bioactive properties of plant species ingested by chimpanzees (Pan troglodytesschweinfurthii) in the Kibale National Park, Uganda. American Journal of Primatology 68: 51-71.

[3]. Kuiate, J. R., Kuete, V., Teponno, R., Bertrand, T., Leon, A. V., Gerard, T. and Gerald, N. (2010). Antidiarrheal activity of extracts and compound from Trilepisium madagascariense stem bark. Indian Journal of Pharmacology 42(3): 157–163.

[4]. Ango, P. Y., Kapche, D. W., Kuete, V., Ngadjui, B. T., Bezabih, M. and Abegaz, B. M. (2012). Chemical constituents of Trilepisium madagascariense (Moraceae) and their antimicrobial activity.Phytochemistry Letters5(3): 524-528.

[5]. Piedrola, G., Novo, E., Escober, F. and Garcia-Robles, R. (2001). White blood cell count and insulin resistance in patients with coronary artery disease.Annals of Endocrinology 62: 7-10.

[6]. Weidmann, P., Boehlen, L. M. and de Courten, M. (1993). Pathogenesis and treatment of hypertension associated with Diabetes mellitus. American Heart Journal125(5): 1498-1513.

[7]. Pandey, A., Tripathi, P., Pandey, R., Srivatava, R. and Goswami, S. (2011). Alternative therapies useful in the management of diabetes: A systematic review. Journal of Pharmacy and Bioallied Sciences3(4): 504-520.

[8]. Habif, S., Turgan, N. and Mutaf, I. (1997). Plasma catalase, glutathione peroxidase and selenium levels in adult diabetic patients. Turkish Journal of Medical Sciences27(2): 139-141.

[9]. Pattanapanyasat, K., Noulsri, E., Lerdwana, S., Sukapirom, K., Onlamoon, N. and Tassaneetrithep, B. (2010). The Use of Glutaraldehyde-Fixed Chicken Red Blood Cells as Counting Beads for Performing Affordable Single-Platform CD4+ T-LymphocyteCount in HIV-1-Infected Patients. Journal of Acquired Immune Deficiency Syndromes (USA) 53: 47-54.

[10]. Ocenna, I. V., Mojica, E. E. and Merca, F. E. (2007). Isolation and Partial Characterization of a Lectin from the seeds of Artocarpuscamansi Blanco.Asian Journal of Plant Sciences 6(5): 757-764.

[11]. Odekanyin, O. O and Kuku, A. (2014) Characterization of galactose-specific lectin from the skin of Africa catfish clariasgariepinus. Burchl, 1822. Scientific Research and Essays 9(20): 869-879.

[12]. Lowry, O. H., Rosebrough, N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

[13]. Cavada, B., Moreira, S., Grangeiro, T., Santos, C., Pinto, V. (1996) Purification and biological properties of a lectin from Canavaliabonariensis. Biology, Biochemistry, Clinical Biochemistry 11th ed. Textop, Hellerup pp 74-80.

[14]. Wang, H., Gao, J. and Ng, T. B. (2000). A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom Pleurotusostreatus.Biochemical and Biophysical Research Communications275(3): 810-816.

[15]. Spande, T. T. and Witkop, B. (1967). Determination of the tryptophan content of proteins with N-bromosuccinimide.Methods in Enzymology11: 498-506.

[16]. Riordan, J. F. (1979). Arginyl residues and anion binding sites in proteins.Molecular and Cellular Biochemistry26(2): 71-92.

[17]. Kuku, A., Odekanyin, O. O. and Okonji, R. E. (2012). Physicochemical properties of a lactose specific lectin from the seeds of Erythrinasenegalensis DC.Ife Journal of Science14(1): 143-153.

[18]. Brand-Williams, W., Cuvelier, M. E. and Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity.LWT-Food science and Technology28(1): 25-30.

[19]. Benzie, I. F and Strain J. J. (1999). Ferric reducing/antioxidant power assay: a direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Method in Enzymology 299: 15-27.

[20]. Xie, Z., Huang, J., Xu, X. and Jin, Z. (2008) Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. Food Chemistry 111:370–376.

[21]. Thirumurugan, K., Bachhawat, A., Shihabudeen, J. M. (2011). Screening of fifteen Indian ayurvedic plants for alpha-glucosidase inhibitory activity and enzyme kinetics.International Journal of Pharmaceutics 3:267-274.

[22]. Apostolidis, E., Kwon, Y. and Shetty, K. (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innovative Food Science and Emergence Technologies 8: 46-54.

[23]. Perez, G. (1998). Isolation and Characterization of a Novel Lectin from Dioclealehmanni (Fabaceae) seeds. Biochemistry and Cell

[24]. Biology 30: 843-853.

[25]. Goldstein, I. J. and Hayes, C. E. (1978). The lectins: Carbohydrate-binding proteins of plants and animals. Advances in Carbohydrate Chemistry and Biochemistry35: 127-340.

[26]. Teixeira-Sa D., Reicher, F.,m Braga, R. C., Beltramini, L. M. and de-Azavedo, M. R. (2009). Isolation of a lectin and a galactoxyloglucan from mucunasloanei seeds.Phytochemistry 70(17): 1965-1972.

[27]. Lacerda, R. R., Moreira, I. C., Nascimento, J. S. J., de-Lacerda, A. C. S., Cabral, N. L et al (2015). Lectinisolated from Brazilian seeds of velvet beans (Mucunapruriens (L) DC.) presents analgesic, anti-inflamatory and antihemolytic action. Journal of Medicinal Plant Research 9(8): 231-242.

[28]. Swamy, J., Marapakala, K., Sultan, A. M. and Kenoth, R. (2015). Galactose-specific seed lectins from Cucurbitaceae.Current Protein and Peptide Science16(1): 17-30.

[29]. Mishra, R. P., Ganaie, A. A. and Allaie, A. H. (2016). Isolation and purification of a galactose specific lectin from seeds of Bauhinia variegata and evaluation of its antimicrobial potential.International Journal of Pharmaceutical Sciences and Research7(2): 804-815.

[30]. Konozy, E. H., Mulay, R., Faca, V., Ward, R. J., Greene, L. J., Roque-Barriera, M. C. and Bhide, S. V. (2002). Purification, some properties of a D-galactose-binding leaf lectin from Erythrinaindicaand further characterization of seed lectin.Biochimie84(10): 1035-1043.

[31]. Sartim, M. A. and Sampaio, S. V. (2015). Snake venom galactoside-binding lectins: a structural and functional overview. Journal of Venomous Animals and Toxins Including Tropical Diseases21(1): 35-52.

[32]. Awoyinka, O. A. and Dada, O. O. (2011). Partial Purification and Characterization of Lectin from the Seeds of Cissuspoplunea.European Journal of Medicinal Plants1(4): 130-139.

[33]. Silva, A. L. C. D., Horta, A. C. G. and Moreira, R. D. A. (2001). Isolation and Partial Purification of a Lectin from Bauhinia pentandra (Bong) Vog. Ex. Steua. RevistaBrasileiraFisiologia Vegetal 13(3): 262-269.

[34]. Damico, D. C. S., Freire, M. G. M., Gomes, V. M., Toyama, M. H., Marangoni, S., Novello, J. C. and Macedo, M. L. R. (2003). Isolation and characterization of a lectin from Annona muricata seeds.Journal of Protein Chemistry22(7): 655-661.

[35]. Li, Y., Zhang, G., Ng, T. B. and Wang, H. (2010). A novel lectin with antiproliferative and HIV-1 reverse transcriptase inhibitory activities from dried fruiting bodies of the monkey head mushroom Hericiumerinaceum. BioMedical Research International2010: 9-10.
[36]. Chan, Y. S. and Ng, T. B. (2013). A lectin with highly potent inhibitory activity towards breast cancer cells from edible tubers of Dioscoreaopposita cv. Nagaimo. PloS One 8(1):54212-54218.
[37]. Singh, R. S., Kaur, H. P. and Singh, J. (2014). Purification and characterization of a mucin specific mycelial lectin from Aspergillus gorakhpurensis: application for mitogenic and antimicrobial activity. PloS One9(10): e109265.
[38]. Kaur, M.,Singh,K.,Rup,P. J.,Saxena, A. K.,Khan, R. H.,Ashraf, M. T.,Kamboj, S. S. andSingh,J. (2006).AtuberlectinfromArisaemahelleborifoliumSchottwithanti-insect activityagainstmelonfruitfly,Bactroceracucurbitae(Coquillett) andanti-cancereffectonhumancancercelllines. Archives of Biochemistry and Biophysics445: 156-165.
[39]. Akinyoola, K. A., Odekanyin, O. O., Kuku, A. and Sosan, M. B. (2016). Anti-insect potential of a lectin from the tuber, Dioscoreamangenotiana towards Eldanasaccharina (Lepidoptera: Pyralidae). Journal of Agricultural, Biotechnology and Sustainable Development8(3): 16-26.
[40]. Sharma, A., Ng, T. B., Wong, J. H. and Lin, P. (2009). Purification and characterization of a lectin from Phaseolus vulgariscv (Anasazi beans). BioMed Research International, 2009: 929568.
[41]. Nadimpalli, S. K. (1999). Chemical modification studies on the glucose/mannose specific lectins from field and lablab beans. International Union of Biochemistry and Molecular Biology Life47(5): 825-834.
[42]. Thakur, A., Rana, M., Lakhanpal, T. N., Ahmad, A. and Khan, M. I. (2007). Purification and characterization of lectin from fruiting body of Ganodermalucidum.BiochimicaetBiophysicaActa (BBA)-General Subjects1770(9): 1404-1412.
[43]. Sargi, S. C., Silva, B. C., Santos, H. M. C., Montanher, P. F., Boeing, J. S., Júnior, S., Oliveira, O., Souza, N. E. and Visentainer, J. V. (2013). Antioxidant capacity and chemical composition in seeds rich in omega-3: chia, flax, and perilla. Food Science and Technology (Campinas)33(3): 541-548.
[44]. Sadananda, T. S., Govindappa, M. and Ramachandra, Y. L. (2014). In vitro Antioxidant Activity of Lectin from Different Endophytic Fungi of Viscum album L. British Journal of Pharmaceutical Research 4(5)**:** 625-643.
[45]. Bahi, A., Necib, Y., Merouane, F., Bouadi, H.and Boulahrouf, K. (2016).Antioxidant, anti-inflammatory and antimicrobial Properties of new lectins purified from roots of Algerian Plants: Morusnigra, Rutagraveolens, Cyperusrotundus and Pistacialentiscus. World Journal of Pharmaceutical Research 5(2): 39-53.
[46]. Valdez-Drtiz, A., Fuentes-Gutiérrez, C. I., Germán-Báez, L. J., GutiérrezDorado, R. and Medina-Godoy, S. (2012). Protein hydrolysates obtained from Azufrado (sulphur yellow) beans (Phaseolus vulgaris): nutritional, ACE-inhibitory and antioxidative characterization. Food Science and Technology 46(1): 91-96.
[47]. Baskar, R., Lavanya, R., Mayilvizhi, S. and Rajasekaran, P. (2008). The free radical scavenging activity of antitumor polysaccharide fractions isolated from Ganodermalucidum (Fr.). P Karst.Natural Product Radiance 7(4): 320-325.
[48]. Nam, K. A., You, S. G and Kim, S. M. (2008). Molecular and physical characteristics of squid (Todarodespacificus) skin collagens and biological properties of their enzymatic hydrolysates. Journal of Food Science.73, C243–C255.
[49]. Poongunran, J., Perera, H. K. I., Fernando, W. I. T., Jayasinghe L. and Sivakanesan, R. (2015). α-Glucosidase and α-Amylase Inhibitory Activities of Nine Sri Lankan Antidiabetic Plants. British Journal of Pharmaceutical Research 7(5): 365-374.
[50]. Keerthana, G., Kalaivani, M. K. and Sumathy, A. (2013). In-vitro alpha amylase inhibitory and anti-oxidant activities of Ethanolic leaf extract of Croton bonplandianum. Asian Journal of Pharmaceutical and Clinical Research6(4): 32-36.
[51]. Jhong, C. H., Riyaphan, J., Lin, S. H., Chia, Y. C. and Weng, C. F. (2015). Screening alpha-glucosidase and alpha-amylase inhibitors from natural compounds by molecular docking in silico.Biofactors41(4): 242-251.
[52]. Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. (2004). Global prevalence of diabetes Estimates for the year 2000 and projections for 2030. Diabetes Care 27: 1047-1053.
[53]. Russo, D., Valentão, P., Andrade, P. B., Fernandez, E. C. and Milella, L. (2015). Evaluation of antioxidant, antidiabetic and anticholinesterase activities of Smallanthussonchifolius landraces and correlation with their phytochemical profiles.International Journal of Molecular Sciences16(8): 17696-17718.
[54]. Bansode, T. S. and Salalkar, B. K. (2016). Strategies in the design of antidiabetic drugs from Terminalia chebula using in silico and in vitro approach. MicroMedicine4(2): 60-67.
[55]. Shai, L. J., Magano, S. R., Lebelo, S. L. and Mogale, A. M. (2011). Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. Journal of Medicinal Plants Research5(13): 2863-286

Table 1: Hemagglutinating Activity of TMSLec

Blood Group	Hemagglutination Titre	
Human	**Non-Trypsinized**	**Trypsinized**
A	2^{12}	2^{15}
B	2^{13}	2^{17}
O	2^{14}	2^{16}
Rabbit	2^{17}	2^{17}
Rat	2^{13}	ND

ND: Not Determined

Each experiment consisted of 100 µl of PBS with a serial dilution of 100 µl of the sample in a U-shaped microtitre plate. 50 µl of 2% erythrocyte suspension (from human A, B, O, rabbit and rat blood) was added to each well. All experiments were carried out in triplicates.

Table 2: Sugar Specificity Test of *T. madagascariense* Seed Crude Extracts

Sugars	A	B	O	Rabbit
Control	2^{11}	2^{11}	2^{10}	2^{18}
Maltose	2^{12}	2^{12}	2^{12}	2^{12}
Mannose	2^{12}	2^{12}	2^{11}	2^{12}
Lactose	2^{12}	2^{12}	2^{10}	2^{11}
Arabinose	2^{10}	2^{11}	2^{10}	2^{11}
Sorbose	2^{11}	2^{12}	2^{11}	2^{11}
Glucose	2^{11}	2^{12}	2^{11}	2^{11}
Galactose	$\mathbf{2^0}$	$\mathbf{2^0}$	$\mathbf{2^0}$	$\mathbf{2^5}$
Glucosamine	2^{12}	2^{12}	2^{12}	2^{12}
Mannitol	2^{11}	2^{11}	2^{11}	2^{12}
N-acetyl-D-glucosamine	2^{12}	2^{11}	2^{7}	2^{12}
2-deoxy-D-Glucose	2^{10}	2^{11}	2^{11}	2^{12}
Sucrose	2^{12}	2^{12}	2^{12}	2^{12}
Dulcitol	2^{6}	2^{6}	2^{5}	2^{10}
α-D-methylglucopyranoside	2^{5}	2^{5}	2^{3}	2^{11}
Xylose	2^{7}	2^{7}	2^{7}	2^{10}

Each experiment consisted of 100 µl of PBS with a serial dilution of 100 µl of the sample in a U shaped microtitre plate. A 0.2 M sugar solution (50 µl) and 2 % erythrocyte (50 µl) suspension (Human blood ABO and rabbit erythrocytes) were added to each well. All experiments were carried out in triplicates.

Table 3: Purification of TMSLec Procedure Summary of TMSLec

Fractions	Volume (ml)	Protein (mg/ml)	Total Protein (mg)	Total Activity(HU)	Specific Activity (HU/mg)	% Yield	Purification Fold
Crude Extract	162	12.15	1967.81	32768	16.65	100	1
Ammonium Precipitate (70%)	57	4.99	284.78	16384	57.53	50	3.89
Gel-filtration (Sephadex G-100)	28	0.82	22.91	4096	178.79	25	11.88

Table. 4: Chemical Modification Assay with DTNB, NBS, and Phenylglyoxal on TMSLec

Treatment	Modified Amino acid	% Residual of Hemagglutinating Activity			
		15min	30min	45min	60min
NBS	Tryptophan	0	0	0	0
DTNB	Cysteine	100	100	100	100
Phenylglyoxal	Arginine	50	25	25	25

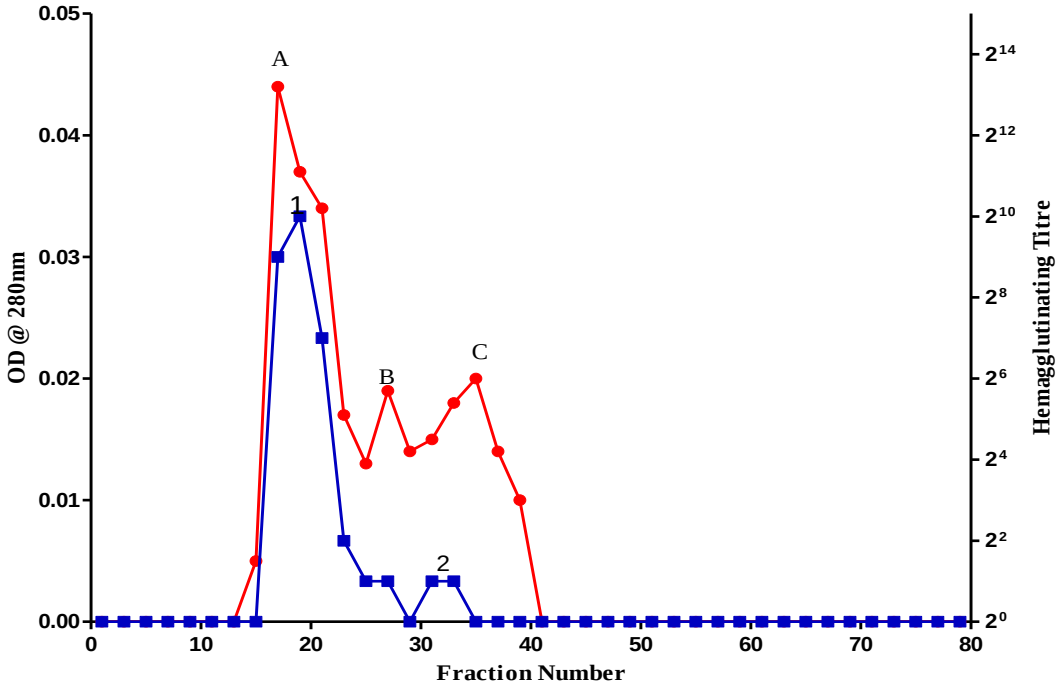

Fig. 1: Gel-Filtration of 70% Ammonium Sulphate Precipitate Dialysate of TMSLec on Sephadex G-100

The column (2.5 X 40 cm) packed with Sephadex G-100 was equiliberated with 25mM phosphate buffered saline (PBS) pH 7.2 containing 10mM sodium chloride (NaCl). 5 ml of ammonium sulphate precipitate dialysate (4 mg) was layered on the column and the lectin was eluted with the same buffer at a flow rate of 15 ml/hr and fractions of 5 ml were collected.

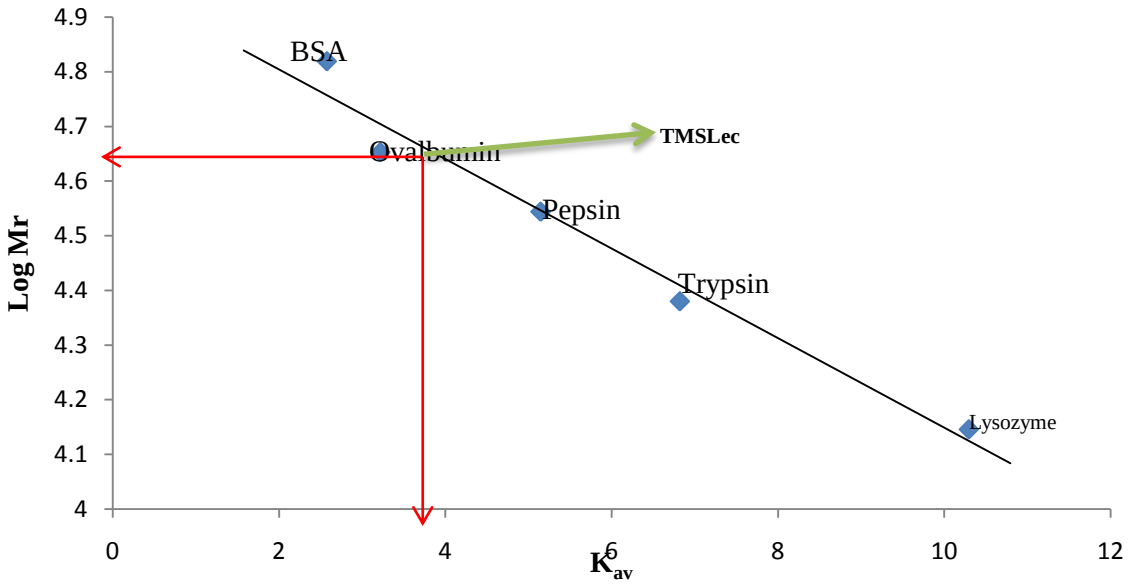

Fig. 2: Calibration Curve for the Determination of Native Molecular Weight of TMSLec on Sephadex G-100

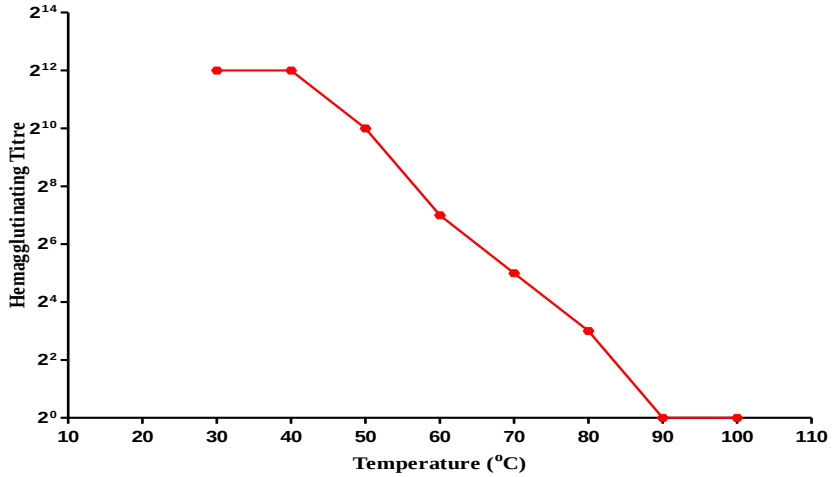

Fig. 3: Effect of Temperature on the Hemagglutinating Activity of TMSLec

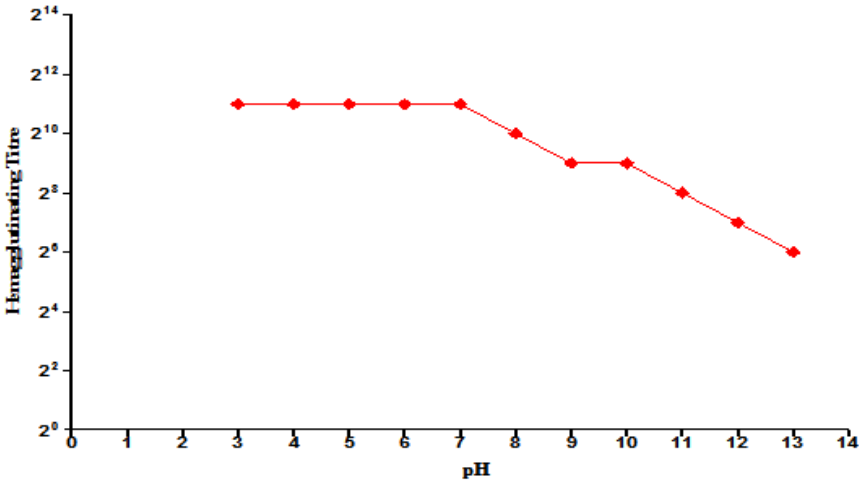

Fig. 4: Effect of pH on the Hemagglutinating Activity of TMSLec

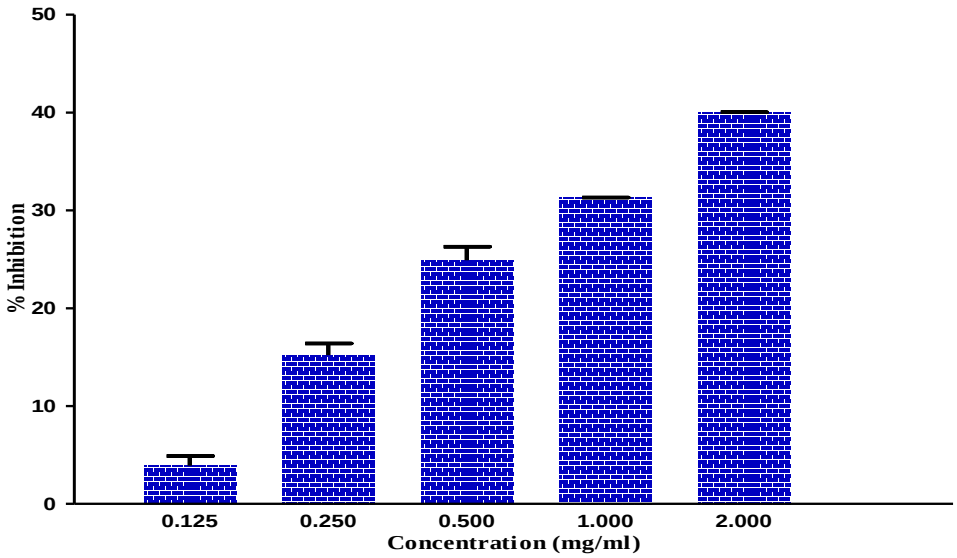

Fig. 5: DPPH Radical Scavenging Activity of TMSLec

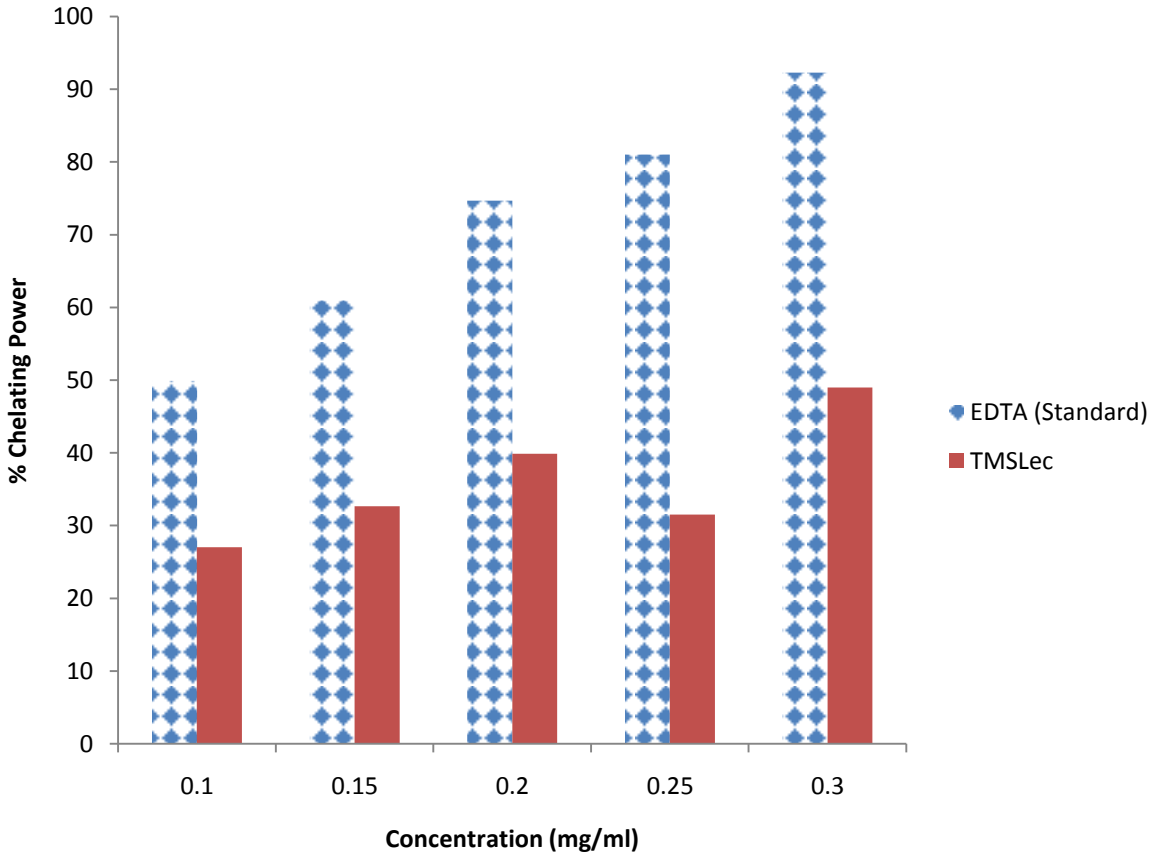

Fig. 6: Metal Chelating Activity of TMSLec

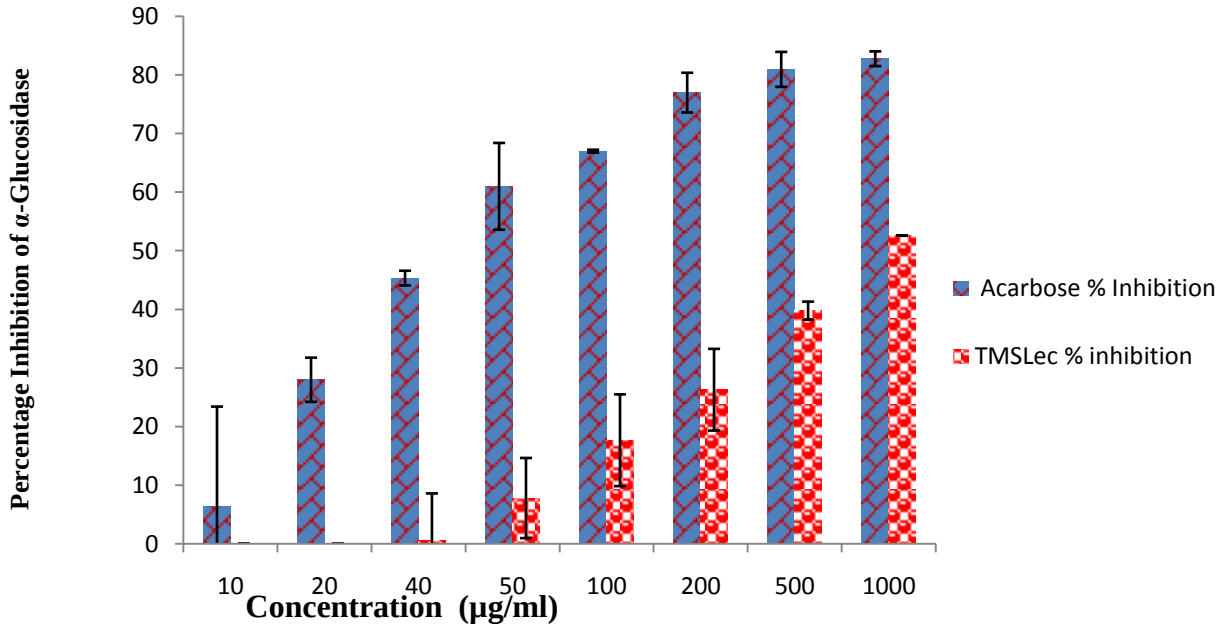

Fig. 6: Inhibitory Potential of TMSLec against α-Glucosidase Activity

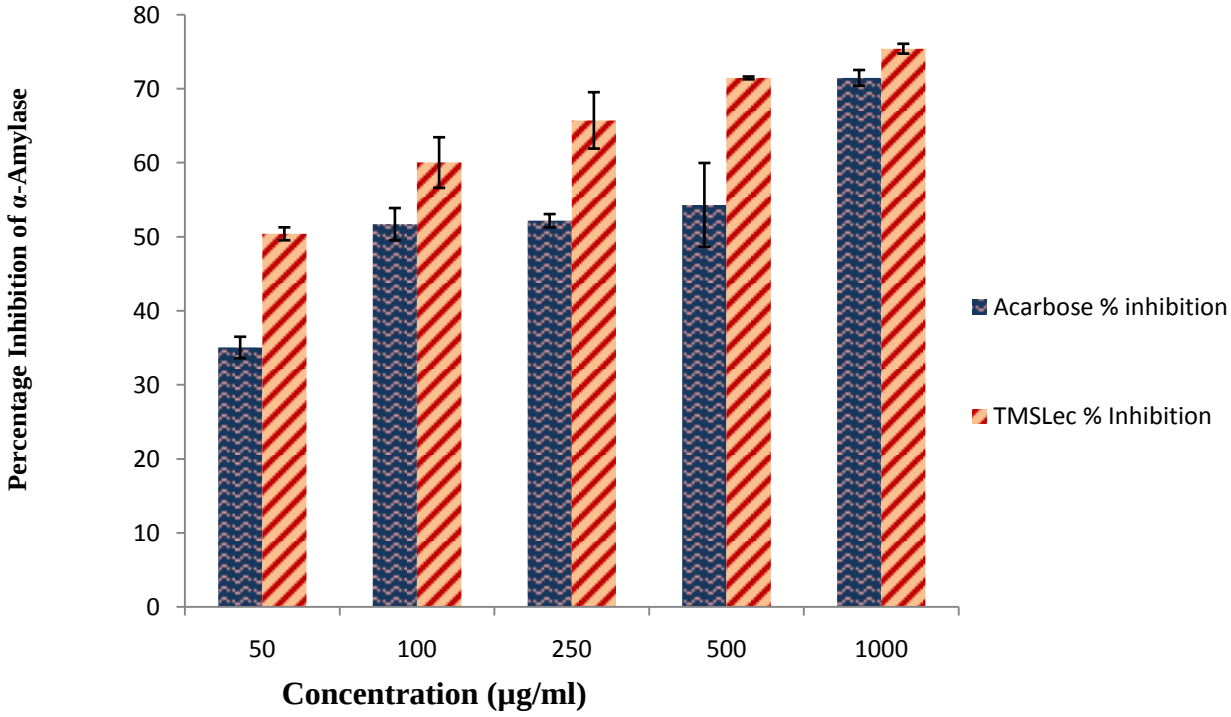

Fig. 7: Inhibitory Potential of TMSLec against α-Amylase Activity

Samuel O.OLODUDE " Evaluation Of Antioxidant And Carbohydrate Hydrolyzing Enzyme Inhibitory Activities Of Trilepisium Madagascarienseficalho Seeds Hemagglutinin." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) , vol. 12, no. 6, 2017, pp. 29-41.