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Purification and erythrocyte-membrane perturbing activity of a ketose-specific lectin from *Moringa oleifera* seeds

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ABSTRACT

This study purified a hemagglutinating protein (MoL) from *Moringa oleifera* seed, and investigated its hemolytic activity. Molecular weight and stability of MoL were also determined. Modification of some amino acid residues was carried out and the effect on MoL hemagglutinating activity determined. Other investigated parameters are the effects of temperature, concentration, incubation period, pH, and sugars on the protein's hemagglutinating and hemolytic activities. The native and subunit molecular weights were estimated as 30 and 27.5 kDa respectively. Hemagglutinating activity of MoL was slightly inhibited by fructose and sucrose, stable at temperature up to 90°C and within pH range of 2–4. Modification of tryptophan and arginine residues resulted in partial loss of hemagglutinating activity. The hemolytic activity of MoL was concentration, temperature, pH, and time-dependent. The study concluded that MoL showed hemolytic (membrane-perturbing) activity in moderate acidic conditions. This suggests its potential exploitation in improved intracellular delivery of bioactive compounds.

1. Introduction

The plasma membrane is one of the most important metabolically flexible biomaterials in living cells, and it plays a vital role against xenobiotics toxins and invading microbial pathogens [1, 2]. Selectively permeable by composition, the bio-membrane is composed of myriads of molecules including carbohydrates, proteins, and lipids. These molecules interact dynamically to facilitate cellular communications, as well as trafficking and clearance of substances across the membrane [3, 4]. From a medical perspective, biomedicine, in particular, the versatility of the plasma membrane has prompted researchers to be on the lookout for protein-based therapeutics which could be a tool for protein-membrane studies to manage certain ailments such as cancer, because of their membrane-perturbing, hemolytic and cytolytic activities [5, 6].

Erythrocytes, also known as red blood cells (RBCs), are the most facile available human cell types, having an average population of approximately 5 billion cells per milliliter [7]. These oxygen-transporting cells are increasingly being exploited as a typical innovative model in understanding unique biochemical processes, including drug delivery, because of the adaptability of their membrane [8, 9]. Efficacy of therapeutic agents can be compromised as a result of drastic changes in temperature and pH during systemic circulation or storage, making novel approach like polymer conjugation and co-administration of plant-based molecules, such as proteins in drug delivery imperative, owing to their biodegradability, physicochemical properties and ease of purification [10, 11].

Although plants and their products are used as curatives, however, unfavorable pharmacokinetics because of poor membrane permeation to a greater extent pose a challenge to their effective application [12]. Of late, efforts are being made at segregating functional components of these therapeutic plants to explain their proposed potential biotechnological uses, especially in the synthesis of nontoxic, biocompatible, non-immunogenic, and biodegradable drug delivery vehicles [13].

Lectins are disparate ubiquitous proteins distributed in a conventional order among microorganisms, plants, and animals, containing at least one non-enzymatic domain enabling them to descry in reversible

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selective comportment to specific sugars or carbohydrate-associated structures without modifying the arrangement of the bound carbohydrates [14-17]. Advances in bio-techniques and genome-wide screenings of plant lectins have unraveled the multitude of different lectin sequences within single plant species suggesting their exploitation for biotechnological purposes [18]. Membrane and cell surface binding of lectins induced by their carbohydrate-binding characteristics allow them to mediate diverse biological activities, encouraging their development as therapeutics because of their remarkable tissue accumulation [19, 20].

Moringa oleifera Lam (Family: *Moringaceae*), is a primordial northeastern India fast-growing softwood tree distributed worldwide and possesses diverse pharmacological potentials [21]. *M. oleifera* is a well-known plant in Nigeria, where it is being exploited for different purposes [22, 23]. Isolation of lectins has been reported from distinct parts of the *M. oleifera* plant, with diverse activities attributed to these quintessential proteins [24-26]. This study, therefore, investigated the hemolytic (membrane-perturbing) activity of a purified lectin from *M. oleifera* seeds, which could be exploited as a protein-based intracellular drug delivery agent.

2. Materials and methods

2.1. Materials and chemicals

Glutaraldehyde, sodium dodecyl sulfate, sugars were purchased from England (BDH Chemical Limited, Poole). Acrylamide, α-chymotrypsinogen, bovine serum albumin (BSA), egg albumin, and standard marker protein are products of Sigma-Aldrich, St Louis, Missouri, USA. Sephadex G-100, Biogel P-100, and Blue dextran were purchased from Sweden (Pharmacia Fine Chemicals, Uppsala). The quality of other used reagents and chemicals was of research-grade.

A, B, O human blood groups were collected with informed consent from healthy subjects. Rabbit erythrocytes were sourced from the blood of rabbits, reared at the College of Health Sciences, Obafemi Awolowo University, Ile–Ife.

Fresh bovine blood collected in a 10% EDTA (w/v) bottle from a municipal abattoir within Ile–Ife was the source of bovine erythrocytes.

2.2. Purification of M. oleifera lectin (MoL)

Extraction of *M. oleifera* crude protein was according to our published report on *Tetracarpidium conophorum* seed lectin [27]. Anhydrous ammonium sulfate (60% saturation) was used to precipitate the crude protein and dialyzed to remove excess ammonium sulfate. Size exclusion chromatography using Sephadex G-100 matrix equilibrated with Phosphate Buffered Saline (PBS, pH 7.2) was used to purify dialysate. Equilibration buffer was used to elute layered protein (≈20 mg), with fractions (4 ml) being collected at a 30 ml/h flow rate. Fractions were monitored at 280nm and hemagglutinating activity assayed. Active fractions were collected, dialyzed, and stored frozen at -20°C. Protein concentration was estimated using BSA as a standard [28].

2.3. Isolation and fixation of erythrocytes

Erythrocytes were isolated and fixed as described by Bing et al. [29]. Briefly, sample blood types were collected in anticoagulant bottles (Lithium heparin) and centrifuged (15 mins at 3,000 rpm). Plasma was removed, and erythrocytes rinsed (four times) with PBS (pH 7.2). Fixing solution (1% v/v glutaraldehyde-Phosphate Buffered Saline) from a 25% glutaraldehyde stock was prepared and chilled at 4°C. The resulting reagent was used to dilute erythrocytes (2% v/v) and incubated at 4°C for 60 min, with intermittent mixing. Erythrocytes were centrifuged and washed as described above. Erythrocytes suspension (2% v/v) were prepared in Phosphate Buffered Saline, pH 7.2 (containing 0.01% w/v sodium azide).

2.4. Hemagglutination and sugar specificity assays

This was performed in a 96-well U-shaped microtitre plate according to the assay experiment of Wang et al. [30]. Typically, Phosphate Buffered Saline (100 µl) was pipetted into each well in a succession order. An aliquot of MoL (100 µl) was introduced into the first well and serially diluted. Fixed erythrocytes suspension (50 µl) was introduced into the wells and plates left placidly for 2 h at 25°C. Hemagglutination titre value was expressed as the reciprocal of highest MoL dilution inducing hemagglutination, while specific activity is expressed as (HU)/mg, which is the amount of hemagglutination unit per expressed protein (mg).

Sugar specificity assay also used a two-fold serial dilution of MoL as described above. Aliquots (50 µl) of the tested sugar solutions (0.2M stock) were introduced into the individual well and left placid for 30 min at 25°C. Erythrocytes suspension (50 µl) was introduced into the respective wells, and hemagglutinating activity was assayed. Reactive sugars decrease MoL activity compared to control (containing Phosphate Buffer Saline and devoid of sugars). Tested sugars were sorbose, galactose, lactose, mannose, fructose, sucrose, sorbitol, glucose, glucosamine-HCl, and mannitol.

2.5. Determination of molecular weight

Denaturing gel electrophoresis was used to estimate the subunit molecular weight of MoL on a 10% acrylamide gel (phosphate gel system) using standard marker proteins of 14.2–66 kDa range, as described by Weber and Osborn [31].

Estimation of the native molecular weight of MoL was by gel filtration chromatography using a Biogel P-100 matrix. In plotting the molecular weight calibration curve, used protein markers were; bovine serum albumin (66 kDa), egg albumin (45 kDa), and α- chymotrypsinogen (25 kDa). Aliquots (5 ml) of the respective proteins were layered independently on the column, eluted at 10 ml/h flow rate using phosphate buffer (10 mM, pH 7.2), and collected fractions (5 ml) monitored for respective proteins at 280nm. Column void volume (Vo) was estimated with blue dextran and elution monitored at 620nm.

2.6. Detection of covalently bound carbohydrate

The assay method of Sun et al. [32] was employed. Briefly, MoL was electrophoresed as detailed by Weber and Osborn [31], and gel fixed at 25°C in 7.5% acetic acid for 60 min. Positive and negative controls used were ovalbumin (glycoprotein) and bovine serum albumin, respectively. Gels were transferred into aqueous periodic acid (0.2%), kept at 4°C for 45 min, and subsequently stained with Schiff's reagent at 4°C for 45 min. Destaining of the gel was done in a 10% acetic acid solution. Glycoprotein bands (if present) stained purplish red, otherwise shows a complete absence of covalently bound carbohydrate.

2.7. Effect of temperature on MoL

An aliquot of MoL was subjected to temperature variation (30–90°C) for 30 min using a water bath [33]. Samples were swiftly iced-cooled, and activity assayed. The activity of MoL at 25°C serves as the control.

2.8. Effect of pH on MoL

An aliquot of MoL was incubated for 1 h with varying buffers of pH 2–13 range (0.2 M Sodium–acetate, pH 2–6; 0.2 M Tris–hydrochloride, pH 7–9; and 0.2 M Glycine–sodium hydroxide, pH 10–11). Control was MoL incubated in Phosphate Buffered Saline, pH 7.2, and results presented as described by Nagakawa et al. [34].

2.9. Effect of chemical denaturants on MoL

Guanidine-Hydrochloride and urea were used to investigate the effect of denaturing agents. An aliquot of MoL (100 µl) was incubated with the equivalent quantity of each denaturing agent (0.5–8.0 M in PBS) at 37°C for 1 h, and hemagglutinating activity assayed. Control was MoL in Phosphate Buffered Saline (pH 7.2) [35].

2.10. Effect of EDTA on MoL

In this assay, an aliquot of MoL was dialyzed against 10 mM ethylene diamine tetraacetic acid (EDTA) for 24 h according to the method of Wang et al. [36], and hemagglutinating activity assayed.

2.11. Determination of metal ions

Presence of Ca^{2+}, Na^{+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+} and Cr^{2+} was investigated by atomic absorption spectroscopy (400, 2010 model). Digestion of MoL was carried out for 30 min with concentrated nitric acid and perchloric acid (1:1 v/v). The resulting digest was thoroughly dialyzed and analyzed using acetylene flame. Standard solutions were prepared using stock solutions of the screened metals.

2.12. Modification of specific amino acid residues

Specific amino acid residues of MoL were modified as described in our previous report on *Dioscorea mangenotiana* lectin [37]. Typically, N-bromosuccinimide (NBS) was used to modify tryptophan residues. Titration of aliquot of MoL (100 µl, 1 mg/ml) in 0.01 M sodium acetate buffer (pH 5.0) against 100 µl 0.01 M NBS was done at 20°C, and activity assayed [38]. Control was MoL without NBS.

For arginine residues, 100 µl MoL (1 mg/ml) in PBS (pH 7.5) was incubated at 25°C for 1 h with 0.01 M phenylglyoxal (10 µl in 0.1 M sodium carbonate, pH 8.0), and hemagglutinating activity assayed. Control was MoL without the modifying reagent, and the percentage residual activity was determined [39].

MoL cysteine residues were modified by incubating 100 µl MoL in 0.05 M phosphate buffer (pH 8.0) with 10 µl 0.1 mM 5,5'-dithiobis- (2-nitrobenzoic acid) (DTNB) at 27°C for 1 h. Residual activity was investigated and the percentage residual hemagglutinating activity was determined.

Phenylmethylsulfonyl fluoride was employed in serine residues modification. An aliquot of MoL (1 mg/ml) in 0.05 M Tris-HCl buffer (100 µl, pH 7.4) was incubated at 25°C for 1 h with phenylmethylsulfonyl fluoride (5 mM). Extensive dialysis was used to remove surplus PMSF, and the percentage residual hemagglutinating activity was determined [39].

2.13. Hemolytic activity

Hemolytic activity was investigated using the assay method of Evans et al. [40]. Briefly, suspension of PBS-washed bovine erythrocytes (4% v/v containing 0.001% sodium azide) in 0.1 M PBS (pH 7.2, 1:9 v/v) containing 10 mM $CaCl_2$ was prepared. Aliquots of MoL (50 µl) were incubated with 950 µl bovine erythrocytes (1:20 v/v) and centrifuged (2000 rpm for 5 min). Absorbance of supernatant (erythrocyte lysate) was read using a microplate reader at 540nm. Erythrocytes incubated with PBS (pH 7.2) served as the control, and percentage hemolysis calculated as described by Taniyama et al. [41].

2.13.1. Effect of varying MoL concentrations on hemolysis

Aliquots (200 µl) of 10% suspension of bovine erythrocytes were incubated at 37°C with varying MoL concentrations (1, 5, 20, and 80 µ5xg/ml) for 4 h. Centrifugation of samples was done, and lysates of the erythrocytes were analyzed at 540nm.

Table 1
Hapten Inhibition of MoL

Sugar	*Hemagglutinating titre*
Control	2^9
Sorbose	2^9
Galactose	2^9
Lactose	2^9
Mannose	2^9
Sucrose	$\mathbf{2^5}$
Fructose	$\mathbf{2^5}$
Sorbitol	2^9
Glucose	2^9
Glucosamine-HCl	2^9
N-acetylglucosamine	2^9
Mannitol	2^9

Experiments comprised MoL (100 µl) diluted serially in a 96-well microtitre plate. Equal volumes (50 µl) of respective sugar solutions (0.2 M) and 2% human blood group O erythrocytes suspension were introduced to the wells. Positive control contained no sugars, and negative control contained neither MoL nor sugars. All experiments were carried out in triplicates.

2.13.2. Effect of varying temperature on MoL-induced hemolysis

Aliquots of MoL (80 µg/ml) were incubated with a 10% suspension of bovine erythrocytes (200 µl) at varying temperatures (10–60°C) for 4 h. Centrifugation of samples was done and lysates of the erythrocytes were analyzed at 540nm

2.13.3. Effect of varying pH on MoL-induced hemolysis

Aliquots of MoL (80 µg/ml) and a 10% suspension of bovine erythrocyte (200 µl) were incubated at 37°C with buffers of varying pH using 100 mM sodium acetate buffer (pH 3.5–5.5), 100 mM sodium phosphate buffer (pH 6.5–7.5), 100 mM Tris–hydrochloride buffer (pH 8.5–9.5) and 100 mM glycine–sodium hydroxide buffer (pH 10.5–11.5) for 4 h. Centrifugation of samples was done and lysates of the erythrocytes were analyzed at 540nm.

2.13.4. Effect of incubation period on MoL-induced hemolysis

A 10% suspension of bovine erythrocytes (200 µl) was incubated with MoL (80 µg/ml) at a time ranging from 0.5–5.0 h. Incubation was carried out at 37°C. Centrifugation of samples was done, and lysates of the erythrocytes were analyzed at 540nm.

2.13.5. Effect of sugars on MoL-induced hemolysis

MoL (80 µg/ml) was incubated with 0.2 M stock solution of galactose, glucose, fructose, sucrose, and lactose respectively at 25°C for 2 h, and subsequently with 10% freshly prepared bovine erythrocytes suspension (200 µl) for a period of 4 h with gentle shaking at 30 mins interval. Centrifugation of samples was done, and lysates of the erythrocytes were analyzed at 540nm. All investigations were done in triplicates.

2.14. Statistical analysis

Data obtained were expressed as mean ± SEM (standard error of mean) of three determinations, and results were subjected to paired t-test using SPSS Statistics 17.0 (2008) software, with < 0.05 p-value considered significant.

3. Results and discussion

3.1. Hemagglutinating activity, sugar specificity, and MoL purification

M. oleifera is a versatile plant enriched with diverse biotechnological relevant compounds, including lectins with distinct applications [42-44]. *M. oleifera* seed lectin (MoL) induced agglutination of the human blood group (A, B, O), bovine, and rabbit erythrocytes, thus,

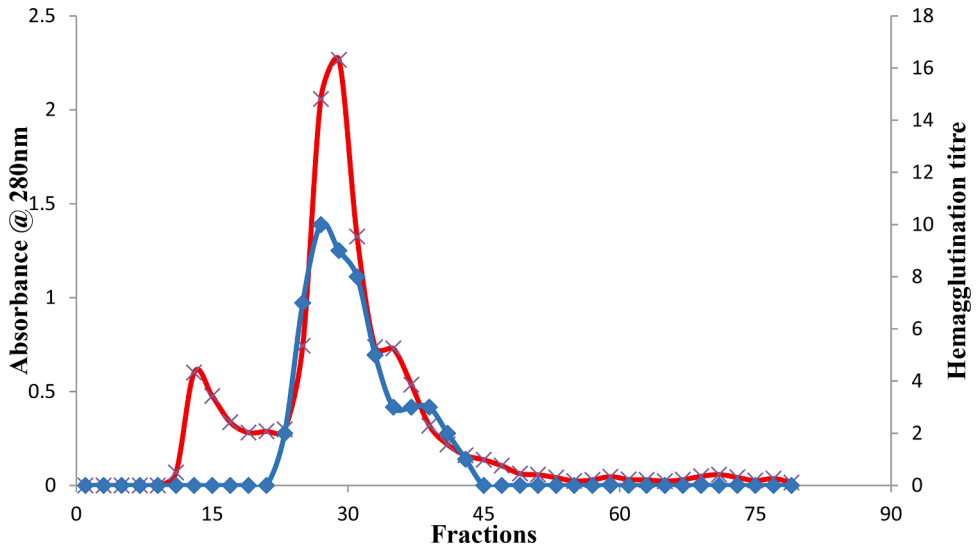

Fig. 1. Size exclusion chromatography of *M. oleifera* ammonium sulfate dialysate. Obtained dialysate (≈20 mg protein) was loaded on the chromatographic column (2.5 × 40 cm) packed with Sephadex G-100 matrix, pre-equilibrated with 10 mM Phosphate Buffered Saline, pH 7.2, and eluted at 30 ml/h flow rate with the same buffer. Approximately 4 ml fractions were collected, with elution monitored at 280nm and hemagglutinating activity assayed.

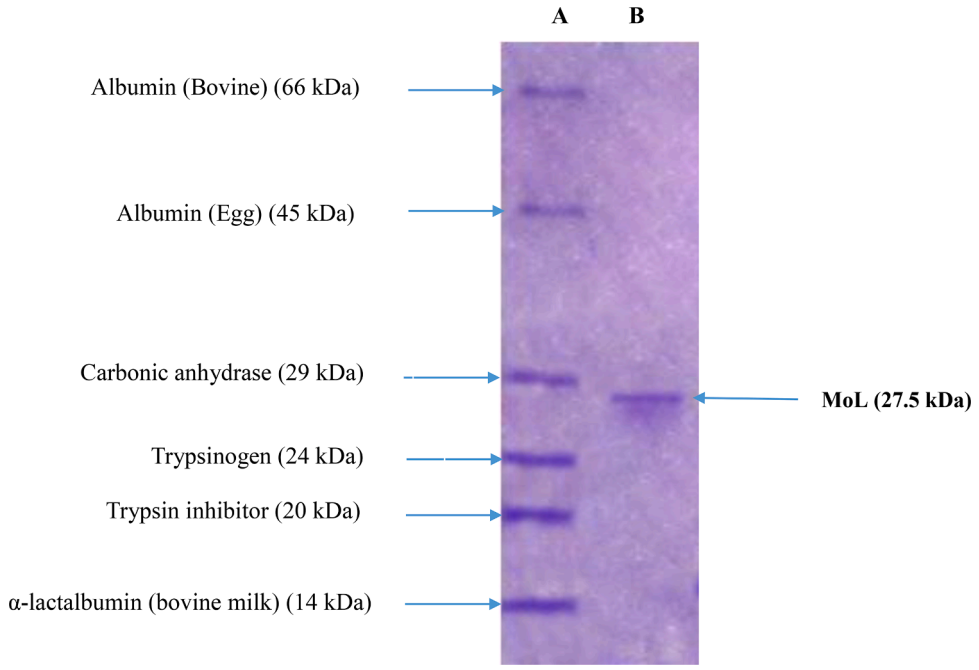

Fig. 2. Electrophoretogram of SDS-Polyacrylamide Gel Electrophoresis of *M. oleifera* seed lectin. Molecular weight standard markers (Lane A) and pooled gel filtration active peak (Lane B). Protein aliquots (≈10 μg) were electrophoresed, and gel stained using coomassie brilliant blue.

could belong to the lectins categorized as pan-agglutinins (non-specific agglutinins). Similar hemagglutinating characteristics have been reported for the anticancer *M. oleifera* coagulant lectin (cMoL) [24, 45], *Dioclea reflexa* lectin [46] and *Tetracarpidium conophorum* lectin [27]. Hapten inhibition test showed ketose sugars (sucrose and fructose) slightly inhibited MoL hemagglutinating activity, while lactose, mannitol, maltose, glucose, mannose, sorbose, N-acetyl-D-glucosamine, galactose, and glucosamine-HCl had a negligible effect (**Table 1**). Fructose specificity has also been reported for the water-soluble *M. oleifera* lectin (WSMoL), which to a greater extent influence the biological activity of the lectin [47, 48], as against the inhibition of the hemolytic lectin from *Sterculia foetida* (SFL) by xylose and arabinose [49].

Application of plant lectins as a distinct source of emerging biopharmaceutical products is placing prominence on the development, optimization, and effective purification of these versatile proteins [50]. Protein purification is often tasking, time-consuming and sometimes result in the generation of unexpected results, however, attempts are being made at simplifying specific purification process taking consideration of yield, purity and biological activity of the targeted protein [51]. In recent times, diverse chromatographic techniques are being used in the purification of lectins, exploiting the size, charge, or ligand affinity of the lectin [52-55]. MoL was purified to homogeneity via a simple and efficient two-step purification process involving ammonium sulfate precipitation and size exclusion chromatography (**Fig. 1**) as confirmed by SDS-PAGE. This purification procedure was quite different

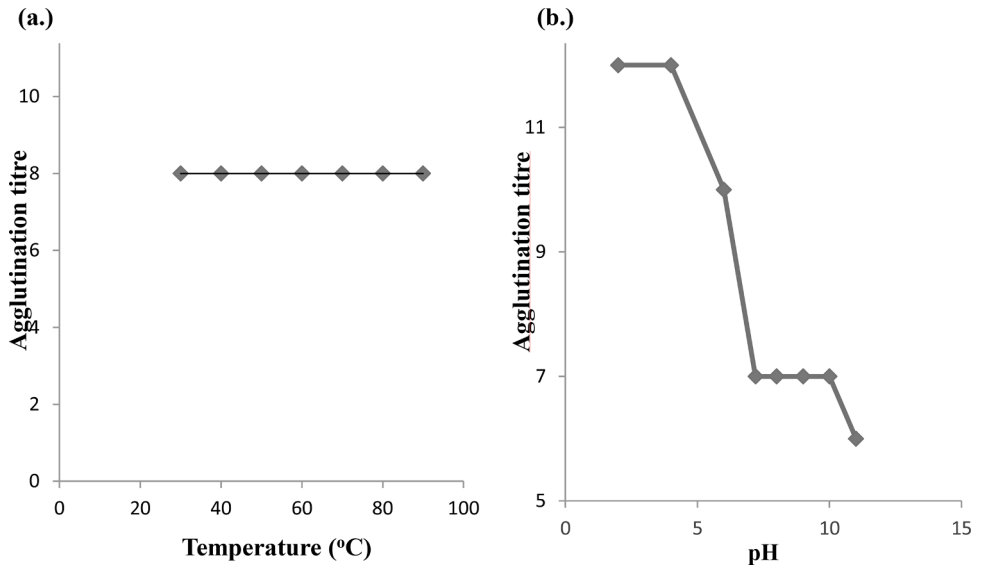

Fig. 3. Effects of temperature (a) and pH (b) on MoL activity

from that of cMoL and WSMoL which was achieved via guar gel chromatography [24] and chitin chromatography [56] respectively.

3.2. Molecular weight, stability, and MoL-bound carbohydrate

The efficacy of drug-delivery agents is largely dependent on their physicochemical properties [57]. Gel filtration on Biogel P-100 matrix and SDS-PAGE analysis of MoL estimated its native and sub-unit molecular weight as 30.3 kDa and 27.5 kDa respectively (**Fig. 2**) hinting at a monomeric arrangement for the MoL. Native and sub-unit molecular weight obtained in this study was closely similar to 30 kDa and 26.5 kDa reported for cMoL [24]. On the contrary, a homodimeric 14 kDa lectin has also been detected from *M. oleifera* seeds [58].

The hemagglutinating activity of MoL was thermostable up to 90°C (**Fig. 3a**). Obtained result was similar to the report on *Holothuria grisea* lectin (HGL) [59], cMoL [24], and *Sterculia foetida* hemolytic lectin [49]. Hemagglutinating activity of MoL was stable within pH 2–4, however, above pH 4, the activity decreased rapidly and was lost at pH 11 (**Fig. 3b**). The pH stability of MoL was quite different from that reported for cMoL and WSMoL which were stable within pH range of 4-9 [24] and 4.5 [47] respectively. Reaction of MoL with Schiff's reagent showed the lectin was not a glycoprotein which supports earlier study that not all lectins are glycoproteins [46, 60].

3.3. Metal chelating, metal content, denaturing agents, and chemical modification

EDTA had a negligible chelating ability on the hemagglutinating activity of MoL, as there was activity after exhaustive dialysis with 10 mM EDTA suggesting these metals might tightly bind to MoL, which hinders their dissociation by mere dialysis, or none of the investigated metal ions were required for its activity. Atomic absorption spectroscopy however showed traces of Ca^{2+}, Mg^{2+}, and Na^{+} in MoL, which could be a case of adventitious metal binding. Traces of detected Ca^{2+} ions in MoL is not surprising, as a similar result was reported for the calcium-dependent hemolytic lectins purified from *Cucumaria echinata* (CEL-III) [61] and *Anadara granosa* (AGL-III) [62].

Hemagglutinating activity of MoL was reduced by urea and guanidine-HCl with residual activity of 43% and 29% respectively, suggesting destabilization of intermolecular bonds and interactions stabilizing MoL as treatment of protein with urea results in the expansion of its hydrophobic core followed by solvation [63]. Denaturation by these agents mostly involves binding the protein directly or exposing its core hydrophobic residues via alteration of the solvent environment, favoring protein unfolding by promoting denatured state stability [64].

Table 2
Effect of Chemical Modification on Hemagglutinating Activity of MoL

Reagents	Amino Acid Modified	Residual Hemagglutinating Activity (%)
Phenyl methyl sulfonyl fluoride (PMSF)	Serine	100
N-Bromosuccinimide (NBS)	Tryptophan	33
5,5′ Dithiobis-(2-nitrobenzoic acid DTNB)	Cysteine	83
Phenylglyoxal	Arginine	67

Relevance of specific amino acid residues in unique protein interactions to the structure-function relationship has been investigated exhaustively via chemical modification [65]. Modification of cysteine and serine residues with DTNB and PMSF respectively showed mild or no change in MoL hemagglutinating activity, suggesting neither of these amino acids was engaged in direct interaction with the sugar-binding pocket of MoL, thus may not have contributed to the hemagglutinating activity. However, upon modification with phenylglyoxal, the percentage of residual hemagglutinating activity was 67% (**Table 2**) showing arginine's involvement in the hemagglutinating activity. This result is similar to the previous study on two galectins isolated from skin mucus of *Conger myriaster* (congerin I and II) that lost their hemagglutinating activity when arginine residues were modified with 1, 2 -cylco-hexanedione [66]. Modification with N-bromosuccinimide (NBS), which induced oxidation of the indole group of tryptophan [65] led to an astonishing decline in the hemagglutinating activity of MoL, suggesting tryptophan was most likely at the carbohydrate-binding domain (CBD) or directly functioning in stabilizing the active site of the lectin. This result is similar to a previous study on *Datura innoxia* seed lectin (*Di*L9) lectin, which lost activity on treatment with NBS [67].

3.4. Hemolytic activity

Erythrocytes are a simple imitation of the endosomal membrane, therefore are a model to study protein-membrane interactions and *in vitro* biocompatibility assessment of vital biotherapeutics [68, 69]. Intravenously administered therapeutic agent can trigger hemolysis which is characterized by the release of hemoglobin into the plasma, induced by disruption, perturbation, or breakdown of the red blood cell

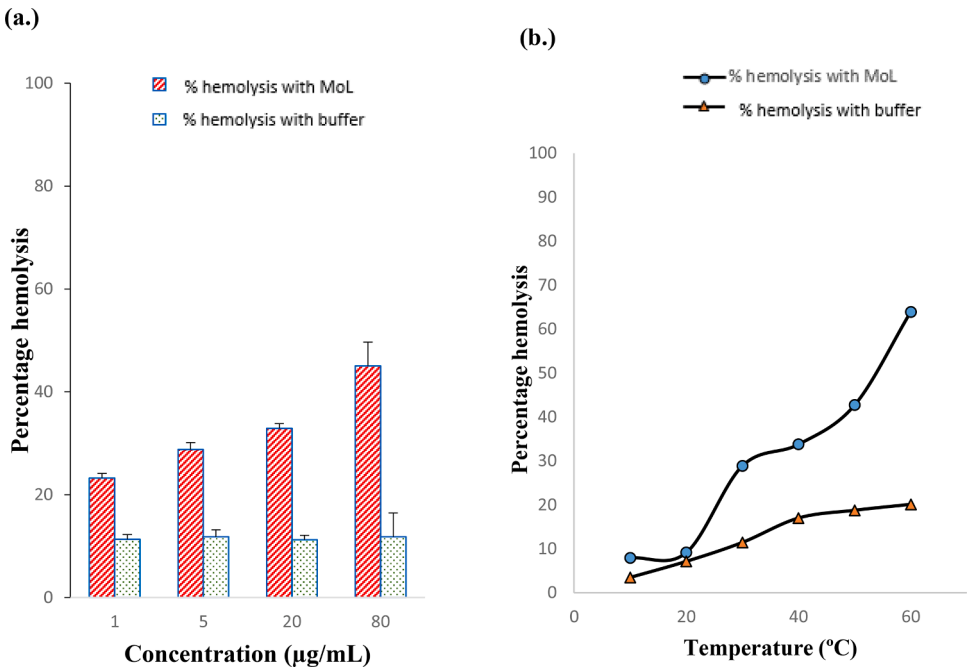

Fig. 4. Effect of varying concentration (a) and temperature (b) of MoL on hemolytic activity

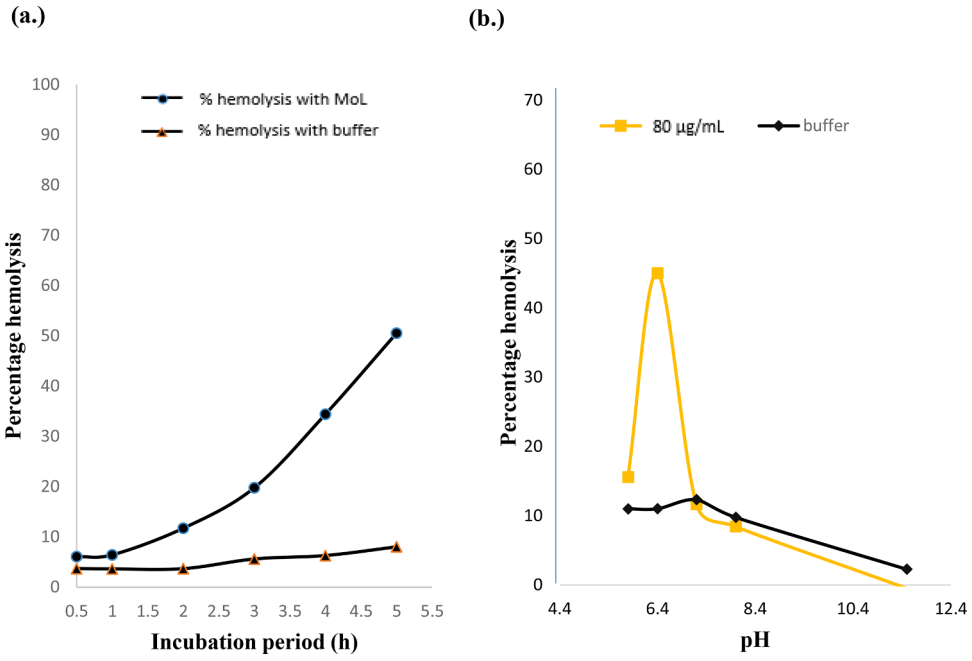

Fig. 5. Effect of varying incubation time (a) and pH (b) of MoL on hemolytic activity

membrane [70, 71], making research into specific proteins as carrier molecules imperative [72, 73]. Cytocompatibility and endosomolytic activity of MoL investigated via hemolytic assay on bovine erythrocytes revealed a concentration-dependent increase in hemolysis. At 80 µg/ml, MoL showed 45% hemolysis compared to the control (11%) **(Fig. 4a).** Obtained result was similar to the hemolytic characteristics of SFL which was reportedly concentration-dependent [49]. Varying the incubation temperature from 10°C to 60°C, MoL showed a temperature-dependent increase in percentage hemolysis. Significant difference ($p < 0.05$) exists in the hemolysis degree observed in MoL-treated erythrocytes (64%) relative to the control (21%), even at 60°C **(Fig. 4b).** This result is similar to what was obtained by Ashraf et al., [74] for galectin isolated from the goat heart muscle.

There was an upsurge in hemolysis of MoL-treated erythrocytes as the incubation period increased. Prolonged incubation above 5 h showed significant ($p < 0.05$) hemolysis (51%) compared to the control (9%) **(Fig. 5a).**

To imitate the extracellular, early endosomal, and late endo-lysosomal environments, MoL, and bovine erythrocytes were incubated together in different buffers at a specific pH range similar to the pH chemistry of these microenvironments. The optimum pH for MoL hemolytic activity was pH 6.4 **(Fig. 5b),** which corresponds to the early endosomal pH range (pH 5.5 – 6.5), as effective cytocompatible candidates for intracellular delivery of therapeutic agents induce hemolysis at

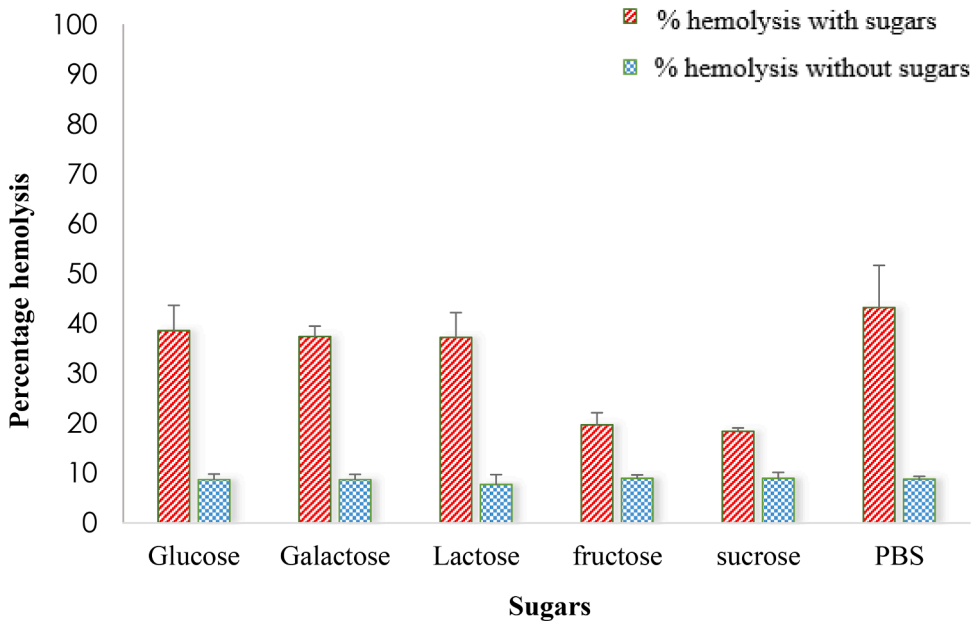

Fig. 6. Effect of Sugars on the hemolytic activity of MoL

pH < 6.5, and have transitional pH nearer to the endosomal pH [40, 75], suggesting the lectin might only perturb the endo-lysosomal membrane at its early phase. Specific drug carrier molecules are engineered to leverage the acidic pH of the endosome, which enhances the activity of the localized enzymes to promote efficient intracellular cargo delivery [40, 76]. The hemolytic activity of MoL declined abruptly at pH 7.2, probably the result of an alteration in MoL ionization state suggesting a weak interaction between MoL and carbohydrate constituents of the erythrocytes membrane. Obtained result supports previous findings of Evans et al. [40].

A significant difference ($p<0.05$) exists in hemolysis of MoL-treated erythrocytes relative to those pre-incubated with fructose and sucrose **(Fig. 6).** Sucrose showed a stronger inhibitory activity which may suggest the lectin possesses an extended binding site. Also, a decrease in the hemolytic activity of MoL in the presence of fructose and sucrose further confirms the fact earlier established that MoL has an affinity for ketose sugars, thus causing an inhibition of the hemolytic activity of MoL. To achieve improved efficiency and minimize the accompanying side effects of specific therapeutic agents, a protein-associated targeted drug delivery system is thus emerging as a propitious strategy [77-79].

4. Conclusion

The purified *M. oleifera* seed lectin is highly thermostable and showed impressive hemolytic (membrane-perturbing) activity in mildly acidic conditions. Further research on the mechanism of the hemolytic activity and structure of the lectin would however need to be ascertained, for possible exploitation of this lectin in improved targeted delivery of therapeutic agents.

Credit authorship contribution statement

NUBI Tolulope: Investigation, Data curation & Formal analysis. **ADEWOLE Scholes Taiwo**: Methodology, Data curation, Writing - review & editing. **AGUNBIADE Titilayo Oluwaseun:** Data curation, Review & editing. **OSUKOYA Olukemi Adetutu:** Methodology, Data curation, **KUKU Adenike:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

Declarations of Competing interest

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