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Detection of water soluble lectin and antioxidant component from *Moringa oleifera* seeds

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Abstract

Seed flour from *Moringa oleifera* is widely used as a natural coagulant for water treatment in developing countries. Extracts obtained by water soaking of *M. oleifera* intact seeds were investigated for the presence of lectin, trypsin inhibitor, tannin as well as antioxidant activity. A water soluble *M. oleifera* lectin (WSMoL) detected was mainly active with rabbit cells at pH 4.5; heat treatment, pH 7.0, fructose and porcine thyroglobulin abolished HA of WSMoL. Trypsin inhibitor or tannins were not detected; the antioxidant component (WSMoAC) reduced 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was slower than catechin and was thermostable. The extracts showed a primary glycopolypeptide band of Mw 20,000; the main native acidic protein showed hemagglutinating activity. WSMoL may be involved in seed coagulant properties.
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Keywords: *Moringa oleifera*; Seeds; Lectin; Trypsin inhibitor; Tannin; Antioxidant activity

1. Introduction

The production of potable water involves coagulant use at a flocculation/coagulation stage to remove turbidity in the form of suspended and colloidal material (Diez et al., 2002); coagulants from *Moringa oleifera* seeds (Moringaceae family) have been recommended for water treatment in African and South Asian countries (Okuda et al., 1999). Natural biodegradable coagulants offer advantages to inorganic or synthetic organic polymers which are associated with human pathological processes (Okuda et al., 2001).

Food antioxidants are important for human nutrition by decreasing the oxidative damage to lipids, proteins and nucleic acids induced by free radicals (Soler-Rivas et al., 2000); however, some water soluble seed compounds are antinutritional factors (Hossain and Becker, 2002). Lectins constitute a heterogeneous protein group of non-immune origin with non-catalytic sites capable of recognizing and reversibly binding to cell surface carbohydrates. Lectins interact with glycoproteins in the mucosa of the intestinal wall and interfere with nutrient digestion and absorption resulting in reduced efficiency of nutrient utilization; also, reduction of amylase activity was demonstrated by interaction of lectin with either the enzyme itself or its substrate (Fish and Thompson, 1991). Long-term study of low lectin doses showed rat pancreas growth in vivo (Kelsall et al., 2002). Protease inhibitors disrupt enzymatic action by formation of stable complexes with trypsin and/or chymotrypsin. Inhibitors have been implicated in reducing protein digestibility in non-ruminants by inhibition of pancreatic proteolytic enzymes. Pancreas hypertrophy (Hanbury et al., 2000) and depression of animal growth rate (Vasconcelos et al., 2001) have been

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associated to trypsin inhibitor in animal diet. Tannins are non-toxic polyphenols and poorly biodegradable, which inhibit growth of fungi, yeasts, bacteria and viruses; they form complexes with proteins which are frequently indigestible (Chung et al., 1998).

Evaluation of biological activity in water is an indication of treatment efficiency used for this purpose (Lindström-Seppä et al., 1998). In this work, lectin activity, trypsin inhibitors, tannins and antioxidant activity were investigated on water obtained after immersion of *M. oleifera* intact seeds.

2. Materials and methods

2.1. M. oleifera extract preparations

Mature seeds of *M. oleifera* were collected in Fortaleza city, State of Ceará, Brazil Northeast in dry season and stored at −20 °C. Three extracts (7.0 g l^{-1}) were prepared by soaking intact *M. oleifera* (Lam.) seeds, without coat removal, in distilled water. The extracts were obtained after 5 (T_5), 15 (T_{15}) and 37 h (T_{37}) at 28 °C, by collecting the supernatant waters and storing at −20 °C. Additionally, seed flour (7.0 g l^{-1}) was mixed with water by 5 h; the obtained water was filtered on paper (Toyo-Filter paper, Toyo Rashi Co., Japan) and used (T_5 flour) for lectin activity and protein determination.

2.2. Protein evaluation

The protein concentration was estimated according to Lowry et al. (1951) using bovine serum albumin (31–500 μg ml^{-1}) as standard; absorbance at 280 nm was also measured.

2.3. Hemagglutinating activity (HA)

Lectin activities of non-treated and heated (100 °C, 15 min) extracts were evaluated according to Correia and Coelho (1995) using glutaraldehyde-treated rabbit or human erythrocytes. The hemagglutinating activity was obtained by mixing a twofold serial dilution of the extracts (50 μl) in 0.15 M NaCl (pH 5.0) followed by the addition of a 2.5% (v/v) suspension of erythrocytes (50 μl), in microtiter plates (Kartell S.P.A., Italy). Titer was defined as the lowest sample concentration after 45 min which showed hemagglutination. A hemagglutinating inhibitory assay was performed by incubation (45 min) of T_5 with 200 mM monosaccharide solutions (D(+)-fructose and D(+)-glucose), trisaccharide (D(+)-raffinose) and porcine thyroglobulin glycoprotein (0.5 mg ml^{-1}), before rabbit erythrocyte suspension addition. The pH effect on HA, 10 mM citrate phosphate buffer containing 0.15 M NaCl (pH 4.5–6.0) and 10 mM sodium phosphate buffer containing 0.15 M NaCl (pH 6.5–7.5), were evaluated.

2.4. Trypsin inhibitory activity

The assay (Kakade et al., 1969) was made with bovine trypsin (10 μg ml^{-1} in 0.1 M Tris-HCl, pH 8.0; 20 μl); pre-incubation (37 °C, 10 min) of enzyme and extracts (30, 40 and 60 μl) was followed by addition (30 μl) of 4 mM *N*-α-benzoyl-DL-arginyl-ρ-nitroanilide (BAPA) dissolved in dimethyl sulfoxide and diluted with tris buffer. After 30 min at 37 °C, 10% (v/v) acetic acid was added (300 μl). The substrate hydrolysis was followed by measurement of absorbance at 405 nm, and the molar extinction coefficient for ρ-nitroanilide used for concentration calculation was 9100 (Oliva et al., 2000). The inhibitory activity evaluated the remaining hydrolytic activity towards BAPA.

2.5. Tannin assay

Tannin was detected with acidified vanillin reaction specific for estimating tannin content (Burns, 1971). The assay used 1 ml of methanolic solutions (2% v/v) of T_5 (1.42 mg), T_{15} (3.12 mg), and T_{37} (4.16 mg), as well as dilutions of catechin solution (100 mg 50 ml^{-1} of methanol) to obtain standard curve (0.078–1 mg).

2.6. Antioxidant activity

The free radical-scavenger capacity of non-treated and heated (100 °C, 15 min) aqueous extracts (50 mg ml^{-1}, 2 μl) and catechin standard (5 mg ml^{-1} of methanol, 2 μl) were determined according to Soler-Rivas et al. (2000) using dot-blots on thin layer chromatography (TLC) stained with a 0.4 mM 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) solution. The dilutions of applied samples were 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256. Positive results were observed on silica sheet which was yellow in color.

2.7. Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) for acidic and basic proteins were made according to Davis (1964) and Reisfeld et al. (1962), respectively. Also, detected protein bands were extracted by cutting the gel, followed by maceration in 0.15 M NaCl and HA evaluation. Denatured electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) and stained to both protein using Coomassie Brilliant Blue (Laemmli, 1970) and glycoprotein with Schiff's Reagent (Pharmacia Fine Chemicals, 1980).

3. Results and discussion

Water treatment by coagulation using flour of *M. oleifera* seeds is common before human consumption. Treated water has been analyzed in relation to pH, conductivity, alkalinity and sulphate concentration (Ndabigengesere and Narasiah, 1998); antibiotic activity was also detected in seed flour extracts (Eilert et al., 1981; Cáceres et al., 1991). Protein isolated from *M. oleifera* seeds showed a characteristic flocculating property (Gassenschmidt et al., 1995).

Tropical seeds often have a high content of antinutritional factors (Hossain and Becker, 2002). In this paper, it was investigated whether water obtained after soaking *M. oleifera* intact seeds contained activities associated with lectin, trypsin inhibitor, tannin or antioxidant compound.

T_5, T_{15}, T_{37} and T_5 flour agglutinated rabbit cells (Table 1); the extracts were clear except for T_5 flour that showed high turbidity. In fact the extract was prepared in distilled water. Seed flour of *M. oleifera* is commonly used to treat water with impurities in suspension, which could induce protein aggregation and precipitation resulting in clear water. To avoid additional methodology for turbidity elimination, only T_5, T_{15} and T_{37} were evaluated. The water soluble *M. oleifera* lectin (WSMoL) showed high solubility (5, 15 and 37 h) and room temperature did not abolish its activity; evaluation of T_5 HA using human erythrocytes also revealed activity with A^+ (16^{-1}) and B^+ (4^{-1}) cells. Other proteins were also solubilized (Table 1, Fig. 1). The different specific HA (SHA) obtained for T_5, T_{15} and T_{37} could reflect solubilization of proteins or compounds which interfere with hemagglutination/protein evaluations. The SHA of WSMoL (T_5) was decreased by carbohydrates and glycoprotein (Table 2); this result revealed the involvement of carbohydrate binding sites in the hemagglutinating activity (Kennedy et al., 1995). WSMoL binding sites, as artocarpin (Rani et al., 2000), interacted with *N*-linked oligosaccharide chain of glycoprotein. WSMoL of T_5 (most active preparation) was mainly active at pH 4.5, but its HA was abolished after treatment with sodium phosphate buffer, pH 7.0. At pH 7.0 the equilibrium of positive and negative charges of protein may lead to coalescence of molecules. HA of WSMoL (T_5, T_{15} and T_{37}) was abolished after heating at 100 °C. Protein denaturation by temperature and pH values has been associated to loss of lectin properties (Ng and Yu, 2001).

Table 1
Water soluble hemagglutinating activity from *M. oleifera* seeds

Sample	Titer $^{(-1)}$	Protein (mg ml^{-1})	SHA
T_5	64	71	0.90
T_{15}	16	156	0.10
T_{37}	32	208	0.15
T_5 flour	64	90	0.70

Hemagglutinating assay used rabbit erythrocytes. SHA, specific hemagglutinating activity (titer/protein).

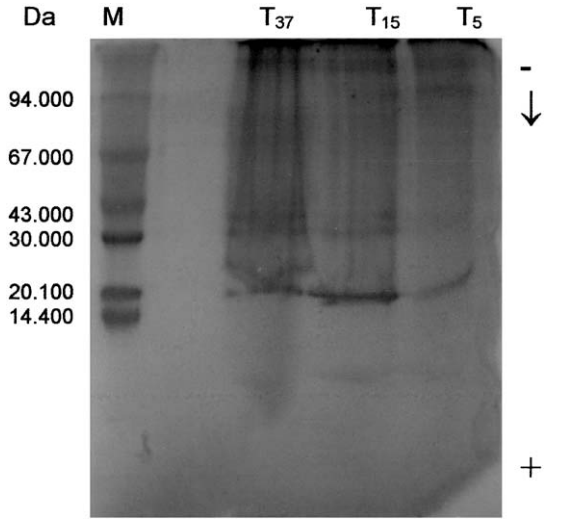


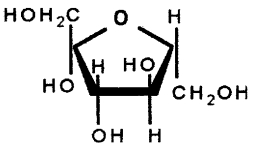
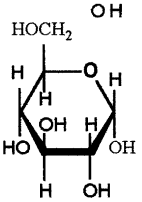
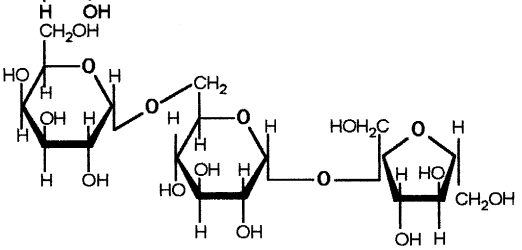
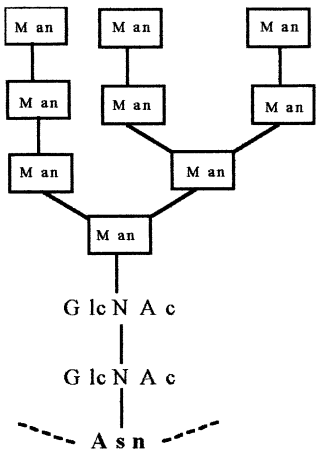
Fig. 1. Denatured PAGE (12%) of *M. oleifera* extract proteins. Molecular weight markers (M) and samples (T_{37}, T_{15} and T_5) containing 100 μg of protein were denatured by heating in the presence of SDS. Protein was stained with Coomassie Brilliant Blue.

When T_5 was submitted to PAGE for native acidic protein, a main band was detected, and extracted from gel (40 μg ml^{-1}) it showed a SHA of 100 (purification factor of 111). SDS-PAGE (Fig. 1) revealed extracts with different electrophoretic patterns and a main glycopolypeptide band of Mw 20,000. A polypeptide with apparent molecular weight of 6500 was also detected; Gassenschmidt et al. (1995) reported the isolation of a 6500 flocculant protein. Okuda et al. (1999) confirmed that the active component of *M. oleifera* coagulant was mainly protein; WSMoL may contribute to this phenomenon.

Hydrolytic activity of bovine trypsin towards BAPA was the same ($A_{405\,nm} = 0.462$) in the presence or absence of T_5, T_{15} and T_{37}; thus, no enzyme inhibition was obtained with *M. oleifera* extracts. Also, tannin was not detected in the preparations. Soaking intact seeds in water has been able to solubilize trypsin inhibitor (Wijaya et al., 2000) and tannin (Viswanathan et al., 1999); however, the protocol used in this paper was not efficient to extract these activities from *M. oleifera*.

Seeds of *M. oleifera* contained an antioxidant component (WSMoAC) detected similarly in T_5, T_{15} and T_{37} through a yellow color obtained by staining silica sheet plate (DPPH reduction, Fig. 2). WSMoAC was revealed after a slow reaction (5 min), in comparison

Table 2
Inhibition of WSMoL (T_5) HA

Inhibitor	SHA	Structure
Fructose	0.06	
Glucose	0.1	
Raffinose	0.1	
Oligosaccharide (thyroglobulin)	0	

Ha was performed with rabbit erythrocytes. SHA of T_5 HA at inhibitor absence was 0.90.

to standard catechin (yellow color immediately observed). WSMoAC activity was retained after heating at 100 °C by 15 min. Compounds with free radical scavenger capacity are able to reduce DPPH and both types and concentration of antioxidant in sample interfere in time of appearance and final intensity of yellow color (Soler-Rivas et al., 2000).

The component purity is essential to elucidate its structural nature and antioxidative mechanism (Tang et al., 2002); WSMoAC isolation is under development in our laboratories. Also, purified and immobilized WSMoL can be of value to isolate thyroglobulin.

4. Conclusions

The purpose of this study was the evaluation of different biological activities on water obtained after immersion of *M. oleifera* intact seeds.

1. The results showed that water solubilized WSMoL, an acidic protein with hemagglutinating activity, and WSMoAC, a component with antioxidant activity. The boiling of Moringa-treated water would destroy the lectin activity while retaining the (heat-stable)

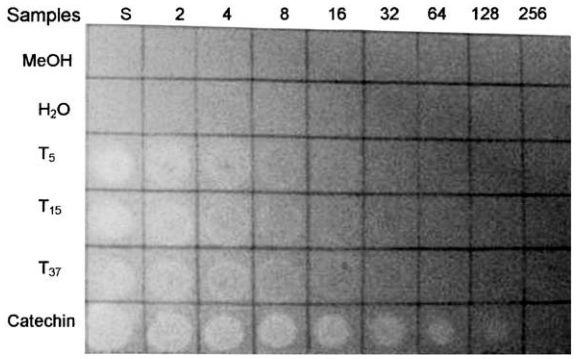


Fig. 2. Dot-blot test of free radical scavenger capacity on a silica sheet stained with a DPPH solution. The dilutions of samples were (from left to right): 1:2 (2), 1:4 (4), 1:8 (8), 1:16 (16); 1:32 (32), 1:64 (64), 1:128 (128) and 1:256 (256). Yellow spots revealed samples with antioxidant activity. MeOH, Methanol; S, undiluted samples.

antioxidant activity, and suggest the testing of health-promoting effects.

2. Trypsin inhibitor and tannin, antinutritional factors, were absent.

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