Partial Characterization and Hydrolysis Procedure of Water Soluble Polysaccharides Extracted from Onesaharian Medicinal Plant: *Malva aegyptiaca* L.

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**Abstract**—*Malva aegyptiaca* L. (Malvaceae), a spontaneous plant used as a traditional medicine in Ghardaïa (Septentrional Sahara Algerian). This paper reports the extraction and partial characterization of water-soluble polysaccharides from *M. aegyptiaca* leaves. These polysaccharides were obtained by elimination of the ethanol extract and sequential extraction in distilled water, followed by precipitation in 75% ethanol. The yield of extract is 1.46% (w/w). The crude water soluble polysaccharide extracts were further characterized and revealed the average values 17.14 ± 1.43% proteins and 78.18±2.04% carbohydrates, among them 30.68±0.42% are uronic acid and 47.49±1.62% are neutral monosaccharides. The acid hydrolysis was studied with trifluoroacetic acid (TFA) at two concentrations, with five durations of hydrolysis and at two temperatures. A single hydrolytic step with 4M TFA at 80°C for 4 h is suggested to be more effective in releasing monomers from polysaccharides than other hydrolysis procedures. The identification of monosaccharide composition by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) methods shows 56.86% of galactose, 8.46% of rhamnose, 9.04% of arabinose, 5.05% of mannose and 20.57% of glucuronic acid.

**Index Terms**—*Malva aegyptiaca*, traditional medicine, polysaccharides, HPAEC-PAD.

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**I. INTRODUCTION**

*Malva aegyptiaca* (Malvaceae) is a spontaneous plant used in traditional medicine prescriptions in Ghardaïa (Septentrional Sahara Algerian) [1]. The macerate offleaves is used in the treatment of dysentry, constipation and fevers. The cataplasm of leaves is also used in treating wounds, sores and skin-eruptions [2]. The Malvaceae family is characterized by the presence of mucilaginous cells that store polysaccharides, allowing the retention of large amounts of water [3]. In recent years, plant producing polysaccharides have been widely studied in order to understand the relationship between physicochemical characteristics and biological activity. Most of the polysaccharides isolated from medicinal plants, have additional non structural activities, such as pectin which possess immunomodulatory, complement-modulating, anti-HSV (Herpes simplex virus) and anti-inflammatory activities [4]. Furthermore, Polysaccharides are the most abundant organic polymers obtained by biosynthesis, available from different plant and animal sources with variable structures. They have attracted researchers because of their advantages as: (I) renewable character, (II) biodegradation, (III) relatively low cost and (IV) possibility of conversion into various derivatives due to their reactivity with many organic molecules [5]. Owing to the commercial and pharmaceutical usefulness of mucilage, physicochemical characterization of these polysaccharides is of significant importance. In the present study, we report the extraction and partial characterization of water soluble polysaccharides from *M. aegyptiaca* leaves. The monosaccharides generated by acid hydrolysis are isolated by high-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

**II. MATERIALS AND METHODS**

**A. Plant Material**

The leaves of *M. aegyptiaca* were harvested from Oued Touzouz (region of Ghardaïa) in March 2010, identified, air-dried at ambient temperature for three weeks and stored in cardboard boxes for later use.

**B. Extraction of Water-Soluble Polysaccharides**

*M. aegyptiaca* leaves were ground into powder using high speed disintegrator and were pre-extracted with 80% ethanol using a soxhlet apparatus in order to remove some colored materials, oligosaccharides and some other low molecular weight compounds. When no more colored material could be observed in the ethanol extract the procedure was ceased and the organic solvent left in the residue was allowed to dry out [4]. The pretreated dry powder was extracted twice with two volumes of deionized water under constant stirring for 3 h in a 60 °C water bath[4][6]. The mixture was centrifuged (2000g, 20 min), then the supernatant was filtered through gauze and Whatman GF/A glass fiber filter, concentrated at 40 °C in vacuum and dialyzed at cut-off 3500 Da [7]. The extract was precipitated by the addition of ethanol to a final
concentration of 75% (v/v) and the precipitates were collected by centrifugation, washed with acetone, dissolved in deionized water and finally lyophilized[4]. Brown crude water-soluble polysaccharides were obtained.

C. Chemical Composition Analysis

Total neutral sugar content was determined by the reaction with phenol in the presence of sulfuric acid using glucose as a standard [8]. The total uronic acid content was colorimetrically determined by the m-hydroxydiphenyl assay using galacturonic acid as a standard [9]. Proteins in the solution were estimated by the method of binding of Coomassie Brilliant Blue G-250 to protein using bovine serum albumin as a standard [10].

D. Analysis of Carbohydrate Composition

Composition analyses of polysaccharides have typically been based on hydrolysis procedures using hydrochloric, sulfuric, or trifluoroacetic acid (TFA) at elevated temperatures. More recently TFA has become the acid of choice for most carbohydrate analysis due to its effectiveness at hydrolyzing glycosidic bonds without causing extensive destruction of thersulting monosaccharide components and due to its volatility, which minimizes its interference with subsequent procedures [11]. Different procedures were tested: (a) 2 M TFA at 100°C for 5 h; (b) 4 M TFA at 100°C for 5 h; (c) 4 M TFA at 80°C for 5 h. Finally, the kinetic of liberation and degradation of oligosaccharides chains as a function of the time were performed. The hydrolysate was cooled and evaporated under reduced pressure at 40 °C, washed with methanol and re-dissolved in distilled water. Hydrolysis procedures were estimated by Thin-layer chromatography. The optimum condition of hydrolysis is used in HPAEC-PAD analysis.

1) Thin-layer chromatography (TLC)

The TLC analyses of hydrolysates were performed on silica gel 60G (Merck) plates with butanol:acetic acid:water (2/1/1, v/v) as the developing phase, and the neutral monosaccharide(s) and uronic acids in the hydrolysate were detected by spraying with diphenylamine:aniline:phosphoric acid (5:5:1, v/v), dried and heated at 80 °C for 15 min [12],[13],[14]. Rf values of different spots were determined by measuring the movement of solvent and movement of solute molecules with a ruler. Movement of solute divided by movement of solvent gives the Rf value.

2) High performance anion exchange-pulse amperometric detection (HPAEC-PAD) chromatography

Monosaccharides resulting from acid hydrolysis are analyzed by HPAEC-PAD method, is currently the most used [15].

3) Sample Preparation

3 mg of polysaccharides was hydrolysed according to procedure (c) for 4 h. The hydrolysate was cooled and evaporated under reduced pressure at 40 °C, washed with methanol and concentrated to dryness. The washing with methanol was repeated several times for removal of the reagent. The hydrolysate was then dissolved in distilled water and fractionated by HPAEC-PAD.

4) Neutral monosaccharides analysis

The neutral monosaccharide compositions of water-soluble polysaccharides were analyzed by HPAEC after acid hydrolysis. A Dionexsystem [Dionex Corporation, Sunnyvale (CA), USA] using a Carbopac PA1 (4mm×250mm) and a guard column (3mm×25mm) was used. Detection was carried out by pulsed amperometry with a gold electrode. The hydrolysates (25 mL) were filtered by passing through a 0.45μm filter before injecting into the column with an autosampler. The monosaccharides were eluted isocratically with 16M NaOH at a flow rate of 1mL min⁻¹. Each carbohydrate concentration was determined after integration of respective areas [Chromeleon management system (Dionex)] and their comparison with standard curves obtained with rhamnose, arabinose, mannose, galactose, glucose and fucose (Sigma) [14].

5) Uronic acid analysis

The Uronic acid composition was determined using HPAEC, on a Carbopac PA-1 analytical column (4mm×250 mm). A 100 μL sample was injected and the column was eluted at 1mL min⁻¹ with a gradient elution from 600mM Sodium acetate (eluent B) in 160mM Sodium hydroxide (eluent A) using the following program: 0–10 min 100% A and 0% B, 10–40 min 0 to 100% B, 40–45 min 100% B, 45–50 min 100 to 0% B. The eluents were degassed by flushing with helium and pressurized continuously using an eluent degas module (EDM-2, Dionex).The uronic acid contents were quantified by comparing with galacturonic acid and glucuronic acid standards and data were processed using Dionex AI 450 software [17].

Yield, proteins, neutral monosaccharide and Uronic acid content, of crude watersoluble polysaccharides from the dried leaves of *M. aegyptiaca* are given in Table 1. The crude water-soluble polysaccharides formed a light brown powder without starch, as confirmed by a negative reaction with iodine, similar to that reported by ATKHAMOVA et al. in six species of Malvaceae family [18]. The yield of water soluble polysaccharides was 1.46%, based on dried leaves. It is slightly less than that reported in *Malvavirgata* (Malvaceae) by ATKHAMOVA et al. of 2% [18].Chemical analysis revealed that crude water-soluble polysaccharides as a heterogeneous mixture of polysaccharides consisted 47.49±1.62% of neutral monosaccharides and 30.68±0.42% of uronic acids such 78.18±0.94% of total carbohydrates, as well as substantial amount of proteins (17.14 ± 1.43%), were greater than that reported by ATKHAMOVA et al., who found in *Malvamavitana* 27.4% of uronic acid and 25% of neutral monosaccharides [18].
B. Acid hydrolysis

The number of spots in TLC Chromatogram and their Rf value were used as a criterion for the selection of the optimal hydrolysis conditions. Hydrolysis with 4 M TFA for 5 h at 100°C shows an effective depolymerization compared to that of 2 M TFA for 5 h at 100°C. The chromatogram of this end (Fig. 1) showed one spot (Rf = 0.24) with training compared to 6 or 7 wide spots with variable Rf value from 0.23 to 0.66 for the hydrolysis by TFA 4 M (Fig. 2). While, hydrolysis by TFA 4 M with low temperature 80°C for 5 h (Fig. 3) shows a significant hydrolysis and much less monosaccharides was degraded appeared in TLC plates by thin spots compared to wide spots in TFA 4 M during 5 h at 100°C. The kinetic of hydrolysis shows that 4 h of treatment with 4 M TFA at 80°C is satisfactorily to the hydrolysis procedure for describing and defining the optimal conditions for the maximal breakdown of polysaccharides to monosaccharides and minimizing the destruction of these monosaccharides due to the thermal and chemical reactions (Fig. 4).

C. Monosaccharide composition

The result of HPAEC profile of acid hydrolysis of water-soluble polysaccharides from the dried leaves of *M. aegyptiaca* is shown in Fig. 5. Significant differences of monosaccharide composition were observed, compared to results reported in other species of Malvaceae. It consisted of galactose, rhamnose, arabinose, mannose and glucuronic acid with the weight percentage of 56.86%, 8.46%, 9.04%, 5.05% and 20.57%, respectively. The monosaccharide contents are summarized in Fig. 6. TOMODA et al. reported that polysaccharides of *M. sylvestris* leaves were composed of 22.2% L-galactose, 40.2% L-rhamnose, 16.0% D-galacturonic acid and 16% D-glucuronic acid [19]. While ATKHAMOVA et al. mentioned that the polysaccharides of *M. mavritana* leaves composed of 10% rhamnose, 5% arabinose, 7.5% galactose, 1.5% mannose, 1% glucose and 27% uronic acid [11]. These last values are considerably different from the values observed in our study. According to ATKHAMOVA et al., Polysaccharides of Malvaceae
consist of a rhamnogalacturonan with uronic acids and galactose ramifications [18]. The monosaccharide content in this study are very similar to that reported in gum arabic (Acacia senegal) by WILLIAMS and PHILLIPS, whose pointed out 44% of galactose, 13% of rhamnose, 27% of mannose.

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IV. CONCLUSION

Water-soluble polysaccharides of Malvaegyptiaca leaves can be effectively extracted by distilled water. The extracted mucilage contains high levels of carbohydrates (78.18%). The use of 4M TFA at 80°C for 4 h is the reagent of choice to liberate monosaccharides from polysaccharides, effectively and least destructively. The polysaccharides were comprised mainly of galactose (56%), glucuronic acid (20%) and contained a little amount of rhamnose, arabinose, and mannose.

ACKNOWLEDGEMENTS

We thank Pr. Philippe MICHAUD from Laboratory of Chemical and Biochemical Engineering at UniversitéBlaire Pascal de Clermont Ferrand for the HPAEC analysis. Acknowledgements also go to Laboratoire Protection des Ecosystèmes en Zones Arides for technical support.

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