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

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***Abstract*—*Malvaaegyptiaca* L. (Malvaceae), a spontaneous plant used as a traditional medicine in Ghardaïa (SeptentrionalSahara 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 and47.49±1.62%are neutralmonosaccharides.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 frompolysaccharides 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*—*Malvaaegyptiaca*, traditional medicine, polysaccharides, HPAEC-PAD.**

I. Introduction

Malvaaegyptiaca (Malvaceae) is a spontaneous plant used in traditional medicine prescriptions in Ghardaïa (Septentrional Sahara Algerian) [1]. The macerate ofleaves is used in the treatment of dysentery, constipationand fevers. The cataplasm of leaves is also used in treating wounds, sore and skin-eruptions [2].The Malvaceaefamily 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 pectinswhich 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 gen erated by acid hydrolysis are isolated by high-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

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II. Materials and Methods

A. Plant Material

The leaves of *M. aegyptiaca*were harvested from OuedTouzouz (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 acidusing 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 typicallybeen based on hydrolysis procedures using hydrochloric,sulfuric, or trifluoroacetic acid (TFA) at elevated temperatures. More recently TFAhas become the acid of choice for most carbohydrateanalysis due to its effectiveness at hydrolyzing glycosidicbonds without causing extensive destruction of theresulting monosaccharide components and due to itsvolatility, which minimizes its interference with subsequentprocedures [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:aceticacid: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 solutemolecules with a ruler. Movement of solute divided by movement of solvent gives the Rf value.

2)High performance anion exchange-pulse amperometric detection (HPAE-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 hydrolysedaccording 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 monosaccharidesanalysis

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 16mM NaOH at a flowrate 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].

III. Results and Discussions

A. Chemical Composition

TABLE I: Chemical Compositionof Crude Water-Soluble Polysaccharides from the Dried Leaves of *M. aegyptiaca*

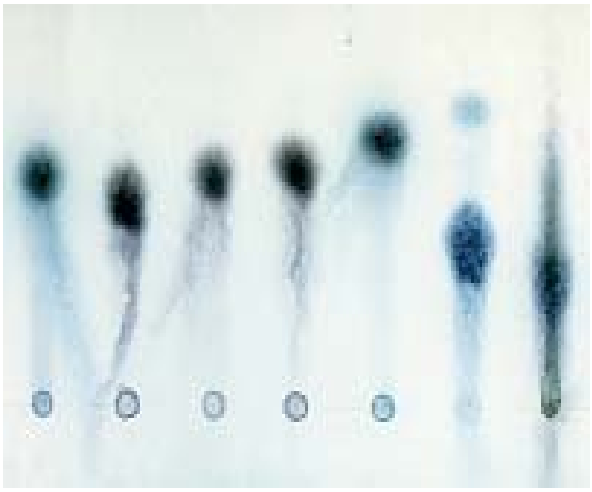
Yield wt.%	Proteinwt.%	Carbohydrate wt.%		
		Total	Neutral	Uronicacid
1.46	17.14 ± 1.43	78.18±2.04	47.49±1.62	30.68±0.42

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 byATKHAMOVA et *al.*in six species of Malvaceae family[18]. The yield of water soluble polysaccharides was1.46%, based on dried leaves. It is slightly less than that reported in *Malvamavritana* (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 *Malvamavritana*27.4%of uronic acid and 25% ofneutralmonosaccharides [18].

B. Acid hydrolysis

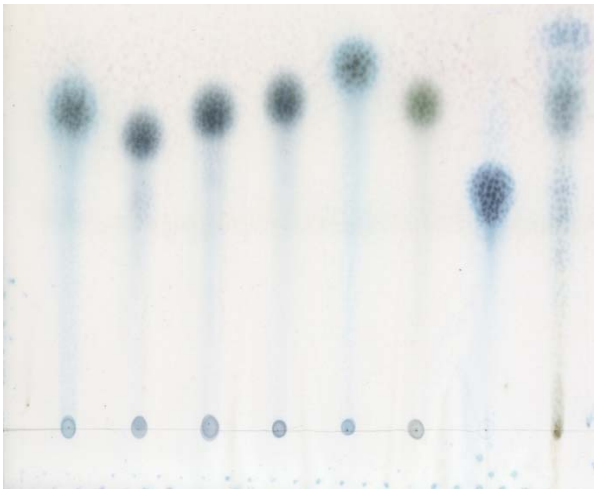
The number of spots in TLC Chromatogram and their Rf value were used as a criterion for the selection of the optimal hydrolysis conditions. Hydrolysis with 4 M TFA for 5 h at 100°C shows an effectivedepolymerization compared to that of 2 M TFA for 5 h at 100°C. the chromatogram of this end (Fig. 1) showed 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



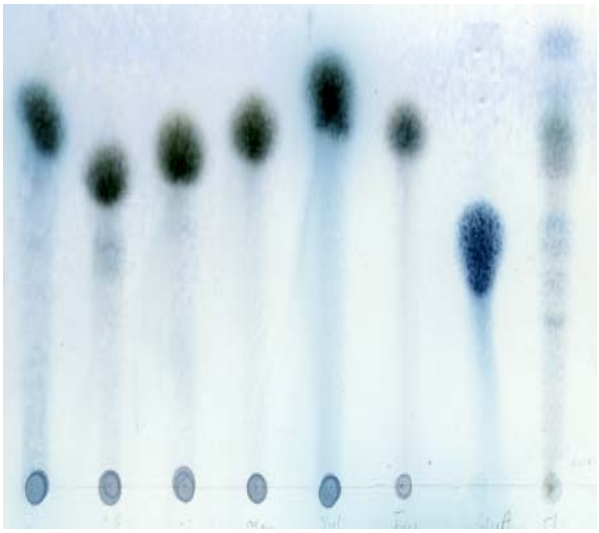
Ara Gal Glc Man Xyl Glc. A M

Fig. 1. Chromatogram of WSP of *M. aegyptiaca*leaveshydrolyzed by TFA 2 M for 5 h at 100°C.



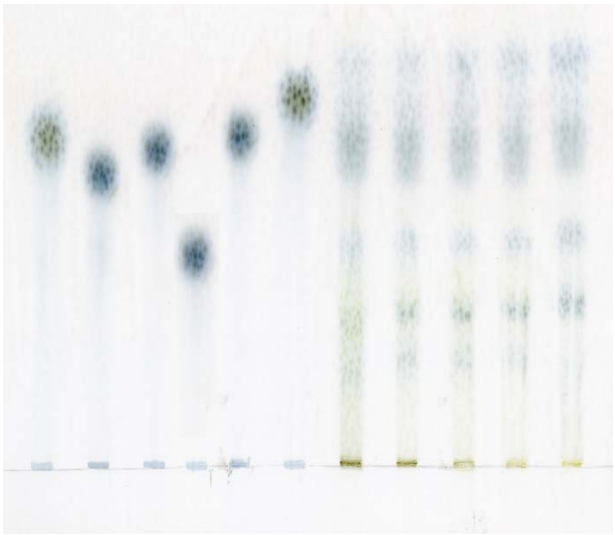
Ara Gal Glc Man Xyl Fru Glc. A M

Fig. 2. Chromatogram of WSP of *M. aegyptiaca*leaveshydrolyzed by TFA 4 M for 5 h at 100°C.



Ara Gal Glc Man Xyl Fru Glc. A M

Fig. 3. Chromatogram of WSP of *M. aegyptiacaleaves* hydrolyzed by TFA 4 M for 5 h at80°C



Ara Gal Glc Glc. A Man Xyl 1h 2h 3h 4h 5h

Fig. 4. Chromatogram of kinetic hydrolysis of WSP of *M.aegyptiacaleaves* hydrolyzed (TFA 4 M/80°C)

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% and20.57%, respectively. The monosaccharide contents are summarized in Fig. 6. TOMODA et *al.*reported that polysaccharides of*M. sylvestris* leaveswere 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 arabinoseand 15% of glucuronic acid[20].

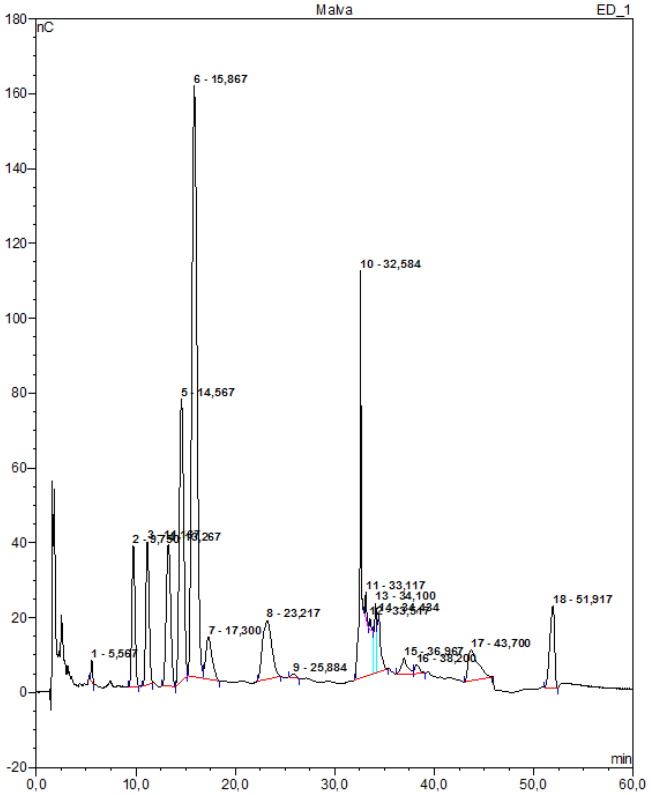


Fig. 5. HPAEC profiles of monosaccharides released from WSP of *M. aegyptiaca leaves* by acid hydrolysis.

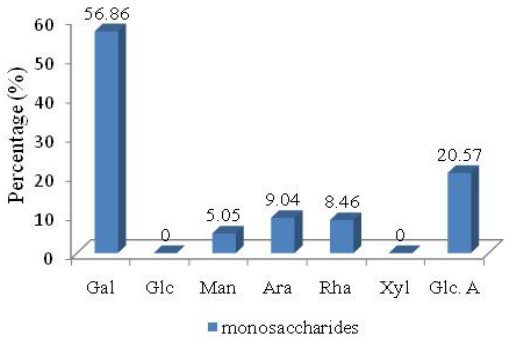


Fig. 6. Percentage of monosaccharides composition from the acid hydrolysate of WSP of *M. aegyptiaca leaves* using HPAEC-PAD.

IV. Conclusion

Water-soluble polysaccharides of*Malvaaegyptiaca leaves* can be effectively extracted by distilled water. The extracted mucilage contains high levels ofcarbohydrates (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 polysaccharideswas comprised mainly of galactose (56%), glucuronic acid (20%) and contained a little amount ofrhamnose,arabinose, and mannose.

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