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Isolation and Partial Characterization of Water Soluble Polysaccharides from One Saharian Medicinal Plant: *Plantago Notata* Lagasca.

Zakaria Boual[1, 2+], Abdellah Kemassi[1], Mustapha Daddi Bouhoun[1], Philippe Michaud[3] and Mohammed Didi Ould El Hadj[1, 2]

1- Laboratoire Protection des Ecosystèmes en Zones Arides et Semi-arides Université KasdiMerbah-Ouargla, Algeria

2- Laboratoire de Biogéochimie des Milieux Désertiques, Université KasdiMerbah-Ouargla, Algeria

3- Laboratoire des Glucides (EPMV) CNRS FRE2779, IUT/GB, UPJV, Avenue des Facultés, Le Bailly, 80025 Amiens Cedex, France

Abstract. *Plantago notata* Lagasca (Plantaginaceae), 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 *Plantago notata* leaves. These polysaccharides were obtained by sequential extraction in distilled water, followed by precipitation in 75% ethanol. The yield of extract is 2.0% (w/w). The crude water soluble polysaccharide extracts were further characterized and revealed the average values12.49±1.61% proteins and 85.03±1,13% carbohydrates, among them 20,34 ± 0,38%are uronic acid and79.65 ± 0.77% are neutral monosaccharides.The identification of monosaccharide composition by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) method shows 43.95% of galactose, 20.28% of rhamnose, 11.30% of glucose 9.55% of arabinose and 12.57% of galacturonic acid.

Keywords: *Plantago notata*, spontaneous, traditional medicine, Polysaccharides, HPAEC-PAD.

1. Introduction

The leaves of *Plantago notata* Lagasca are used in wound healing in traditional medicine. The powder of leaves is used directly on wounds of all kinds to stop bleeding, keep the wound clean and to enhance the healing process (VOISIN, 1987).The macerate of leaves is used in the treatment of dysentery and constipation(BATTANDIER, 1882).The species of the genus *Plantago* have various medicinal and other economic uses (PRAMANIK and RAYCHAUDHURI,1997). The seeds of *P. ovata, P. psyllium* and *P.major* are used as laxatives (WASIEKY, 1961). *P. ovata* seed husk and gum acacia can be used as ice cream stabilizer (UPADHYAY et *al.*, 1978). *P. lanceolata* and *P. major* have been used as external antirheumatic herbal remedies in the traditional medicine of Italy (CANIATO, 1982). Polysaccharide fractions have been isolated from the leaves of *P. major* and tested for anti-complementary activity (SAMUELSEN et *al.*,1998). *P. lanceolata* has been used as an antifertility agent (HERRERA et *al.*, 1984). Antitumor activity of *P. asiatica* has been demonstrated by RAVN and BRIMER (1988). The polysaccharide of some of these plants is well known to science and has been studied by pharmacologists and found to possess biologically active principles (MORTON, 1990). Furthermore, Polysaccharides 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 (MATTHEWS and ENDRESS, 2006). Owing to the commercial and pharmaceutical usefulness of mucilage, physicochemical

+ Corresponding author. Tel.: +213 07 95 94 51 44; fax: +213 029 88 7945.
E-mail address: biozakaria@yahoo.fr (Zakaria Boual)

characterization of these polysaccharides is of significant importance. In the present study, we report the extraction and partial characterization of water soluble polysaccharides from *P. notata* leaves. The monosaccharides generated by acid hydrolysis are isolated by high-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

2. Materials and Methods

2.1. Plant Material

The leaves of *P. notata* were harvested from Oued Nechou (region of Ghardaïa) in April 2010, authenticated, air-dried at ambient temperature for three weeks and stored in cardboard boxes for later use.

2.2. Extraction of Water-Soluble Polysaccharides

P. notata 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 (WU et *al.*,2007). The pretreated dry powder was extracted twice with two volumes of deionized water under constant stirring for 3 h in a 60 ℃ water bath(CIPRIANI et *al.*, 2009). The mixture was centrifuged (2000g, 20 min), then the supernatant was filtered through gauze and Whatman GF/A glass fiber filter, concentrated at 40 ℃ in vacuum and dialyzed at cut-off 3500 Da (NERGARD et *al.*,2004). The extract was precipitated by the addition of ethanol to a final concentration of 75% (w/w) and the precipitates were collected by centrifugation, washed with acetone, dissolved in deionized water and finally lyophilized(WU et *al.*,2007). Crude water-soluble polysaccharides were obtained.

2.3. Chemical Composition Analysis

Total neutral sugar content was determined by the reaction with phenol in the presence of sulfuric acidusing glucose as a standard (DUBOIS et *al.*, 1956). The total uronic acid content was colorimetrically determined by the m-hydroxydiphenyl assay using galacturonic acid as a standard (BLUMENKRANTZ and ASBOE-HANSEN, 1973). 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 (BRADFORD, 1976).

2.4. 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 the resulting monosaccharide components and due to itsvolatility, which minimizes its interference with subsequentprocedures (MATTHEWS and ENDRESS, 2007).Monosaccharides resulting from acid hydrolysis are further analyzed by HPAEC-PAD method, is currently the most used (YU IP et *al.*, 1992).

2.4.1. Sample Preparation

An equivalent of the polysaccharides powder in deionized water (3 mg)mixed with 4 M TFAand incubated for 4 h at 80 ℃. The hydrolysate was cooled and evaporated under reduced pressure at 40 ℃, washed with methanol and concentrated to dryness. The washing with methanol was repeated several times to remove the reagent. The hydrolysate was then dissolved in deionized water and fractionated by HPAEC-PAD (JOHANSSON et al., 2006).

2.4.2. 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) (SAMUELSEN et *al.*, 1999).

2.4.3. 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(KAMERLING et *al.*, 2007).

3. Results and Discussions

3.1. Chemical Composition

Table 1- Chemical composition of crude water-soluble polysaccharides from the dried leaves of *P. notata*

Yield wt.%	Proteinwt.%	Carbohydrate wt.%		
		Total	Neutral	Uronicacid
2.0	12.49±1.61	85.03±1,13	79.65 ± 0.77	20.34 ± 0,38

Yield, proteins, neutral monosaccharide and uronic acid content, of water soluble polysaccharides from the dried leaves of *P. notata*are given in Table 1. The extraction and purification with ethanol and acetone dissolve part of the chlorophyll present in the leaves produces a clear yellow mucilage powder, similar to other commercial gums used in the food industry. The yield is 2.0%, based on dried leaves. The mucilage content was less than that reported by GORIN (1965) in *P. major* leaves10% of nondemineralized polysaccharide.Chemical analysis revealed that water-soluble polysaccharides as a heterogeneous mixture of polysaccharides consisted 79.65 ± 0.77% of neutral monosaccharides and 20.34 ± 0,38% of uronic acids such 85.03±1,13% of total carbohydrates, as well as substantial amount of proteins (12.49±1.61%). The carbohydrate content was higher than that reported by CRAEYVELD et *al.*(2009) in seed huskof *P.ovata* (70.7%).Therefore, The proteins content value foundwere greater than that reported by CRAEYVELD et *al.* (2009) in seed huskof *P. ovata* (7.1%). SAMUELSEN et *al.*(1998) reported 1.5% of proteins in fraction of water-soluble polysaccharides from the leaves of *P.major*.

3.2. Monosaccharide Composition

The result of HPAEC profile of acid hydrolysis of water soluble polysaccharides from the dried leaves of *P. notate* is shown in Fig. 1(a). Significant differences of monosaccharide composition were observed, compared to results reported in other species of *Plantaginaceae*. It consisted of galactose, rhamnose, glucose, arabinose and galacturonic acid with the weight percentage of 43.95%, 20.28%, 11.30%, 9.55% and12.57%, respectively. The monosaccharide contents are summarized inFig. 1(b). CRAEYVELD et *al.*(2009) mentioned that polysaccharides of seed husk from *P. ovata*composed of arabinose (20.7%), xylose (50.3%), galactose (4.8%), glucose (2.0%), mannose (1.1%), rhamnose (1.1%) and uronic acid (5.0%). According to GUO et *al*., (2007) water soluble polysaccharides of psyllium gum (*P. ovata*) consist of 83.2±0.2% of neutral monosaccharides including; Rhamnose (9.86%), Arabinose (15.97%), Galactose (2.63%), Xylose(68.94%),Mannose(2.26%), with 15.9±0.2% of uronic acid. These last values are considerably different from the values observed in our study. The monosaccharide content in our study are very similar to that cited by SAMUELSEN et *al.* (1998) whose reported that biological active fraction of polysaccharides

from *P.major* leaves were composed of 49% of galactose, 38% of arabinose6% of rhamnose, 7% of galacturonic acid.

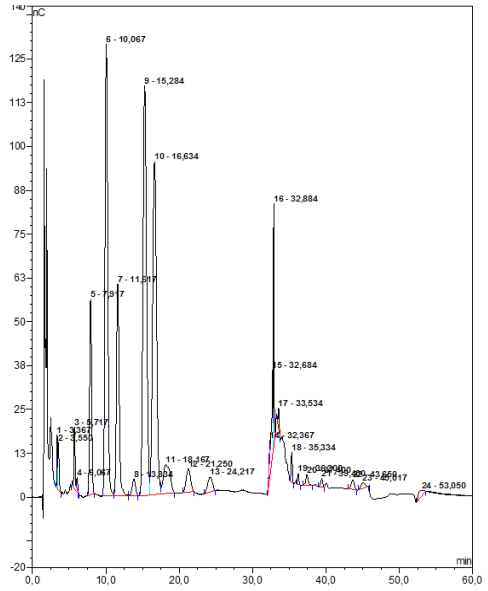

Fig. 1(a). - HPAEC profiles of monosaccharides released from water soluble polysaccharidesof P. notata leaves by acid hydrolysis.

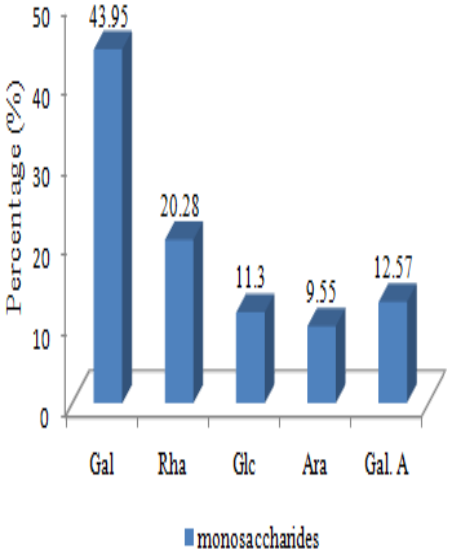

Fig. 1(b). - Percentage of monosaccharides composition from the acid hydrolysate

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5. References

[1] A.VOISIN. Utilisation des plantes m édicinales dans le souf au 19 ème si ècle, 1987, Le sahara, 1er trimestre, 100:25-28.
[2] A.BATTANDIER. Le droguier d'un mozabite. Imprim és de biblioth èque nationale de la r épublique fran çaise, 1882, Paris, pp. 3-12.
[3] S. PRAMANIK and S. S. RAYCHAUDHURI. DNA Content, Chromosome Composition, and Isozyme Patterns in Plantago L. the botanical review, 1997, 63: 124-127.
[4] R. WASIEKY. Investigations of the seeds ofPlantago ovata, P. psyllium and P.. major variety cruneta as laxatives, 1961, Pl. Med. 9: 232-244.
[5] K. G. UPADHYAY, A. R. PATEL AND S. H. VYAS. Evaluation of isabgul (psyllium) husk and gum acacia as ice cream stabilizers. 1978, Gujarat Agric. Univ. Ges, 4: 45-50.
[6] R.CANIATO. External antirheumatic and antineuralgic herbal remedies in the traditional medicine of northeastern Italy. J. Etlmopharm, 1982, 6: 161-190.
[7] A. B. SAMUELSEN, B. S.PAULSEN, J. K. WOLD, S. H. KNUTSEN and H. YAMADA. Characterization of a biologically active arabinogalactan from the leaves of Plantago major L. Carbohydrate polymers, 1998, 35: 145-153.
[8] C. L. HERRERA, E. V. RAMOS and B. A. VILLANNEVA. Philippine plants as possible sources of antifertility agents. Philippine J. Sci, 1984, 113: 91-129.
[9] H. RAVN and L. BRIMER. Structure and antibacterial activity of plantamajoside, a caffeic acid sugar ester from Plantago major subsp, major. Phytocbemislry. 1988, 27: 3433-3437.
[10] J.F. MORTON. Mucilaginous plants and their uses in medicine. Journal of Ethnophannacology, 1990, 29: 245-26.
[11] M. L.MATTHEWS and P. K.ENDRESS. Floral structure and systematics in four orders of rosids, including a broad survey of floral mucilage cells. Pl. Syst. Evol.2006, 260: 199–221.
[12] Y. WU, S. W. CUI, J. TANG, Q. Wang and X.GU.Preparation, partial characterization and bioactivity of water-soluble polysaccharides from boat-fruited sterculia seeds. Carbohydrate polymers. 2007, 70: 437–443.
[13] T. R. CIPRIANI, C. G. MELLINGER, L. M. DE SOUZA, C. H. BAGGIO, C.S. FREITAS, M. A. MARQUES, P. J. GORIN, G. L. SASSAKI and M. IACOMINI. Polygalacturonic acid: Another anti-ulcer polysaccharide from the medicinal plant Maytenus ilicifolia. Carbohydrate Polymers, 2009, 78:361–363

[14] C. S. NERGARD, D. DIALLO, T. E. MICHAELSEN, K. E. MALTERUD, H. KIYOHARA, T. MATSUMOTO, H. YAMADA and B. S. PAULSEN. Isolation, partial characterisation and immunomodulating activities of polysaccharides from Vernonia kotschyana Sch. Bip. ex Walp. Journal of Ethnopharmacology, 2004, 91: 141–152.
[15] M.DUBOIS, K. A.GILLES, J. K.HAMILTON, P. A.REBERSandF.SMITH. Colorimetric method for determination of sugars and related substances. Ana. Chemistry. 1956, 28: 350–356.
[16] N. BLUMENKRANTZ and G.ASBOE-HANSEN. New method for quantitative determination of uronic acids. Analytical biochemistry. 1973, 54: 484-489.
[17] M. M.BRADFORD. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976,72: 248–252.
[18] C. C. YU IP, V. MANAM, R. HEPLER andJ. P. HENNESSEY. Carbohydrate Composition Analysis of Bacterial Polysaccharides: Optimized Acid Hydrolysis Conditions for HPAEC-PAD Analysis. ANALYTICAL BIOCHEMISTRY, 1992, 201:343-349.
[19] L. JOHANSSON, L. VIRKKI, H. ANTTILA, H. ESSELSTROM, P. TUOMAINEN, T. SONTAG-STROHM. Hydrolysis of β-glucan. 2006, Food Chemistry,97: 71–79.
[20] A. B. SAMUELSEN, E. H. COHEN, B. S. PAULSEN, L. P. BRULL, J. E. THOMAS-OATES. Structural studies of a heteroxylan from Plantago major L. seeds by partial hydrolysis, HPAEC-PAD, methylation and GC–MS, ESMS and ESMS: MS. Carbohydrate Research, 1999, 315:312–318.
[21] L.KAMERLING, G. L.BOONS, Y. C.LEE, A.SUZUKI, N. TANIGUCHI and A.VORAGEN. Comprehensive glycoscience. Ed. Elsevier. 2007, 02, Paris: 654-681.
[22] A. G. Gorin. Chemical Investigation of the polysaccharides of the leaves of Plantago major L. Khimiya Pirodnykh Soedinenii, 1965, 1: 297-302.
[23] V.V.CRAEYVELD, J. A. DELCOUR, C. M. COURTIN. Extractability and chemical and enzymic degradation of psyllium(Plantago ovata Forsk) seed husk arabinoxylans. Food Chemistry, 2009, 112 : 812–819.
[24] A. B. SAMUELSEN, B. S.PAULSEN, J. K. WOLD, S. H. KNUTSEN And H. YAMADA. Characterization of a biologically active arabinogalactan from the leaves of Plantago major L. 1998, Carbohydrate polymers, 35: 145-153.
[25] Q.GUO, S.W. CUI, Q.WANG and J.C.YOUNG. Fractionation and Physicochemical Characterization of Psyllium Gum. Carbohydrate Polymers, 2007, 3614:11-37.