EXTRACTION AND PRELIMINARY CHARACTERIZATION OF ALKALI-SOLUBLE POLYSACCHARIDES FROM *Plantago ciliata* DESF. SEEDS

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Abstract.- *Extraction of crude alkali-soluble polysaccharides from* Plantago ciliata *seeds, using hot alkaline solution KOH at 80°C for 2h generate tow fractions GPSAI and GPSAII. The mass yield of each one is 3.63% and 3.19% (w/w), respectively refering to the dry weight of the seeds. They are contained 43.77±0.19, 73.77±0.17% neutral sugars, 0.00, 21.95±0.07% uronic acid and 10.42±0.01, 10.54±0.01% protein, for GPSAI and GPSAII respectively. Crude alkali-soluble polysaccharides consist of an hetero-neutral polysaccharides. It was mainly composed of arabinose and xylose in addition to the traces of galactose using Thin Layer Chromatography analysis.*

Key words: *Extraction, characterization, alkali-soluble, polysaccharides, seeds,* Plantago ciliata.

EXTRACTION ET CARACTÉRISATION PRÉLIMINAIRE DES POLYSACCHARIDES ALCALI-SOLUBLES À PARTIR DES GRAINES DE *Plantago ciliata* DESF

Résumé.- *L'extraction des polysaccharides bruts alcali-solubles à partir de graines de* Plantago ciliata *par macération dans une solution alcaline chaude de KOH à 80°C pendant 2h, génère deux fractions GPSAI et GPSAII. Le rendement massique de chacune entre elles est de 3,63% et 3,19% (p/p), respectivement, par rapport au poids sec des graines. Ils contiennent 43,77±0,19, 73,77±0,17% des oses neutres et 10,42±0,01, 10,54±0,01% des protéines pour GPSAI et GPSAII, respectivement. L'acide uronique est de 21,95±0,07% pour la fraction GPSAII, alors que GPSAI n'a montré aucun acide uronique. Les polysaccharides bruts alcali-solubles se consistent d'un hétéro neutres polysaccharides. Il était principalement composé d'arabinose et de xylose en plus des traces de galactose en utilisant la chromatographie sur couche mince.*

Mots-clés: *Extraction, caractérisation, alcali-soluble, polysaccharides, graines,* Plantago ciliata.

Introduction

Plantago ciliata Desf. is a spontaneous plant from Plantaginaceae family [1]. This species is widely used in folk medicine due to the biomedical effects of mucilage extracted especially from their seeds [2]**.** In recent years, several researches were investigated to explore structural feature and biological activities of polysaccharides extracted from *Plantago* species. An arabinogalactan was extracted from *Plantago major* L. leaves. It was composed of 49% galactose, 38% arabinose, 7% galacturonic acid and 6% rhamnose. This

polysaccharide showed a powerful anti-complement activity [3]. Water-soluble polysaccharides from *Plantago palmata* leaves were studies for its immunomodulatory properties by stimulating NO and TNF-α production from macrophages cells [4]. The functional maturation of dentritic cells was performed by their treating with *Plantago asiatica* polysaccharides from seeds. This later is an heterpoxylan composed of rhamnose, arabinose, xylose, mannose, glucose and galactose with molaire ratio 0,05: 1,00: 1,90: 0,05: 0,06: 0,10 [5,6].

However, it is worthwhile to characterize polysaccharides of *Plantago ciliata* species. The main objective of this study was to extract and to characterize the monosaccharide composition of the alkali-soluble polysaccharide from *Plantago ciliata* seeds.

1.- Materials and methods

1.1.- Plant materials

Plantago ciliata seeds were collected from Intissa zone, located in M'zab valley (Septentrional Algerian Sahara). They were dried at ambient temperature, in the shadow for three weeks and then crushed into fine powder.

1.2.- Extraction of GPSA

After deffated of *Plantago ciliata* seeds (12.22g) and water-soluble extraction, the marc was soaked in hot alkaline solution of 0.5M KOH (80°C, 2 h, Twice) with periodic stirring followed by centrifugation at 4000 rpm [7,8] for 10min [9]. In order to precipitate alkali-soluble polysaccharides, combined supernatants were made neutral with 2M hydrochloric acid; succeed by centrifugation [10] for obtained GPSAI fraction. The alkaline polysaccharides fraction GPSAII not precipitated by neutralization was gotten by addition of three volume 99.5% ethanol [11] at 5°C during a night [10]. The crude extract polysaccharides were recovered by centrifugation [12] after successive washing with 99% acetone [13]. Both of collected alkali-soluble polysaccharides GPSAI and GPSAII were frozen and lyophilized [8].

1.3.- Composition of crude alkali-soluble polysaccharides extract

The chemical composition of GPSAI and GPSAII were determined colorimetrically. It's about neutral sugar, uronic acid, in addition to the protein rate.

Firstly**,** total carbohydrates were measured by the phenol sulphuric acid method [14], with slight modifications. Briefly, 200µl of 0.01% of GPSAI and GPSAII solutions were added to 200µl of phenol aqueous solution (5%, w/v). After making homogenized by stirrer, 1ml of concentrated sulfuric acid was quickly added. The mixtures were placed in a water bath (100°C, 5min) followed with cooling at the darkness during half an hour. Optical densities were measured afterwards by UV-visible spectrophotometer at 490nm. The standard curve was prepared from glucose with concentrations varied from 0.001 to 0.01%.

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Secondly, the modified method of MONSIGNY *et al*. (1988) [15] was adopted to determine neutral sugars. Shortly, 200µl of 0.01% samples was added to 200µl of 0.6% resorcinol with 1ml of concentrated sulfuric acid. The mixture was heated at 90°C in water bath for 30min, and then the absorbance were determined at 480nm after cooling in dark ice bath during half-hour. A standard of glucose from 0.001 to 0.01% was used as a reference.

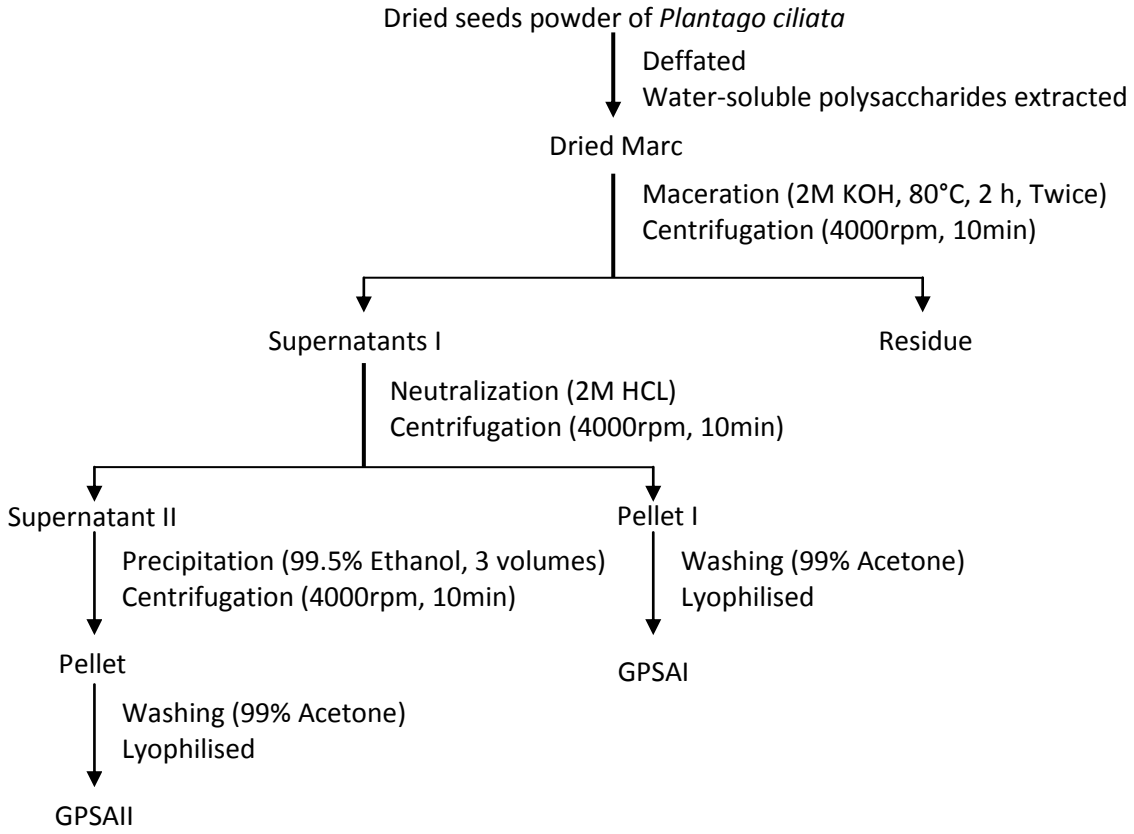

Figure 1.- Scheme for alkali-soluble polysaccharides extraction from *Plantago ciliata* seeds

Thirdly, for assess uronic acid, Blumenkrantz et Asboe-Hansen protocol was used [16]. 200 µl of crude alkali-soluble polysaccharides extracts and 1.2ml of 0.0125M sodium tetraborate solution were mixed and stirred. Then, they were heated for 5min at 100°C after incubated in cold water bath few of minutes. After that, samples were cooling before 20µl m-HDP was added. Optical densities were read at 520nm. Glucuronic acid (0.001 à 0.01%) was used as standard.

Finally, BRADFORD (1976) [17] method was performed to quantify protein in *Plantago ciliata* extracts. 200 µl of each sample was homogenized with 2ml of Coomassie reagent for 30s. Absorbances were measured at 595nm, in time interval between 2min and one hour in the max. Bovine Serum Albumin was used to prepare standards range from 0.001% to 0.01%.

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1.4. Characterization of carbohydrate composition

Monosaccharides components was analysed with Thin Layer Chromatography (TLC). An acid glycosidic bonds hydrolysis was performed. Twenty five milligrams of alkali polysaccharides samples were mixed with 1ml of 2M Trifluoroacetic acid. Then, they were left in laboratory oven (100°C, 4 h). Next, few drops of 99.7% methanol were added to supernatants. After 24h, 1ml of distilled water was used to dissolve dry products [18]. Two systems were chosen with different developing phases [19, 20]. Nigrum was used as revealing [21].

2.- Results and discussion

2.1.- Yield and chemical composition

Mass yields and chemical composition as carbohydrate and protein content of crude alkali-soluble polysaccharides from *Plantago ciliata* seeds were summarized in Table I.

Table I.- Chemical and biochemical Composition of alkali-solubles polysaccharides from *Plantago ciliata* seeds

Fraction	Yield (%)	Carbohydrates (%)			Protein (%)
		Total	Neutral	Uronic acid	
GPSAI	3.19	46.32±0.10	43.77±0.19	0	10.42±0.01
GPSAII	3.63	97.63±0.03	73.77±0.17	21.95±0.07	10.54±0.01

The yield content of GPSAI was 3.19%, while it was a little higher 3.63% for GPSAII fraction. They are slightly less than that founded by hot alkaline extraction and ethanol precipitation in *Plantago asiatica* L. by Gong *et al*. of approximately 5.45% (w/w) [22].

Chemical analysis showed that crude alkali-soluble polysaccharides consisted 46.32±0.10% and 97.63±0.03% of total carbohydrates, 43.77±0.19% and 73.77±0.17% of neutral monosaccharides for GPSAI and GPSAII, respectively. For uronic acid, GPSAII has a rate of 21.95±0.07%. Whereas, no trace has been reported for GPSAI. In other hand, protein rate was almost similar for both of them.

2.2.- TLC characterization

Qualitative analysis of alkali-soluble polysaccharides GPSAI and GPSAII has determined the nature of constituent monosaccharides; per refer to Rf of standards (fig. 2).

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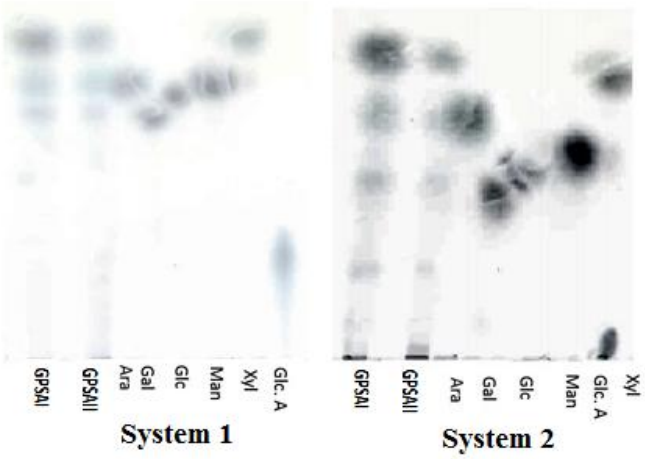

Figure 2.- Chromatogram of GPSAI and GPSAII of *Plantago ciliata* seeds with tow systems

The result of TLC Chromatogram is summary in Table II.

Table II.- Retentions factor Rf for samples and standard

Fraction	System 1						System 2					
Standard	**Ara**	**Gal**	**Glc**	**Man**	**Xyl**	**Glc.A**	**Ara**	**Gal**	**Glc**	**Man**	**Xyl**	**Glc.A**
GPSAI	**X**	**X**			**X**		**X**	**X**			**X**	
GPSAII	**X**				**X**		**X**	**X**			**X**	

Generally, it was noticed an accordance of results for the two systems. However, glucuronic acid standard didn't move in second system. In other hand, GPSAI fraction has three spots. It was arabinose, galactose and xylose, in both of systems. Whereas, GPSAII fraction showed two spots in first system. It was corresponding of arabinose and xylose. While, a third spot of galactose was appear in the system 2. An unidentified spot with 0,140 Rf was presented in second system for GPSAI and GPSAII togother. The monosaccharide components in this work are very similar to that reported by Gong *et al*. in *Plantago asiatica* L. alkali polysaccharides PLP. The result of GC analysis showed that the monosaccharides composition of PLP was xylose, arabinose, glucose, and galactose [22].

Conclusion

This work is interested to analyze crude alkali-soluble polysaccharides that were extracted from seeds of *Plantago ciliata* Desf. (Plantaginaceae). The alkali treatement was generating tow fraction GPSAI and II. Chromatography analysis with tow systems showed that both of fractions composed in the majority of arabinose and xylose, besides a trace of galactose.

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