EXTRACTION OF POLYSACCHARIDES FROM GRASS OF *Ampelodesmos mauritanicus*

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Abstract. *- The Diss plant* (Ampelodesmos mauritanicus) *is an herbaceous perennial plant of the family Gramineae that lives on arid and sandy soils, this species which is adapted to arid areas, the species is widespread in North Africa growing in wild state. In the past, it was used as building material because of its mechanical and hydrous qualities. Polysaccharides are macromolecules derived from various living organisms, including the plants, its stems are composed of cellulosic filaments linked by lignin, pectins and hemicellulose. The aim of this study was to evaluate quantitatively some parietal polysaccharides like cellulose and noncellulosic substances such as pectin, lignin, and hemicelluloses of Ampelodesmos mauritanicus Grass which biodegradable ecological, and renewable plant source. The results indicate that the studied specie is a plant potential that can be exploited in various fields, especially in pharmaceutical, cosmetic industries efficient conversion of lignocellulosic materials to fuel ethanol and other value-added fermentation products.*

Keywords: Ampelodesmos *mauritanicus, polysaccharides cellulose, hemicellulose, pectin.*

EXTRACTION DES POLYSACCHARIDES À PARTIR D'HERBE D'*Ampelodsmos mauritanicus* (DISS)

Résumé. *- La plante Diss* (Ampelodesmos mauritanicus) *et une plante herbacée vivace de la famille des Graminées, qui vit dans les sols arides et sableux, cette espèce qui est adaptée aux zones arides, l'espèce est répandue en Afrique du Nord poussant à l'état sauvage. Au passé, il était utilisé comme matériau de construction en raison de ses qualités mécaniques et hydriques. Les polysaccharides sont des macromolécules provenant de divers organismes vivants, y compris les plantes, dont les tiges sont composées de filaments cellulosiques liés par la lignine, les pectines et l'hémicellulose. Cette étude est une évaluation quantitativement certains polysaccharides pariétaux comme la cellulose et les substances non cellulosiques telles que la pectine, la lignine et les hémicelluloses. Les résultats indiquent que l'espèce étudiée est un végétal potentiel qui peut être exploité dans divers domaines, en particulier dans les industries pharmaceutiques et cosmétiques, la conversion efficace de matériaux lignocellulosiques pour la production de l'éthanol et d'autres produits de fermentation à valeur ajoutée.*

Mots clés: Ampelodesmos mauritanicus, *cellulose, polysaccharides, hémicellulose, pectine.*

استخلاص السكريات من النبات العشبي أمبيلديسموس موريتانيكوس (الديس)

ملخص: نبات الديس (أمبيلوديسموس موريتانيكوس) هو نبات عشبي دائم من عائلة غرا ميناي التي تعيش على التربة القاحلة والرملية، وهذا النوع الذي يتكيف مع المناطق القاحلة، وينتشر هذا النوع في شمال أفريقيا في الحالة البرية. في الماضي، كان يستخدم كمادة بناء بسبب صفاته الميكانيكية والمائية. السكريات هي جزيئات مشتقة من الكائنات الحية المختلفة، بما في ذلك النباتات، ويتألف ساقها من خيوط سلولوزية مرتبطة باللجنين، البكتين وهيميسيلولوز.
الهدف من هذه الدراسة هو تقييم كميا بعض السكريات مثل السليلوز والمواد غير السلولوزية مثل البكتين،

اللجنين، وهيميسيلولوز من أمبيلوديسيموس موريتانيكوس. تشير النتائج إلى أن الخواص المدروسة تتيح إمكانات استغلال نبات أمبيلوديسيموس موريتانيكوس في مختلف المجالات وخاصة في الصناعات الصيدلانية والصناعات التجميلية وامكانية التحويل الفعال للمواد اللنجوسيلولوزية إلى وقود الإيثانول وغيرها من منتجات التخمير ذات القيمة المضافة.

.كلمات دالة: أمبيلوديسموس موريتانيكوس، السلولوز، السكريات، هيميسيلولوز، البكتين

Introduction

Cellulose is the main component of several natural fibers such as cotton, flax, hemp, jute and sisal within others. This natural polymer represents about one-third of plant tissues and it can be restocked by photosynthesis.

Cellulose is a linear polymer of β-(1 →4)-D glucopyranose units. There are several types of cellulose (I, II, III, IV and V) [1]. Hemicelluloses are the second most common group of polysaccharides in nature, represent about 20-35% of lignocellulosic biomass.

In recent years, bioconversion of hemicellulose has received much attention such as efficient conversion of hemicellulose biomass to fuels and chemicals, delignification of paper pulp, digestibility enhancement of animal feedstock, clarification of juices.

The utilization of hemicellulosic sugars is essential for efficient conversion of lignocellulosic materials to fuel ethanol and other value-added fermentation products. Other potential applications of hemicellulases include bio pulping of wood, coffee processing, fruit and vegetable maceration, and preparation of high fiber baked goods [2-5].

Pectin, the main heteropolysacharide component of the primary walls of nonwoody plant cells, is a valued commercial hydrocolloid, whose excellent health and functional properties justify a wide and increasing use in the food, cosmetic, medical and pharmaceutical industries [6] Pectin, is mainly used in food industries for its gelling and stabilizing properties. In the food industry, pectin is widely employed in the production of jams and jellies, confectionary products and bakery fillings.

The other major use of pectin concerns the stabilization of acidified milk drinks and yogurts [7].

Production of other value-added products from hemicellulose hydrolyzates Lactic acid is used in the food, pharmaceutical, and cosmetic industries. It is a component of biodegradable plastic polylactate, the market for which is expected to grow significantly. Production of other value-added products from hemicellulose hydrolyzates Lactic acid is used in the food, pharmaceutical, and cosmetic industries. It is a component of biodegradable plastic polylactate, the market for which is expected to grow significantly [2]. Lignin is one of the most abundant aromatic biopolymers and a major component of plant cell walls, imbedded in a carbohydrate polymer matrix of cellulose and hemicellulose. Ampelodesma mauritanica (Diss); family of Poaceae is a plant native of northern Africa and southern Europe; is perennial and luxuriant growing spontaneously in wild state around the Mediterranean basin. The antiparasitic property is the only traditional use of this plant [8].

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This plant previously used in the realization of the old homes of North Africa regions because of its Mechanical qualities. Possibly the use of such a fibrous plant in cements paste offers resistances very interesting, which make this material as an excellent filling Lightweight for structures subjected to seismic [9]. Recently *Ampelodesmos Mauritanicus* used for production of biogas from the pyrolysis biochar at 450℃-550℃ [10].

The crude methanolic and butanolic extracts of the aerial parts of Ampelodesma mauritanica were examined for antibacterial and antifungal activity in vitro using the disc diffusion method. Activity against five bacterial strains (gram positive bacteria and gram-negative bacteria) and one fungal strain is discussed. Phytochemical screening shows that this plant is particularly rich in flavonoids and saponins which might be responsible for its antimicrobial activity. The hypoglycemic effect and antioxidant activity of the methanol extract of Ampelodesma mauritanica roots was studied.

The levels of total phenolic and the total flavonoid content in *Ampelodesmos mauritanicus* roots were determined. Also, its antioxidant capacity. Methanol root extract could be a valuable source of hypoglycemic compounds the phytochemical screening revealed the presence of flavonoids, saponins, cardenolides and tannins. It is observed that in some regions of Algeria *Ampelodesmos mauritanicus* has been used for reducing the blood glucose levels in diabetics [8,11].

Polysaccharides have the anti tumoral, immunostimulant and antioxydant activity, they characterized by a broad therapeutic spectrum and a low toxicity [12] They play a big role in the industry of materials and health human and animal as a dietetic fiber [13].

The main purpose of this study was to evaluate quantitatively some parietal polysaccharides like cellulose and noncellulosic substances such as pectin, lignin, and hemicelluloses.

1.- Materials and methods

Plant material

Ampelodesmos mauritanicus was collected from Batna in the east of Algeria during September 2016.Purify all of the Diss fibres after removing the yellow and violet limbs.

The aerial parts were air dried in shade at room temperature, then washed with water and Javelle water (12°) then were crushed [14]. The proportions of fibres contents were determined by treated fibers by soda and Sulfuric acids concentrate to obtain lignin, the cellulose obtained by bleaching and KOH treatment, the filtrate obtained adjusted by acetic acid to obtain hemicelluloses.

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Figure 1.- Grass *Ampelodesmos mauritanicus* (Diss)

Taxonomy

Reign: Vegetal
Branch: Magnoliophyta
Class: Liliopsida
Order: Cyperales
Family: Poaceae
Genre: Ampelodesma
Botanical Name: *Ampelodesmos mauritanicus*

Chemical characterization

Water and Volatile content

water and volatile matter content it corresponds to the loss of mass undergone by the sample after Drying in an oven at 100°C until constant weight (for 4 hours). Content Water and volatile matter (denoted W) is expressed as:

$$W = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

m_0 (g): mass of empty crucible.
m_1 (g): mass of crucible and test portion before heating.
m_2 (g): mass (crucible + residue) after heating up to constant weights.

Dry matter

The dry matter is determined from a raw sample, which is introduced into Ceramic crucible, then weighed (m_0) and placed in the oven at 105 ° C. until A constant weight. After cooling in a desiccator, the crucible containing the material Dry is weighed (m_1). The moisture content is then obtained from the equation below:

$$M\% = [(m_0 - m_1) / m_0] \times 100$$

m_0 = 2 g of Diss fiber was placed in the oven at a temperature of 103-105° C for 4 hours.

Then the sample was allowed to cool in a desiccator containing $CaCl_2$ and the sample obtained was weighed.

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Mineral content

The content of mineral substances (including mineral ash) is determined, the dry sample is calcined at 500°C till constant weight. The calcined residue obtained is weighed.

The content of mineral matter (M_m) is expressed by:

$$M_m = \frac{m_3 - m_0}{m_1 - m_0} \times 100$$

m_3: is the weight of the crucible and of the residue after calcination to constant weight (g). The weight difference between the mass of dry matter and the mass of Mineral content corresponds to the mass of organic matter.

Figure 2.- Raw *Ampelodesmos mauritanicus* Stem

Extraction of wax and grease

20 g of mashed *Ampelodesmos mauritanicus* was put in Methylene chloride solution for 24 hours then filtrated, the extract was obtained by evaporate the filtrate using a rotary evaporate.

Extraction of pectin

10 g of crushed amplodesma mauritanica were extracted with water (1:25, w: v), acidified with HCl, at 95 °C, for 1 hour [15]. A filtration after extraction, was done through four layers of gauze and then the filtrate was passed through a Buchner funnel under vacuum with a Whatman No.1. Pectins were precipitated by adding seven volumes of boiling ethanol 99.8%. The precipitate was filtered under vacuum and washed with ethanol solution (65%). Coagulated pectin was finally solubilized with 0.05 N NaOH [16].

Extraction of Lignin

15 g of the crushed *Ampelodesmos mauritanicus* was treated with 300 ml of sulfuric acid (80 wt. %) for 3 hours at 25°C. To the mixture 200 ml of Sulfuric Acid (4 wt.%) was added, then refluxed for 5 hours at 100°C, before filtration was left for 12 hours. The residue washed with cold and hot water several times, the obtained residue was desiccated in oven at 105C° for 5 hours.

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Extraction of cellulose and hemicellulose

Extraction with NaOH

10 g of Ampelodesma Mauritanica sample was put in sufficient quantity of water for two hours and then the sample was dried after filtration. The sample was put in 1 liter of NaOH (2 wt. %) and then in water bath at 80°C for 2 hours, distilled water was used to wash sample till neutralization of pH after filtration.

Bleaching

The samples obtained from NaOH extraction (brown colour) were bleached using sodium hypochlorite and acid buffer solution (30 g of soda in 50 ml of distilled water and 70 ml of acetic acid completed to 1 liter with distilled water). The pulp was put in solution of (H_2O_2/Acetic tampon/ NaClO) (3:1:1) It is processed in a water bath at 80°C for 2 hours then filtered, washed till a white pulp were obtained (hollocellulose).

Extraction of cellulose

The hollocellulose obtained were dissolved in 80 ml of KOH (25 wt. %), the mixture was kept under stirring for 15 hours, then the paste was filtrated and washed till eliminate all NaOH with distilled water, second wash was done with diluted acetic acid then ethanol finally the cellulose was obtained after dried.

Figure 3.- *Ampelodesmos mauritanicus* after chemical treatment

Extraction of Hemicellulose

The filtrate obtained from cellulose extraction was neutralized with (60 wt.%) of acetic acid with stirring for 15 minutes, a small portion of ethanol was added with continuous stirring till filtration, finally the hemicellulose was obtained after filtration, then was desiccated at room temperature.

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Spectroscopic analysis

Analysis of extracted cellulose fibers by infrared spectroscopy (FTIR - ATR) FTIR 8300 Fourier transformed SHIMADZY. Allowed us to determine the groups functional properties present in the fibers. The results obtained are presented in Figure 4.

X-ray diffraction

Crystallinity rate (the crystalline structure) as a function of the chemical treatment is studied by X-ray diffraction figure 5. Carried out on samples mechanically crushed using an electric mill, sieved to a size less than 125 µm. The diffraction was carried out by a Bruker D8 apparatus. The scanning speed is 0.2s / step the angle of Diffraction is taken between 5 and 75 °.

The monochromatic incident beam is center on the copper Kα1 line (λ = 1.5418A). In this approach, the X-ray apparent crystallinity (%) of cellulose is calculated from the height ratio between the intensity of the crystalline peak and the total intensity after the subtraction of the background signal (non-crystalline) measured without cellulose [16] according to the following equation:

$$C = 100.\frac{I_{200} - I_{non-cr}}{I_{200}}$$

2.- Results and discussion

Plant Analysis

After *Ampelodesmos mauritanicus* extraction and bleaching, the cellulose was obtained.

The chemical composition of *Ampelodesmos mauritanicus* is shown in Table 1. Cellulose is the major component, followed by hemicelluloses and lignin. The smallest components are extractives and ashes.

Table1.- Pourcent of each composant of extracted *Ampelodesmos mauritanicus* grass

Ampelodesmos mauritanicus	**Wt.% for each composant**
Cellulose	41.5
Lignin	27.5
Hemicellulose	22
Extractives	7
Pectin	4.5
Ashes	3
Fats and waxes	1.3

Mineralogical Analysis of ashes after calcinations of the *Ampelodesmos mauritanicus* stem:

The mineral analysis of dissolved ash determined by using Atomic Absorption A.A-6200 SHIMADZU5.

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Table 2.-Ashes mineral components of *Ampelodesmos mauritanicus*

Elements	SiO_2	CaO	Fe_2O_3	MgO	K_2O	Na_2O
%	72.25	4.50	2.31	1.45	3.60	0.70

Chemical composition of ash results in its very variable mineral composition. The silica is very present in the composition of the *Ampelodesmos mauritanicus*; it constitutes even one of the reasons for which the delignification of this grass is carried out by the alkaline processes.

FT-IR Spectra

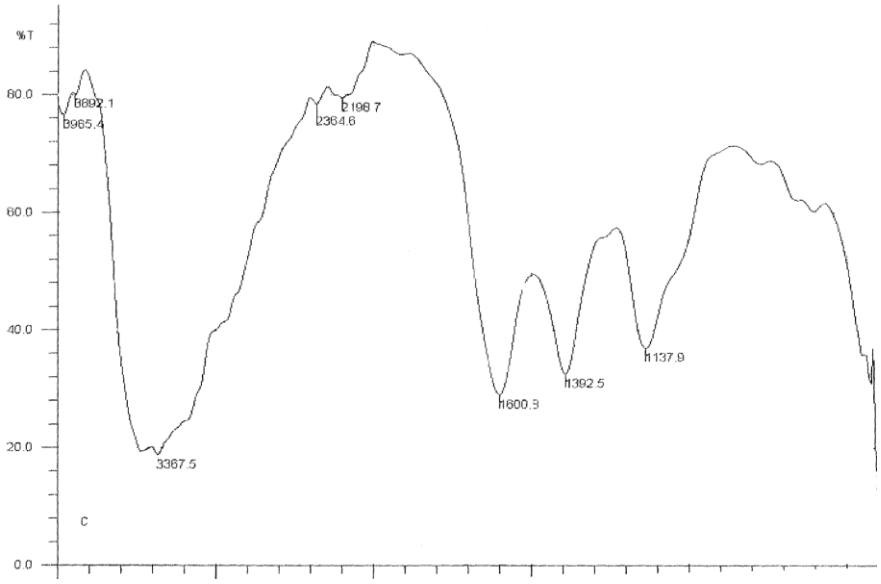

Figure 4.- FTIR spectrum of *Ampelodesmos mauritanicus* cellulose

In FT-IR spectrum of *Ampelodesmos mauritanicus* (fig.4) a broad absorption band at 3367.5 cm^{-1} is mainly due to OH groups in the existing structure of the cellulose.

We also note the presence of a band at 1137 cm^{-1}, and a second one at 1600 cm^{-1} which indicates the existence of single C–O and double C═O bonds. At 1392 cm^{-1} liaison C -O cycle aliphatic of cellulose, pick at 2340 cm^{-1}Indicate the presence of OH acid carboxylic [17, 18].

X-Ray Analysis

Diffractogram of cellulose obtained from *Ampelodesmos mauritanicus* (figure 5), have a very similar diffraction pattern. Crystalline peak appears at I (200) correspond to the intensity diffracted at 2θ = 22.7°. It is understandable that the cellulose content increases, whereas the amorphous hemicellulose content decreases during the Physico-chemical process. I (AM) corresponds to the intensity diffracted at 2θ = 15.2°. The crystallinity index of the crude *Ampelodesmos mauritanicus* cellulose is of the order of 34.27 %. It is higher than that of esparto grass which is of the order of 25% [19,20].

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Conclusion

The cellulose, hemicelluloses and pectins were extracted from *Ampelodesmos mauritanicus* stem. Quantitatively, the cellulose remains the major component (41.5 %) This is followed by lignin
(27.5 %) a hemicellulose (22%) and pectin (4.5%).

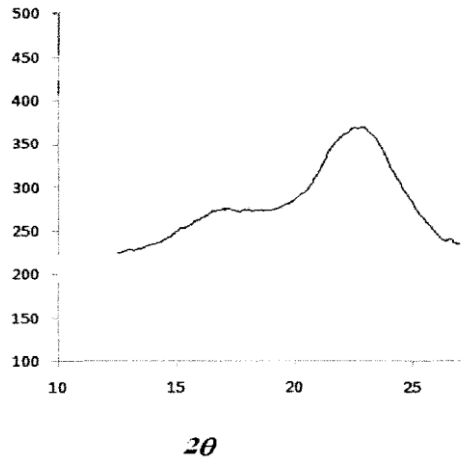

Figure 5.- X-ray Diffractograms of cellulose obtained from *Ampelodesmos mauritanicus*

A further study will be due to determine the monosaccharides composition of hemicellulose and pectins extracts.

Grasse such as *Ampelodesmos mauritanicus* is attractive feedstock because it's perennial that can grow on less fertile or marginal lands not used for food crops, require little fertilizer and modest pest and disease management, and are not a human food source.

This study emphasizes the use of Ampelodesma mauritanica for different useable products in order to improve its value into high-added value compounds for sustainable development.

References

[1].- Morán J. I., Alvarez V. A., Cyras V. P. and Vázquez A. (2008.-. Extraction of cellulose and preparation of nanocellulose from sisal fibers. Cellulose, 15(1): 149-159.

[2].- Saha B. C., 2003.- Hemicellulose bioconversion. Journal of Industrial Microbiology and Biotechnology, 30(5): 279-291.

[3].- Wong K. K., Tan L. U., and Saddler J. N., 1988.- Multiplicity of beta-1, 4-xylanase in microorganisms: functions and applications. Microbiological reviews, 52(3), 305 p.

[4].- Zeikus J. G., Lee C., Lee Y. E., et Saha B. C., 1991.- Thermostable Saccharidases: New sources, uses, and biodesigns.

Tel/Fax : (+213) 029 71 56 89 e-mail : polysac2017@gmail.com

[5].- Coughlan M. P., et Hazlewood G. P., 1993.-. beta-1, 4-D-xylan-degrading enzyme systems: biochemistry, molecular biology and applications. Biotechnology and Applied Biochemistry, 17(3): 259-289.

[6].- Fidalgo A., Ciriminna R., Carnaroglio D., Tamburino A., Cravotto G., Grillo G. and Pagliaro M., 2016.- Eco-friendly extraction of pectin and essential oils from orange and lemon peels. ACS Sustainable Chemistry and Engineering, 4(4): 2243-2251.

[7].- Nasseri A. T., Thibault J. F., and Ralet M. C., 2008.- Citrus Pectin: Structure and Application in Acid Dairy Drinks. Tree and Forestry Science and Biotechnology, 2: 60-70.

[8].- Toudert N., Djilani S. E., Djilani A., Dicko A., and Soulimani R., 2009.- Antimicrobial activity of the butanolic and methanolic extracts of Ampelodesma mauritanica. Advances in Natural and Applied Sciences, 3(1), 19-21.

[9].- Merzoud M., et Habita M. F., 2008.-. Elaboration de composite cimentaire à base de diss « Ampelodesma Mauritanica ». Afrique Science: Revue Internationale des Sciences et Technologie, 4(2).

[10].- Krope J., Olabi A. G., Goričanec D. and Božičnik, S., 2017.- *Ampelodesmos Mauritanicus* Pyrolysis Biochar in Anaerobic Digestion Process: Evaluation of the Biogas Yield.

[11].- Djilani A., Toudert N., and Djilani S., 2011.- Evaluation of the hypoglycemic effect and
Antioxidant activity of methanol extract of Ampelodesma mauritanica roots. Life Sciences and
Medicine Research, 31: 1-6.

[12].- Wasser S. P., 2002.- Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied microbiology and biotechnology, 60(3): 258-274.

[13].- Zeitoun R., 2011.-. Procédés de fractionnement de la matière végétale: application à la Production des polysaccharides du son et de la paille de blé (Doctoral dissertation, INPT).

[14].- Sbiai A., 2011.-. Matériaux composites à matrice époxyde chargée par des fibres de palmier Dattier: effet de l'oxydation au tempo sur les fibres (Doctoral dissertation, Lyon, INSA).

[15].- Mollea C., Chiampo F., and Conti R., 2008.-. Extraction and characterization of pectins from cocoa husks: A preliminary study. Food Chemistry, 107(3): 1353-1356.

[16].- Terinte N., Ibbett R. and Schuster K. C., 2011.- Overview on native cellulose and microcrystalline cellulose I structure studied by X-ray diffraction (WAXD): Comparison between measurement techniques. Lenzinger Berichte, 89: 118-131.

Tel/Fax : (+213) 029 71 56 89 e-mail : polysac2017@gmail.com

[17].- Grobe. A., 1989.- Polymer handbook, edition J. Bandruo; E.H. Immergut New York, pp.117-170.

[18].- Fan M., Dai D. and Huang B., 2012.- Fourier transform infrared spectroscopy for natural fibres. In Fourier Transform-Materials Analysis. InTech.

[19].- Maghchiche A., Haouam A. and Immirzi B., 2013.- Extraction and characterization of Algerian Alfa grass short fibers (*Stipa Tenacissima*). Chemistry and Chemical Technology, (7, № 3): 339-344.

[20].- Benyahia A. and Merrouche A., 2014.- Effect of chemical surface modifications on the properties of alfa fiber-polyester composites. Polymer-Plastics Technology and Engineering, 53(4): 403-410.

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