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Chemical And Biological Studies of Polysaccharides Extracted from the Seeds of *Cassia hypophallus* (FABACEAE)

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ABSTRACT: The genus *Cassia hypophallus* belongs to the Fabaceae family. The unity of the Fabaceae family is due to its fruit, called a pod or legume, hence the other denomination of Leguminosae by which this family is better known. Although rich in polysaccharides like other plants in the same family, this plant has not yet been exploited for polysaccharide production. Our work aims to define the conditions for extracting polysaccharides from the seeds of this plant, identify different secondary metabolites, determine the structure, characterize physicochemical properties, and assess antioxidant properties. Our work led to the obtaining of galactomannan-type polysaccharides with a yield of 12%, composed of 23% neutral sugars, 0.7% acidic sugars, 19.6% sulfates, and 10% proteins, with a molar mass of 1,337,000 g/mol. This polysaccharide is mainly composed of mannose (2.28%) and galactose (1%), along with four other minor monosaccharides (rhamnose, glucose, xylose, and arabinose) in its structure. The polysaccharide fractions do not form a gel regardless of the ionic strength. Both polysaccharide and oligosaccharide fractions exhibited antioxidant properties.

KEYWORDS: *Cassia hypophallus*, galactomannan, antioxidant activity

I. INTRODUCTION

Plants constitute a reservoir of biologically active compounds that have been used by humans since the dawn of humanity. The growing interest in naturally derived compounds revives ethnobotanical investigations to valorize plants traditionally used for their medicinal properties (Meriane 2018). Madagascar is globally known for its natural riches and abundant medicinal plants. Among these, many are endemic, and numerous have not yet been thoroughly studied (Rasoanaivo, 2000). This endemic flora, which has not yet revealed all its secrets, is a wealth still undervalued for the Malagasy population. Moreover, it is strongly threatened by logging, agriculture, and the arrival of numerous invasive foreign species. Polysaccharides are the most abundant macromolecules on earth, originating from various renewable and abundant sources such as plants, microorganisms, and animals. These biopolymers are generally highly hydrophilic, biodegradable, and biocompatible. They are extensively used in the agri-food and pharmaceutical industries for their technological, physiological, and pharmaceutical properties, playing significant roles in the global economy (Efstathiou, *et al*, 2012).

In this context, Madagascar has been the subject of relatively few studies on plant-derived polysaccharides. Acknowledging this, the Laboratory of Natural Substances Chemistry at the Faculty of Sciences, University of Diego Suarez, focuses on the chemical and biological valorization of polysaccharides produced by higher plants.

In this context, we chose to conduct "Chemical and Biological Studies of Polysaccharides Extracted from *Cassia hypophallus* Seeds (Fabaceae)."

This thesis work has two objectives: to extract, purify, and characterize water-soluble polysaccharides from *Cassia hypophallus* seeds, as well as to study their rheological and biological properties in particular. This study is part of a collaboration between the University of Antsiranana (Diego Suarez, Madagascar) and the Institute Pascal laboratory (UMR CNRS 6602) at Blaise Pascal University (Clermont-Ferrand, France).

II. RELATED WORK

Dry pods of *Cassia hyppophallus* filled with seeds are stored in kraft paper bags. They are transported to the laboratory and stored in a cool, dry place away from light. The seeds were manually isolated from the dry pods.

The seeds and husks were ground into fine powder (<500 µm) using a mechanical grinder (Blended 800 ES). The powders were stored in a cool, dry place away from light.

II.1. Biological Materials: *Cassia hyppophallus* (Capuron, 1968)

II.1.1. Classification**

- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiosperms
- Class: Dicotyledons
- Order: Fabales
- Family: Fabaceae
- Subfamily: Caesalpinioideae
- Genus: *Cassia*
- Species: *hyppophallus*
- Vernacular Name: Latatsoavaly



*Figure 1: Photo of pods, seeds, and seed powder of *Cassia hyppophallus* (Author)*

II.2. Geographical Distribution

II.2.1. Harvest and Studied Parts

For this study, *Cassia hyppophallus* species were harvested in the Sakaramy region, rural commune of Antsiranana located in the north of Madagascar in August 2016.

In this study, *Cassia hyppophallus* pods were selected for polysaccharide study. They were transported to the laboratory and stored in a cool, dry place away from light, at room temperature (OMS, 2003).



Figure 2: Location of study sites.

III.METHODOLOGY

III.1. Preliminary Studies of *Cassia hypophallus***

Phytochemical screening is a qualitative analysis based on precipitation and/or coloration reactions. These reactions help to define the presence or absence of secondary metabolites such as coumarins, saponins, quinones, flavonoids, alkaloids, anthocyanins, and tannins in different plant organs (Daira . *et al*, 2016).

III.2. Structural Studies of Polysaccharides from the Genus *Cassia hypophallus*

The structural study of polysaccharides from the genus *Cassia hypophallus* must be carried out with pure extracts, requiring optimization of extraction and purification conditions (Nwokocha *et al*, 2014). The chemical characterization of these polysaccharides primarily involves determining their chemical structure. Typically, the structural characterization of polysaccharides relies on the assessment of their overall composition. Thus, the proportions of neutral sugars (Dubois *et al.*, 1956), uronic acids (Blumenkrantz and Asboe-Hansen, 1973), as well as proteins and sulfate groups, will be estimated through this method.

Subsequently, a chromatographic method (HPAEC: high-pressure anion exchange chromatography) was performed to specify the composition of monosaccharides and uronic acids. The structure and their substituents are determined by IR spectroscopy (Infrared). The determination of molar masses was carried out by size exclusion chromatography coupled with multi-angle laser light scattering (SEC/MALLS). The identification of organic compounds was carried out using a thin-layer chromatography-based analysis method (TLC: Thin-Layer Chromatography) (Delattre, 2005). The measurement of the antioxidant activity of *Cassia hypophallus* polysaccharides was conducted by the determination of ascorbic acid or vitamin C.

IV. EXPERIMENTAL RESULTS

IV.1. Phytochemical Screening Results

Phytochemical screening is essentially based on precipitation reactions and specific coloration reactions for different secondary metabolites. The table below summarizes the overall results of the chemical screening of two organs of the *Cassia hypophallus* species, knowing that:

- The presence of the substance is represented by: +
- The absence of the substance is represented by: -

Table 1: Overall results of phytochemical screening of the species

Test	Reagents	Cold Seed Extracts				Hot Seed Extracts			
		EtOH	MetOH	Ac	Aq	EtOH	MetOH	Ac	Aq
Alkaloid	Mayer	+	-	-	-	+	-	-	-
	Wagner	+	-	-	-	+	-	-	-
Tannin & other polyphenols	Gelatin	-	-	-	-	-	-	-	-
	Selective gelatin	-	-	-	-	-	-	-	+
	FeCl ₃ MetOH	+	-	+	-	+	-	+	+
Polysaccharides	-	-	-	+	-	-	-	+	
Ferric chloride	+	+	+	-	+	+	+	-	
Leucoanthocyanin	+	-	+	-	+	-	+	-	
Saponins	+	-	+	-	+	-	+	-	
Deoxyhexoses	+	+	+	+	+	+	+	+	
Iridoids	+	-	+	-	+	-	+	-	
Flavonoids	+	+	+	-	+	+	+	-	
Reducing sugars	+	+	+	+	+	+	+	+	
Triterpene & Steroids	Sakowski Test	+	+	+	+	+	+	+	+
	Lieberman-Burchard Test	+	+	+	-	+	+	+	+
Oses and Holosides				+				+	

IV.2. Polysaccharide Extraction Results from *Cassia hypophallus* Seeds

The extraction of water-soluble polysaccharides from *Cassia hypophallus* seeds was followed by their purification through ethanol precipitation and acetone washing. The obtained product is described as depigmented.

Table 2: Polysaccharide extraction results from *Cassia hypophallus* seeds

Extraction Type	Cold Aqueous Extraction	Hot Aqueous Extraction
Color	Off-white	White
Yield (%)	4,5	10

Table 3: Oligosaccharide production results

Degradation	Acid Hydrolysis
Color	White
Yield (%)	70

IV.3. Neutral Ose Quantification

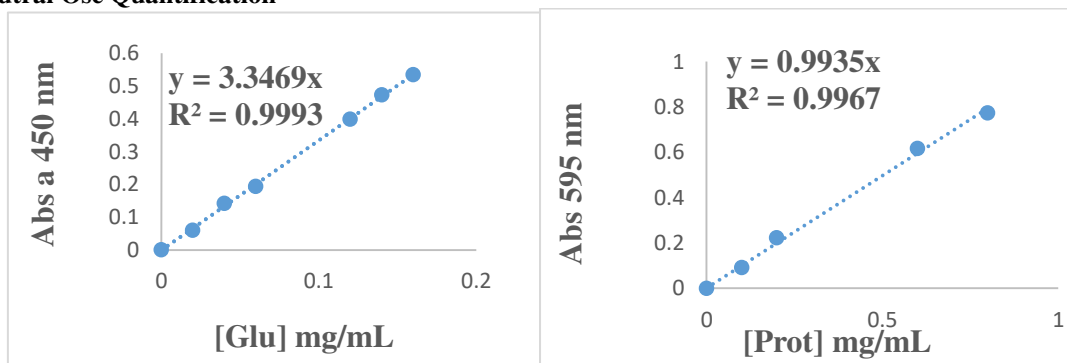


Figure 3: Absorbance standard curve as a function of neutral ose concentration ((Abs)= f [ON]) and Absorbance standard curve as a function of protein concentration ((Abs)=f [Pro]).

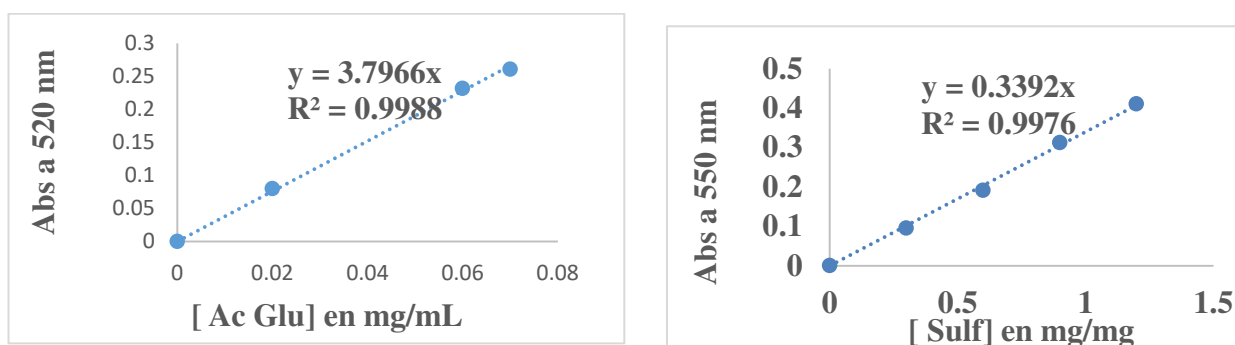


Figure 4: Absorbance standard curve as a function of sulfate concentration ((Abs)=f [Sul]) and Absorbance standard curve as a function of glucuronic acid concentration ((Abs)=f [Ac. Glu]).

IV.4. Monosaccharide Composition

The monosaccharide composition study of *Cassia hypophallus* polysaccharide extract was conducted by HPAEC after total hydrolysis of 10 mg of polysaccharides with 4M trifluoroacetic acid (TFA) at 100°C for 4 hours in a water bath. The hydrolysates were neutralized with a 4M ammonia solution after cooling, then diluted to 1/10 in Milli-Q water.

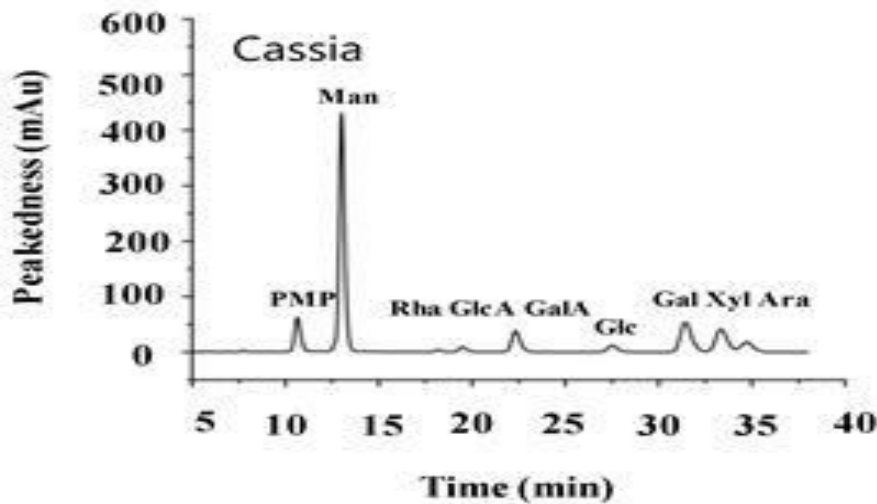


Figure 5: Chromatogram of *Cassia hypophallus* polysaccharides hydrolyzed and analyzed by HPAEC.

Table 5: Analysis of constitutive monosaccharides of polysaccharide extracted from *Cassia hypophallus* seeds

Identified Oses	Molar %
Mannose	2,28
Rhamnose	0,07
Glucose	0,17
Galactose	1,00
Xylose	0,79
Arabinose	0,29
Glucuronic Acid	0,12
Galacturonic Acid	0,52

IV.5. Infrared Spectroscopy of *Cassia hypophallus* Polysaccharide and Oligosaccharide**

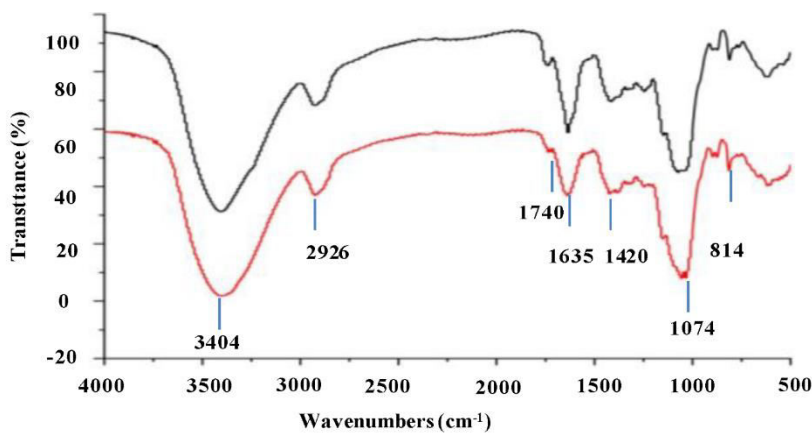


Figure 6: Spectrum of polysaccharides extracted from *Cassia hypophallus* seeds. Black: *Cassia* - P; Red: *Cassia* - O.

IV.6. Determination of Molar Mass by Size Exclusion Chromatography Coupled with Multi-Angle Laser Light Scattering (SEC/MALLS) of *Cassia hypophallus* Seed Polysaccharides

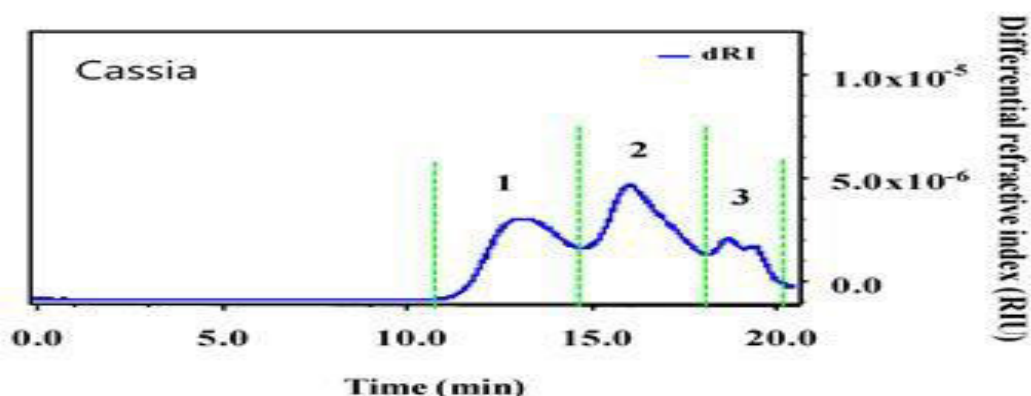


Figure 7: Elution profile in size exclusion chromatography of *Cassia hypophallus* polysaccharide at 0.5g/L in 0.1M NaNO₃. Refractometry detection (DRI: – solid blue). Mw (weight-average molar mass), Mn (number-average molar mass), Ip (polydispersity).

Table 6: Weight and number average molecular masses of *Cassia hypophallus* polysaccharide

Samples	Polysaccharides peaks		
	Fraction1	Fraction 2	Fraction 3
pics			
Mw(Da)	1 337 000 (± 1,55%) ^a	98 380 (± 1,88%) ^b	25 140 (± 3,66) ^b
Mn(Da)	855 900	69 100	20 030
Ip= Mw/Mn	1,562	1,423	1,255
[η](mL/g)	281± 0,05 ^a		

IV.7. Study of Gelling Properties

In this study, 0.5 g of *Cassia hypophallus* polysaccharides were dissolved in 20 ml of a 1 M ionic solution, and the results are summarized in the table below.

Table 7: Gelation test results

Sample	Ion	Solubilization	Gelation
Polysaccharide	K ⁺	Viscous	No
Polysaccharide	Na ⁺	Viscous	No
Polysaccharide	Ba ²⁺	Viscous	No

IV.7. Study of Antioxidant Properties of Polysaccharides Extracted from *Cassia hypophallus* Seeds and Husks

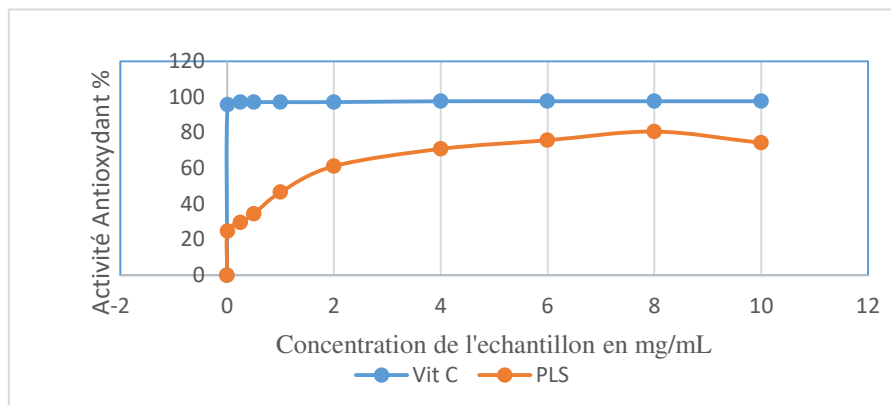


Figure 8: Evolution of the reducing power of polysaccharide fractions from *Cassia hypnophallus* seeds and vitamin C.

V. CONCLUSION

Table 4 shows the results obtained from the study of the composition of polysaccharides extracted from *Cassia hypnophallus* seeds, where neutral oses (23%) dominate compared to acidic oses (0.7%). By comparing our result with the water-soluble polysaccharides of *Astragalus gombo Bunge* seeds (Chouana, 2017), we observed that the polysaccharide of *Cassia hypnophallus* has a significantly higher protein content (10%) compared to that of *Astragalus gombo Bunge* (1.8%). However, regarding neutral oses, for the seed extract of *Astragalus gombo Bunge*, the neutral ose content (85%) predominates compared to the neutral ose extracted from *Cassia hypnophallus* seeds (23%). Polysaccharides isolated from *Astragalus armatus* seeds contain 6.54% protein content (Boual, 2015), which is lower than that found with *Cassia hypnophallus* seeds, namely 10%. The sulfate content (19.6%) is higher compared to the protein content (10%) in our extract. According to this table, the monosaccharide composition consists of mannose (2.28%) and galactose (1%), as well as 4 other minor monosaccharides (rhamnose, glucose, xylose, and arabinose) in its structure. According to Table 5, the polysaccharide from *Cassia hypnophallus* is therefore of the Galactomannan type with a ratio: Man/Gal=2.28. The molar percentages of neutral oses and acidic oses were also detected. They are respectively 4.6% and 0.64%. These results are comparable to those of other published seed polysaccharides. The purity of GMs and the Manp/Galp ratio appear to be quite variable among Fabaceae seeds depending on the plant origin and the extraction processes (Kodiralieva and Rakhmanberdyeva 2011; Muschin and Yoshida, 2012, Srivastava and Kapoor, 2005). In comparison with other GMs from *Astragalus sp* seeds, the Ip of 1.56 is close to that of the GM from *Astragalus armatus* seeds (Boual *et al.*, 2015). The intrinsic viscosity indicating the hydrodynamic volume occupied by macromolecules in a diluted solution was estimated for the GM of *Cassia hypnophallus* at 281mL/g. This value is lower than the $[\eta]$ values of *A. gombo* at 860 mL/g but it is close to those of other intrinsic viscosity values of GMs extracted from seeds of the *Astragalus sp* genus ranging from 471 to 1337 mL/g (Mestechkina *et al.*, 2000; Olennikov and Rokhin, 2008, 2009, 2010, 2011). These differences are related to the plant origin and the extraction/purification processes used, which influence the molecular weight and intrinsic viscosity.

VI. ACKNOWLEDGEMENTS

This study is part of a collaboration between the University of Antsirananana (Diego Suarez - Madagascar) and the laboratory of the Pascal Institute (UMR CNRS 6602) of Blaise Pascal University (Clermont Ferrand France).

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