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Original article

Preliminary Extraction and Characterization of Galactomannan from *Cajanus cajan* and *Mucuna pruriens* Seeds

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ABSTRACT:
Galactomannan is a natural polymer, namely polysaccharides isolated from endosperm seeds, especially from the Leguminoceae family which consists of mannan as the main chain and galactose in its side chain. The galactomannan application covers a variety of industrial fields from the food industry to biomedicine. This makes the demand for galactomannanis high. However, to date, only the main commercial galactomannan sources have originated from the locust bean and guar gums. There are still many galactomannan sources that have not been explored and characterized by their physicochemical properties. Therefore, there is still an opportunity to obtain galactomannan sources including *Cajanus cajan* and *Mucuna puriens*. In this study, exploration was carried out to obtain new galactomannan sources from C. *cajan* and *M. puriens* using extraction and centrifugation results from flocculation using ethanol. Galactomannan isolate then analyzed the mannose/galactose ratio. The results of the analysis of *C. cajan* and *M. puriens* seeds using HPLC showed that galactose/mannose ratios were 2.75 and 3.21 respectively. The presence of a galactose group in a mannose molecule functions as a steric hindrance between galactomannan polymer molecules to influence the solubility properties of galactomannan in water. In other words, galactomannan solubility increases if galactose substitution is also high.

Keywords: galactomannan, galactose-mannose ratio, *Cajanus cajan, Mucuna puriens*

1. INTRODUCTION

Galactomannan is a polysaccharide composed of β- (1–4) -d-mannan as the main chain and d-galactose side chain in α- (1–6) (Cerqueira, 2011). Galactomannan was isolated from endosperm of dicotyledon seeds, planted in the Leguminoceae family. Galactomannan is mainly isolated from the endosperm of the seeds of the family plant Leguminosae. Galactomannan was also identified in plants belonging to Annonaceae, Ebenaceae, and Loganiaceae. The galactomannan produced by each species is different in terms of the ratio of mannose/galactose (M/G ratio) to the structure [1].

The molar ratio of galactose to mannose in the galactomannan structure varies between plant species ranging from 1.0: 1.0–1.1, 1.0: 1.6–1.8, 1.0: 3.0 [2].The presence of a galactose group in a mannose molecule functions as a steric hindrance between galactomannan polymer molecules to influence the solubility properties of galactomannan in water. In other words, galactomannan solubility increases if galactose substitution is also high. The variation of galactomannan structure, especially in the case of mannose/galactose ratio and the arrangement of galactose substitution in the main chain results in changes in the solubility, viscosity, and interactions between galactomannan and other polysaccharides[1, 3, 4].

Polysaccharides with high galactose content, for example in galactomannan from guar gum, are soluble in water and tend to form low gels, compared to galactomannan produced by locust bean gum with a lower mannose/galactose ratio. The higher the number of side-chain substitutions on galactomannan, the higher the solubility because the substitution prevents the main chain mannose intermolecular interactions. Whereas galactomannans which have lower galactose substitution or in other words high mannose/galactose ratio, can interact with other polysaccharides due to the absence of steric hindrance between mannose chain molecules [1-5].

The galactomannan used to meet the needs of the food, pharmaceutical, and cosmetics industries to date only come from two main sources, guar gum (*Cyamopsis tetragonoloba*, M/G ratio: 2: 1) and locust bean gum (Ceratonia siliqua with M/G ratio 3.5:1). Actually, there are two other sources, namely tara gum (*Caesalpinia spinosa*

Kuntze with M/G ratio 3:1) and fenugreek (*Trigonella foenum-graecum* L.) both of which are expensive so that it is not feasible to use the scale of production. Various plants have been studied but no one has provided a commercial prospect [2, 4].Meanwhile, the industrial needs are very large, so we need a galactomannan alternative source. In addition to meeting the needs in terms of quantity, the search for new galactomannan sources is also needed to obtain new functionalities, especially for applications in the fields of pharmaceuticals, biomedicine, or cosmetics. This is considering that the characteristics of the galactomannan produced by different plants can have different physicochemical properties as well. Especially in the biomedical field, galactomannan can be used to form hydrogels that can support the management of tissue engineering, stem cell delivery matrices, hydrogel membranes for wound healing, drug delivery matrices, and cosmetic applications. In addition to being supported by its varied physicochemical properties, galactomannan is also non-toxic so it is suitable for biomedical applications[6, 7, 8, 9, 10, 11]. Therefore, efforts are still needed to obtain galactomannan sources that have commercial prospects and have functionalities for various applications.This study aims to explore new sources that can produce galactomannan, which is among them from bean seeds *C. cajan* and *M. puriens*. The galactomannan isolates obtained from the *C. cajan* and *M. puriens* then analyzed for mannose/galactose ratio.

2. MATERIAL AND METHODS

2.1 Materials

Milled C. cajan and M. puriens. The pods were collected from a region in East Java, Indonesia, ethanol 96%, and aquadest.

2.2 Extraction

The polysaccharide extraction method used in this study refers to a procedure that was used by Cerqueira *et al*. [4]. Dry bean seeds were crushed and the endosperm was taken and then milled. The milled seeds were then sifted to obtain fine powder then suspended in 96% ethanol with a ratio of 1: 3 (gram of seeds: milliliter of ethanol 96%) at 70 ^{o}C for 15 minutes to inactivate the enzyme. Ethanol is decanted, then distilled water is added in a ratio of 1: 5 (endosperm:water). This suspension is then left to stand for 24 hours. Next, add water in a ratio of 1:10 (suspension:water) and homogenize using a blender for 5 minutes. The result of this homogenization was then filtered using a cloth. The filtering result was centrifuged at 3800 g for 20 minutes at 20 ^{o}C. Supernatant from the centrifugation was added with 96% ethanol (ratio 1: 2) to precipitate the galactomannan. Ethanol was then decanted and galactomannan sedimentwas then dried using the lyophilization method and stored until the next utilization.

2.3 Determination of galactose and mannose content

The analytical method used is HPLC. A sample of 5 ml was put in 10 ml conical and centrifuged at a speed of 8500 rpm for 5 minutes. The filtrate obtained was then filtered using a 0.45 micrometer membrane filter. The test conditions were using the Metacharb 87C HPLC column, sample volume 20 microliter, flow rate 0.5 ml/min, eluent H_2O at temperature 85 ^{o}C, and RID detector.

3. RESULTS AND DISCUSSION

The extraction of *C. cajan* and *M. puriens* seed obtaining galactomannan isolates 2.77% and 11.26% respectively. The two isolates were still in thick form as a result of the flocculation using ethanol with ratio of 1:2. The galactomannan obtained from *M. puriens* has a black color (Figure 1). This was probably because of theendosperm was not completely separated from its skin so there were still components of the skin that were oxidized during the process. It is suggested that increasing the efficiency of sieving seed powder will able in minimizing the remaining seed-skin. This may result clearer isolates of galactomannan. On the other hand, the isolates obtained from *C. cajan*has brighter colors.

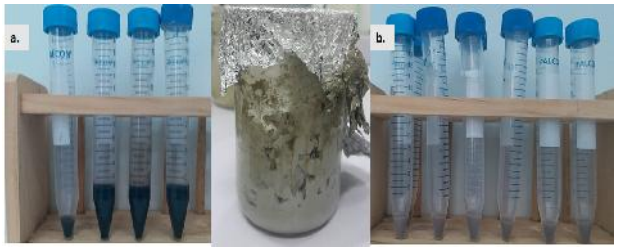

Fig 1: Galactomannan obtained from a) *M. puriens*, and b) *C. cajan*

Galactomannan isolates obtained were then analyzed using HPLC method to determine the ratio of galactose components to mannose (Fig. 2a and 2b). The ratio of galactose to mannose in the galactomannan structure varies between plant species ranging from 1.0: 1.0–1.1, 1.0: 1.6–1.8, 1.0: 3.0[2].The presence of a galactose group in a mannose molecule has a function as a steric hindrance between galactomannan polymer molecules to influence the solubility properties of galactomannan in water. In other words, galactomannan solubility increases if galactose substitution is also high. The results of the analysis of *C. cajan* and *M. puriens* seeds resulted in this study of galactose/mannose ratios were 2.75 and 3.21 respectively.

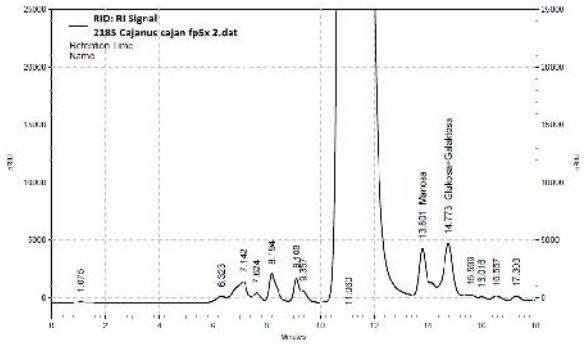

Fig 2a: Results of analysis of galactose and mannose components in *C. cajan*

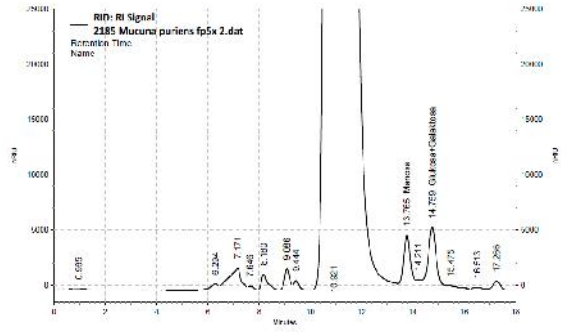

Fig 2b: Results of analysis of galactose and mannose components in *M. puriens*

The resulting galactomannan from each *C. cajan* and *M. puriens* were lyophilized. In this study, the process of lyophilization was not able in resulting powder but semisolid mass. This was probably caused by the cooling temperature and pressure during sublimation were not optimal. Therefore it is necessary to optimize this lyophilization process. Another analysis to characterize the galactomannan was the ash content of galactomannan deposits in *C. cajan* and *M. puriens*. The method used in this study was gravimetric. The data obtained were the ash content for galactomannan deposits from *C. cajan* was 2.10% and *M. puriens* was 2.21%. This data shows the presence of impurities or the presence of mineral materials in the sample.

4. CONCLUSIONS

Galactomannan was successfully isolated from *C.cajan* and *M. puriens*resulting galactose/mannose ratio of 2.75 and 3.21 respectively. The yield of the galactomannan isolates obtained from the two samples was different where *M.puriens* was higher than *C.cajan*. Nevertheless, we cannot conclude that the best galactomannan source is *M.puriens* because currently the galactomannan isolate still needs to be optimized for the drying process to produce a powder.

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