ORIGINAL ARTICLE

Development and validation of visible spectrophotometric method for the determination of total D-glucose and D-mannose levels calculated as glucomannan in porang powder *(Amorphophallus oncophyllus)* and Konjac powder *(Amorphophallus konjac)*

Sudjarwo, G. Fransiska, A. Prawita

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

Abstract

Objective: Porang or konjac is one type of tubers that are widely used in industry, food, cosmetics, and medical. Porang and Konjac contain glucomannan, a polysaccharide consisting of D-glucose and D-manosa. **Materials and Methods:** One way to determine the content is using phenol-concentrated sulfuric acid reagent with visible spectrophotometric method. Before determinate how much glucomannan in porang, the color stability test and method validation were performed. **Results:** From the color stability test, the absorbance was observed after 180 min. For the validation result, the selected wavelength was 492.0 nm, and the equation of regression was y = 0.0266x+0.0019 (r = 0.9992 > r table = 0.834) and Vxo 2.16%, while the precision gave the coefficient of variation 0.54% and accuracy gave average recovery for porang powder (99.60 ± 0.86% w/w) and konjac powder (100.48 ± 0.56% w/w). **Conclusion:** The determination of glucomannan was carried out in two samples, which were taken from Indonesia (porang powder) and China (konjac powder). The result showed that the concentration of glucomannan in porang powder was 52.33 ± 0.74% (w/w) and in konjac powder was 61.24 ± 0.60% (w/w).

Key words: Glucomannan (D-glucose and D-mannose), konjac powder (*Amorphophallus konjac*), phenol-sulfuric acid reagent, porang powder (*Amorphophallus oncophyllus*), validation method, visible spectrophotometry

INTRODUCTION

Porang or konjac plant is one of the tuber plant species that widely used in industry, food, cosmetics, and health. Utilization is due to the carbohydrate content of glucomannan. Glucomannan is a polysaccharide consisting of 33% D-glucose units and D-mannose of 67% with a molar ratio of 1:1.6 with a β-1,4 glycosidic bond form.[1] Both have the $C_6H_{12}O_6$ molecular formula, in which D-mannose itself is an isomeric form of D-glucose having a difference in the position of the hydroxy group (-OH) at the number two C atom.[2]

Glucomannan content in porang can reach 45–65%. Glucomannan plays a role in health as a diet,[3] reduce cholesterol,[4,-6] increasing the mass of the stool,[7] prebiotics,[6,8,9] and enhancing the immune system.[10,11]

The determination of carbohydrate levels can be done by high-performance liquid chromatography and gas chromatography methods, but these two methods are rarely used given that the price is quite expensive. Enzymatic methods can also be done but require specific enzymes of each type of sugar, require a large cost, and have a long process. There is also a

Address for correspondence:
Sudjarwo, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia. E-mail: sudjarwo0958@gmail.com

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method of reducing sugar, but it is necessary to hydrolyze it first to become a monosaccharide. Other research uses visible spectrophotometry by adding phenol-sulfuric acid without hydrolysis.[12,13]

With the addition of phenol-sulfuric acid reactants to D-glucose and D-mannose, it will produce a yellow-orange complex compound so that it can be assayed with a visible spectrophotometry at 490 nm wavelength.[14] The addition of this phenol-sulfuric acid is considered easy to apply, reproducible, and sensitive.[15]

The phenol-sulfuric acid reaction will react with the D-glucose and D-mannose monosaccharides so that it will form a complex compound of a furfural derivative named 5-hydroxymethyl furfural which gives a yellow-orange color.[13,16,17]

MATERIALS AND METHODS

Instrument

UV-visible spectrophotometer (Hitachi UH-5300), analytical scales, and glass tools were commonly used in laboratories.

Chemicals

Porang powder (The Ministry of Agriculture Research and Development Agency Postharvest Agriculture, Indonesia). Konjac powder (Seatech Conyaku Jelly), China; phenol p.a from Merck; sulfuric acid p.a from Merck; and aquadest.

Procedures

Water content determination

The determination of moisture content is carried out by gravimetric method according to the procedure at AOAC (2005)[18]

Sample preparation

The powder sample was weighed 50.0 mg, added aquadest 40.0 ml, and stirred for 4 h with a magnetic stirrer, put in a 50.0 ml measuring flask and augmented the aquadest to the exact mark. Centrifuge at 4000 rpm for 40 min and plugged a 2.0 ml supernatant and placed in a 10.0 ml measuring flask. Then, 1.0 ml of 5% (w/w) phenol solution, 5.0 ml of concentrated sulfuric acid, and aquadest to the appropriate marks were added. After 3 h, its absorption was observed at selected wavelengths.[14]

Color stability

The stability of the color is done by reading the absorption of the prepared D-glucose solution at 180, 240, and 300 min at the selected wavelength.

Validation Method

Selectivity

The selectivity of porang and konjac powder is done by looking at the spectra of the sample, D-glucose solution, and the sample having added D-glucose solution at 400–550 nm wavelength to determine the selected wavelength.

Linearity

A series of standard D-glucose solutions with an increased concentration were added, each of which added 1.0 ml of phenol solution of 5% (w/w), 5.0 ml of concentrated sulfuric acid, and aquadest to the exact mark. After 3 h, the absorption was observed at the selected wavelength.

Table 1: Water content of porang and konjac powder

Replication	Porang powder (%, w/w)	Konjac powder (%, w/w)
1	13.34	10.69
2	13.42	11.04
3	13.42	10.79
Mean±SD	13.39±0.0462	10.84±0.1803
CV	0.34	1.66

SD: Standard deviation, CV: Coefficient of variation

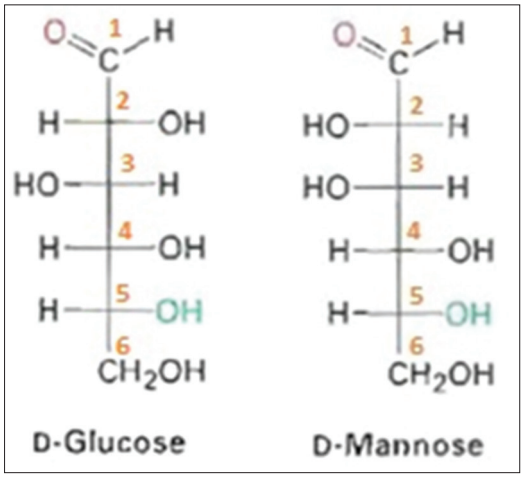

Figure 1: D-glucose and D-mannose structures

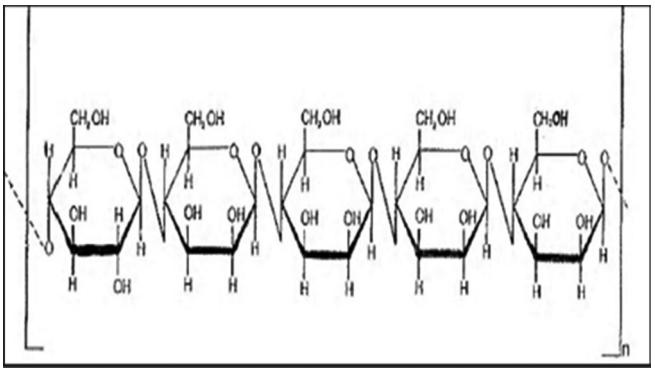

Figure 2: Structure of glucomannan

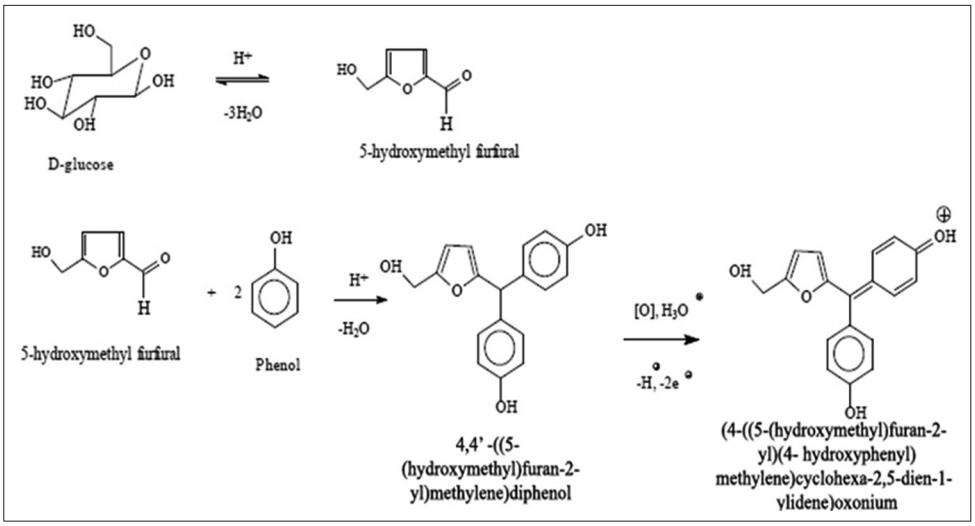

Figure 3: Reaction between D-glucose and phenol-sulfuric acid

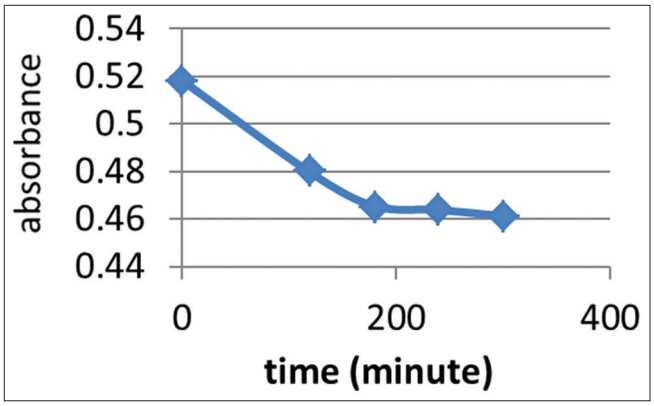

Figure 4: Color stability

Table 2: Precision

Replication	Absorbance (λ 492.0 nm)
1	0.3526
2	0.3550
3	0.3544
4	0.3551
5	0.3507
6	0.3557
7	0.3543
8	0.3531
9	0.3498
10	0.3524
Mean±SD	0.3533±0.0019
CV (%)	0.54

SD: Standard deviation, CV: Coefficient of variation

Precision

A standard D-glucose solution, each adding 1.0 ml of 5% (w/w) phenol solution, 5.0 ml concentrated sulfuric acid, and aquadest to 10.0 ml. After 3 h, the absorption was observed at the selected wavelength of 10 times.

Accuracy

Accuracy of porang and konjac powder was performed with 80%, 100%, and 120% (D-glucose 80.0 mg, 100.0 mg, and 120.0 mg), each of which added porang powder solution, 50.0 mg, 60.0 mg, 70.0 mg, 80.0 mg, and 100.0 mg. Then, each of the 2.0 ml was added 1.0 ml of 5% (w/w) phenol solution, 5.0 ml of concentrated sulfuric acid, and aquadest to a volume of 10.0 ml. After 3 h, the absorption was observed at the selected wavelength. Accuracy requirements if percent of recovery is 98-102%.[19]

Determination of Total D-Glucose and D-mannose Levels Calculated as Glucomannan

Porang and konjac powder 100.0 mg, each added with a solution equivalent to D-glucose 50.0 mg; 60.0 mg; 70.0 mg; 80.0 mg and 100.0 mg. Then pipette 2.0 ml add 1.0 ml of 5% (w/w) phenol solution, 5.0 ml concentrated sulfuric acid and aquadest to a volume of 10.0 ml. After 3 h, the uptake was observed at selected wavelengths.[14]

RESULTS AND DISCUSSION

Water Content Determination

The determination of the water content is carried out gravimetrically with the amount of porang powder weighed for 1.0 g. The results of the determination of water content are shown in Table 1.

Water content of porang powder was obtained 13.39±0.0462% (w/w) and konjac powder 10.84±0.1803% (w/w). The other

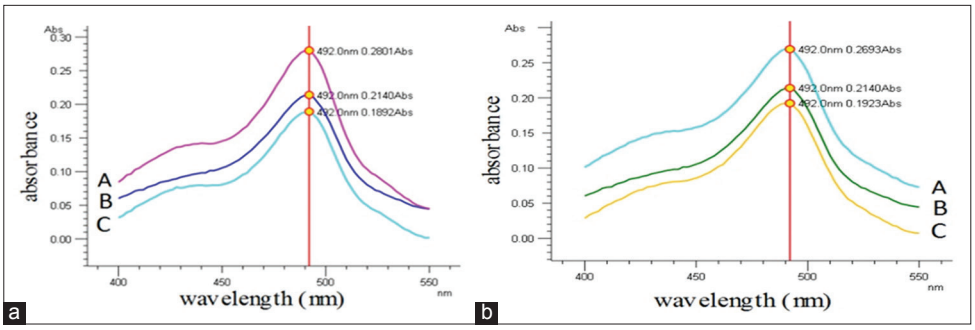

Figure 5: (a) Selectivity (A) porang+D-glucose addition, (B) Standard D-glucose; (C) Porang. (b) Selectivity, (A) Konjac+Standard D-glucose, (B) Standard D-glucose, (C) Konjac

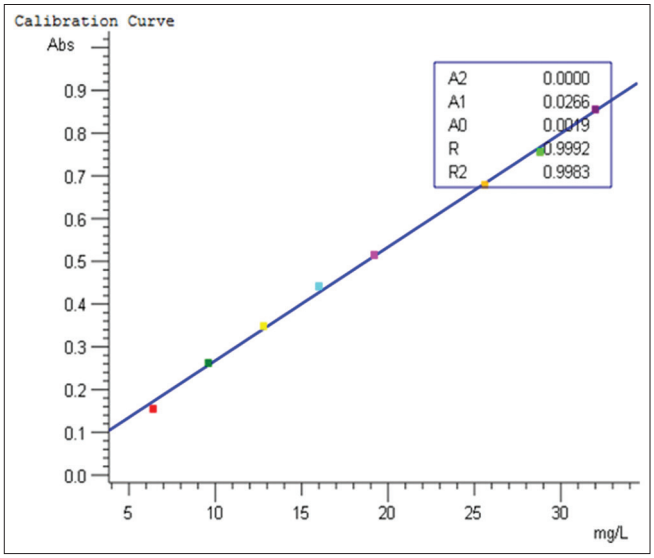

Figure 6: Linearity D-glucose solution

Table 3: Accuracy of porang powder *(Amorphophallus oncophyllus)*

(%) Content	Added (g)	Obtained (g)	Recovery (%; b/b)
80%	0.0830	0.0830	100.00
	0.0799	0.0798	99.87
	0.0815	0.0822	100.86
100%	0.1000	0.0996	99.60
	0.0980	0.0985	100.51
	0.1001	0.0992	99.10
120%	0.1204	0.1191	98.92
	0.1204	0.1198	99.50
	0.1203	0.1179	98.00
Mean±SD			99.60±0.8610
CV			0.86

SD: Standard deviation, CV: Coefficient of variation

research reported, not mentioned the determination of moisture content.[14] However, differences in water content can occur and can be due to differences in the type of tubers used, the age of the plant, as well as the process of making porang/porang powder itself from the porang bulb, especially in the drying process.

Color Stability

The reaction product between glucose and phenol-sulfuric acid will form a yellow-orange compound as shown in Figures 1-3.

The stability of the color of the reaction is shown in Figure 4. This color stability test is performed to see the stability of the color of the sample reaction with phenol-sulfuric acid. The color stability (Anova one way), and the result no siginificant difference at 180–300 minute $p = 0.326$ ($p > 0.05$). Thus, the absorption measurements were made at 180–300 min after the sample was reacted.

Meanwhile, when compared with other studies such as research of Chua *et al*. (2012), which examined glucomannan concentration in konjac *(A. konjac)* with the same method, it was not mentioned whether the color stability test was done first, but it is known that the measurement of absorption is done after 10 min of silence at room temperature, so there is a difference of absorbance reading time of 180 min. The time difference impacts the absorption value so that it affects the next step.

Selectivity

Selectivity test is performed to determine the selected wavelength. Selectivity is done by looking at sample spectra, glucose working standard, and samples with glucose addition [Figure 5a and b].

From the selectivity of porang and konjac, wavelength selected 492.0 nm because gives maximum absorption. Hence, the next absorption readings are done at 492.0 nm wavelength, while in the article absorption, readings were carried out at 490.0 nm wavelength.[14] The wavelength difference of 2 nm is considered the same.

Linearity

Linearity was performed to see a linear relationship between concentration and absorption through the correlation coefficient (r). The test was performed using eight different concentrations.

The result of regression equation is y = 0.0266x+0.0019 (r count = 0.9992>r_{table} = 0.834), with Vxo (coefficient of variation [CV] of function) 2.16% (Vxo <5%), so this method has eligible linearity parameter (r count > r table) [Figure 6].

Table 4: Accuracy of Konjac powder *(Amorphophallus konjac)*

(%) Content	Added (g)	Obtained (g)	Recovery (%; b/b)
80%	0.0801	0.0812	101.37
	0.0800	0.0804	100.50
	0.0800	0.0809	101.12
100%	0.1001	0.1002	100.10
	0.1000	0.1000	100.00
	0.1001	0.1012	101.10
120%	0.1200	0.1200	100.00
	0.1201	0.1200	99.92
	0.1203	0.1206	100.25
Mean±SD			100.48±0.5655
CV			0.56

SD: Standard deviation, CV: Coefficient of variation

From the article, known glucose standard curve with five kinds of concentration obtained correlation coefficient value of 0.9978.[14] The difference of correlation coefficient is due to different glucose concentrations, but both has a linear relationship between concentration and absorption.

Precision

Precision was performed to test the method to be used to measure samples with adjacent results measured by the value of CV.

Obtained coefficient of variation 0.54%, so that precision fulfill requirement because requirement CV < 2% [Table 2]. The precision difference is due to differences in glucomannan concentration and the observation time of uptake.[14]

Accuracy

Accuracy tests were performed at levels of 80%, 100%, and 120% using addition techniques. It is said to be accurate if the percent recovery is obtained between 98% and 102%.[19]

Table 5: Total D-glucose and D-mannose levels calculated as glucomannan on porang powder *(Amorphophallus oncophyllus)*

Replication	Heavy porang powder (g)	Heavy D-glucose and D-mannose (g)	Percentage (%; w/w)
1	0.1000	0.0522	52.20
2	0.1000	0.0521	52.10
3	0.1000	0.0529	52.90
4	0.1001	0.0529	52.85
5	0.1003	0.0524	52.24
6	0.1005	0.0524	52.14
7	0.1002	0.0520	51.90
Mean±SD			52.33±0.3860
CV			0.74

SD: Standard deviation, CV: Coefficient of variation

Table 6: Total D-glucose and D-mannose levels calculated as glucomannan on Konjac powder (*Amorphophallus konjac*)

Replication	Heavy konjac powder (g)	Heavy D-glucose and D-mannose (g)	Percentage (%; w/w)
1	0.1000	0.0611	61.10
2	0.1000	0.0612	61.20
3	0.1001	0.0609	60.84
4	0.1003	0.0612	61.02
5	0.1003	0.0614	61.22
6	0.0995	0.0617	62.01
7	0.1000	0.0613	61.30
Mean±SD			61.24±0.3711
CV			0.60

SD: Standard deviation, CV: Coefficient of variation

The average yield obtained in the porang powder was 99.60 ± 0.86% (w/w) [Table 3] and on the konjac powder was 100.48 ± 0.56% (w/w) [Table 4]. When compared with the accuracy results the other article at 83.9%, that difference caused not do optimization and validation method in the other research.[14]

Total D-Glucose and D-Mannose Levels are Calculated as Glucomannan

To determine the total D-glucose and D-mannose levels calculated as glucomannan, the validated method was used.

The content of glucomannan in porang powder (52.33 ± 0.74% w/w) [Table 5] and konjac powder (61.24 ± 0.60% w/w) [Table 6] was obtained. The other article 83.9%, caused not do optimization and validation method.[14] Variations in levels can be attributed to a variety of factors, ranging from the type of plant, the age of the plant, the length of time after harvest, the treatments, to the tools, and methods used.[20]

CONCLUSION

The results showed that the methods used for the determination of total D-glucose and D-mannose levels calculated as glucomannan in porang powder *(A. oncophyllus)* and konjac powder *(A. konjac)* met the validation requirements of the analysis methods including selectivity, linearity, precision, and accuracy so that it can be applied to the determination of total D-glucose and D-mannose levels calculated as glucomannan in porang powder (*A. oncophyllus*) of 52.33 ± 0.74% (w/w) and konjac powder *(A. konjac*) of 61.24 ± 0.60% (w/w).

Suggestion

Determination of glucomannan level can be done using phenol-sulfuric acid reagent by visible spectrophotometric method.

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