Glucohealth: The potential of porang (*Amorphophallus muelleri*) acid hydrolysed glucomannan as an inhibitor of SARS-CoV-2 interaction with ACE2

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KEYWORDS

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ABSTRACT

The novel SARS-CoV-2 that causing global pandemic COVID-19 known to enter the host cell using the hACE2 as cell receptor. SARS-CoV S1 protein cleaves the ACE2 receptor, then the S2 subunits facilitates the cell membrane fusion, the inhibition of S1-ACE2 interaction can help develop anti SARS-CoV-2 medication.

Porang glucomannan is a polysaccharide known as immunomodulator but never reported as anti-virus by direct inhibition of viral entry. Glucohealth was developed to investigate its potential. Method: Glucohealth is a glucomannan hydrolysate (HGM) that made from porang (*Amorphophallus muelleri*). Hydrolysis was carried using HCl in different concentration (0.25N, 0.5N, 1N) then analyzed its inhibitor activity using ELISA kit. Result: Higher HCl concentration produced HGM with smaller average particle size and lower glucomannan content. However, ELISA studies showed that glucomannan, including its hydrolysates, have the potency to bind with S1 protein and inhibit the binding activity of S1-ACE2. Degraded glucomannan proven to have better bioactivity and able to interact with pathogen to inhibit its cell entry. This project should be a gateway for further biomedical study of glucomannan from Indonesia’s local tuber and new approach to produce more natural therapy against COVID-19.

Introduction

Glucomannan (GM) is a water-soluble polysaccharide abundant in nature, commonly isolated from tubers of *Amorphophallus* sp. This polysaccharide composed of β-1,4 linked d-mannose and d-glucose monomers (Alonso-Sande et al., 2009). Until now, *Amorphophallus konjac* has become one of the main sources of glucomannan commercially and has been used as a source of glucomannan by the Chinese people 2000 years ago before being introduced to the Japanese community 500 years later (Srzednicki and Borompichaichartkul, 2020). The high demand for glucomannan was not balanced with the production of konjac plants which have low yields due to their long growth cycle. This is the beginning of porang or *Amorphophallus muelleri* as native Indonesian tubers began to be cultivated as the main source of commercial glucomannan besides konjac. Porang is considered to have disease resistance, high coefficient of propagation, and adequate glucomannan content.

GM reportedly have promising biopharmaceutical benefits and been widely used in pharmaceutical, chemical, and food technology industries (Wardhani et al., 2020). In some cases, degraded glucomannan proved to have better bioactivity, including its antioxidant activity and immunomodulatory function (Wardhani et al., 2020; Suzuki et al., 2010; Onishi et al., 2005). However, most of these studies used glucomannan from konjac, while research on porang glucomannan was still limited. The development of glucomannan from porang in the biomedical field can increase the added value and competitiveness of porang as a local Indonesian product.

Currently, we are in a crisis situation due to the global pandemic caused by the coronavirus infection. Coronaviruses were pathogens relevant in veterinary medicine before the 21st century but...
not considered hazardous to human health before several outbreaks caused by the virus (da Costa et al., 2020). Coronaviruses reported as the perpetrator of several major respiratory disease outbreaks in the last 20 years, including the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003, the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, and recently the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that first identified at the end of December 2019. Unlike previous coronavirus-linked outbreaks, the Coronavirus Disease 2019 (Covid-19) caused by SARS-CoV-2 resulted in massive global pandemic that until 12 months after the virus first identified in Wuhan, China it has reached more than 71 million confirmed cases with more than 1.5 million confirmed deaths caused by the virus in 220 countries and areas (World Health Organization, 2021). Rapid disease spread of Covid-19 most likely resulted from asymptomatic carriers that spread the virus throughout the world via travel and physical contacts (Machhi et al., 2020). The person-to-person spread made SARS-CoV-2 more contagious than SARS-CoV and became a severe threat to human health in this century (Chen et al., 2020).

On the other side, Covid-19 indicated less virulent than diseases caused by influenza viruses. However, most humans own certain degrees of immunity against influenza due to a long history of human exposure to the viruses but not with SARS-CoV-2 (Abdulamir and Hafidh, 2020). The lack of records in the human immune system towards human coronaviruses especially with novel SARS-CoV-2 causing dangerous situation where the human body cannot immediately generate adequate immune response towards SARS-CoV-2 to tackle the infection. Albeit testing and treatment technologies, including the vaccine, have been quickly developed, pharmacologic and immune-modulatory strategies are still urgently needed to control this pandemic’s spread and fatality (Abdulamir and Hafidh, 2020).

Coronavirus belongs to family Coronaviridae, which are enveloped viruses with a positive-sense and single-stranded RNA (da Costa et al., 2020; Chen et al., 2020). Typical coronavirus consists of major structural components: spike, envelope, membrane, and nucleocapsid (Zheng et al., 2018). The spike glycoprotein (S) covered the outer layer of lipid of coronavirus virions, hence the name corona that means "crown" in Latin (Chorba, 2020). The S protein has a significant role in the pathogenesis of coronavirus, where it acts as a mediator to facilitate viral invasion of coronavirus into the host cell (Arias-Reyes et al., 2020). Angiotensin converting enzyme 2 (ACE2) was identified as the cellular receptors where the extracellular part of ACE2 interacts with the SARS-CoV S protein with high affinity to mediate the binding and entry of coronavirus (Jia et al., 2005).

ACE2 is a membrane glycoprotein expressed in most tissues, mostly in the kidney, endothelium, lungs, and heart (Tikellis and Thomas, 2012). Its interaction with SARS-CoV-2 may be the key to develop Covid-19 medication by producing the inhibitor of SARS-CoV-2 and ACE2 interaction. The inhibition of the interaction between SARS-CoV-2 and ACE2 may prevent virus invasion into the host cell and potentially help prevent the downregulation of ACE2 in the body. Low levels or activity of ACE2 will increase the ratio between ACE and ACE2, leading to a condition where the human body may be at more risk of having worse Covid-19 infection (Pagliaro and Penna, 2020).

This creates conditions where methods of prevention and treatment of viral infections are urgently needed. Despite its popularity, glucomannan as an antiviral, especially with direct inhibition mechanism of S protein and ACE2 interaction was never investigated before. Glucomannan can be used as anti-viral by indirect action as immunomodulator showed by its ability to affect the regulation of pro-inflammatory cytokines such as IL-1β, TNFα, and NFκB (Zheng et al., 2019; Zhao et al., 2020). One of the main obstacles in glucomannan processing is its particle size and high viscosity, making it difficult to handle. Various studies have shown an increase in the biological activity of smaller glucomannans (Onishi et al., 2005; Ojima et al., 2009; Connolly et al., 2010; Suzuki et al., 2010). This makes the glucomannan size reduction process important before it is further modified such as sulfation, oxidation, or so on.

Glucohealth is developed from glucomannan hydrolysate that will explore its potential as SARS-CoV and ACE2 interaction inhibitor. In this study, glucomannan derived from Indonesia's
local porang tuber (Amorphophallus muelleri) hydrolysed by acid hydrolysis in various HCl concentrations to determine its effect SARS-CoV-2 inhibitor activity. The limitation of Glucohealth referred short-chain polysaccharide molecules from the hydrolysis of glucomannan by acid hydrolysis method.

Research Methods
Time and Location
The research was carried out at the Pilot Plant Laboratory and the Food and Agricultural Product Processing Laboratory of the Faculty of Agricultural Technology, the Biomolecular Laboratory of the Faculty of Mathematics and Natural Sciences, and the Central Laboratory of Biological Sciences, Universitas Brawijaya in September 2020 – June 2021.

Materials
The material used in the manufacture of glucomannan is porang flour from local varieties of porang tubers. Chemicals used in the manufacture of HGM and analysis of biological activity include HCl (PA), NaOH (PA), distilled water (distilled water), aquabides (double distilled water), ethanol (PA), Nelson A, Nelson B, arsenomolybate solution, formic acid (PA), glucose, immuno buffer, blocking buffer, and PBS.

Preparation of Glucomannan
Porang tubers, provided by Pusat Penelitian dan Pengembangan Porang Indonesia (P4I), were extracted according to (Wootton et al., 1993) method with slight modification to collect the porang glucomannan (GM) flour. Briefly, porang tubers were peeled and cut into small pieces. Those pieces then blended, followed by filtration and extracted sequentially with ethanol 96%. The glucomannan would precipitate and separated before it went into the drying process. Dried porang flour then grounded into a powder.

Preparation of Hydrolysed Glucomannan
Hydrolysis of glucomannan was carried based on (Suzuki et al., 2010) using HCl in various concentrations. About 2% of porang glucomannan flour dissolved in distilled water and put in shaker water-bath for 20 minutes at 75°C, then hydrolysed with HCl (0.25N, 0.5N, and 1.0 N). Hydrolysis carried on a magnetic stirrer for 60 minutes at 75°C. The glucomannan hydrolysate (HGM) solution then neutralised using 4N NaOH at room temperature followed by addition of 1x volume double distilled water. The solution then filtered in order to collect the supernatant. Next, the supernatant was added with twice of the volume ethanol 99% until the glucomannan precipitated. The HGM was collected and dried using a fluidized bed dryer at 50°C.

Characteristics of HGM
Characteristics of HGM measured by percentage of its glucomannan content and its particle size. In this study, glucomannan was analysed based on the content of reducing sugars in the hydrolysate and sample extracts according to the method (Chua et al., 2012). The particle size measured based on ImageJ measurement of SEM images according to (Igathinathane et al., 2009).

Measurement of SARS-CoV-2 and ACE2 Interaction Inhibition
The inhibitory activity of HGM of SARS-CoV-2 and ACE2 were tested using enzyme-linked immunosorbert assay (ELISA) kit #79954 purchased from BPS Bioscience (San Diego, CA, USA), following the manufacturers' instruction (Figure 1). Blanko (no inhibitor nor ACE2-Biotin) was used as negative control and buffer with no inhibitor used as positive control of S1-ACE2 interaction. Briefly, 50 μL of 2 μg/ml recombinant Spike S1 protein (#100678, BPS Bioscience) was coated overnight at 4°C onto transparent 96-well microplate. The plate then was washed with 100 μL of 1x Immuno Buffer (#79311, BPS Bioscience), blocked for 1 h at room temperature with Blocking Buffer 2 (#79728, BPS Bioscience), and washed again. Then, 10 μL of the HGM (150 μg/ml), as test inhibitors was added into all wells, except for the wells that designated as negative and positive control, and the plate was incubated for 1 h at room temperature with slow shaking. Next, 20 μL of 1 μg/mL of biotinylated ACE-2 (#100665, BPS Bioscience) was added to the wells, except for negative control, and the plate was incubated again for 1 h, washed by immuno buffer and blocked for 10 minutes. After that, HRP-conjugated streptavidin (#79742, BPS Bioscience) was added to each well and the plate was incubated for 1 h. After incubation, the plate was washed and colorimetric HRP substrate was added onto each well. The plate was incubated at room temperature until blue color was developed in positive control. The reaction was then stopped by 100 μL of 1N HCl and the absorbance was read at 450 nm using UV/Vis spectrophotometer.
microplate reader (BioTek, ELx808). All samples were tested in duplicate. Negative control consisted of Spike S1 protein without test inhibitor and ACE-2. Positive control consisted of Spike S1 protein and ACE-2 without the test inhibitor. Percentage of binding activity (% BA) and inhibition (% I) were calculated by:

\[
\% \text{ BA} = \frac{\text{abs of samples} - \text{abs of negative control}}{\text{abs of positive control} - \text{abs of negative control}} \times 100\% \quad (1)
\]

\[
\% \ I = 100 - \% \text{ BA}
\]

Figure 1. ELISA could be used to examine activity of HGM abrogate interaction between ACE2 and Spike protein.

Table 1. Samples characteristics

| Hydrolysis concentration | Porang glucomannan flour (g) | HGM yield (g) | Characteristics |
|--------------------------|-------------------------------|---------------|----------------|
|                          |                               |               | Average particle size (µm) | Glucomannan content (%) |
| 0 N                      | 1.5                           | -             | 286.7 ± 8.9               | 56.46 ± 0.4               |
| 0.25 N                   | 1.5                           | 0.86 ± 0.3    | 72.7 ± 7.1                | 52.57 ± 3.3               |
| 0.50 N                   | 1.5                           | 0.44 ± 0.1    | 50.9 ± 7.9                | 46.75 ± 1.7               |
| 1.00 N                   | 1.5                           | 0.16 ± 0.1    | 45.3 ± 9.4                | 36.17 ± 2.4               |

Results and Discussion

Glucomannan is a water-soluble polysaccharide with high molar mass, and its aqueous solution has a very high viscosity that makes it hard to handle (Ojima et al., 2009). The most popular glucomannan source came from the konjac plant’s roots and tuber (Amorphophallus konjac) and the tuber of porang plant (Amorphophallus muelleri). Due to its high molecular weight and non-caloric nature, glucomannan is known for its significant health benefits: anti-obesity, anti-diabetic, prebiotic, laxative, and anti-inflammation (Devaraj et al., 2019).

Several studies reported the glucomannan modification into the smaller molecular size and lower viscosity to have better biological activities, including immunomodulatory function by suppression of IgE production (Suzuki et al., 2010)(Onishi et al., 2005), inhibition of bacterial growth (Al-Ghazzewi and Tester, 2010), ACE inhibitory activity (Song et al., 2018), and higher anti-inflammatory activity (Zheng et al., 2019). In general, glucomannan degradation can be carried by physicochemical degradation using acid, heat, oxidation, ultrasound, or irradiation and biodegradation by an enzyme. Acid hydrolysis effectively breaks down the glycosidic chain and gives greater viscosity reduction than enzyme hydrolysis (Pederson, 2017). While non-acid physicochemical hydrolysis still faces several limitations, such as broad molecular distribution with irradiation treatment and lower molecular degradation with the ultrasound method (Jiang et al., 2018).

Characteristics of HGM

HCl concentrations in glucomannan hydrolysis shown as a significant factor for each HGM characteristics (Table 1). Higher hydrolysis concentrations produced HGM with lower yield, average particle size, and glucomannan content. HGM with low hydrolysis concentration (0.25N) produced flakes appearance before grinded (Figure 2).
The viscosity of the glucomannan solution is strongly influenced by the molecular chain length and the pH of the solution, where at neutral pH (5-7) the viscosity of the glucomannan solution is very high (Srzednicki and Boromphichaiarchkul, 2020). This is due to the ability of glucomannan to absorb and retain water with an absorption index of up to 100 grams of water per gram of sample (Yan et al., 2012; Xiao et al., 2015). The degradation of glucomannan particles can reduce the ability of glucomannan to bind water and its viscosity in solution (Onishi et al., 2005; Takigami et al., 2009). Based on this, the characteristics of 0.25N HGM can be influenced by the reaction between glucomannan and water which is higher than the degradation by acid due to too low HCl concentration. Acid concentration greatly affects hydrolysis, where the higher the concentration of HCl, the higher the degradation of glucomannan molecules, including increasing purity and decreasing glucomannan particle size (Suzuki et al., 2010; Kumoro et al., 2018). Hydrolysis concentration that is too low causes degradation of glucomannan which is also low so that when the sample is neutralized (pH 7.2) water sorption on glucomannan causes the viscosity of the solution to be high again.

**Average Particle Size**
Polysaccharide hydrolysis is carried out with the main objective as a size reduction method. Based on the SEM images, the shape of the particles in HGM looks much smaller than glucomannan (Figure 3). Hydrolysis of glucomannan molecular chains with HCl occurs randomly and causes a decrease in molecular weight (Mw) which is higher as the HCl concentration increases, but this process is known to not cause changes in the chemical structure of glucomannan and does not produce significant new compounds (Ojima et al., 2009). Based on the results of making HGM, the higher the concentration of HCl used causes a decrease in the average particle size (µm). Acid conditions and high temperatures in the hydrolysis process also affect other compounds in glucomannan flour, especially starch as the main impurities (Wardhani et al., 2015; Manab et al., 2016). Precipitation process utilize the lower solubility of glucomannan than starch in ethanol solution so that glucomannan can be further separated from impurities (Kumoro et al., 2018; Wang et al., 2020). The separation of glucomannan granules from other compounds causes the particle size to be smaller and the yield produced is less.

**Glucomannan Content**
In general, the rate of hydrolysis and formation of reducing sugars increases with the concentration of acid used, this can be caused by the increase in the activity of hydrogen ions in the hydrolysis reaction (Tasić et al., 2009). Glucomannan levels in samples are showed to have decrease with the increase of acid concentration used. Acid in hydrolysis process will degrade starch into reducing sugars and glucomannan into its monomers (Kumoro et al., 2018).
The decrease in glucomannan content in HGMs indicates an excess of decomposition due to too high hydrolysis concentration (Tanaka et al., 2013). Acid concentration is one of the hydrolysis factors that must be controlled to prevent the decomposition of excess sugar into by-products, where in hydrolysate of glucomannan furfural compounds are often found after excessive hydrolysis which causes the purity of glucomannan to decrease (Tasić et al., 2009; Bo et al., 2013).

**Spike Protein and ACE2 Interaction Inhibitor Activity of HGM**

The results of the trial with HGM showed the potential for a direct interaction between HGM and S1. Based on the test results, HGM 1N was able to inhibit the bond between S1 and ACE2 up to 50.56% (Figure 4). The ability of HGM to bind to S1 could be the main potential of glucomannan as an anti-SARS-CoV-2 directly considering the fundamental role of S protein in SARS-CoV-2 infection.

Research by (Hassanzadeh et al., 2020) showed a difference in charge on S SARS-CoV-2 which was more positive than SARS-CoV due to the different number of charged residues. The difference in charge becomes even more influential due to the high number of S proteins on the virus particles so that the electrostatic potential of SARS-CoV-2 tends to be positive. Meanwhile, hACE2 as a whole has a negatively charged surface and binds to the positive S residue of SARS-CoV-2 especially on salt bridges and hydrogen bonds between them (Hassanzadeh et al., 2020; Xie et al., 2020). A more positive SARS-CoV-2 load will have a higher binding affinity for ACE2 than SARS-CoV.

The positive charge characteristics of the virus can be used as a target for interaction with negatively charged inhibitors. Glucomannan is essentially a neutrally charged polysaccharide and can be modified molecularly by sulfation to create a negatively charged glucomannan (Li et al., 2016; Srzednicki and Borompichaichartkul, 2020). However, based on (Jian et al., 2015) glucomannan which was treated with acid with HCl showed a negative charge based on the zeta potential. Zeta potential itself is a method of measuring electrostatic charge that can be used as a parameter of molecular interactions through electrostatic forces (Saito et al., 2013).
The greater inhibitory effect obtained by HGM produced by more significant hydrolysis, which suggested rather than the glucomannan content, its molecular weight of porang glucomannan plays a significant role in its bioactivity. The data indicate that hydrolysis potency with higher than 1N HCl concentration can be done to produce glucomannan with much smaller particle size to provide a more significant to optimal S1 – ACE2 interaction inhibitory effect. This experiment becomes a gateway for further glucomannan antiviral study, including glucomannan sulphation that follows after hydrolysation, especially towards inhibition of SARS-CoV-2 infection.

**Conclusion**
Glucohealth as hydrolysed porang glucomannan exhibits potential of antiviral activity against SARS-CoV-2. Different concentrations of acid hydrolysis affect glucomannan particle size degradation and lead to a better biological function. In this study, the result implied that higher acid hydrolysis concentration (0.5N - 1N) best carried out to degrade porang glucomannan flour to enhance its ability to interact with positive virus. This study also suggested rather than glucomannan content, the molecular charge and size of glucomannan plays more significant role in direct interaction with spike protein of SARS-CoV-2. This paper reported for the first time, HGM can bind with spike protein of coronavirus. Further studies of the Glucohealth mechanism of action and methods to increase the antiviral activities are needed to develop further the potency of natural virus treatment from Indonesia’s local porang tuber.

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