Isolation oligosaccharides from gembili (*Dioscorea esculenta* Lour. Burkill) as prebiotics

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Abstract. Oligosaccharides is a potential prebiotic and used as an alternative diet therapy for diabetes mellitus. Gembili (*Dioscorea esculenta* (Lour.) Burkill) is known as functional food that contains oligosaccharides. This study was done to isolate oligosaccharides from gembili and evaluate its prebiotics potential. Oligosaccharides was extracted and analysed by High Performance Liquid Chromatography (HPLC). Potential of prebiotics was analysed using faecal samples from healthy volunteers in anaerobic condition at certain time interval (0, 24, and 48 hours) and bacterial growth (Bifidobacteria and Lactobacillus as probiotic bacteria, Bacteroides and Clostridium as pathogen bacteria), prebiotic index were analysed. Short Chain Fatty Acid (SCFA) production (acetic acid, propionic acid, and butyric acid) analyzed using HPLC. The results showed that oligosaccharides from gembili contains lactulose (0,231 %); inulin (2,541 %) and rafinose (1,485 %). Prebiotic index increased from 0,86 ± 0,20 (24 hours incubation) to 1,12 ± 0,05 (48 hours). This oligosaccharides increased probiotics and decreased pathogen bacteria. It could be concluded that oligosaccharides from gembili were potential as prebiotics.

1. Introduction
Diabetes mellitus (DM), as an international public health concern and it is one of the five leading causes of death in the world and about six deaths per minute are attributable to diabetes complications [1]. In 2015, about 415 million adults had diabetes, a 4-fold increase from 108 million in the 1980s. If there are no precautions, this number will continue to increase and in 2040 estimated it will increase to 642 million sufferers [2].

Prebiotic may play important role in prevention rather than in the treatment of human type 2 diabetes [3]. Disaccharides (lactulose and lactylol), oligosaccharides (oligosaccharides include
rafinose, oligofructose, palatimose, isomaltose, lactosu-
(ose) and polysaccharides (inulin and resistant starch) are prebiotics [4] [5].

Gembili (Dioscorea esculenta (Lour.) Burkill) is a functional food and contains oligosaccharide inulin [6]. Gembili also have several bioactive compounds such as dioscorin, diosgenin that can improve the body’s defense mechanism (immunomodulator), prevention of metabolic disease (hypercholesterolemia, dyslipidemia, diabetes and obesity), inflammation and cancer [7]. Because of its functional content especially on oligosaccarides, this study was conducted to isolation oligosaccharides from gembili and evaluate its prebiotic potential.

2. Methods

2.1. Materials

Gembili flour, glucose standard (inulin, lactulose, glucose, galactose and fructose), chemical (for analysis), agar media (Plate count agar, columbia agar, rogossa agar, reinforced clostridial agar and tryptone soya agar), antibiotic (kanamycin, vancycin and collistin) and feses from healthy people.

2.2. Oligosaccarides extraction

Oligosaccharides extraction Using ethanol (70%) by stirring for 15-20 hours at room temperature, filtered and washed with ethanol (70%), evaporated and drying at 60°C [8]. Total Dissolve Solids (TDS) from extract was analysed using vacuum oven method [9] and oligosaccharides analysed using HPLC with Metacharb 87°C with RID detector, eluent flow rate H2O, 0.6 ml/min and temperature 85°C [10].

2.3. The potential of prebiotic

Faecal samples were obtained from a healthy human volunteer who had not been prescribed antibiotics for at least 6 months prior to the study and had no history of any gastrointestinal diseases from a healthy volunteer (35 – 37 years old). Fresh faecal dissolved in 0.1 M phosphate buffer saline (PBS) sterile, pH 7 with ratio 1: 10 (w / v), and homogenised with magnetic stirrer (200 rpm, 2 min). The liquid was taken and used as an inoculum for subsequent testing [11].

For preparation microbial culture, faecal slurry (10 ml) and oligosaccharide extract (1 % v/v) with the basal nutrient medium (90 ml, peptone water 2 g/l), yeast extract 2 g/l, NaC 10.1 g/l, K2HPO4 0.04 g/l, KH2PO4 0.04 g/l, MgSO4, 7H2O 0.01 g/l, CaCl2.6H2O 0.01, NaHCO3 2 g/l, Tween 80 2 ml, haemin 0.02 g/l, vitamin K 110 μL, cysteine.HCl 0.5 g/l, and bile salts (sodium glycocholate andsodium taurocholate) 0.5 g/l, pH 7.0). The culture temperature was set at 37°C and with CO2 gas for 10 minutes (anaerobic). The sample is taken at certain time interval (hours 0, 24, and 48) [11], and parameters are bacterial growth (Bifidobacteria and Lactobacillus) and microbial pathogens (Clostridium and Bacteroides) [12][13], prebiotic index [14], pH [10], and SCFA production (acetic acid, propionic acid, and butyric acid) by HPLC [13].

3. Result and Discussions

3.1. Oligosaccharides extraction

Extraction gembili flour (200 g) in ethanol 70% (2000 ml) yielded 165 ml of concentrated extract. It was analysed for total disolve solids (TDS) to determine amount of disolve sugar which represented the content of oligosaccharides. The TDS content were 19.5%. Oligosaccharide test using HPLC result the parameter of oligosaccharides were lactulose, inulin and rafinose (table 1). The presence of rafinose show the monosaccharides content (glucose, galactose and fructose), so needs to be known (table 1).
Table 1. HPLC test results for analysis of oligosaccharides and monosaccharides from gembili

| Parameter of oligosaccharides | Results (%) | Parameter of monosaccharides | Results (%) |
|-------------------------------|-------------|-------------------------------|-------------|
| Lactulose                     | 0.231       | Glucose                       | 15,411      |
| Inulin                        | 2.541       | Galactose                     | 1,122       |
| Rafinose                      | 1.485       | Fructose                      | 9,042       |

The highest oligosaccharide in gembili was inulin (2.541%), but it’s lower than the other studies (14.629%) [15]. The differences is due to extraction method, using single extraction using ethanol (70%) [8] and double extraction using thanol (30%) [16]. The highest monosaccharide content is glucose (15.411%), affect the prebiotic potential of gembili. It’s related to microorganisms both probiotic and pathogens bacteria using glucose for metabolism. The fructose content (9.042%) proof of high inulin, because inulin is a polymer of fructose units which is associated with β- (2-1) glycosidic bonds with glucose terminal group [17].

3.2. The potential of prebiotic

3.2.1. Bacterial growth

The diversity of colon bacteria in faeces at the beginning showed (figure 1). *Bifidobacteria, Lactobacillus* and *Clostridium* have same population of 4 logs, while Bacteroides are 2 logs. This is influenced by many factors, especially diet, type of food consumed, illness, antibiotic exposure, etc. Colon microbiota in infant dominated by Actinobactera and Proteobacteria. However, getting older, more diverse compositions of colonic microbiota, and 65 years old there is an increase in Bacteroides and Clostridium custer IV [18][19].

![Figure 1](image-url)

**Figure 1.** Colon bacterial growth in basal media containing oligosaccharides extract of gembili for 48 hours of incubation time

Incubation 24 hours increase Total bacteria, *Bifidobacteria* and *Lactobacillus* 2 logs and *Clostridium* 1 logs. The high growth of *Bifidobacteria* and *Lactobacillus* is supported by pH decrease (acidity) of the media (figure 2.). *Bifidobacteria* and *Lactobacillus* will produce lactic acid as the primary metabolite, and reduce the pH media. Bacteroides decrease (48 hours), because of acidic media environment and the inability of Bacteroides to ferment oligosaccharides for their growth.
Total bacteria and *Clostridium* decrease at 48 hours, because substrate in medium begins to diminish and pressure of the acidic media environment. The oligosaccharides can support the growth of probiotic bacteria and inhibit growth of pathogenic bacteria in the colon. Inulin, rafinosa and lactulosa in gembili extract can be a prebiotic source and support probiotics growth in human digestive tract [20][21].

![Figure 2](image)

**Figure 2.** pH on the fermentation oligosaccharides extract of gembili for 48 hours of incubation time

### 3.2.2. Prebiotic index

The prebiotic index oligosaccharide extract of gembili increase from 0.86 ± 0.20 (24 hours) to 1.12 ± 0.05 (48 hours) (Table 2). Substrate from oligosaccharide extracts of gembili supports the growth of probiotic bacteria and decrease the pathogenic bacteria. The prebiotic index optimal at 24 hours and decreases at 48 hours, in accordance with the availability of substrate and environmental conditions [11]. Oligosaccharides extract of gembili used in this study is crude extract, where the purity of oligosaccharides is low and there is high glucose. The presence of glucose still be used by pathogenic to grow, especially in the first 24 hours.

#### Table 2. Prebiotic index on the fermentation oligosaccharides extract of gembili for 48 hours of incubation time

| Incubation time | Prebiotic index     |
|-----------------|---------------------|
| 24              | 0.86 ± 0.20         |
| 48              | 1.12 ± 0.05         |

### 3.2.3. Production of short chain fatty acid (SCFA)

There was an increase on SCFA at the end of fermentation. It show the activity of prebiotic consumption and the production of metabolites in the form of SCFA. The highest concentration of acetic acid compared to propionic acid and butyric acid. The highest concentration of acetic acid was followed by the concentration of propionic acid and butyric acid [22]. This can be caused by other probiotic bacteria that are able to produce SCFA, but pathogenic bacteria such as members of the *Bacteroides* also have the potential to produce health by producing SCFA, example, *Bacteroides vulgatus* which is capable of producing acetic acid and formic acid.
Table 3. SCFA composition on the fermentation oligosaccharides extract of gembili

| Fatty acid      | 0 hours | 48 hours |
|-----------------|---------|----------|
| Acetic acid     | 21.07   | 32.34    |
| Pronionic acid  | 4.75    | 8.18     |
| Butyric acid    | 0       | 0.23     |

4. Conclusions
Oligosaccharides from gembili contains lactulosa (0.231 %); inulin (2.541 %) and rafinosa (1.485 %) and prebiotic index increased from 0.86 ± 0.20 (24 hours) to 1.12 ± 0.05 (48 hours). This oligosaccharides increased probiotics and decreased pathogen bacteria. So, oligosaccharides from gembili were potential as prebiotics.

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