THE POTENTIAL OF BUTON FOREST ONION Eleutherine bulbosa (Mill.) Urb. EXTRACT AS A PREBIOTIC AND AN ANTIOXIDANT

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ABSTRACT

The aim of this study was to analyze the potential of Buton forest onion extract (BFOE) Eleutherine bulbosa (Mill.) Urb. as a prebiotic and an antioxidant. Buton forest onion was extracted using 96% ethanol by maceration method. The prebiotic content of BFOE was determined using high performance liquid chromatography (HPLC). The ability of BFOE as a prebiotic was analyzed the growth of Pseudalteromonas piscicida 1Ub and Bacillus sp. NPs and its resistance to artificial gastric acid and α-amylase. Antioxidant activities were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Results indicate that the content of the oligosaccharides of BFOE including inulins, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and raffinose was 2.1%, 10%, 1% and 7.5%, respectively. The mixed oligosaccharides were found capable to stimulate the growth of P. piscicida 1Ub and Bacillus sp. NPs. The optimum BFOE concentration to stimulate the growth of probiotics was 1.25 mg/mL (0.17 ± 0.02 and 0.21 ± 0.00). The mixed oligosaccharides were also resistant to hydrolysis of artificial gastric acid and α-amylase, with maximum hydrolysis percentages of 8.25 ± 0.25% and 27.52 ± 1.68%, respectively. The BFOE was capable to DPPH radical scavenger with IC50 of 1.48 µg/mL. The BFOE of this study has the potential to be a prebiotic and an antioxidant.

Keywords: Eleutherine bulbosa, extract, prebiotic, probiotic, antioxidant

INTRODUCTION

Buton forest onion Eleutherine bulbosa (Mill.) Urb. of the family Iridaceae, has green leaves, white flowers and red bulbs that resemble the shape of red onion bulbs. Buton forest onions are known various names in Indonesia, such as bawang saubang, bawang dayak, babawan be Besteem, bawang hantu, bawang siyem, bawang kapul, bawang sayap, and bawang liuk (Febrinda, 2014). E. bulbosa (Mill.) Urb. is also known by such names as E. americana, E. anomala Herb., E. longifolia Gagnep., Bermudiana bulbosa (Mill) Molina, Cipura plicata (Sw.) Griseb., E. plicata (Sw.) Herb., Ferraria parviflora Salisb., B. congesta (Klatt) Kunz, Galatea americana (Aubl.) Kunz, G. bulbosa (Mill.) Britton, G. plicata (Sw.) Baker, Isia americana Aubl., E. Subaphylla Gagnep, Sisyrinchium americanum (Aubl) Lemee, S. bulbosum Mill., S. capitatum Pers., S. congestum Klatt, S. eklatum, S. latifolium Sw., S. plicatum, and S. racemosum Pers. (http://www.theplantlist.org/tpl/record/kew-327974).

E. bulbosa contain bioactive compounds of naphthalene, naphthoquinone, anthraquinone (Mahaburasakam et al., 2010; Iyama et al., 2011), flavonoid, tanin, saponin, steroid, triterpenoid groups (Munaeni et al., 2017), securixantone (Munaeni et al., 2019a). The E. bulbosa is anti-microbial, anti-inflammatory, anti-hypertensive, anti-diabetic, and capable of being virus replication inhibitors (Insanu et al., 2014). Buton forest onion extract (BFOE) is capable to inhibit Staphylococcus aureus (Iesan et al., 2009; Subramaniam et al., 2012), and Vibrio harveyi (Munaeni et al., 2017), and potential to increase the growth performance of vannamei shrimp (Liopeneus vannamei) (Munaeni et al., 2019b). The E. bulbosa is able to reduce the occurrence of lipid peroxidation in diabetic rat liver (Febrinda et al., 2014). In addition, according to Phoen & Voravuthikunchai (2013). E. americana is potential as a prebiotic because it can increase the growth of intestinal microbiota (Bacteroides population). Prebiotics are foods that cannot be utilized by the host because the amylase or other hydrodases in the intestinal tract cannot be digested (Carabin et al., 1999) but they can be digested or fermented selectively by the microbiota in the digestive tract that will benefit the host (Gibson et al., 2004). Some criteria of prebiotics include their being resistant to hydrolysis by artificial gastric acid, α-amylase, and bile salt being able to be fermented by intestinal microbes and being able to stimulate the growth of probiotics in the digestive tract (Wichienchoot et al., 2010; Sawangwan et al., 2018). Oligosaccharides are prebiotic sources that have been widely used in mammals and fish (Mahinios et al., 2006). Prebiotic oligosaccharides include fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), isomalt-oligosaccharides (IMO), raffinose, lactosucrose, soy-oligosaccharides (SOS), palatinose, inulins, oligofructose (OF), isomaltulose, arabinoxyloligosaccharides, sololigosaccharides, galactoolactose, and pyrodextrins (Mohani et al., 2018). Antioxidants are substances that can significantly prevent oxidation on the substrate at a low concentration (Halliwell, 1990). Antioxidants are able to stabilize free radicals by donating of electrons, inhibiting the occurrence of chain reactions from the formation of free radicals; acting as free radical scavenger, and converting free radicals into compounds that are nonreactive and relatively stable by donating hydrogen atoms (Kim et al., 2014). Phytochemical compounds from plants are able to contribute as natural antioxidants, making it necessary to conduct a study to obtain an optimum antioxidant efficiency (Sahren et al., 2010; Mihaylova et al., 2019). However, no studies to observed the content of oligosaccharides in Buton forest onion Eleutherine bulbosa (Mill.) Urb. extract and their role as prebiotics. Thus, the aim of this study was to analyze the potential of the oligosaccharides in Buton forest onion Eleutherine bulbosa (Mill.) Urb. extract as a prebiotic and an antioxidant.

MATERIAL AND METHODS

Collection and identification of plant material

The bulbs of Buton forest onion were obtained from Kumbewaha Village, Buton Regency, South East Sulawesi, Indonesia, then identified at the Botany Division, Research Center for Biology, Indonesian Institutite of Sciences (No. 1000/1PH.1.011/1.07/V/2018).
Processing of plant materials and extract preparation

Extraction of Buton forest onion bulbs was carried out using the maceration method (Munaeni et al. 2017). Buton forest onion bulbs were cleaned and thinly sliced, then placed in an oven at a temperature of 60 °C for 48 hours. The sample was powdered using a blender (Miyako, Japan), then extracted using 96% ethanol at a ratio of 1:4 (w/v) for 24 hours at room temperature using a magnetic stirrer. The maceration yield was filtered using Whatman’s filter paper No. 41, then the filtered leaving was re-macerated twice using the same method. The maceration yield was thickened using a vacuum evaporator at 40 °C. The extract was then stored in a freezer at -20 °C for further analysis.

Microorganism preparation

Stimulation test on the growth of probiotics was done using two types of bacteria, namely probiotic and enteric bacteria. The probiotic bacteria used were P. piscicida 1Ub and Bacillus sp. NP5, while the enteric bacteria used Vibrio parahaemolyticus. P. piscicida 1Ub, were probiotics isolated from vannaeim shrimp nauplii (Widanarni et al., 2009). Bacillus sp. NP5 were probiotics isolated from the digestive tract of tilapia (Putra et al., 2015). V. parahaemolyticus were collected from the Research Center of Brackishwater Aquaculture and Fisheries Counseling, Maros, South Sulawesi. All these bacteria were grown at 37 °C and reared at 4°C on SWC agar slant (sea water complete: 1 g of yeast extract, 3 mL of glycerol, 5 g of bactopeptone, 250 mL of distilled water, and 750 mL of seawater).

Oligosaccharide Content of Buton Forest Onion Extract (BFOE)

The type and concentration of oligosaccharides in BFOE were determined using high performance liquid chromatography (HPLC). The galactooligosaccharides (GOS) and raffinoses analysis was based on AOAC 2001.02 method, while the inulins and fructooligosaccharides (FOS) analysis was based on AOAC 997.08 method. Twenty μL of sample was injected in a gel TSK Amido-8 column (4.6 × 250 mm, Tosoh, Tokyo, Japan) and LC-10ADVP pumps (Shimadzu, Kyoto, Japan). A Refractive Index (RI) detector was used, and the column temperature was 35 °C. Mobile phase was acetonitrile and water (ratio 80:20) with a flow rate of 1 mL/minute. The sugar concentration in the sample was obtained by comparing the peak area of the standard curve.

Activity of Buton Forest Onion Extract (BFOE) in Probiotic Growth Stimulation

The activity of BFOE in the stimulation of probiotic growth was analyzed using the spectrophotometric methods, refers to Huebner et al., (2008) with minor modifications. The BFOE was diluted using sterile distilled water at concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.156 mg/mL (b/v), each repeated triplicate. One mL of BFOE from each concentration was added into 10 mL of liquid SWC medium, while the control only used the medium. The bacterial suspension was culture in liquid SWC medium at 28-29 °C for 140 rpm for 18 h for P. piscicida 1Ub and Bacillus sp. NP5 and 14 h for V. parahaemolyticus. The bacterial suspension (density of 10^7 CFU/mL) was inoculated into each treatment (v/v) by 5%, then incubated at a temperature of 28-29 °C at 140 rpm. The optical density (OD) of the supernatant was analyzed using a spectrophotometer UV-200-RS at a wavelength of 600 nm, then the blanks used liquid SWC medium without bacteria. Observations of BFOE to the stimulation of probiotic growth were conducted for 14, 18, and 24 h. The blanks used media without bacteria. Prebiotic activity scores were expressed quantitatively by the following formula (Huebner et al., 2008):

\[
\text{Prebiotic activity scores} = \frac{\log \text{Ppt} - \log \text{Pp0}}{\log \text{Ept} - \log \text{Ep0}} = \frac{\text{Log Ppt} - \text{Log Pp0}}{\text{Log Ept} - \text{Log Ep0}}
\]

where Ppt was probiotic log OD on the BFOE at t-h (t: time; h: hour), Pp0 was probiotic log OD on the BFOE at 0-h, Ppt was probiotic log OD on control/media at t-h, Pp0 was probiotic log OD on control/media at 0-h, Ept was enteric log OD on the BFOE at t-h, Ep0 was enteric log OD on the BFOE at 0-h, Ept was enteric log OD on control/media at t-h, and Ep0 was enteric log OD on control/media at 0-h.

High prebiotic activity scores indicate that the substrates of the oligosaccharide samples had increased the growth of probiotics while the enteric bacteria yielded lower growth.

Resistance of Buton Forest Onion Extract (BFOE) to hydrolysis by artificial gastric acid

Testing of the resistance of BFOE to hydrolysis by artificial gastric acid was conducted according to Wichienchot et al. (2010) method. The artificial gastric acid used was hydrochloric acid buffer (composition in g/L: NaCl: 8; KCl: 0.2; NaHPO4·2H2O: 8.25; NaH2PO4: 14.35; CaCl2·2H2O: 0.01; MgCl2·6H2O: 0.18).

BFOE samples were dissolved in distilled water. The concentration of BFOE used to testing of the hydrolysis resistance by artificial gastric acid were the three concentrations that yielded the highest values in the probiotic growth stimulation test, each repeated triplicate. Commercial fructooligosaccharide (FOS) was used as a reference at the same concentration as BFOE. The pH of HCL buffer was adjusted to 2.5 using 5 M HCL then mixed with BFOE at each concentration in a ratio of 1:1 (v/v). The reaction mixture was incubated in water bath for 3 h at 37 °C. The total carbohydrate was determined by phenol-sulfuric acid method before incubation. Reduction of sugar content was carried out for 0, 1, 2, and 3 h by DNS method. Each concentration was repeated triplicate. The percent hydrolysis of the samples was calculated using this formula (Wichienchot et al., 2010): hydrolysis (%) = (reducing sugar at final h - reducing sugar at initial h) x 100 / (total sugar content - initial reducing sugar content).

Resistance of Buton Forest Onion Extract (BFOE) to hydrolysis by α-amylase

Two units/mL of α-amylase (Novozymes, Denmark) was dissolved in a buffer of 20 mM sodium phosphate at pH 8.5. The concentration of BFOE used to testing of the hydrolysis resistance by α-amylase were the three concentrations which yielded the highest values in the probiotic growth stimulation test. The three concentration of BFOE was mixed with α-amylase at a ratio of 1:1 (v/v) then incubated in water bath at 37 °C for 3 h. The total carbohydrate was determined by phenol-sulfuric acid method and reducing sugar content was determined by DNS method.

Buton Forest Onion Extract (BFOE) as an Antioxidant

An analysis of DPPH free-radical scavenger (2,2-diphenyl-1-picrylhydrazyl) was conducted according to the method of Mayur et al. (2010). The concentrations of BFOE used to analysis of DPPH free-radical scavenger were the three concentrations which yielded the highest values in the probiotic growth stimulation test, each repeated triplicate. The BFOE was dissolved with ethanol. Two mL of BFOE was mixed with 1 mL of 0.5 mM DPPH then shaken and left for 25 minutes at 27-29 °C in a dark room. The control used 1 mL of 0.5 mM DPPH solution added with 2 mL of ethanol and ascorbic acid as a comparison. Absorbance was measured at a wavelength of 518 nm using a spectrophotometer. The percentage of inhibition was calculated using the formula: % inhibition = 1 - ([absorbance control - absorbance of sample] / (absorbance control)) x 100. In addition, an IC50 analysis was carried out to determine the concentration of BFOE which has the ability to inhibit DPPH free-radical scavenger by 50%.

Data Analysis

The data were analyzed using one-way analysis of variance (ANOVA) then follow up with Duncan’s test using the SPSS (Statistical Program Software System) and expressed as mean ± standard deviation (SD). A significant difference were obtained if P < 0.05.

RESULTS AND DISCUSSION

Oligosaccharide Content of Buton Forest Onion Extract (BFOE)

The results of the analysis of oligosaccharide content of forest onion extract using high performance liquid chromatography (HPLC) in the present study for milunits, fructooligosaccharides (FOS), galactooligosaccharides (GOS) and raffinoses were at 2.1%, 1%, and 7.5%, respectively. According to Ritsema & Smeekens (2003), various type of onion plant are able to produce prebiotic oligosaccharides. Prebiotics are foods that can be selectively utilized by beneficial intestinal microbosis (Mohanty et al., 2018), generally short-chain and indigestible by enzymes of the host (Sekhon et al., 2010). The compound of oligosaccharides with the highest percentage found in BFOE in the study was fructooligosaccharide (FOS) than other oligosaccharides. Thus, the use of oligosaccharides from BFOE can be used as prebiotics which are expected to stimulate the host immune response. According to Saad et al. (2013), the function of fructooligosaccharides were modulate the body's immunity in the intestine in association with lymphoid tissue, increase the production of anti-inflammatory cytokines, increase the natural activity of killer cells, and produce antibodies.

Activity of Buton Forest Onion Extract (BFOE) in Probiotic Growth Stimulation

All concentrations of BFOE was capable to stimulate growth of P. piscicida 1Ub (Figure 1a) and Bacillus sp. NP5 (Figure 1b). After 24 h of incubation, all the concentrations of BFOE was decrease in cell density of the probiotic. The concentration of 1.25 mg/mL was capable to yield the highest prebiotic activity scores after 24 h of incubation in both of the probiotics, (0.17 ± 0.02 and 0.21 ± 0.00). The concentration of 0.156 mg/mL was value lowest prebiotic activity score after 14 h of incubation in P. piscicida 1Ub, which was not significantly
different (P > 0.05) at the concentration of 0.313 mg/mL but significantly different (P < 0.05) from all other concentrations. The prebiotic activity scores of *P. piscicida* 1Ub at the concentrations of 1.25 and 2.5 mg/mL for 18 h were significantly (P<0.05) higher than those at other concentrations. The concentrations of 2.5, 5, and 10 mg/mL yielded prebiotic activity scores that were significantly (P<0.05) lower than the concentration of 0.625 and 1.25 mg/mL after 24 h of incubation. The stimulation of BFOE against *Bacillus* sp. NPS showed that the concentration of 2.5 and 5 mg/mL yielded prebiotic activity scores significantly (P < 0.05) higher than the other concentrations after 14 h of incubation but significantly (P<0.05) lower compared than the concentration 1.25 mg/mL after 24 h of incubation.

![Figure 1](image1.png)

**Figure 1** The scores of the activity of Buton forest onion extract (BFOE) in the growth stimulation on (a) *P. piscicida* 1Ub and (b) *Bacillus* sp. NPS. Data (mean ± SD) with different letters at the same h indicating significant different (P < 0.05).

The use of different concentrations of BFOE showed differently in the prebiotic growth stimulation. The higher of the BFOE concentration (2.5, 5, and 10 mg/mL) and the increase of incubation time was the lower the both prebiotic activity. It is assumed that the role of the phytochemical compounds in BFOE as antibacterial agents is greater than that of prebiotics. The growth of *P. piscicida* 1Ub and *Bacillus* sp. NPS at the concentrations of 5 and 10 mg/mL decreased after 24 h of incubation. The concentration of 1.25 mg/mL was the optimal concentration of BFOE for stimulating the growth of *P. piscicida* 1Ub and *Bacillus* sp. NPS for 14 h to 24 h of incubation. Sawangwang et al. (2018) showed that the prebiotic oligosaccharides mushroom extracts are capable to stimulate the growth of *Lactobacillus acidophilus* and *Lactobacillus plantarum* for 24 h of incubation. Another study by Azmi et al. (2012) which used extracts of the *Gigantochloa levis* shoots, show the activity of prebiotics which could act as a carbon source so as to stimulate the growth of prebiotic. The ability of oligosaccharides from BFOE to stimulate the growth of probiotics in this study is expected to improve host health. The fructooligosaccharides have function to improve the intestinal health by stimulating intestinal probiotics and increase short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (Saad et al. 2013). The use of BFOE has been capable to increase the microbiota diversity in the intestinal of white shrimp *Litopenaeus vannamei*, digestibility, growth, immune response and resistance to the pathogenic *V. parahaemolyticus* (data under review).

### Resistance of Buton Forest Onion Extract (BFOE) to α-amylase

The percentage of α-amylase hydrolysis in this study became higher with the increase in the concentration of BFOE. The largest percentage of hydrolysis in 2 h of incubation was found at the concentration yielded 2.5 mg/mL (74.0%). Meanwhile, the concentrations of 0.625 and 1.25 mg/mL yielded 4.85% and 2.33%, respectively. Thus, when the oligosaccharides from BFOE are in the stomach for 2 h, an estimated 92% of them will reach the intestine. Another study shows that the percentage of gastric acid hydrolysis in dragon fruit after 2 h of incubation at pH 2 was 2.43%, while that of the reference (inulin) was 23.22% (Wichienchot et al., 2010). The gluco-oligosaccharides produced by *Glucobacter oxydans* NCIMB 4943 are capable of being resistant to 98.4% of human gastric acid conditions (Wichienchot et al., 2006). The highest percentages of hydrolysis by α-amylase of the oligosaccharide in BFOE at the concentrations of 0.625, 1.25, and 2.5 mg/mL after 3 h of incubation were 17.58 ± 1.46%, 23.48 ± 2.13% and 27.52 ± 1.68, respectively (Figure 3). The percentage of α-amylase hydrolysis of BFOE at 2.5 mg/mL was significantly higher (P < 0.05) than the other at those concentrations during the incubation period, with that at 1.25 mg/mL being the second highest. The lowest percentage of hydrolysis by α-amylase was at the concentration of 0.625 mg/mL. The percentages of hydrolysis of reference prebiotic from commercial fructooligosaccharides (FOS) at the concentrations of 0.625, 1.25, and 2.5 mg/mL after 3 h of incubation was 23.11 ± 2.48%, 40.73 ± 1.60%, and 50.34 ± 5.05%, respectively. The percentage of α-amylase hydrolysis in this study was lower than that in the study by Wichienchot et al. (2010), which used dragon fruit (32%). Prebiotics can be tolerant of stomach acid and α-amylase in the digestive tract and can be utilized by beneficial microbes (Chowdhury et al., 2015).

![Figure 2](image2.png)

**Figure 2** The resistance of Buton forest onion extract (BFOE) and fructooligosaccharides (FOS) to α-amylase at different concentrations. Data (mean ± SD) with different letters at the same h indicate significant difference (P < 0.05).

### Resistance of Buton Forest Onion Extract (BFOE) to α-amylase

The ability of BFOE at different concentrations as compared with ascorbic acid can be seen in Table 1. The ability of an extract concentration to inhibit or reduce DPPH free radical activity by 90.09 ± 1.3% – 99.39 ± 0.35%, than the ability of ascorbic acid by 81.02 ± 0.28% – 86.71 ± 0.08%. The percentage of inhibition of BFOE at 2.5 mg/mL was significantly higher (P < 0.05) than the other concentrations. Furthermore, test results of antioxidant activity in IC50 are expressed in μg/mL. The IC50 value in the BFOE in this study (1.48 ± 0.35 μg/mL) was higher than that in ascorbic acid (0.32 ± 0.23 μg/mL). This indicates that the ability of BFOE was lower than that of ascorbic acid. However, the IC50 value of BFOE was very strong. As explained by Molyneux (2004), the antioxidant activity of a compound can be categorized as very strong if the IC50 value < 50 μg/mL. The high antioxidant ability in this study assumed to be the effect of the phytochemical compounds contained in BFOE such as xanthones, isoquinolines, naphtalenes, and phenolics groups. The compound of xanthones was highest than the other compounds (Munaeni et al. 2019a). Mahaburasakam et al. (2009) and Ieyama et al. (2011) explain that the phytochemical compounds...
in the naphtalenes, napthoquinones, antiahaquinones group are antioxidant compounds that have the ability to inhibit free radical activity. Xanthones and phenolics compounds are capable to inhibit the growth of cancer cells (Zhang et al., 2018; Yang et al., 2019). Phenolic compounds are capable of donating hydrogen atoms to free radicals to form more radicals derived from phenols (Jongberg et al., 2013).

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<tr>
<th>Concentrations (mg/mL)</th>
<th>5% inhibition</th>
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<tr>
<td></td>
<td>BFOE</td>
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<tr>
<td>0.625</td>
<td>90.09 ± 1.31a</td>
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<tr>
<td>1.25</td>
<td>96.31 ± 1.48b</td>
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<tr>
<td>2.5</td>
<td>99.39 ± 0.35c</td>
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<tr>
<td>IC50 (µg/mL)</td>
<td>1.48 ± 0.35</td>
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Data (mean ± SD) (n=3) with different letters at the same column indicate significant difference (P<0.05).

**CONCLUSION**

The content of the oligosaccharides of Buton forest onion extract (BFOE) was included inulins, fructooligosaccharides (FOS), galactooligosaccharides (GOS) and raffinose. The oligosaccharides of BFOE were found capable to stimulate the growth of P. piscicida 1Ub and Bacillus sp. NPS. The optimum BFOE concentration to stimulate the growth of probiotics. The Buton forest onion extract (BFOE) was resistant to artificial gastric acid and α-amylase. The BFOE was capable to inhibit proangiogenic activity with IC50 of 1.48 µg/mL. The BFOE in this study has the potential to be a prebiotic and an antioxidant.

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