JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

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

*Waode Munaeni*[1,2], *Widanarni Widanarni*[1], *Munti Yuhana**[1], *Mia Setiawati*[1], *Aris Tri Wahyudi*[3]

***Address(es):***
[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University (Bogor Agricultural University), Bogor 16680, Indonesia.
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232, Indonesia.
[3]Department of Biology, Faculty of Mathematics and Natural Science, IPB University (Bogor Agricultural University), Bogor 16680, Indonesia.

*Corresponding author: yuhanamunti@gmail.com

**doi: 10.15414/jmbfs.2020.10.1.107-111**

ARTICLE INFO

Received 18. 5. 2019
Revised 24. 3. 2020
Accepted 31. 3. 2020
Published 1. 8. 2020

Regular article

OPEN ACCESS

**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 *Pseudoalteromonas piscicida* 1Ub and *Bacillus* sp. NP5.and its resistance to artificial gastric acid and α-amylase. Antioxidant activities were determined using 2,2-diphenyl-1-picrylhidrazyl (DPPH). Results indicate that the content of the oligosaccharides of BFOE including inulins, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and raffinoses 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. NP5. 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 gastrice 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 scavenge 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 sabrang, bawang dayak , babawangan beureum, bawang hantu, bawang siyem, bawang kapal, bawang sayup,* and *bawang lubak* **(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) Kuntze, *Galatea americana* (Aubl.) Kuntze, *G. bulbosa* (Mill.) Britton, *G. plicata* (Sw.) Baker, *Ixia americana* Aubl, *E. Subaphylla* Gagnep, *Sisyrinchium americanum* (Aubl) Lemee, *S. bulbosum* Mill., *S. capitatum* Pers., *S. congestum* Klatt, *S. elatum, 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; Ieyama *et al*., 2011)**, flavonoid, tannin, saponin, steroid, triterpenoid groups **(Munaeni *et al*., 2017), securixantone (Munaeni *et al*., 2019a)**. The *E. bulbosa* are 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* **(Ifesan *et al*., 2009; Subramaniam *et al*., 2012)**, and *Vibrio harveyi* **(Munaeni *et al*., 2017),** and potential to increase the growth performance of vannamei shrimp (*Litopenaeus 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 **Phoem & Voravuthikunchai (2013)**, *E. americana* is potential as a prebiotic because it can increase the growth of intestinal microbiotas (Bacteroides population).
Prebiotics are foods that cannot be utilized by the host because the amylase or other hydrolases in the intestinal tract cannot be digested **(Carabin *et al*., 1999)** but they can be digested or fermented selectively by the microbiotas 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 **(Wichienchot *et al*., 2010; Sawangwan *et al*., 2018)**. Oligosaccharides are prebiotic sources that have been widely used in mammals and fish **(Mahiuos *et al*., 2006)**. Prebiotic oligosaccharides include fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), isomalto-oligosaccharides (IMO), raffinoses, lactosucroses, soy-oligosaccharide (SOS), palatinoses, inulins, oligofructoses (OF), isomaltuloses, arabinoxyoligosaccharides, soligosaccharides, galactosil lactose, and pyrodextrinsares **(Mohanti *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 oigosaccharides in Buton forest onion *E. 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 *E. 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, Reseach Center for Biologi, Indonesian Institutite of Sciences (No. 1000/1PH.1.01/11.07/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 dried in an oven at a temperature of 60 $^{o}$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 $^{o}$C. The extract was then stored in a freezer at -20 $^{o}$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 vannamei 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 $^{o}$C and reared at 4 $^{o}$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 analyze was based on **AOAC 2001.02** method, while the inulins and fructooligosaccharides (FOS) analyze 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 $^{o}$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 at 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 $^{o}$C at 140 rpm for 18 h for *P. piscicida* 1Ub and *Bacillus* sp. NP5 and 14 h for *V. parahaemolyticus*. The bacterial suspension (density of $10^{8}$ CFU/mL) was inoculated into each treatment (v/v) by 5%, then incubated at a temperature of 28-29 $^{o}$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)**:

$$\text{Prebiotic activity scores} = \frac{\text{Log Ppt} - \text{Log Pp0}}{\text{Log Pgt} - \text{Log Pg0}} - \frac{\text{Log Ept} - \text{Log Ep0}}{\text{Log Egt} - \text{Log Eg0}}$$

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, Pgt was probiotic log OD on control/media at t-h, Pg0 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, Egt was enteric log OD on control/media at t-h, and Eg0 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; $Na_2HPO_4.2H_2O$, 8.25; $NaH_2PO_4$, 14.35; $CaCl_2.2H_2O$, 0.01; $MgCl_2.6H_2O$, 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 $^{o}$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 $^{o}$C for 3 h. The total carbohydrate was determined by phenol-sulfurc 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 followed 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 inulins, fructooligosaccharides (FOS), galactooligosaccharides (GOS) and raffinoses were at 2.1%, 10%, 1% and 7.5%, respectively. According to **Ritsema & Smeekensy (2003)**, various type of onion plant are able to produce prebiotic oligosaccharides. Prebiotics are foods that can be selectively utilized by beneficial intestinal microbiotas **(Mohanti *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. NP5 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.

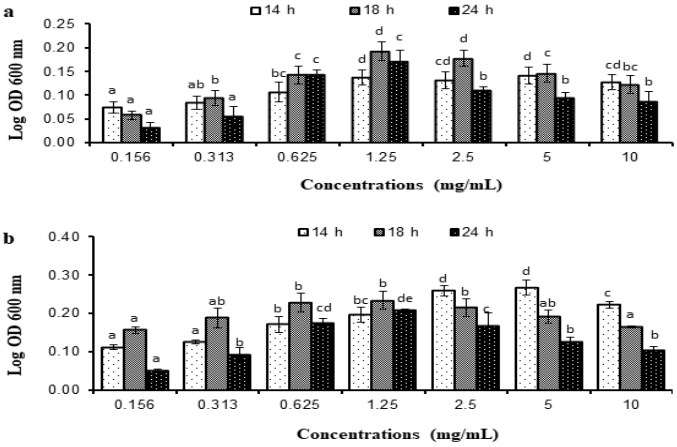


**Figure 1** The scores of the activity of Buton forest onion extract (BFOE) in the growth stimulation on (a) *P. piscicida* 1Ub and (b) *Bacillus* sp. NP5. Data (mean ± SD) with different letters at the same h indicating significant different (P < 0.05).

The use of different concentrations of BFOE showed differently in the probiotic 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. NP5 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. NP5 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 probiotic. 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 capability 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 artificial gastric acid**

The highest percentage of gastric acid hydrolysis of the oligosaccharide of BFOE at concentrations of 0.625, 1.25, and 2.5 mg/mL after 3 h of incubation were 2.53 ± 0.80%, 4.72 ± 0.36%, and 8.25 ± 0.25%, respectively (Figure 2). The percentage of hydrolysis of reference prebiotic (FOS) was higher than the BFOE at the same concentration for 3 h of incubation. The BFOE was more resistant compared to the reference prebiotic (FOS). The hydrolysis percentage of artificial gastric acid of commercial FOS increased after 3 h of incubation. The degrees of hydrolysis at the concentrations of 0.625, 1.25, and 2.5 mg/mL were 7.09 ± 0.54%, 19.83 ± 2.26%, and 28.73 ± 0.83%, respectively.

In addition to being able to stimulate the growth of probiotics, the oligosaccharides in BFOE are also resistant to artificial gastric acid and α-amylase. This was also found in previous study by **Azmi *et al*. (2012)**, which showed that the prebiotic extract of *G. levis* shoots, not only is able to stimulate the growth of probiotics, but also is resistant to artificial gastric acid at up to 99%. The percentage of oligosaccharide 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 (7.94%). Meanwhile, the concentrations of 0.625 and 1.25 mg/mL yielded 4.45% 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 *Gluconobacter oxydans* NCIMB 4943 are capable of being resistant to 98.4% of human gastric acid conditions **(Wichienchot *et al*., 2006)**.

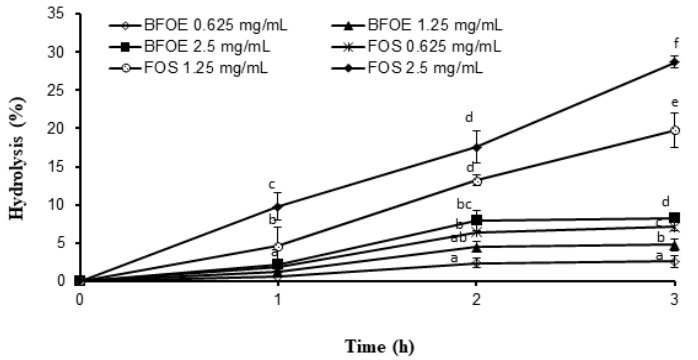


**Figure 2** The resistance of Buton forest onion extract (BFOE) and fructooligosaccharides (FOS) to artificial gastric acid at different concentrations. Data (mean ± SD) with different letters at the same h indicate significant difference (P < 0.05).

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

The 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 those at other 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 were 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)**.

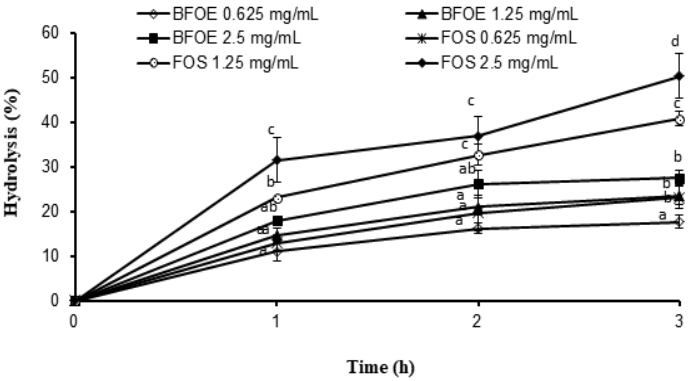


**Figure 3** The resistance of Buton forest onion extract (BFOE) and fructooligosaccharides (FOS) to α-amylase at different concentrations. Data (mean ± SD) with different letters at the same h indicate significant difference (P < 0.05).

**Buton Forest Onion Extract (BFOE) as an Antioxidant**

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 is < 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 compunds **(Munaeni *et al*. 2019a)**. **Mahaburasakam *et al*. (2009)** and **Ieyama *et al*. (2011)** explain that the phytochemical compounds

in the naphtalenes, naphtoquinones, anthraquinones 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).**

**Table 1** The DPPH free-radical scavenging activity of Buton Forest Onion Extract (BFOE) and ascorbic acid at different concentrations.

| Concentrations (mg/mL) | % inhibition | |
|---|---|---|
| | BFOE | Ascorbic acid |
| 0.625 | 90.09 ± 1.31a | 81.02 ± 0.28a |
| 1.25 | 96.31 ± 1.48b | 84.17 ± 0.33b |
| 2.5 | 99.39 ± 0.35c | 86.71 ± 0.08c |
| IC50 (µg/mL) | 1.48 ± 0.35 | 0.32 ± 0.23 |

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 raffinoses. The oligosaccharides of BFOE were found capable to stimulate the growth of *P. piscicida* 1Ub and *Bacillus* sp. NP5. 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 DPPH radical scavenge with IC50 of 1.48 µg/mL. The BFOE in this study has the potential to be a prebiotic and an antioxidant.

**Acknowledgments:** This article is part of the dissertation by Waode Munaeni in the Department of Aquaculture, Faculty of Fishery and Marine Science, Bogor Agricultural University. The research is financially supported by the Ministry of Research, Technology, and Higher Education and the Lembaga Pengelola Dana Pendidikan (LPDP, Indonesia Endowment Fund for Education) for funding the present study in the form of the BUDI-DN (Beasiswa Unggulan Dosen Indonesia- Dalam Negeri, The Indonesian Superior Lecturer Scholarship-Domestic) scholarship.

## REFERENCES

AOAC (Association of Analytical Communities) Method 997.08. Fructans in Food Products.

AOAC (Association of Analytical Communities) Method 2002.02. Determination of Trans-Galactooligosaccharides in Foods by AOAC Method 2001.02. https://tools.thermofisher.com/content/sfs/brochures/AN-155-IC-Trans Galactooligosaccharides-Foods-LPN1547-EN.pdf.

AZMI, A. F. M. N., MUSTAFA, S., HASIM, A. M. D., & MANAP, Y. A. (2012). Prebiotic Activity of Polysaccharides Extracted from *Gigantochloa Levis* (Buluh beting) Shoots. *Molecules*, 17, 1635-1651. https://doi:10.3390/molecules17021635.

CARABIN, I. G., & FLAMM, W. G. (1999). Evaluation of safety on inulin and oligofructose as dietary fiber. *Regul. Toxicol. Pharmacol*, 30, 268-282. https://doi:10.1006/rtph.1999.1349.

FEBRINDA, A. E. (2014). Antioxidants and Antidiabetic Potency of Aqueous and Ethanolic Extracts of *Bawang Dayak* Bulbs (*Eleutherine palmifolia*) in vitro and in vivo. [Thesis]. Bogor (ID): Institut Pertanian Bogor.

FEBRINDA, A. E., YULIANA, N. D., RIDWAN, E., WRESDIYATI, T., & ASTAWAN, M. (2014). Hyperglycemic control and diabetes complication preventive activities of Bawang Dayak (*Eleutherine palmifolia* L. Merr.) bulbs extracts in alloxan-diabetic rats. *International Food Research Journal*, 21(4), 1405-1411.

GIBSON, G. R., PROBERT, H. M., RASTALL, R. A., & ROBERFROID, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, 17, 259-275. https://doi:10.1079/NRR200479.

HALLIWELL, B. (1990). How to characterize a biological antioxidant. *Free Radical Research Communications*, 9(1), 1-32. https://doi:10.3109/10715769009148569.

HUEBNER, J., WEHLING, R. L., PARKHURST, A., & HUTKINS, R. W. (2008). Effect of processing conditions on the prebiotic activity of commercial prebiotics. *International Dairy Journal*, 18, 287-293. https://doi:10.1016/j.idairyj.2007.08.013.

IFESAN, B. O. T., HAMTASIN, C., MAHABUSARAKAM, C., & VORAVUTHIKUNCHAI, S. P. (2009). Inhibitory effect of *Eleutherine americana* Merr. extract on *Staphylococcus aureus* isolated from food. *Journal of Food Scienc*, 7(1), 31-36. https://doi:10.1111/j.1750-3841.2008.01004.x.

INSANU, M., KUSMARDIYANI, S., & HARTATI, R. (2014). Recent studies on phytochemicals and pharmacological effects of *Eleutherine americana* Merr. *Procedia Chemistry*, 13, 221-228. https://doi:10.1016/j.proche.2014.12.032

IEYAMA, T., GUNAWAN-PUTERI, M. D. P. T, & KAWABATA, J. (2011). α-Glucosidase inhibitors from the bulb of *Eleutherine americana*. *Food Chemistry*, 128, 308-311. https://doi:10.1016/j.foodchem.2011.03.021

JONGBERG, S., TORNGREN, M. A., GUNVIG, A., SKIBSTED, L. H., & LUND, M. N. (2013). Effect of green tea or rosemary extract on protein oxidation in Bologna type sausages prepared from oxidatively stressed pork. *Meat Sci*, 93, 538-546. https://doi:10.1016/j.meatsci.2012.11.005.

KIM, H. S., QUON, M. J., & KIM, J. A. (2014). New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. *Redox Biol*ogy, 2, 187-95. https://doi:10.1016/j.redox.2013.12.022.

MAHABUSARAKAM, W., HEMTASIN, C., CHAKTHONG, S., VORAVUTHIKUNCHAI, S. P., & OLAWUMI, I. B. (2010). Naphthoquinones, Anthraquinones and Naphthalene Derivatives from the Bulbs of *Eleutherine americana*. *Planta Med*, 76, 345-349. https://doi:10.1055/s-0029-1186143.

MAHIOUS, A. S., GETESOUPE, F. J., HERVI, M., METAILLER, R., & OLLEVIER, F. (2006). Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C.1758). *Aquaculture Internasional*, 14(3), 219-229. http://dx.doi.org/10.1007/s10499-005-9003-4.

MAYUR, B., SANDESH, S., SHRUTI, S., & SUNG-YUM, S. (2010). Antioxidant and α- glucosidase inhibitory properties of *Carpesium abrotanoides* L. *J Med Plants Res*, 4, 1547-1553. http://dx.doi.org/10.5897/JMPR10.210.

MIHAYLOVA, D., VRANCHEVA, R., IVANOV, I., & POPOVA, A. (2019). Phytochemical Profile and Antioxidant Activity of Water Pepper (*Polygonum hydropiper* L.). *Journal of Microbiology, Biotechnology and Food Science*, (5), 1205-1208. http://dx.doi:10.15414/jmbfs.2019.8.5.1205-1208.

MOHANTI, D., MISRA, S., MOHAPATRA, S., & SAHU, P. S. (2018). Prebiotics and synbiotics: Recent concepts in nutrition. *Food Bioscience*, https://doi.org/10.1016/j.fbio.2018.10.008.

MOLYNEUX, P. (2004). The use of stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activities. *Songklanakarin Journal Science Technology*, 26(2), 211-219.

MUNAENI, W., PARIAKAN, A., YUHANA, M., SETIAWATI, M., & ABIDIN, L. O. B. (2017). In Vitro Phytochemical and Inhibitory Potential Tests of Buton Forest Onion Extract (*Eleutherine palmifolia* L. Merr.) on *Vibrio harveyi*. *Microbiology Indonesia*, 11(3), 75-80. doi.org/10.5454/mi.11.3.1.

MUNAENI, W., WIDANANRI, W., YUHANA, M., SETIAWATI, M., & WAHYUDI, A. T. (2019). Phytochemical analysis and antibacterial activities of *Eleutherine bulbosa* (Mill.) Urb. extract against *Vibrio parahaemolyticus*. *Asian Pacific Journal of Tropical Biomedicin*, 9(9): 397-404. doi: 10.4103/2221-1691.267669.

MUNAENI, W., DISNAWATI , YUHANA, M., SETIAWATI, M., ABIDIN, L. O. B., & KURNIAJI A. (2017).Buton Forest Onion Extract (*Eleutherine bulbosa* (Mill.) Potential on Growth Performance of Vannamei Shrimp (*Litopenaeus vannamei*). *Pakistan Journal of Biological Sciences*, 22(1), 15-20. DOI: 10.3923/pjbs.2019.15.20.

PHOEM, A. N., & VORAVUTHIKUNCHAI, S. P. (2013). *Eleutherine americana* as a growth promotor for infant intestinal microbiota. *Anaerobe*, 20, 14-19.

PUTRA, A. N., UTOMO, N. B. P., & WIDANARNI. (2015). *Growth Performance of Tilapia (Oreochromis niloticus) Fed with Probiotic, Prebiotic and Synbiotic in Diet. Pakistan Journal of Nutrition*, 14, 263-268. https://doi.org/10.3923/pjn.2015.263.268.

RITSEMA, T., & SMEEKENS, S. (2003). Fructans: Beneficial for plants and humans. *Current Opinion in Plant Biology*, 6, 223-230. https://doi.org/10.1016/S1369-5266(03)00034-7.

SAHREEN, S., KHAN, M. R., & KHAN, R. A. (2010). Evaluation of antioxidant activities of various solvent extracts of *Carissa opacus* fruits. *Food Chem*, 122, 1205-1211. https://doi.org/10.1016/j.foodchem.2010.03.120.

SAWANGWAN, T., WANSANIT, W., PATTANI, L., & NOYSANG, C. (2018). Study of prebiotic properties from edible mushroom extraction. *Agriculture and Natural Resources*, 52, 519-524. https://doi.org/10.1016/j.anres.2018.11.020.

SEKHON, B. S., & JAIRATH, S. (2010). Prebiotics, probiotics and synbiotics: An overview. *Indian Journal of Pharmaceutical Education and Research*, 1, 13-36.

SUBRAMANIAM, K., SURIYAMOORTHY, S., WAHAB, F., SHARON, F.B., & REX, G. R. Antagonistic activity of *Eleutherine bulbosa* (Mill.) Urb. Linn. *Asian Pacific Journal of Tropical Disease*, S491-S493.

WICHIENCHOT, S., PRASERTSAN, P., HONGPATTARAKERE, T., GIBSON, G. R., & RASTALL, R. A. (2006). In vitro fermentation of mixed linkage gluco-oligosaccharides produced by *Gluconobacter oxydans* NCIMB 4943 by the human colonic microflora. *Current Issues in Intestinal Microbiology*, 7, 7-12.

WICHIENCHOT, S., JATUPORNPIPAT, M., & RASTALL, R. A. (2010). Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chemistry*, 120, 850-857. https://doi:10.1016/j.foodchem.2009.11.026.

WIDANARNI, TEPU, I., SUKENDA, & SETIAWATI, M. (2009). Selection of probiotic bacteria for biocontrol of vibriosis on tiger shrimp (*Penaues monodon*) larvae using co-culture method. *Jurnal Riset Akuakultur*, 4(1), 95-105.

YANG, H. X., LI, W., LI, Q., AI, H.L., LI, Z. H., HUANG, R., FENG, T., LIU, J. K. (2019). Piperidine alkaloids and xanthone from the roots of *Caulophyllum robustum* Maxim. *Fitoterapia*, 132, 22-25. https://doi.org/10.1016/j.fitote.2018.07.014.

ZHANG, B. J., FU, W. W., WU, R., YANG, J. L., YAO, C. Y., YAN, B. X., TAN, H. S., ZHENG, C.W., SONG, Z. J., XU, H. X. (2018). Bioactive scalemic caged xanthones from the leaves of *Garcinia bracteata*. *Bioorganic Chemistry*, doi: https://doi.org/10.1016/j.bioorg.2018.10.041.