Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.4623

Chemical composition, bioactive compounds, antioxidant activity, and inhibitor alpha-glucosidase enzyme of *Sargassum* sp.

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Abstract

Sargassum sp. has the potential to be a source of bioactive components. The purpose of this study was to determine the chemical composition, bioactive compounds, antioxidant activity, and α-glucosidase enzyme inhibitory activity of *Sargassum* sp. extract. The exploratory research method involved extracting *Sargassum* sp. for 72 h using solvents of different polarities. The chemical composition of *Sargassum* sp. was analyzed using parameter analysis as well as the identification of phytochemicals and total phenolics. Antioxidant activity was measured using the DPPH method as well as inhibition of α-glucosidase enzyme activity. The chemical composition of *Sargassum* sp. was found to be 15.67, 17.56, 13.46, 0.6, 57.82, and 13.62% moisture content, ash, protein, fat, carbohydrates (by difference), and total crude fiber, respectively. The bioactive compounds of *Sargassum* sp. include saponins and phenolic compounds that were extracted with methanol (617.778 GAE/100 g) and ethyl acetate (309.55 GAE/100 g). The antioxidant activity with IC_{50} value of *Sargassum* sp. extract successively extracted 648.33 mg/mL of methanol, 864.22 mg/mL of ethyl acetate, and 884 mg/mL of n-hexane. *Sargassum* sp. extract inhibited the activity of α-glucosidase enzyme methanol extract (9.44–0.35%), n-hexane extract (11.72–0.46%), and ethyl acetate did not. Except for the ethyl-acetate extract, *Sargassum* sp. extract has antioxidant and enzyme α-glucosidase inhibitor properties.

Keywords: activity; bioactive compounds; inhibitor; *Sargassum* sp.; polarity

Practical Application: Extract technique by maceration on brown Sargassum sp seaweed can produce yields that serve as guidelines for functional testing and alpha-glucosidase inhibition.

1 INTRODUCTION

Seaweed is one of the many biological resources found in the Indonesian coastal sea. Seaweed has primarily been cultivated in red and brown grades. Seaweed is classified into four classes based on pigment content: red, brown, green, and blue. Brown seaweed is still exported as raw material, as the domestic processing industry has not yet developed. Although its production potential is enormous, the price of brown seaweed remains relatively low. Brown seaweed has the potential to be used as a functional food ingredient because it contains bioactive components.

Several studies have found bioactive components in brown seaweed, such as phenolic compounds, natural pigments, sulfated polysaccharides, fiber, and halogen compounds (Farvin & Jacobsen, 2013; Holdt & Kraan, 2011; Kim & Pangestuti, 2011). Bioactive compounds derived from seaweed serve as nutraceuticals (Leandro et al., 2020). Bioactive components include polysaccharides (e.g., alginate, fucoidan, Ulva, agar, and carrageenan). The protein contents in seaweed are phycobiliprotein and carotenoids (beta-carotene and fucoxanthin). It also contains phenolic compounds (such as phlorotannin) and vitamins (especially vitamins A, B, C, D, E, and K). Aside from protein, seaweed contains essential minerals such as iron, iodine, magnesium, and potassium, as well as polyunsaturated fatty acids (W-3) (Bouga & Combat, 2015; Cherry et al., 2019; Mišurcová, 2011; Pereira, 2016; Rajapakse & Kim, 2011; Rebours et al., 2014). These bioactive compounds serve as drugs to prevent diseases like hyperglycemia, diabetes, metabolic disorders, cancer, pathogenic diseases, aging, obesity, bone diseases, neurodegenerative diseases, and cardiovascular diseases (Kim & Pangestuti, 2011; Shannon & Abu-Ghannam, 2019; Cotas et al., 2020; Murray & Lopez, 2013; Pereira, 2018; Pereira et al., 2018). According to Edison et al. (2020), many other studies on the bioactive components of *Sargassum* sp. have been conducted, but they are still in the form of crude extracts. *Sargassum plagyophyllum* crude extract, with ethyl acetate, produced phenolic bioactive components, alkaloids, flavonoids, steroids/terpenoids, and saponins, but not all of the solvent n-hexane and bioactive methanol components were detected.

Brown seaweed contains bioactive alkaloids, glycosides, tannins, and steroids that are used in the pharmaceutical industry (Sathya et al., 2017). The bioactive compounds are phlorotannins, phenolics (Tanniou et al., 2014), flavonoids that can inhibit

Received Feb. 2, 2023
Accepted Jun. 5, 2023
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LDL oxidation, angiotensin-converting enzyme, α-amylase, and α-glucosidase (Nagappan et al., 2017). Moreover, other research results state that red and brown seaweeds contain antioxidants (Brown et al., 2014). Seaweed is also used to treat various diseases. The findings of alginate activity in brown seaweed and in vitro tests as antioxidants and antidiabetics were also promising (Addina et al., 2020).

The discovery of new antioxidant and antidiabetic ingredients derived from the sea holds great promise for use as a new commodity in the food industry, particularly in the field of functional food. Currently, the development of functional food is a trend in many countries, and demand for it is growing (Westrate et al., 2002). It is critical to use new ingredients as the primary ingredient in the development of functional foods. Therefore, this study was carried out to investigate the chemical composition, bioactive compounds, antioxidant activity, and inhibitory α-glucosidase enzyme of *Sargassum* sp.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Raw materials

The seaweed used in this research was *Sargassum* sp. The harvested age was approximately 45 days and originated from cultivation along the southern coast of Gunung Kidul, Yogyakarta Province. The harvested *Sargassum* sp. was washed with running water to remove dirt and excess salts before being dried at room temperature (25°–28°C) for 48 h until it had a moisture content of ±16%. Before extraction and analysis, *Sargassum* sp. was dried, cleaned of any attached dirt, and floured.

2.1.2 Chemical reagents

The solvents used in the chemical reagent were n-hexane, ethyl acetate, and methanol. The reagents for phytochemical analysis include 2% aluminum chloride, quercetin, sulfuric acid, chloroform, acetic anhydride, sulfuric acid, magnesium, amyl alcohol, alcohol, ethanol, 5% $FeCl_3$, and 2N HCl. The analysis materials for the total content of phytochemical compounds were Folin-Ciocalteu reagent 50%, gallic acid, ethanol, distilled water, and 2% sodium carbonate. Antioxidant activity with 1,1-diphenyl-2-picrylhydrazil (DPPH), methanol p.a., and vitamin C as standards, as well as the activity test of α-glucosidase inhibitory α-glucosidase enzyme derived from Saccharomyces cerevisiae recombinant (Sigma Aldrich, USA), p-nitrophenyl-α-D-substrate glucopyranoside (PNPG) (Sigma Aldrich, USA), dimethyl sulfoxide (DMSO) (Merck, Germany), acarbose (Dexa Medica), and sodium carbonate (Merck, Germany) were performed.

2.2 Extraction of Sargassum *sp.*

Lee et al. (2017) used a modified method to extract seaweed. At room temperature (26°–29°C), 300 g samples of dried seaweed (powder) were macerated with 900 mL of methanol, ethyl acetate, and n-hexane as solvents. The sample-to-solvent ratio was 1:3 (w/v). During the maceration process, maceration was carried out for 72 h. Every 4 h, the mixture was stirred. After soaking for 72 h, it was filtered through a 500 μm cloth filter. The filtrate was evaporated using a rotary vacuum at 50°C, yielding a paste-like crude extract.

2.3 Proximate composition

The protein, ash, crude lipid, moisture, and total fiber content of *Sargassum* sp. were determined using AOAC (2005) procedures.

2.4 Phytochemical Test (Harborne, 1996)

2.4.1 Flavonoid test

The 0.5 g sample was mixed with 0.1 mg of magnesium powder, 0.4 mL of amyl alcohol (a mixture 37 and 95% ethanol in the same volume of hydrochloric acid), and 4 mL of alcohol and then shaken. The formation of a red, yellow, or orange color on the amyl alcohol layer indicated the presence of flavonoids.

2.4.2 Saponin test (foam test)

The foam test in hot water was performed to detect saponins. Foam that was stable for 30 min and did not disappear on the addition of 1 drop of 2N HCl indicated the presence of saponins.

2.4.3 Alkaloid test

Several samples were dissolved in a few drops of 2N sulfuric acid before being tested with three alkaloid reagents: Dragendorff's, Meyer's, and Wagner's reagents. The test results were positive when a yellowish-white precipitate was formed with Meyer's reagent, a brown precipitate was formed with Wagner's reagent, and a red-to-orange precipitate was formed with Dragendorff's reagent.

2.4.4 Steroid/triterpenoid test

The steroid/triterpenoid test was performed in a dry test tube by dissolving 0.5 g of the sample in 2 mL of chloroform. The mixture was then treated with 10 drops of acetic anhydride and 3 drops of concentrated sulfuric acid. The red solution formed for the first time and then turned blue and green, indicating a positive reaction.

2.4.5 Hydroquinone phenol test

The phenol hydroquinone test ($FeCl_3$ reagent) was performed by extracting 1 g of the sample with 20 mL of 70% ethanol. The resulting solution was diluted to 1 mL, and two drops of a 5% $FeCl_3$ solution were added. The green or blue-green color indicated the presence of phenolic compounds in the material.

2.4.6 Tannin test

The content of bioactive tannins was determined by extracting a thick methanol extract with hot water. The extract was then dripped with 10% NaCl solution and filtered, and $FeCl_3$ solution was added. The formation of a dark blue solution indicated the presence of a positive bioactive tannin compound in the sample.

2.5 Analysis of total phenol content

The phenol content was determined using the Folin-Ciocalteu method of Toga et al. (1984) modification. A 10 mL volumetric flask was used to dilute 100 mg of sample extract. A 1 mL of the sample solution was pipetted and diluted again in a 10 mL volumetric flask. Then, 0.2 mL of the sample solution was pipetted and 15.8 mL of distilled water was added, followed by 1 mL of Folin-Ciocalteu reagent and 3 mL of 10% Na_2CO_3, and the solution was allowed to stand for 2 h at room temperature. The absorbance of the standard solution was then measured with a UV-Vis spectrophotometer at a wavelength of 765 nm.

2.6 Standard curve

A total of 50 mg of gallic acid was mixed with 1 mL of ethanol and then diluted with distilled water in a 50 mL flask (a 1,000 ppm gallic acid solution). The standard solution of 1,000 ppm was then diluted to 100, 125, 150, 175, and 200 ppm in a 10 mL volumetric flask. A total of 0.2 mL of the standard solution of each concentration was mixed with 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent, then left to stand for 8 min. Then, 3 mL of 10% Na_2CO_3 was added and the solution was allowed to stand at room temperature for 2 h. The absorbance of the standard solution was then measured with a UV-Vis spectrophotometer at a wavelength of 765 nm.

2.7 Analysis of Saponin

2.7.1 Standard solution

A total of 10 mg of standard saponins were mixed with 5 mL of distilled water, vortexed for ±5 min, then mixed with 50 mL of anisaldehyde, homogenized, and allowed to stand for 10 min. Then, 2 mL of 50% H_2SO_4 was added, the solution was heated at 60°C for 10 min, and the volume was adjusted to 10 mL (standard solution, 1000 ppm). Then, the standard solution of 1,000 ppm was diluted to 6.25, 12.5, 25, 50, 100, and 200 ppm, and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 435 nm.

2.7.2 Sample

A total of 100 mg of sample was mixed with 2 mL of 25% H_2SO_4, autoclaved at 120°C, and extracted with ether, and then the filtrate was dried. The dry extract was mixed with 1 mL of distilled water and vortexed for ±5 min before adding 50 mL of homogenized anisaldehyde and allowed to stand for 10 min. After that, 2 mL of 50% H_2SO_4 was added and the solution was heated at 60°C for 10 min. The volume was reduced to 10 mL and diluted 10 times. The absorbance was then measured with a UV-Vis spectrophotometer at a wavelength of 435 nm.

2.7.3 Determination of antioxidant activity

The antioxidant activity of the extract can be done by the DPPH method (1,1-diphenyl-2-picrylhydrazil). The antioxidant activity of the extract was analyzed using the DPPH method (1,1-diphenyl-2-picrylhydrazil) and then measured using a UV-Vis spectrophotometer with a wavelength of 517 nm (Zhang et al., 2006, with modifications). This test had several stages. First, the extraction results were weighed at 50 mg, which were then dissolved in 50 mL of methanol to obtain a concentration of 1,000 mg/mL/methanol dilution (1,000, 500, 250, 125, and 62.5 ppm). Antioxidant activity was determined by pipetting 4.5 mL with a micropipette into a measuring flask and then adding 0.5 mL of a 100 ppm DPPH solution. The mixture was homogenized and left in the dark for 30 min before measuring the absorption using a UV-Vis spectrophotometer with a wavelength of 517 nm. The antioxidant activity of the sample was determined by the magnitude of the DPPH radical absorption inhibition by calculating the percentage of DPPH absorption inhibition using the Equation 1:

$$\text{Inhibition (5)} = \frac{\text{(A control-A sample)}}{\text{(A control)}} \times 100\% \quad (1)$$

A control: absorbance without sample;

A sample: absorbance of a sample.

2.7.4 Analysis of the activity of alpha-glucosidase enzyme

This activity test was based on the method developed by Sancheti et al. (2009) with modification. Samples of the active ingredient fraction were diluted in distilled water to 312.5, 625, 1,250, 2,500, and 5,000 ppm and sonicated to remove any air bubbles. The α-glucosidase solution was prepared by diluting the enzyme 40 times with 0.1 M sodium phosphate buffer, pH 6.9. A total of 10 mL of sample solution was put into the microplate, added 50 mL of phosphate buffer, 25 mL of PNPG substrate (4-nitrophenyl α-dglucopyranoside), and 25 mL of enzyme solution. After 30 min of incubation at 37°C, 100 mL of 0.2 M Na_2CO_3 was added to stop the enzymatic reaction. The absorbance of the solution was measured at 410 nm using an ELISA reader (Epoch Biotech). The percent inhibition of α-glucosidase was calculated as follows (Equation 2):

$$\%\ \alpha\text{-glucosidase inhibitors} = 100 \times \{1 - ([\text{glucose}]S / [\text{glucose}]B)\} \quad (2)$$

[Glucose]S and [glucose]B represent the glucose concentrations produced in the presence and absence of the test sample, respectively.

The IC_{50} values were calculated by fitting data from plots of percent α-glucosidase inhibition against acarbose concentrations of 0.1, 0.5, 1, 5, and 10 ppm (final test concentration) to a regression equation. Acarbose was used as the reference inhibitor (positive control).

2.8 Data analysis

All data were obtained from three replicates. All data were calculated based on equations, analyzed descriptively, and presented in the form of tables and graphs. The data are presented as the mean and standard deviation.

3 RESULT AND DISCUSSION

3.1 Proximate composition of Sargassum *sp.*

Table 1 shows the results of the chemical composition analysis of *Sargassum* sp., which includes moisture content, ash, protein, fat, carbohydrate (by difference), and total crude fiber. The highest content of *Sargassum* sp. was carbohydrates, followed by ash and moisture content. The fat content was the lowest because *Sargassum* sp. is not a source of fat.

The moisture content of *Sargassum* sp. was calculated according to the Indonesian National Standard (BSN, 2009). The total mineral content (ash) of *Sargassum* sp. was lower than that of previous studies. Seaweed is a source of carbohydrates and fiber. The protein content in the study was 13.48%. This protein content was similar to that reported by Fleurence et al. (2012), who stated that the protein content of seaweed ranged between 3 and 15% dry weight. According to Ismail (2017), the protein content of *S. linifolium* was higher (14.89%) than that of U. fasciata (9.56%) and *C. officinalis* (5.91%) of algal dry weight. Varying protein content may be proportional to temperature values, seasonal periods, or consumption by seaweeds in terms of growth, reproduction, and species differences. The cause of these variations could be that the protein content of seaweed varies between species and within the same species due to maturity levels, time of year, and different habitats (Gressler et al., 2010).

The carbohydrate content in the study was high (57.82%). According to El-Sheekh et al. (2021), another type of brown seaweed, *Padina pavonia*, had a carbohydrate content of 20.4%. There is a difference in carbohydrate content because environmental conditions such as currents, nutrients, pH, and temperature are thought to influence the chemical composition of seaweed species. Brown algae contain more carbohydrates than red seaweed (El-Shenody et al., 2019).

3.2 Identify qualitative bioactive compounds of Sargassum *sp. extract*

Sargassum sp. was extracted using organic solvents with varying polarities. Table 2 shows the identification of bioactive compounds used with methanol, n-hexane, and ethyl acetate.

Table 2 shows that extracting *Sargassum* sp. with polar, polar, and semi-polar solvents resulted in a positive sign that the color changed to dark green/dark green. Steroids are typically in the form of glycosides, which are compounds made up of sugars and aglycones. The bound sugar is polar, so it can dissolve in polar solvents (methanol). Aglyco is non-polar, so steroids are more soluble in semi-polar and non-polar solvents (ethyl acetate and n-hexane) (Purwatresna, 2012). Terpenoids have non-polar properties and are easily soluble in non-polar solvents because they are made of long chains of C30 hydrocarbons. Some terpenoid compounds have a cyclic structure in the form of alcohol with a –OH group, which causes them to become semi-polar and attracted to polar and semi-polar solvents (Hayati et al., 2010).

Other bioactive components found in *Sargassum* sp. extract are phenolic. A change in the extract's color from green to dark blue indicated the presence of phenolic compounds. Phenolics can be found in extracts that are polar, non-polar, or semi-polar. Phenolics are most attracted to polar and semi-polar solvents. Phenolic compounds are generally more easily attracted by semi-polar and polar organic solvents (Septiana and Asnani, 2012). *Sargassum* sp. extract also contains saponins. The analysis results of saponins were indicated by foam after being shaken for 10 min and left for 15 min. Saponin compounds contain a hydrophilic group that binds to water. The hydrophobic group, on the contrary, binds oxygen in the air, where the polar group is outside the micelle and the non-polar group is inside the micelle. Because semi-polar solvents have little ability to attract polar compounds caused by the oxidation process, the polarity of polar compounds can be reduced when saponins are dissolved in ethyl acetate solvent (semi-polar).

3.2.1 Total phenolics Sargassum *sp. extract*

The total phenolic content of *Sargassum* sp. extracts extracted with methanol and ethyl acetate was 617.778 mg GAE/100 g and 309.555 mg GAE/100 g, respectively. Ismail et al. (2019) showed that *Turbinaria decurres* was extracted with an acetone phenolic content value of 4.32 mg GAE/g DW, while the ethanol extract of *Sargassum muticum* and *Sargassum acinarium* had total phenolic contents of 3.31 and 2.88 mg GAE/g DW,

Table 1. Chemical composition of *Sargassum* sp.

Parameters	Mean±SD (%)
Moisture*	15.67±0.17
Ash**	17.56±0.24
Protein**	13.48±0.16
Fat**	0.60±0.13
Carbohydrate (by difference)*	57.82±0.45
Total crude fiber*	13.62±0.16

*Wet basis; **dry basis.

Table 2. Bioactive compounds of *Sargassum* sp.

Bioactive Compounds	Methanol extract	Ethyl Acetate	n-Hexane
Alkaloid			
a. Meyer	-	-	-
b. Dragendorff	-	-	-
Flavonoids	-	-	-
Phenolic	+	+	+
Saponin	+	+	+
Steroids	+	+	-
Terpenoids	+	+	+
Tannin	+	+	+

+: present; -: absent.

respectively. According to Ganapathi et al. (2013), brown seaweed contains phenolic compounds that act as antioxidants. Brown seaweed contains 20–30% phenol by dry weight. In this study, the phenolic content of the extract of methanol was higher than that of the extract of ethyl acetate. This result is significantly higher than the *Sargassum vulgare* ethyl acetate extract, which is 5.77 mg GAE/g (Khaled et al., 2012).

3.2.2 Antioxidant activity

The antioxidant activity of *Sargassum* sp. extract differed depending on the solvents used. Each solvent achieved an IC_{50} value (Table 3). Methanol extract had a high antioxidant activity with an IC_{50} of 648.33 mg/mL, whereas ethyl acetate had an IC value of 863.22 mg/mL, and n-hexane had an IC_{50} value of 884.72 mg/mL.

The IC_{50} value of the antioxidant activity affected the solvent. In this study, *Sargassum* sp. extract with methanol had the highest antioxidant activity when compared to the ethyl acetate and n-hexane extracts. El-Sheekh (2021) reported a similar result, stating that methanol extract had the highest antioxidant activity for all tested (*P. pavinia* and *J. rubens*) seaweeds, except *T. atomaria*. The highest antioxidant activity was found in *T. atomaria*. Hexane extract showed the lowest antioxidant activity for all tested (*J. rubens* and *T. atomaria)* seaweeds except *P. pavonia*, which had the lowest antioxidant activity with diethyl ether extract (0.5±0.1 mg ascorbic acid equivalents/g crude extract). In hexane extract, *J. rubens* had the lowest antioxidant activity, with 0.14±0.0 mg ascorbic acid equivalents/g crude extract. Methanol was the most effective solvent for extracting antioxidant compounds from seaweeds. Methanol had a larger dielectric constant than ethanol.

The compound will be immersed in solvents of the same polarity (Harborne, 1996). Polar compounds dominated the extracted content of *Sargassum* sp. Yudiati et al. (2018a, 2018b) demonstrated that alginate extract from *Sargassum* sp. could also inhibit free radicals via their hydroxyl groups.

The IC_{50} value in this research was lower than that of Hidayati et al. (2019) (72.95 mg/mL). The different results were due to variations in the activity of antioxidants (Parthiban et al., 2013), their location (Mirghani et al., 2018), and their seasons (Khairy & El-Sheikh, 2015).

3.2.3 Activity inhibitory alpha-glucosidase enzyme

Table 4 shows the results of the analysis of the inhibitory activity of *Sargassum* sp. extract against the α-glucosidase enzyme.

Table 3. Antioxidant activity (IC_{50}) *Sargassum* sp. extract with different solvents (methanol, ethyl acetate, and n-hexane).

Treatment	y=ax+b	R^2	R	IC_{50}
Ascorbate acid*	y=7.118x+12.013	0.9994	0.9997	5.40
Methanol	y=5.038x+17.582	0.9681	0.9839	648.33
Ethyl acetate	y=10.5x-20.987	0.9879	0.99393	863.22
m-Hexane	y=3.5352x-14.699	0.9826	0.99126	884.73

*Ascorbate acid as standard.

Table 3 shows that each extract with different solvents inhibits the α-glucosidase enzyme in a different way. The methanol and n-hexane extracts inhibited α-glucosidase enzyme activity between 9.44 and 0.248 ppm (from 5,000 to 312.5 ppm), while the n-hexane extract inhibited it between 11.72 and 0.464 percent. In contrast, the ethyl acetate extract had no enzyme-inhibitory activity, α-glucosidase, due to negative percent inhibition.

4 CONCLUSION

Sargassum sp., a brown seaweed, has a relatively high chemical composition (ash and carbohydrates). Secondary metabolites such as phenolics, steroids/terpenoids, and saponins make up the content of bioactive compounds. A crude extract of *Sargassum* sp. has relatively low antioxidant activity and α-glucosidase inhibitor activity. In the future, the extract of *Sargassum* sp. will be separated for purer bioactive compounds and increased activity.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Laboratory of the Center for iopharmaceutical Studies, Research and Community Service, Bogor Agricultural University, the Laboratory of Agricultural Products, the Faculty of Agriculture, the Laboratory of Natural Materials Chemistry, the Faculty of Mathematics and Natural Sciences, and the University of Riau for their assistance in facilitating our research. Also, to thank the Director General of Higher Education, the Ministry of Research and Technology, and Higher Education of the Republic of Indonesia for funding the National Competitive Research Grant Program 2022.

Table 4. Percent inhibition of *Sargassum* sp. extract against the alpha-glucosidase enzyme.

Sample	Concentration (ppm)	% inhibition
Methanol	5,000	9.435±0.241
	2,500	2.088±0.506
	1,250	0.309±0.439
	625	0.348±0.201
	312.5	0.348±0.116
n-Hexane	5,000	11.717±0.307
	2,500	8.507±0.354
	1,250	1.083±0.067
	625	0.464±0.418
	312.5	-0.387±0.439
Ethyl acetate	5,000	0.193±0.469
	2,500	-0.116±0.232
	1,250	0.116±0.418
	625	0.193±0.241
	312.5	0.232±0.307
Acarbose*	10	97.866±0.474
	5	93.785±0.189
	1	73.509±0.605
	0.5	63.026±0.474
	0.1	46.642±2.074

*Acarbose as standard.

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