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In Vitro Antidiabetic Activity of Herbal Beverage Containing Turmeric and Guava Leaves

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Abstract: Herbs have widely used in beverages. This study aimed to develop an herbal beverage for diabetics and to study the effect of guava leaves (G) and turmeric (K) proportions in an herbal beverage mixture on the inhibition of α-amylase and α-glucosidase enzyme activity and the organoleptic quality of the beverage. The proportions of G and K used in the beverage were C1: 0% G and 100% K; C2: 20% G and 80% K; C3: 40% G and 60% K; C4: 60% G and 40% K; C5: 80% G and 20% K; C6: 100% G and 0% K. The greater the proportion of K, the inhibition of the α-amylase enzyme increased. Increasing G proportion from 0% to 20% increased the inhibition of the α-glucosidase enzyme by 38.49% (from 21.05% to 59.54%), but no changes were observed on G proportion higher than 20%. Panelists reacted 'dislike' or 'neither like nor dislike' to the taste, aroma, and color of the beverages. The best beverage formulation with high inhibitory activities against α-amylase and α-glucosidase enzymes (64.15% and 68.58%, respectively) was a mixture of 40% G and 60% K, which was assessed to be 'neither liked nor disliked' in terms of taste, aroma, and color.

Keywords: antidiabetic, guava leaves, herbal beverage, turmeric, α-amylase and α-glucosidase

INTRODUCTION

Herbal teas have been used as traditional medicine for centuries and are now also a popular beverage choice worldwide [1]. Research on the bioactive content of plants and their health benefits encourage the development of technology for processing various types of plants, especially herbs, into herbal teas. The formula of this product was developed based on a compromise between a possibly unpleasant taste and the expected efficacy. Because herbal teas are served as beverages, the sensory properties of brewed teas will greatly determine consumer acceptance [2].

Herbs have been known as sources of bioactive compounds able to control blood glucose levels of diabetic patients without adverse side effects [3]. Guava leaf as one of the herbs rich in phenolic compounds [4] has the potential to be developed as raw material for functional and pharmaceutical foods [5] due to its α-glucosidase and α-amylase enzyme inhibitory activity [6]. The use of guava leaves as the main ingredient of herbal beverages will result in less attractive color and taste. Therefore, other ingredients are needed and are expected to improve the beverage's organoleptic quality as well its anti-diabetic efficacy. One of the herbs that has this is turmeric (*Curcuma longa* L.).

Turmeric has been used for thousands of years in various parts of the world as herbal medicine, seasoning, additives, and food coloring. Turmeric is a source of curcumin which, based on pre-clinical and clinical trials, has the potential to prevent and treat type II diabetes and its complications [7]. Turmeric juice has been used in citrus-based beverages and the addition of up to 10% juice resulted in a sensory score of 8.6 (the maximum score was 9.0) [8].

Type II Diabetes Mellitus (DM) patients usually use synthetic α-amylase and α-glucosidase enzyme inhibitors such as acarbose. However, these drugs cause various gastrointestinal side

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effects such as bloating, nausea, diarrhea and flatulence [9], [10]. The use of natural ingredients as α-amylase and α-glucosidase enzyme inhibitor tends to be safer, cheaper, and easier to find than the synthetic ones [11], [12]. Therefore, research to develop herbal beverages from a mixture of guava leaves and turmeric was of interest. The purpose of this study was to determine the effect of the proportion of guava leaves and turmeric in an herbal beverage on the inhibitory activity of α-amylase and α-glucosidase enzymes as well as its hedonic quality.

MATERIALS AND METHODS

Materials

The guava leaves were obtained from a local farmer in Way Halim, Bandar Lampung. The turmeric was bought from a local market in Kemiling, Bandar Lampung. The α-amylase (EC 3.2.1.1) and α-glucosidase enzymes (EC 3.2.1.20) were from Sigma. The phosphate buffer, starch soluble, 4-Nitrophenyl-β-D- glucopyranoside (PNPG) substrate (p-nitrophenyl-α), methyl a-D-glucopyranoside, 3,5-Dinitrosalicylic acid (DNS) reagent, Folin Ciocalteu reagent, and gallic acid were all analytical grade.

Experiment design and data analysis

This study was a non-factorial arranged in Completely Randomized Block Design with four replications. The treatment in this study was the proportion of guava leaves and turmeric in the beverage (Table 1).

Table 1 Proportion of guava leaves and turmeric in the herbal beverage (w/w)

Formula	Guava leaves (G)	Turmeric (K)
C1	0%	100%
C2	20%	80%
C3	40%	60%
C4	60%	40%
C5	80%	20%
C6	100%	0%

Homogeneity data was tested using the Bartlet test, and additional data were tested with the Tuckey test. Analysis of variances (ANOVA) and least significant test were applied at a 5% significance level.

Preparation of Herbal Beverage

Fresh turmeric rhizomes were sorted, washed, and thinly sliced. Fresh guava leaves were washed thoroughly, then soaked at 50°C water for 5 minutes. Then, the processed turmeric and guava leaves were dried at 40°C for 48 hours. The dried herbs were then crushed using a blender then sieved to obtain turmeric powder and guava leaf powder. The herbal beverages were made by mixing guava leaf powder and turmeric powder according to the formula, then brewed in 100 mL of hot water and stirred. The beverages were then allowed to stand for 5 minutes and filtered. For the antidiabetic test, the beverages were centrifuged at 7 000 rpm for 5 minutes. For the sensory test, 3.99 g of zero-calorie sweetener was added for every 300 mL of the beverage.

Sample analysis

Total phenolic content

The total phenolic content was analyzed using the method of Fattahi et al. [13]. The steps carried out started with preparing a 0.2 mL sample of the herbal beverage added with 0.2 mL of distilled water and 0.2 mL of Folin Ciocalteu reagent, and then vortexed for 1 minute. After that, the mixture was added with 4 mL of 2% sodium carbonate (Na_2CO_3) solution and vortexed again for one minute, then allowed to stand in a dark room at room temperature for 30 minutes. Next, the absorbance was read at a wavelength of 760 nm. The total phenolic content is expressed as ppm Gallic Acid Equivalent (GAE).

Total flavonoid content

The total flavonoid content was measured by a colorimetric assay [13]. Five hundred micro litre of herbal beverage was added to 2 mL of distilled water and 0.15 mL 5% sodium nitrite and kept for 6 minutes. Then, 0.15 mL of 10% aluminium chloride was added. After 6 minutes, 2 mL of 4% sodium hydroxide was added to the mixture. Immediately, the mixture was diluted with the addition of 0.7 ml distilled water, mixed thoroughly and incubated for 15 minutes. The absorbance was determined at 510 nm. Quercetin was used as the standard and the total flavonoid content of the beverage was expressed as ppm quercetin equivalents.

α-Amylase inhibition assay

The inhibition of α-amylase enzyme activity was tested using the method of Thalapaneni et al. [14]. A sample of 500 µl of herbal beverage was put into 500 µl of α-amylase enzyme solution, then incubated at 25°C for 10 minutes. After that, 500 µl of starch solution was added, then incubated at 25°C for 10 minutes. After the second incubation, 1 mL of DNS reagent was added, then incubated in boiling water for 5 minutes. Next, 10 mL of distilled water was added to the reaction mixture, then centrifuged at 7000 rpm for 3 minutes. After that, the absorbance was read with a spectrophotometer at a wavelength of 540 nm. The inhibitory activity of the sample was calculated using the following formula

$$\text{Inhibitory activity (\%)} = \left[\frac{A_1 - A_2}{A_1} \right] \text{x } 100\%$$

where:

A_1 = Control absorbance

A_2 = Sample absorbance

The determination of IC_{50} was done by making samples of the best treatment, turmeric treatment, and guava leaf treatment with a dilution series of 0%, 20%, 40%, 60%, 80%, and 100%.

α-Glucosidase inhibition assay

Assays for the inhibition of α-glucosidase enzyme activity were determined using the method of Thalapaneni et al.[14] with modification. A 10 µl herbal beverage solution was added to a reaction mixture consisting of 250 µl of 20 mM PNPG and 495 µl of 100 mM phosphate buffer (pH 7). Next, it was incubated at 37°C for 5 minutes, and the reaction was started by adding 250 µl of α-glucosidase enzyme (0.075 units). After that, it was re-incubated at 37°C for 15 minutes. After the second incubation, the reaction was stopped by adding 2 mL of 0.1 M Na_2CO_3, then the absorbance was read with a spectrophotometer at a wavelength of 400 nm. The percentage of inhibitory activity against α-glucosidase enzyme was calculated as followed:

$$\text{Inhibitory activity (\%)} = \left[\frac{(A_o - A_s)}{A_0} \right] \text{x } 100\%$$

Where:

A_s = Sample absorbance

A_o = Control absorbance

The IC_{50} of beverage containing the best formula and single herb was assayed.

Sensory test

The sensory test was conducted to achieve a herbal beverage that was accepted by consumers. The sensory test used was a hedonic central location test [15]. Fifty untrained panelists consisting of students in the Faculty of Agriculture, University of Lampung who visited the student center were involved in the sensory evaluation. The panelists evaluated the taste, aroma, and color of the herbal beverages with the hedonic scale of 1 (very dislike), 2 (dislike), 3 (neither like nor dislike), 4 (like) and 5 (very like).

RESULT AND DISCUSSION

Total phenolic and flavonoid content

The total phenolic and flavonoid content of the herbal beverages increases when guava leaf (G) proportion increases (Table 2). Beverage made from 100% guava leaves (C6) contained 5 327.7 ± 60.7 ppm (GAE) and 508.1 ± 36.9 ppm (QE) of total phenolic and flavonoid content, much higher than the beverage with 100% turmeric (666.7 ± 87.7 and 63.5 ± 8.4 of total phenolic and flavonoid content).

The decrease in the proportion of guava leaves tends to reduce the total phenolic content of the beverage because guava leaves (C6) contain higher total phenolic content than turmeric (C1). Previous research showed that the total phenolic content of guava leaves reached 511 mg/g (GAE) [16], while turmeric reached 496 mg/g (GAE). According to De Cerio et al. [17], guava leaf extract contains phenolic components such as catechins, gallic acid, guaijaverin, gallocatechin, and quercitrin. In guava leaf extract, catechins were found to be more abundant than other phenolic components, which was 846.19 mg/100 g [18].

Table 2 Total phenolic and flavonoid content of guava leaf and turmeric herbal beverages

Proportion of guava leaves and turmeric in the herbal beverage (w/w)		
Formula	Total phenolic content (ppm GAE)	Total flavonoid content (ppm QE)
C1	666.7 ± 87.7	63.5 ± 8.4
C2	1 590.8 ± 79.5	132.3 ± 9.4
C3	2 361.3 ± 250.2	237.3 ± 31.9
C4	3 063.8 ± 307.4	314.5 ± 23.7
C5	3 330.8 ± 141.0	460.3 ± 41.1
C6	3 527.5 ± 60.7	508.1 ± 36.9

C1 : 0% G and 100% K; C2 : 20% G and 80% K; C3: 40% G and 60% K; C4: 60% G and 40% K; C5: 80% G and 20% K; C6: 100% G and 0% K.

α-Amylase enzyme inhibition

The proportion of guava leaves and turmeric the herbal beverages significantly affected the inhibition of α-amylase enzyme activity. Figure 1 shows the inhibition of α-amylase enzyme activity in herbal beverages containing guava leaves and turmeric ranging from 28.16% to 78.95%. Mixing guava leaves and turmeric produced an additive effect in inhibiting the activity of the α-amylase enzyme.

Figure 1 shows that turmeric has a significant effect on the inhibitory activity against α-amylase enzyme. The greater the proportion of turmeric in the herbal beverage, the higher the inhibitory activity against α-amylase enzyme was. A more than 40% decrease of turmeric proportion reduced the inhibitory activity against α-amylase enzyme by 14.80% to 50.78%. The inhibitory activity of C2 (72.93%) was not significantly different from C1 (78.95%).

However, due to C2 containing more types of herbs than C1, C2 was considered as the best treatment against α-amylase enzyme. Herbal beverages containing more herb types is suggested to have more bioactive components and higher efficacy [19]. Based on the figure 1, the α-amylase enzyme inhibitory activities of the phenolic compounds found in guava leaves and turmeric are competitive [20].

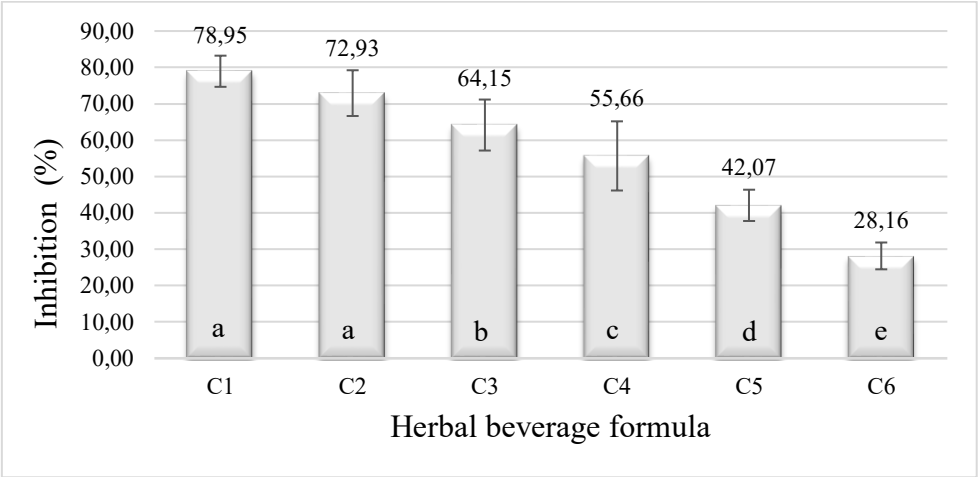

Figure 1 α-amylase inhibition of herbal beverages containing turmeric and guava leaves. C1 : 0% G and 100% K; C2 : 20% G and 80% K; C3: 40% G and 60% K; C4: 60% G and 40% K; C5: 80% G and 20% K; C6: 100% G and 0% K.

The IC_{50} of α-amylase inhibitory activity was determined for the best formulation (C2) as well as the beverage containing a single herb (C1 and C6). The IC_{50} of C1, C2, and C6 were 1 036.05 ppm (GAE), 2 782.48 ppm (GAE), and 7 074.55 ppm (GAE) respectively. These results indicate that the inhibitory activity against α-amylase enzyme of turmeric beverage (C1) is 700% stronger than that of guava leaf beverage (C6).

The effect of phenolic compounds on the activity of α-amylase enzymes can be seen through several mechanisms depending on the concentration, types of the phenolic compounds [21] and compositions of the phenolic compounds[22]. The phenolic compounds can interact directly with the α-amylase enzyme and inhibit its activity [22] or interact with starch, making the phenolic-starch complex difficult to digest [22], [23]. Moreover, the addition of phenolic compounds can reduce the crystalline structure of starch due to the decrease in water content or molecular interactions in the lamellae[23].

α-Glucosidase enzyme inhibition

The proportion of guava leaves and turmeric in herbal beverages have a significant effect on the inhibition of α-glucosidase enzyme activity (Figure 2). Figure 2 shows the inhibitory activities against α-glucosidase enzyme of herbal beverages made from guava leaves and turmeric, ranging from 21.05% to 73.68%. The mixture of guava leaves and turmeric produced an additive effect in inhibiting the activity of α-glucosidase enzyme.

Figure 2 shows that guava leaves in herbal beverages contribute more to increasing the inhibitory activity of the α-glucosidase enzyme compared to turmeric. The addition of 20% guava leaves to turmeric increased the inhibition of the α-glucosidase enzyme by 38.49%. However, further addition of guava leaves (40%-100%) did not have a significant effect on increasing the inhibitory activity of the α-glucosidase enzyme. This is due to the non-competitive nature of the inhibitory power of guava leaf's phenolic compounds against α-glucosidase enzymes [20]. When the proportion of guava leaves was increased to 40%, the

reaction between phenolic compounds and enzymes was assumed to be saturated. Thus, increasing the proportion of guava leaves no longer affected the enzyme activity.

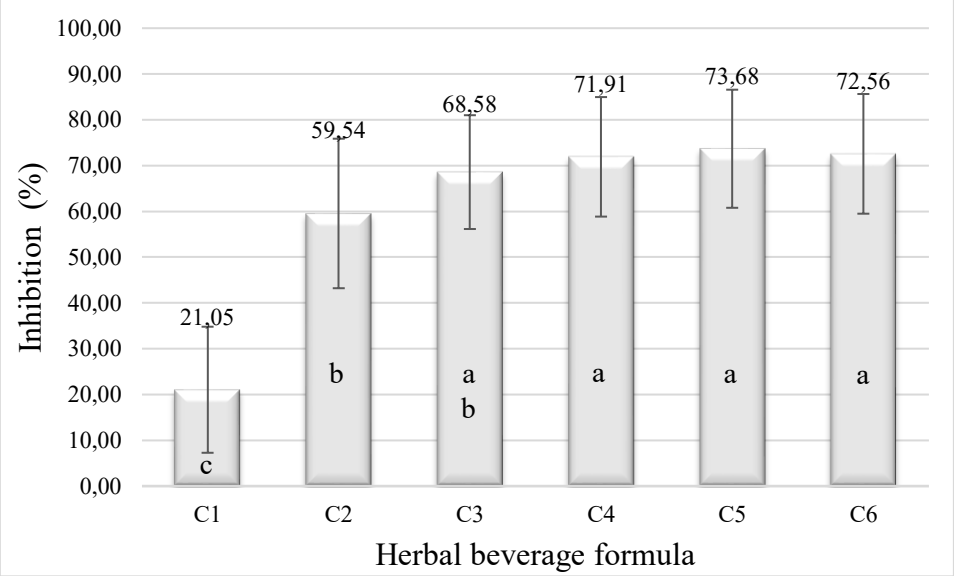

Figure 2 α-glucosidase inhibition of herbal beverages containing turmeric and guava leaves. C1 : 0% G and 100% K; C2 : 20% G and 80% K; C3: 40% G and 60% K; C4: 60% G and 40% K; C5: 80% G and 20% K; C6: 100% G and 0% K.

The IC_{50} of treatment C3, C1, and C6 were 59.39 ppm, 314.02 ppm, and 53.54 ppm respectively. The IC_{50} of C3 is smaller than C1, indicating that a mixture of 40% guava leaves and 60% turmeric (C3) inhibits the α-glucosidase enzyme activity more than turmeric (C1) does. However, the C3 treatment required a higher concentration of 50% inhibition of the α-glucosidase enzyme than the C6 treatment (guava leaf).

Several studies have reported that the inhibition of α-glucosidase enzyme activity is caused by the presence of phenolic compounds present in plants[24]. The greater the proportion of guava leaves in the beverage, the greater the total phenolic content and the inhibition of α-glucosidase enzyme. Phenolic compounds which are thought to contribute to the inhibition of α-glucosidase enzymes are catechins. According to Simao et al. [18], the highest phenol component in guava leaf extract was catechins, followed by gallic acid. The research of Yilmazer-Musa et al.[25] showed catechins (IC_{90} > 290 g/mL) had more potential to inhibit α-glucosidase enzymes than acarbose (IC_{90} > 645 g/mL) while gallic acid was able to inhibit α-glucosidase enzymes by 43.9% [26].

The relationship between total phenolic content and enzyme inhibition

Table 3 shows that the inhibitory activity against α-amylase enzyme is negatively correlated with the total phenolic content with a Pearson correlation value of -0.831. The lower the total phenolic content, the higher the inhibition of α-amylase enzyme activity. Based on the total phenolic concentration (Figure 1), it is suggested that the inhibition of the α-amylase enzyme does not depend on the total phenolic concentration, but on the type of the phenolic compounds. The phenolic compounds in turmeric have a greater inhibitory effect on the α-amylase enzyme than guava leaves, but the total phenolic content of turmeric is much lower than that of guava leaves (Figure 1). When the proportion of guava leaves increased, the total phenolic content increased. However, the phenolic compounds with high inhibitory activity against α-amylase

decreased. As a result, the inhibition of the total phenolic content against α-amylase enzyme in the herbal beverage decreased.

The phenolic compound in turmeric which is thought to strongly inhibit the α-amylase enzyme is curcumin. According to Jhong et al. [27], curcumin in turmeric (IC_{50} 130 M) has a greater inhibition against α-amylase enzyme than catechins in guava leaves (IC_{50} 380 M). Therefore, the greater the proportion of turmeric in herbal beverages, the greater the inhibitory activity against the α-amylase enzyme was observed. Nurdin et al.[28] also reported that the inhibitory activity against α-amylase enzyme was negatively correlated with the total phenolic concentration of the aqueous extract of a mixture of bay, pandan, and citrus leaves.

Table 3 Coefficient correlation between total phenolic content of herbal beverage and inhibitory activity against α-amylase and α-glucosidase enzymes

Inhibitory Activity	Correlation value	Total phenolic	Total flavonoid
α-amylase	*Pearson correlation*	-0.919*	-0.986*
	P-value	0,010	0.000
α-glucosidase	*Pearson correlation*	0.900*	0.770
	P-value	0,015	0.073

Inhibitory activity against α-glucosidase enzyme was positively correlated with total phenol of herbal beverages (Table 2). The result is in accordance with previous studies which showed that total phenol was positively correlated with the inhibition activity against α-glucosidase enzyme. Research by Wu and Xu [29] showed that total phenolic content in boiling water extracts was positively correlated with the inhibitory power of α-glucosidase enzyme with a Pearson correlation value of 0.92 ($p < 0.05$). This positive correlation also occurred in grape pulp extract with a Pearson correlation value of 0.882 ($p < 0.01$) [30]. Guava leaves contain catechins, which have a higher inhibitory activity against α-glucosidase enzyme than acarbose [25].

Organoleptic quality

The proportion of herbs in the beverage determines the panelists preference for the taste, aroma, and color of the mixed herbal beverages (Table 4). The sensory test on taste has the lowest value for the C5 mixture formulation, which is 2.885 (dislike), while the highest value is for the C1 formulation, which is 3.470 (neither like nor dislike). The average value of preference for the aroma of the herbal beverages ranged from 3.225-3.535 (neither like nor dislike) (Table 4). The highest average value for the aroma of the herbal beverages was found in treatment C6, which was 3.535 (neither like nor dislike), while the lowest value was for treatment C5 with 3.225 (neither like nor dislike). These data showed that although statistically there were differences between treatments, all formulas received a 'neither like nor dislike' response.

Table 4 Hedonic levels of herbal beverages containing turmeric and guava leaves.

Formula	Hedonic attributes		
	Taste	Aroma	Color
C1	3.5^{a}	3.4^{ab}	3.5^{b}
C2	3.3^{ab}	3.3^{b}	3.5^{b}
C3	3.3^{ab}	3.4^{ab}	3.4^{b}
C4	3.0^{cd}	3.3^{b}	3.4^{b}
C5	2.9^{d}	3.2^{b}	3.5^{b}

Formula	Hedonic attributes		
	Taste	Aroma	Color
C6	3.2^{bc}	3.5^{a}	3.8^{a}

C1: 0% G and 100% K; C2: 20% G and 80% K; C3: 40% G and 60% K; C4: 60% G and 40% K; C5: 80% G and 20% K; C6: 100% G and 0% K. Hedonic scale: 1 (very dislike), 2 (dislike), 3 (neither like nor dislike), 4 (like) and 5 (very like).

Consumer acceptance of an herbal beverage is determined by various attributes such as taste, aroma, and flavor [31]. However, color is the most important factor in the quality of appearance of the product [32]. The highest average value for the color attribute in this study was for treatment C6, which was 3.815 (neither like nor dislike), and the lowest value was for treatment C4 with 3.350 (neither like nor dislike) (Table 3). Panelists preferred guava leaf herbal beverage to turmeric or a mixture of guava leaves and turmeric, presumably because the color produced in the formulation of 100% guava leaves and 0% turmeric (C6) was mostly clear and slightly brown.

CONCLUSION

The greater the proportion of turmeric in the herbal beverages, the higher the inhibitory activity against α-amylase enzyme was. The addition of guava leaves up to 20% in herbal beverages increased the inhibition of the α-glucosidase enzyme by 38.49% (21.05% to 59.54%), but further increase had no significant effect on the inhibition of α-glucosidase enzyme. Panelists gave an assessment of 'dislike' to 'neither like nor dislike' to the taste, aroma, and color of the herbal beverages. The best herbal beverage formulation that produced high inhibition of α-amylase and α-glucosidase enzymes was a mixture of 40% guava leaf and 60% turmeric (C3) with 64.15% inhibition against α-amylase enzyme and 68.58% inhibition against α-glucosidase enzyme, with the quality of taste, aroma, and color that was neither liked nor disliked.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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