Formula optimization of functional beverages from Sargassum sp., Cinnamomum burmannii, and Curcuma xanthorrhiza Roxb with α-Glucosidase inhibitor activity

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Abstract. Sargassum sp., cinnamon, and C. xanthorrhiza contain high polyphenols, which play an essential role in inhibiting α-glucosidase activity. The purpose of this study was to determine the optimization of the formula by mixing Sargassum sp. extract, cinnamon extract, and C. xanthorrhiza extract as functional beverages of α-glucosidase inhibitors. This research using the Response Surface Method (RSM) and Box-Behnken with a combination of three factors, namely the concentration of Sargassum sp. extract (A), the concentration of cinnamon extract (B), and the concentration of C. xanthorrhiza extract (C). The response variables consisted of total polyphenol (Y1), and percentage inhibition of α-glucosidase (Y2). The optimum formula was followed by UPLC-MS/MS assay. The best formulation for functional beverages is Sargassum sp. 20%, cinnamon 12.773%, and C. xanthorrhiza extract at 18.5% with of total polyphenols (108.834 mgGAE/100mL) and inhibition of α-glucosidase (64.323%).

1. Introduction

Diabetes is one of the most severe and chronic diseases globally, and its incidence and severity increase with obesity and aging [1]. The disease can be classified as a multifactorial metabolic syndrome with hyperglycemic. The hyperglycemic is due to impaired insulin secretion, insulin action, or its combination. There are three ways to manage hyperglycemia (high blood glucose): a). performing physical exercises, 2). taking hypoglycemic agents and diet planning [2]. For reducing the blood glucose, α-glucosidase activity should be inhibited by the natural or synthetic agent [3].

Polyphenol has α-glucosidase inhibitory activity. This compound plays a role in the interaction with the active side of α-glucosidase. As a result, the sugar can not bind to the α-glucosidase [4].

Sargassum sp. contains a high phenolic compound [5]. Sargassum sp. has been reported for antioxidative, antimicrobial, anticoagulant, antithrombotic, antiinflammatory, and antitumor activities [6,7,8]. Cinnamon contains many phytochemical compounds from phenylpropanoids in the form of cinnamic acid [9]. The extraction of cinnamon bark has antioxidant compounds in the form of polyphenols (tannins, flavonoids) and essential oils of phenol groups. Curcuma widely used for medicinal purposes or as a herbal drink. Curcuminoid is one of the main bioactive groups of polyphenols, which gives a yellow color to the Curcuma rhizome [10].
Sargassum spp., cinnamon, and curcuma have been reported for high polyphenol. Hence, they are a potential source for an α-glucosidase inhibitor. This study aims to formulate Sargassum sp, cinnamon, and curcuma extract as functional beverages by inhibiting α-glucosidase.

2. Methods
Design Expert v 11.0 software was used for Response Surface analysis using the Box Behnken Design method. The relative proportions of the concentration of extract Sargassum sp., cinnamon, and curcuma extract data. Furthermore, the minimum limit of each extract (lower limit) and the maximum relative proportion of each extract (upper limit) are used as input data before the experimental design model is obtained.

2.1. Total polyphenol assay
2.1.1. Gallic acid curve standard.
Gallic acid served as a standard solution. The concentration of 20; 40; 60; 80; 100 ppm was provided for a standard solution. Each concentration of 200 µL was inserted into the test tube, then reacted with 1.5 mL of 10% folin ciocalteu and then homogenized and left for 5 min. Next, 1.5 mL of 5% Na₂CO₃ solution was homogenized and re-incubated for 30 minutes at room temperature. The solution was read at 760 nm.

2.1.2. Total polyphenol.
Each formula was taken as much as 200 µL and put into the test tube, then reacted with 1.5 mL of 10% folin ciocalteu and then homogenized and left for 5 min. Next, 1.5 mL of 5% Na₂CO₃ solution was homogenized and re-incubated for 30 minutes at room temperature. The further method was similar to the analysis of gallic acid. The values were then analyzed using Design Expert v 11 software for analysis [11].

2.2. α-Glucosidase inhibition assay
The procedure for inhibiting α-glucosidase activity with a total volume of 200 µL is presented in Table 1 [12].

| Reagent                        | Volume (µL) | B₁ | B₀ | S₁ | S₀ |
|-------------------------------|-------------|----|----|----|----|
| Sample/standard               |             | -  | -  | 30 | 30 |
| DMSO 2% inphosphate buffer pH 6,8 | 30          | 30 | -  | -  | -  |
| phosphate buffer pH 6,8       | 36          | 36 | 36 | 36 | 36 |
| PNPG 5 mM                     | 17          | 17 | 17 | 17 | 17 |
| **Incubation at 37°C for 5 min** |             |    |    |    |    |
| Enzyme (0,15 U/mL)            | 17          | -  | 17 | -  | -  |
| phosphate buffer pH 6,8       | -           | 17 | -  | 17 | 17 |
| **Incubation of 37°C for 15 min** |             |    |    |    |    |
| Sodium carbonate (200 mM)     | 100         | 100| 100| 100| 100|

Measure the absorbance of p-nitrophenol formed on the wavelength of 405 nm

% inhibition of α-glucosidase can be calculated using the following formula.

\[
\text{% inhibition} = \left(\frac{(B₁-B₀)-(S₁-S₀)}{(B₁-B₀)}\right) \times 100\%
\]

where:

\( B₀ = \text{Blank} \)
B₁ = Blank control  
S₀ = Sample control and standard control (acarbose)  
S₁ = Sample and standard (acarbose)

2.3. Response analysis

Each research phase variable was analyzed ANOVA in the same software. ANOVA models found in this design are Linear, Quadratic, Special Cubic, and Cubic[13].

2.4. Optimization phase

The optimization phase was carried out after the analysis of the optimum response was complete. The optimal goal determined each response (total polyphenols and inhibition of α-glucosidase). The results of the optimization phase are recommendations for several new formulas that are optimal according to the program. The most optimal formula is a formula with maximum desirability values [13]. The optimal formulation is a nearly one in a single response.

3. Results and Discussion

3.1. Determination of Box-Behnken design limits

In the preliminary study, three factors of extracts with a different concentration were applied. Each factor consists of 3 levels. The level of each factor to determine the lower limit value (-1) and the upper limit value (+1), the remainder as the middle value (0) is the average of values (-1) and (+1) for each factor (Table 2.).

| Table 2. The limit factor of the formula. |
|------------------------------------------|
| Factor | Lower limits (%) | Mid point (%) | Upper limits (%) |
|--------|------------------|---------------|------------------|
| Sargassum sp. | 15 | 17.5 | 20 |
| Cinnamon | 12.5 | 15 | 17.5 |
| Curcuma | 17.5 | 20 | 22.5 |

The formulation of the research can be found in Table 3.

| Table 3. Combination of functional beverages formula. |
|------------------------------------------------------|
| Run | Sargassum sp. (%) | Cinnamon (%) | Curcuma (%) |
|-----|-------------------|--------------|-------------|
| 1   | 20                | 12.5         | 20.5        |
| 2   | 15                | 15           | 18.5        |
| 3   | 17.5              | 17.5         | 22.5        |
| 4   | 15                | 12.5         | 20.5        |
| 5   | 15                | 17.5         | 20.5        |
| 6   | 17.5              | 15           | 20.5        |
| 7   | 20                | 15           | 18.5        |
| 8   | 17.5              | 12.5         | 22.5        |
| 9   | 17.5              | 12.5         | 18.5        |
| 10  | 20                | 17.5         | 20.5        |
| 11  | 17.5              | 17.5         | 18.5        |
| 12  | 15                | 15           | 22.5        |
| 13  | 20                | 15           | 22.5        |
3.2. Response formulation phase

Then the response analysis phase includes the total response polyphenols (Y1) and α-glucosidase (Y2) inhibition. The results of each response analysis conducted in 13 formulations were then included in the variable response. The results of the formulation response and response analysis are presented in Table 4.

| Run | A  | B  | C  | Y1     | Y2     |
|-----|----|----|----|--------|--------|
| 1   | 20 | 12.5 | 20.5 | 113.451 | 64.04  |
| 2   | 15 | 15  | 18.5 | 98.971  | 46.29  |
| 3   | 17.5 | 17.5 | 22.5 | 119.989 | 33.55  |
| 4   | 15 | 12.5 | 20.5 | 91.977  | 42.98  |
| 5   | 15 | 17.5 | 20.5 | 101.429 | 63.26  |
| 6   | 17.5 | 15  | 20.5 | 125.897 | 47.92  |
| 7   | 20 | 15  | 18.5 | 116.469 | 62.87  |
| 8   | 17.5 | 12.5 | 22.5 | 87.554  | 50.26  |
| 9   | 17.5 | 12.5 | 18.5 | 100.091 | 43.17  |
| 10  | 20 | 17.5 | 20.5 | 98.834  | 50.07  |
| 11  | 17.5 | 17.5 | 18.5 | 126.469 | 60.8   |
| 12  | 15 | 15  | 22.5 | 108.583 | 56.7   |
| 13  | 20 | 15  | 22.5 | 124.446 | 47.4   |

Note:
A  Sargassum sp. (%)  
B  Cinnamon (%)  
C  Curcuma (%)  
Y1 Total Polyphenol (mg GAE/100 mL)  
Y2 α-glucosidase inhibition (%)  

3.2.1. Total Polyphenol Response
The results of the variance analysis (ANOVA) showed that the quadratic model did not have a significant effect on the total response of functional beverage polyphenols with a p-value of more than 0.05, which was 0.720. The p-value for each factor and its interaction also showed no significant (P > 0.05) response to the total polyphenols.

Mixing extract Sargassum sp., extract cinnamon, and extract curcuma did not have a significant effect on the total response of functional beverage polyphenols. Each content of bioactive compounds in Sargassum sp, cinnamon, and curcuma has strong polyphenol properties. However, the polyphenol compounds from each component do not interact with each other, besides the phenol content of each ingredient can decrease due to the processing carried out. The average yield of functional polyphenols was 108.78 mg GAE / 100 mL.

Phenol is easily oxidized, evaporates, is sensitive to light. Phenol levels will decrease, among others, by the treatment of washing, boiling, and further processing to become products that are ready for consumption [14]. Mixing of several active compounds of plants can provide effects such as synergistic, antagonistic, and neutral [15].

3.2.2. α-Glucosidase Inhibition Response
The quadratic model had a significant influence on the inhibition response of α-glucosidase functional drinks with a value of 0.038 Sargassum sp., Cinnamon, and curcuma extract had a significant effect on the α-glucosidase inhibitory. Besides, the interaction of factors has a significant effect on α-glucosidase inhibition. The polynomial equation for the α-glucosidase inhibition response is as follows:
\[ Y = 47.92 + 1.89A + 0.904B - 3.15C - 8.56AB - 6.47AC - 8.58BC + 6.77A^2 - 0.398B^2 - 1.37C^2 \quad (2) \]

Note:
A : *Sargassum* sp. extract
B : cinnamon extract
C : curcuma extract

Interaction of *Sargassum* sp. with cinnamon, reduce inhibition of \( \alpha \)-glucosidase. A negative value indicates it. Contour graph plot and three-dimensional surface response curve interaction of *Sargassum* sp. and cinnamon are shown in Figure 1.

**Figure 1.** (a) contour plot and (b) three-dimensional response surface curve in the \( \alpha \)-glucosidase inhibition response of the quadratic model of extract interaction *Sargassum* sp. and cinnamon extract.

Moreover, Similar trend was also shown in *Sargassum* and curcuma interaction (Figure 2). And Cinnamon and curcuma (Figure 3.).

**Figure 2.** (a) Contour plot and (b) three-dimensional surface response curve in the \( \alpha \)-glucosidase inhibition response of the quadratic model of extract interaction *Sargassum* sp. and curcuma extract.
Figure 3. (a) Contour plot and (b) three-dimensional surface response curve in the α-glucosidase inhibition quadratic model of extract interaction cinnamon and curcuma extract.

The increase in interactions of *Sargassum* sp. and extract cinnamon (AB), extract interaction *Sargassum* sp. and extract curcuma (AC), and extract cinnamon and extract curcuma (BC) interactions will significantly reduce α-glucosidase inhibition. The combination of active ingredients either shows a synergistic effect or antagonistic effect [16]. The antagonistic effect of the formulation occurred by competitive and non-competitive matter[18].

### 3.3. Optimization of functional beverages formula

The optimization process is done to get a formula that produces the optimal response according to the desired optimization target. The components and target criteria for functional drinks are presented in Table 5.

| Component                  | Criteria  | Lower Limits | Upper Limits |
|----------------------------|-----------|--------------|--------------|
| *Sargassum* sp. Extract    | In Range  | 15           | 20           |
| Cinnamon Extract           | In Range  | 12.5         | 17.5         |
| Curcuma Extract            | In Range  | 17.5         | 22.5         |
| Total Polyphenol           | Maximum   | 87.55        | 126.47       |
| α-Glukosidase Inhibition   | Maximum   | 31.4         | 64.04        |

The concentration of functional beverage ingredients consisting of extract *Sargassum* sp., cinnamon, and curcuma are components that are optimized as in Table 5. The pH of functional drinks is expected to be in accordance with the SNI pH of packaged water. Organoleptic parameters in the form of preferences for taste, color, appearance, and aroma are expected to be the most preferred by consumers so that they are optimized with the maximum criteria target.

| A  | B  | C  | Y1 | Y2 | Desirability |
|----|----|----|----|----|--------------|
| 20.000 | 12.773 | 18.500 | 108.837 | 64.323 | 0.861        |

The best formulation for functional beverages is *Sargassum* sp. 20%, cinnamon 12.773%, and *C. xanthorrhiza* extract at 18.5% with of total polyphenols (108.834 mgGAE/100mL) and inhibition of α-glucosidase (64.323%). The higher the desirability value indicates the higher suitability of the combination of process parameters.
4. Conclusion

The optimum formula for functional beverages is concentrations of Sargassum sp. 20%, cinnamon 12.773%, and C. xanthorrhiza extract at a value of 18.5%. The optimum formula has a predicted total polyphenols inhibition of α-glucosidase, and desirability value of 108.834 mgGAE/100mL, 64.323%, 0.861, respectively.

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