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# Development of New Antidiabetic Drink from *Sargassum* sp.: Total Phenolic Content, α-Glucosidase Inhibition, and Sensory Analysis

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**ABSTRACT**

*Diabetes is a major global health challenge, with α-glucosidase inhibitors playing a key role in managing blood glucose levels by delaying carbohydrate digestion and absorption. This study developed an antidiabetic functional drink combining Sargassum extract with cinnamon (Cinnamomum burmannii) and temulawak (Curcuma xanthorrhiza). A Completely Randomized Design (CRD) was used with four treatments: a control (F0) and formulations with 15% (F1), 17.5% (F2), and 20% (F3) Sargassum extract. Data on pH, polyphenols, and α-glucosidase inhibition were analyzed using ANOVA, while sensory attributes were evaluated with nonparametric tests. The addition of Sargassum extract from 15% to 17.5% significantly increased the phenolic content by 15%. However, it did not affect α-glucosidase inhibition. This suggests that not all phenolic compounds in Sargassum contribute to the inhibition of α-glucosidase. The formulation with 20% Sargassum, 15% cinnamon, and 18.5% temulawak extract exhibited the highest phenolic content (315.21 mg GAE/100 ml) and α-glucosidase inhibition (62.27%). The sensory analysis shows that adding up to 20% Sargassum is still acceptable for the panelists, with a hedonic value of 3.34 to 3.43 (neutral). This study discovers that the bitterness of Sargassum at the highest concentration can be mitigated by adding cinnamon and temulawak without reducing the antidiabetic potential. These results contribute to conveying the potential of this drink as a natural anti-diabetic product with good sensory acceptability. Further research is needed to evaluate bioactive interactions and phenolic composition to optimize efficacy.*

**KEYWORDS**

α-glucosidase inhibition; Antidiabetic; Functional drink; *Sargassum* sp.; Phenolic compounds

## 1. INTRODUCTION

Diabetes is recognized as a chronic disease that significantly impacts individual health and societal resources [1]. It is characterized by its prolonged duration, requiring ongoing management and care. This disease remains a major contributor to global morbidity and mortality, accounting for 63% of deaths worldwide [2]. One approach to diabetes management, especially for type 2 diabetes, involves controlling blood glucose levels. In this context, α-glucosidase inhibitors play a crucial role by slowing down carbohydrate digestion and glucose absorption in the small intestine [3]. This inhibition helps reduce postprandial (after-meal) spikes in blood sugar levels, making α-glucosidase inhibitors essential in antidiabetic strategies.

The demand for functional drinks has significantly increased as consumers seek products that offer health benefits and enjoyable sensory experiences [4]. Functional drinks are designed to deliver bioactive compounds to help prevent or manage various health conditions. Recently, research has focused on


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functional drinks fortified with plant extracts as antidiabetic agents [5]. For example, functional drinks formulated from kenikir leaf extract (*Cosmos caudatus*) and *belimbing buluh* (*Averrhoa bilimbi*) contain ascorbic acid, quercetin, and chlorogenic acid, which are reported to exhibit high antioxidant activity [6]. Similarly, the combination of avocado (*Persea americana* Mill.) peel and red ginger (*Zingiber officinale* var. *Rubrum*) in functional drinks has shown high antioxidant capacity and α-amylase inhibition [3]. Most of these beverages use plant and herb extracts as raw materials.

*Sargassum*, a genus of brown seaweed, has attracted interest as a potential ingredient in antidiabetic beverages due to its high polyphenol content [7]. Notably, *Sargassum* contains phlorotannins, a unique class of polyphenols found exclusively in brown seaweeds [8]. Phlorotannins are known to effectively inhibit α-glucosidase, making *Sargassum* a promising candidate for developing natural antidiabetic products [8]. A functional tisane combining *Sargassum* with butterfly pea flowers and sappan wood has been developed, demonstrating favorable sensory attributes and high antioxidant content [9]. Additionally, *Sargassum hystrix* juice has been produced at varying concentrations, showing significant antioxidant activity and consumer preference [10]. However, despite its potent bioactive properties, the use of *Sargassum* in functional beverages presents a significant challenge due to its inherent bitterness. This bitter taste can limit consumer acceptance, necessitating the addition of other ingredients to enhance both flavor and health benefits.

To address this challenge, cinnamon (*Cinnamomum verum*) and *temulawak* (*Curcuma xanthorrhiza*) are proposed as complementary ingredients. These herbs are widely recognized for their roles in traditional medicine and functional beverages due to their pleasant flavors and health-promoting properties [11]. Cinnamon is known for its sweet and warming taste, while *temulawak* contributes an earthy and slightly spicy flavor [11]. Both herbs also contain bioactive compounds, including polyphenols, which have been reported to inhibit α-glucosidase. The study by Anggriawan et al., (2015) [12] revealed that aqueous and ethanol extracts of *C. burmannii* inhibited α-glucosidase enzyme activity, with an inhibition rate of 94.88%. Curcuminoids, one of the main bioactive compounds in *temulawak*, are responsible for the rhizome's yellow color [13]. *Temulawak* curcuminoids have been shown to have antihyperglycemic potential through the inhibition of α-glucosidase activity [14]. The combination of *Sargassum*, cinnamon, and *temulawak* could thus provide a dual benefit: improving the sensory quality of the beverage while enhancing its antidiabetic potential through synergistic α-glucosidase inhibition.

The aim of this study was to develop a novel antidiabetic functional drink by combining cinnamon and *temulawak* with *Sargassum* in various concentrations. This study explored how different concentrations of *Sargassum* impacted polyphenol content, α-glucosidase inhibition, and sensory acceptance of the functional drink. The results will contribute to the understanding of formulating functional beverages that achieve a balance between bioactive efficacy and sensory quality.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Brown seaweed (*Sargassum* sp.) was collected from the sea in Sumenep, Madura, Indonesia, and harvested between January and March. Cinnamon *(Cinnanomum burmanii)* and *temulawak* (*Curcuma xanthorrhiza Roxb*) were bought from traditional market in Malang, Indonesia. Gallic acid standard, folin ciacolteu reagent, p-nitrophenyl-α-D-glucopyranoside substrate, and α-glucosidase enzyme were purchased from Sigma Aldrich (St. Louis, MO, USA). The tools used in this study were pH meter (Mettler Toledo, Polaris Parkway Columbus OH, USA), spectrophotometer UV-Vis (UV-1650PC, Shimadzu, Kyoto, Japan), microplate reader (BioTek ELX800, Santa Clara, CA, USA), and other glass tool.

### 2.2. Formulation of Functional Drink

The functional drink was formulated using three main ingredients: *Sargassum*, cinnamon, and *temulawak*. The active components of each ingredient were extracted using the decoction method. For *Sargassum*, 200 g was cut to increase surface area and decocted in 600 ml of distilled water at 90 °C. The decoction was filtered to obtain a 100% extract, which was then diluted to concentrations of 15%, 17.5%, and 20%. Cinnamon was prepared by washing, drying, and weighing 200 g, followed by decoction in 600

ml of distilled water for 30 minutes. The 100% cinnamon extract was then diluted to a 15% concentration. *Temulawak* was cleaned, sliced to 3 mm thickness, and 200 g was decocted in 600 ml of distilled water at 90 °C for 30 minutes. The 100% *temulawak* extract was diluted to a concentration of 18.5%. The diluted extracts of each ingredient were then mixed in equal volumes (20 ml each) to produce a 60 ml functional beverage [15].

### 2.3. Total Phenolic Content

The total polyphenol content was calculated using the Folin-Ciocalteu method according to the procedure by Damayanti et al., (2021) [10] with slight modification. Each functional beverage formulation (1 ml) was diluted threefold with distilled water. From the final dilution, 2 ml was taken and placed into a test tube, followed by adding 1 ml of 10% Folin-Ciocalteu reagent. The mixture was homogenized and left for 5 minutes. Then, 0.25 ml of 5% $Na_2CO_3$ solution and 1.75 ml of distilled water were added, homogenized again, and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at a wavelength of 760 nm using a UV-Vis spectrophotometer. The results were expressed as mg gallic acid (GA) equivalent (E)/100 ml functional drink using standard curve gallic acid (0-100 µg/ml). Gallic acid equivalent was calculated according to equation (1). The total phenolic content (TPC) of the functional drinks was calculated according to equation (2).

$$GAE\ (\mu g\ GAE) = phenolic\ concentration\ \left(\mu g\ \frac{GAE}{ml}\right) x\ amount\ of\ sample(ml) \quad (1)$$

$$TPC\ (mg\ \frac{GAE}{100\ ml}) = \frac{GAE\ (mg\ GAE)\ x\ 100\ ml}{ml\ of\ sample\ (ml)} \quad (2)$$

### 2.4. α-glucosidase Inhibition

The inhibition of α-glucosidase enzyme was calculated based on the procedure presented by Umam and Firdaus (2020) [15]. The sample testing involved adding 30 µl of the functional beverage sample to 36 µl of phosphate buffer (pH 6.8) and 17 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG). The mixture was incubated at 37 °C for 5 minutes. Then, 17 µl of 0.15 U/ml enzyme solution was added, and the mixture was incubated for another 15 minutes at 37 °C. After incubation, 100 µl of 267 mM sodium carbonate solution was added. The mixture without the sample was used as a blank (B0), while the mixture without the sample and enzyme was used as a control blank (B1). The sample without adding the enzyme was used as a control sample (S0). The absorbance of blank, blank control, control sample, and sample was then measured using a microplate reader at 405 nm to calculate the percentage of inhibition against α-glucosidase according to equation (3).

$$T\%inhibition = \frac{(B1 - B0) - (S1 - S0)}{(B1 - B0)} \quad (3)$$

where B0 is the absorbance of the blank, B1 is the absorbance of the control blank, S1 is the absorbance of the sample, and S0 is the absorbance of the control sample.

### 2.5. Sensory Analysis

This study employed the hedonic test. Analysis of product acceptability was conducted by 35 untrained panelists to assessed 4 attributes including taste, color, aroma, and appearance. Panelists rated these attributes on a scale of 1 to 5, where 1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, and 5 = strongly like. The average score was calculated to determine which formulation was most acceptable to the panelists. The organoleptic test assessed consumer responses to functional beverages [10].

### 2.6. pH Analysis

The pH testing for functional beverages was conducted based on the method from Maulida et al., (2024) [16] regarding the procedure for pH testing in bottled drinking water.

### 2.7. Qualitative Phytochemical Composition

Phytochemical composition in the functional drink was determined qualitatively, including flavonoid, tannin, saponin, phenol, alkaloid, terpenoid, and steroid according to the procedure by Umam and Firdaus (2020) [15]. For the flavonoid test, 1 ml of the functional drink was added to a test tube, followed by the addition of 1-2 ml of hot methanol and magnesium metal powder. Then, 0.5 ml of concentrated HCl was added. If a red or orange color appeared, the extract was considered positive for alkaloids. The tannin test was performed by adding 1 ml of the functional drink to a test tube, followed by a few drops of hot distilled water. The mixture was then cooled and filtered. Next, 3 drops of 10% NaCl were added, followed by filtration. Then, 2 drops of $FeCl_3$ were added. The sample was considered positive for tannins if a dark green or deep blue color appeared. For the saponin test, 1 ml of the functional drink was added to a test tube, followed by adding 5 ml of distilled water and shaking for 30 seconds. If foam formed and persisted for at least 30 seconds, the extract was considered positive for saponins. To maintain the foam, 1 M HCl could be added. For the phenol test, 1 ml of the functional drink was added to a test tube, and then 10 drops of 1% $FeCl_3$ were added. The extract was considered positive for phenols when a green, red, purple, blue, or dark black color appeared. For the alkaloid test, 1 ml of the functional drink was added to a test tube, followed by 3-5 ml of Dragendorff's reagent. A positive reaction was indicated by forming a brown or orange precipitate. For the terpenoid and steroid test, 1 ml of the functional drink was added to a test tube, followed by adding 0.5 ml of chloroform and 0.5 ml of acetic anhydride. Then, 3-5 drops of concentrated $H_2SO_4$ were added along the inner wall of the test tube. If a green or blue color appeared, the extract was considered positive for steroids, while a purple-red color indicated the presence of terpenoids.

### 2.8. Experimental Design and Data Analysis

This study used a Completely Randomized Design (CRD) with 4 treatments. The treatments applied consisted of a control variable (F0) without the addition of *Sargassum* extract, the addition of 15% *Sargassum* extract (F1), the addition of 17.5% *Sargassum* extract (F2), and the addition of 20% *Sargassum* extract (F3). The formulations with different *Sargassum* concentrations were evaluated for total polyphenols, phytochemical composition, and α-glucosidase inhibition and organoleptic evaluation (taste, color, appearance, and aroma).

The data on pH, total polyphenols, and α-glucosidase inhibition were statistically tested using ANOVA at a 5% significance level with further testing using Tukey's test. The scores for organoleptic properties and hedonic quality were analyzed using the nonparametric Kruskal-Wallis test at a 5% significance level, followed by the Mann-Whitney test. Data analysis was calculated with the of SPSS 27.0 for Windows.

## 3. RESULTS AND DISCUSSION

### 3.1. Total Phenolic Content

Polyphenols are active components in *Sargassum*, cinnamon, and *temulawak,* which have been widely reported to inhibit α-glucosidase enzyme activity. This inhibition is expected to help suppress postprandial blood glucose levels. The total polyphenol content of the functional beverage formulations with varying concentrations of *Sargassum* extract is shown in Figure 1. The highest total phenolic content (315.20 mg GAE/100 ml) was observed in the F3 functional beverage by adding 20% *Sargassum* extract. This result was higher than functional beverages from liang tea bay leaves with the addition of ginger and *secang* wood extract (250 mg GAE/100 ml) [17]. The functional beverage without *Sargassum* extract (F0) had a total phenolic content of 219.05 mg GAE/100 ml, indicating that both cinnamon and *temulawak* also contain active phenolic compounds. Cinnamon is rich in polyphenolic compounds, including phenolic acids (caffeic acid, gallic acid, vanillic acid, and p-coumaric acid), flavonoids (flavonols, anthocyanins, flavan-

3-ols, and procyanidins), and monophenols (cinnamaldehyde and eugenol) [18]. The polyphenol composition of *temulawak* (*Curcuma xanthorrhiza*) is primarily characterized by curcuminoids, with curcumin being the main polyphenolic compound (68-76% of total curcuminoids), along with smaller amounts of demethoxycurcumin and bisdemethoxycurcumin [19].

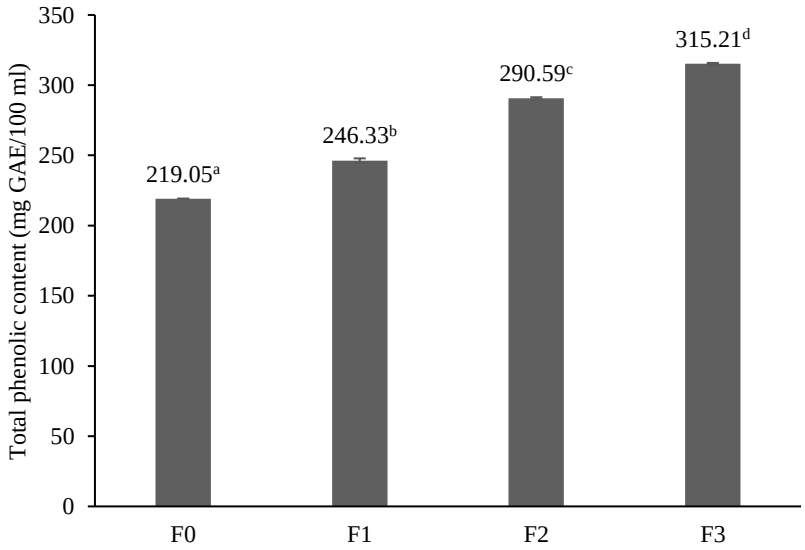


Figure 1. Total phenolic content of functional beverages with various addition of *Sargassum* extract concentration (F0=0% *Sargassum*; F1=15% *Sargassum*; F2=17.5% *Sargassum*; and F3=20% *Sargassum*).

The addition of 15% *Sargassum* extract (F1) increased the total phenolic content to 246.32 mg GAE/100 ml, while 17.5% *Sargassum* extract (F2) further raised it to 290.58 mg GAE/100 ml. The main phenolic compounds found in *Sargassum sp.* are tannins and flavonoids [20]. The increase in total phenolic content with the addition of *Sargassum* extract suggests that the combination of active phenolic compounds in *Sargassum*, cinnamon, and *temulawak* exhibits a synergistic relationship, with the phenolic effects being enhanced as the concentration of *Sargassum* in the functional beverage increases. The phenolic compounds in the individual ingredients can have different molecular structures, such as flavonoids, tannins and phenolic acids, which do not form strong bonds or complexes with each other [21]. Therefore, the phenolic compounds of the individual ingredients are additive rather than interactive, so the more *Sargassum* extract is added, the higher the total phenolic content. This supports the idea that the formulation of the functional beverage benefits from a synergistic yet independent contribution of phenolics that enhances its nutritional and functional properties. Combining several active plant compounds can lead to synergistic, antagonistic or neutral effects [22]. In a plant extract, besides the main active compounds, there are usually many other less active compounds whose presence may reduce, increase, or have no effect on the overall activity of the extract [21].

### 3.2. α-glucosidase Inhibition

α-glucosidase is an enzyme involved in the hydrolysis of carbohydrates into glucose in the digestive system, particularly in the small intestine. Inhibition of this enzyme can suppress the rise in postprandial blood glucose levels [4]. The inhibition activity of α-glucosidase in functional beverages ranged between 30.85% and 62.26% (Figure 2). The formulation of the functional beverage without the addition of *Sargassum* exhibited α-glucosidase inhibition activity of 30.85%. This indicates that the polyphenol content in cinnamon and *temulawak* plays a role in inhibiting α-glucosidase activity. Cinnamaldehyde and cinnamic acid are the main polyphenolic compounds found in cinnamon, which have been reported to possess α-glucosidase inhibitory activity [23]. *Temulawak* contains the active polyphenolic compound curcumin,

which has both antioxidant and α-glucosidase inhibitory activities [24], [25].

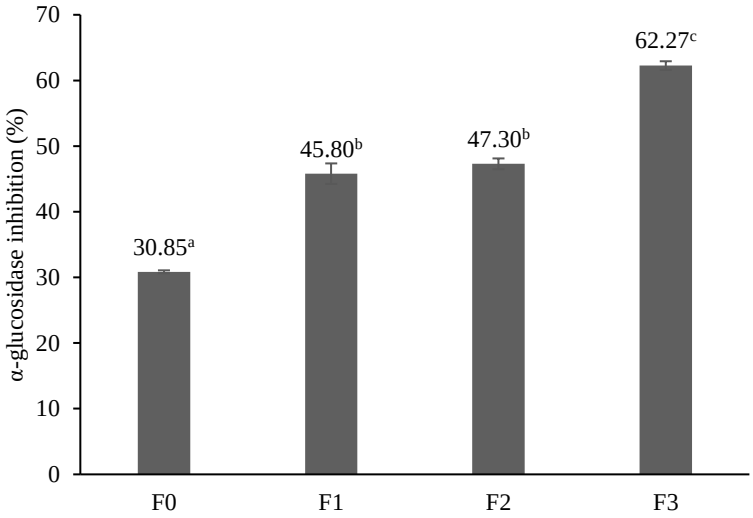


Figure 2. α-glucosidase inhibition of functional beverages with various addition of *Sargassum* extract concentration. (F0=0% *Sargassum*; F1=15% *Sargassum*; F2=17.5% *Sargassum*; and F3=20% *Sargassum*).

The addition of 15% *Sargassum* extract increased α-glucosidase inhibition to 48.46%. As shown in Figure 1, although the total phenolic content increased significantly from 246.32 mg GAE/100 ml (F1) to 290.58 mg GAE/100 ml (F2), the α-glucosidase inhibition did not increase significantly ($p<0.05$). This suggests that not all phenolic compounds in *Sargassum* extract contribute to the inhibition of α-glucosidase enzyme activity. Meanwhile, the highest α-glucosidase inhibition obtained in the functional beverage formula F3 with the addition of *Sargassum* extract 20%. This result higher compared to inhibition of α-glucosidase in functional beverages from fenugreek and ginger which reported in range between 10.52%-27.89% [26]. According to Henri et al., (2023) [20], the phenolic content in brown algae constitutes 20-30% of the dry weight. Studies have shown that phlorotannins are the primary phenolic compounds detected in brown algae. Phlorotannins are a group of phenolic compounds formed by the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) units and are synthesized via the acetate-malonate pathway in macroalgae. The mechanism of phlorotannin's antihyperglycemic activity involves binding to the active sites of α-glucosidase and α-amylase [8].

### 3.3. Sensory Analysis

The addition of *Sargassum* extract to a functional drink aimed to develop a new antidiabetic beverage with α-glucosidase inhibition properties. Hedonic attributes were evaluated across four formulas, including taste, color, appearance, and aroma. Taste was the most crucial attribute for consumer preference in the functional drink. Table 1 shows that the functional drink had an average hedonic scale of 2.5 (dislike to neutral). The highest score was observed in formula F3, with a score of 2.57. Statistical analysis using the Kruskal-Wallis method showed no significant difference among the four functional drink formulas. This may be because only a small concentration of *Sargassum* was added, even in the highest concentration for F3 (20%). This result suggests that the addition of *Sargassum* extract at 20%, which increased α-glucosidase inhibition up to 62.26%, maintained nearly the same level of consumer liking. This functional drink consists of three raw materials: *Sargassum*, cinnamon, and *temulawak*, all of which contribute to its flavor profile. The basic taste of *Sargassum* exhibits sourness and bitterness [7]. Cinnamon is often described as sweet and pungent, with high-quality varieties displaying a strong flavor without astringency [27]. *Temulawak* has a naturally bitter flavor, which is often considered unpleasant [28]. The bitterness of

*Sargassum* and *temulawak* can be masked by the sweetness of cinnamon. Thus, to enhance the taste hedonic score, increasing the concentration of *temulawak* may be beneficial.

Table 1. The hedonic score of functional drink.

| Formula | Taste | Color | Aroma | Appearance |
|---|---|---|---|---|
| F0 | 2.49±0.89[a] | 3.29±0.79[a] | 3.03±1.04[a] | 3.34±0.64[a] |
| F1 | 2.51±0.74[a] | 3.26±0.74[a] | 3.11±0.87[a] | 3.40±0.74[a] |
| F2 | 2.43±0.65[a] | 3.26±0.78[a] | 2.97±0.92[a] | 3.34±0.64[a] |
| F3 | 2.57±0.88[a] | 3.43±0.61[a] | 3.11±0.99[a] | 3.43±0.70[a] |

Note: F0=0% *Sargassum*; F1=15% *Sargassum*; F2=17.5% *Sargassum*; and F3=20% *Sargassum*.

Color results from visual perception and is important in evaluating a product, especially for food and beverages, as it represents freshness and consumer preference. The hedonic color score ranged from 3.26 to 3.43 (neutral to slightly liked), with the highest score observed in the formula containing 20% *Sargassum* extract (F3). However, statistical analysis showed no significant differences among all formulas, indicating similar color preferences with the addition of *Sargassum*. The color of the functional drink in all four formulas was yellowish-brown, likely influenced by the *Sargassum* extract, which produces a brownish hue due to fucoxanthin, a significant carotenoid pigment [10]. The hedonic score for aroma ranged from 2.97 to 3.11 (neutral), and aroma also showed no statistically significant difference. This result suggests that adding *Sargassum* extract up to 20% is still acceptable. The overall score combines these sensory attributes to evaluate the entire functional beverage. The hedonic test results for overall liking showed that panelists were neutral toward the functional drink. As presented in Table 1, the average overall scores ranged from 3.34 to 3.43 (neutral).

### 3.4. pH and Qualitative Phytochemical Composition

The functional beverage formulation with the highest inhibition activity and the most consumer preference (F3) was further tested for pH and qualitatively analyzed for its phytochemical composition. pH is a key parameter related to the functional beverage's acidity or alkalinity. The pH of the functional beverage was 7.09, indicating that the beverage is neutral in terms of acidity. The phytochemical components analyzed included flavonoids, tannins, saponins, phenols, alkaloids, terpenoids, and steroids. The qualitative analysis results of the functional beverage's phytochemical composition are presented in Table 2.

Table 2. Phytochemical analysis of functional beverages.

| Phytochemical | Results |
|---|---|
| Flavonoids | - |
| Tannin | + |
| Saponin | + |
| Phenol | + |
| Alkaloid | + |
| Terpenoid | - |
| Steroid | - |

Based on the phytochemical testing results in Table 2, the functional beverage contained tannins, saponins, phenols, and alkaloids. Flavonoids, terpenoids, and steroids were not detected in the functional beverage. This is likely due to the polar nature of the solvent used for the beverage's decoction (distilled water), which only allowed polar compounds (tannins, saponins, phenols, and alkaloids) to be detected in the beverage. This is consistent with research reported by Petchidurai et al., (2023) [29], which noted that *Sargassum* contains 20.62% tannins, with significant levels of hydrolyzable tannins and soluble

phlorotannins. Saponins and phenols are also present in *Sargassum* aqueous extracts [20]. Cinnamon extracts have been reported to contain alkaloids, phenols, saponins, and tannins [30]. Alkaloids are found in high concentrations, particularly in *Curcuma*, with levels reaching 41.20 mg/100 g [31].

## 4. CONCLUSION

The antidiabetic functional drink formulated with 20% *Sargassum* extract, 15% cinnamon extract, and 18.5% *temulawak* extract exhibited the highest phenolic content (315.21 mg GAE/100 ml) and α-glucosidase inhibition (62.27%) while remaining acceptable to the panelists. However, not all phenolic compounds in each raw material inhibit α-glucosidase. The interaction of compounds responsible for α-glucosidase inhibition in each raw material requires further evaluation. Additionally, the composition of phenolic compounds in the functional drink should be analyzed to better understand the underlying antidiabetic mechanism of this beverage.

## AUTHOR CONTRIBUTION

**Nada Itorul Umam**: Writing (review & editing), writing (original draft), formal analysis, conceptualization, funding acquisition. **Rina Rifqie Mariana**: Writing (review & editing), writing (original draft), investigation, formal analysis. **Aditia Gustiana Gunawan**: Investigation, writing (review & editing). **Asep Awaludin Prihanto:** Supervision, conceptualization. **Hidayatun Muyasyaroh**: Writing (review & editing), writing (original draft), investigation.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest to declare.

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