Antioxidant, antidiabetic activities and consumer acceptance of *Sargassum hystrix* tea combined with cinnamon powder

1Setiyawan, A. and 1,2,*Husni, A.

1Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
2Center for Seafood Security and Sustainability, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

**Article history:**
Received: 31 March 2021
Received in revised form: 2 May 2021
Accepted: 11 July 2021
Available Online: 20 March 2022

**Keywords:**
Antidiabetic, Antioxidant, Functional drink, *Sargassum hystrix*

**DOI:**
https://doi.org/10.26656/fr.2017.6(2).226

**Abstract**

*Sargassum hystrix* is a species of brown seaweed containing bioactive compounds and has the potential to be used as a functional drink. This study was aimed to determine the fucoidan content, antioxidant, antidiabetic activities, and consumer acceptance rate of combining cinnamon powder to manufacture *S. hystrix* seaweed tea. The process was carried out by washing, drying, cutting, blending, weighing, roasting, and packaging the seaweed. Furthermore, the seaweed was tested to determine the total phenolic content, antioxidant (scavenging of hydroxyl radical and superoxide anion), antidiabetic (inhibition of α-glucosidase and α-amylase) activities and sensory evaluation. The results showed that the addition of cinnamon powder affected the total phenol content, antioxidant and antidiabetic activities, and consumer acceptance of *S. hystrix* tea. Meanwhile, an increase in the amount (0 ~ 5%) of cinnamon powder reduced levels of antioxidants (46.90±0.92 to 39.53±0.11% for hydroxyl radical scavenging activity, and 82.00±1.19 to 73.56±1.23% for superoxide anion scavenging activity), with a rise in antidiabetic activity (22.27±1.74 to 83.98±2.37% for inhibition activity of α-glucosidase and 72.94±1.55 to 95.83±1.06% for inhibition activity of α-amylase). In addition, the use of 5% and 2% cinnamon powder has the ability to overcome the fishy smell and taste, respectively.

**1. Introduction**

Indonesia is a country rich in biological resources, such as seaweed. According to Eriningisih *et al*. (2014), one of the most common seaweeds naturally found in Indonesian waters is the brown *Sargassum* sp. Unfortunately, there is limited use of this species, especially in the industrial sector. Budhiyanti *et al*. (2012) reported that the *Sargassum hystrix* has the highest antioxidant activity than brown seaweed on the South coast of Yogyakarta and North of Central Java. Furthermore, besides its use as an antioxidant, this species is used as an antidiabetic substance in the medical field (Husni *et al*., 2018; Gotama *et al*., 2018; Azizi *et al*., 2019; Azizah *et al*., 2019; Husni *et al*., 2020). Lailatussifa *et al*. (2017) reported that *S. hystrix* powder has antioxidant properties due to the presence of bioactive compounds such as steroids, alkaloids, terpenoids, tannins, phenols, and saponins which neutralizes free radicals. Therefore, *S. hystrix* powder is widely used as a supplement in food production.

Functional drinks positively influence the body due to their numerous nutritional and non-nutritional elements (Palupi and Widyamingsih, 2015). These types of drinks are made from a variety of brown seaweeds, such as *Sargassum* sp. According to Alura *et al*. (2016), China has significantly used seaweed to eliminate the use of phlegm for decades. However, one of the disadvantages associated with the use of *Sargassum* as a tea product is its fishy smell. This led to the process of adding natural ingredients such as cinnamon, to eliminate the rotten fishy odour (Sahara, 2019). Therefore, this study examined the effect of adding cinnamon powder in the manufacture of *S. hystrix* seaweed tea.

**2. Materials and methods**

**2.1 Materials**

The materials used in this research are seaweed *S. hystrix* obtained from the coastal of Gunungkidul Yogyakarta Indonesia. Others include methanol, ethanol, ethyl acetate, and sodium carbonate (Merck, USA). Furthermore, the materials used to analyze the α-glucosidase inhibition activities were α-glucosidase from...
Saccharomyces cerevisiae type I (Sigma-Aldrich, Germany), p-nitrophenyl-α-d-glucopyranoside (Sigma-Aldrich), and acarbose (Bayer Pharmaceuticals, Indonesia). Other essential ingredients include CaCl2, KBr, ethanol, phenols (Merck, USA), H2SO4, L-fucose, (Sigma-Aldrich, Germany), BaCl2 (Merck, USA), xylene (Sigma-Aldrich, Germany), gelatine, K2SO4 (Merck, USA), trichloroacetic acid (Merck, USA), DPPH (Merck, USA), FeCl3 (Merck, USA), vitamin C (Merck, USA) and commercial food-grade fucoidan (Fucophilix, Kalbe Farma, Indonesia).

2.2 Seaweed tea preparation and manufacture

The seaweed *S. hystrix* used in this research was obtained from Drini Beach Gunungkidul Yogyakarta in August 2019. Sample preparation was carried out by washing in freshwater with the sample dried in an oven (Eyela WFO-601SD, Japan) for 72 hrs at a temperature of 40°C. The seaweed was cut into small pieces, blended (Philips, Netherlands) until a powdered form was obtained, and stored at 4°C before use.

The manufacturing process by Lim *et al.* (2017) was used to prepare *S. hystrix* with the addition of cinnamon powder in percentages of 0 ~ 5% (w/w). Seaweed tea formula was made consisting of *S. hystrix* seaweed powder and cinnamon powder with a formula combination of 100 : 0%; 99: 1%; 98: 2%; 97: 3%; 96: 4%; and 95: 5%. The composition of the formula for *S. hystrix* seaweed tea is shown in Table 1. In this process, the cinnamon powder was first dissolved in mineral water (8:10, w/v). Then a solution of cinnamon powder and cinnamon powder solution was completely mixed and dry. The mixture of *S. hystrix* powder and cinnamon powder was put into a tea bag (2 g/teabag), then covered with a sealer.

<table>
<thead>
<tr>
<th>Formula (%)</th>
<th>Sargassum hystrix (g)</th>
<th>Cinnamon powder (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>2.00</td>
<td>0.00</td>
</tr>
<tr>
<td>99:1</td>
<td>1.98</td>
<td>0.02</td>
</tr>
<tr>
<td>98:2</td>
<td>1.96</td>
<td>0.04</td>
</tr>
<tr>
<td>97:3</td>
<td>1.94</td>
<td>0.06</td>
</tr>
<tr>
<td>96:4</td>
<td>1.92</td>
<td>0.08</td>
</tr>
<tr>
<td>95:5</td>
<td>1.90</td>
<td>0.10</td>
</tr>
</tbody>
</table>

2.3 Total phenolic content

The total phenolic test refers to Sinurat and Suryaningrum (2019). Approximately 1 mL of the samples (2 g/200 mL) was put in the bottle then 1 mL of 96% ethanol, 5 mL of distilled water and 0.5 mL of Folin Ciocalteau reagent 50% were added. The mixture was left to stand for 5 min and 1 mL of 5% Na2CO3 was added. The mixture was homogenized and then incubated in the dark for one hour. The standard used was gallic acid with a concentration of 0, 20, 40, 60, 80, and 100 ppm. Standard solutions and samples were measured with a UV-Vis spectrophotometer at a wavelength of 725 nm.

2.4 Hydroxyl radical (-·OH) scavenging activity

Lim *et al.* (2017) proposed method was used to determine the hydroxyl radical scavenging activity (-·OH) in this research. This was further analyzed using the Fenton reaction, by adding 0.5 mL of 9 mM ferrous sulphate (FeSO4) to 1.0 mL of 8.8 mM hydrogen peroxide (H2O2). Furthermore, 1 mL of seaweed tea samples (2 g/200 mL) or cinnamon powder (2 g/200 mL) and 0.2 mL of 9 mM salicylic acid solution were sequentially added to the mixture, and allowed to stand at a temperature of 37°C for 1 hr. This was followed by measuring the absorbance in a 96-well plate (200 μL) at 510 nm using a microplate reader (Heales MB-580, China). Finally, the hydroxyl radical scavenging activity was regulated in triplicate (n = 3) and calculated as followed:

$$
\text{Hydroxyl radical scavenging rate (\%) = \left[1 - \frac{(A_0 - A_1)}{A_0}\right] \times 100}
$$

Where *A*0 and *A*1 denote the absorbance of the blank without/with the samples, while *A*2 is the absorbance containing the sample without salicylic acid.

2.5 Superoxide anion scavenging activity

The inhibited pyrogallol auto-oxidation was used to determine the superoxide anion scavenging activity (Lim *et al.*, 2017). Approximately 0.3 mL of each sample (2 g/200 mL), were dropped into 2.6 mL phosphate buffer (50 mM, pH 8.24), with 90 μL of 3 mM pyrogallol. Furthermore, blank samples were prepared using distilled water with a microplate reader (Heales MB-580, China) used to determine the inhibition rate of pyrogallol auto-oxidation, at a wavelength of 325 nm in a 96-well plate (200 μL), with the absorbance recorded every 1 min for 10 mins. In addition, the sample’s percentages were calculated using n value of 3 as follows:

$$
\text{Scavenging rate (\%) = \left[1 - \frac{(A_2 - A_1)}{A_0}\right] \times 100}
$$

Where *A*1 and *A*2, *A*0 denote the absorbance of the sample at zero, tenth min, and the autoxidation rate of pyrogallol for the blank.
2.6 Inhibition of the α-amylase activity

Husni et al. (2018) modifications were used to actuate the inhibitory activity of α-amylase. Acarbose, an antidiabetic drug, was used as the standard at the same concentration as the sample. This was carried out using varying concentrations of the 25 mL sample extract (2 g/200 mL) or standard (2 g/200 mL) as well as 25 mL of 0.02 M sodium phosphate buffer containing 13 U/mL α-amylase with pH 6.9 and 0.006 M NaCl. A vortex mixer was used to mix the test solution, which was incubated at 37°C for 10 mins. This was followed by adding 25 mL of 1% soluble starch in 0.02 M sodium phosphate buffer to the test solution, which was incubated at 37°C for 10 mins. Furthermore, the solution was treated by adding 50 mL of 96 mM 3,5-dinitrosalicylic acid (DNS), which was incubated for 5 mins in a water bath. The solution was cooled at room temperature, and wavelength of 550 nm with the absorbance values calculated as follows:

\[
\text{Percentage inhibition} = \left(\frac{K - S_1 - S_0}{K}\right) \times 100\%
\]

Where K, S1, and S0 denote the absorbance of the control -blank, the sample with and without enzymes, respectively.

2.7 Inhibition of the α-glucosidase activity

An inhibition test of α-glucosidase was carried out in accordance with Azizi et al. (2019) research. Acarbose, an antidiabetic drug, was used as the standard. The test consists of 50 mL of 0.1 M phosphate buffer (K2HPO4), pH 7, 25 mL of 0.5 mM p-nitrophenyl-α-D-glucopyranoside (PNP-G, the substrate), 10 mL of the sample extract (2 g/200 mL) or standard (2 g/200 mL), and 0.2 U/mL α-glucosidase of 25 mL. Furthermore, the sample was also mixed and incubated at 37°C for approximately 30 mins, with the reaction stopped at 100 mL of 0.2 M Na2CO3. The p-nitrophenol formed using a microplate reader was used to determine the enzyme activity, which was inhibited at a wavelength of 405 nm. Furthermore, the absorbance values were used to analyze the enzyme’s percentage inhibition.

\[
\text{Percentage inhibition} = \left(\frac{K - (S_1 - S_0)}{K}\right) \times 100\%
\]

Where K, S1, and S0 denote the absorbance of the control -blank, as well as a sample with and without enzyme.

2.8 Sensory evaluation of Sargassum hystrix seaweed tea

A discriminative model was used to determine the minimum cinnamon powder concentration needed to overcome the seaweed smell and taste in the tea. The samples with and without cinnamon powder were evaluated by a panel comprising 75 undergraduate and postgraduate students from Universitas Gadjah Mada. This sensory test was conducted from August 25 to September 2, 2020. Furthermore, the sensory evaluation method was used in the laboratory to determine the proper panels separating the tables with samples comprising of screw caps at room temperature served in 20 mL universal bottles. The universal bottles were individually filled with 10 mL samples (2 g/200 mL), and the panel was used to identify the seaweed smell and taste from the reference sample without and with cinnamon powder. The panels choose more than one sample without smell/taste in the reference. Samples were randomly arranged and labelled to minimise errors in accordance with Lim et al. (2017). The results are shown as a score graph, with chosen and not chosen samples denoted by 1 and 0.

2.9 Statistic analysis

The analysis phase was conducted using three replications (n = 3), with 75 panellists involved in the sensory evaluation process. Furthermore, the mean and standard deviation of the data were obtained and analyzed using the one-way ANOVA, followed by Duncan’s multiple range test (DMRT) and SPSS Version 21. Mean differences were considered significant when p is below 0.05.

3. Results and discussion

3.1 Total phenolic content

The total phenolic content of S. hystrix seaweed tea was combined with cinnamon powder in percentages of 0 ~ 5%, as shown in Figure 1. The combination of the addition of cinnamon powder has an effect on the total phenol content of S. hystrix seaweed tea. The total phenolic content of S. hystrix seaweed tea combined with 1-5% cinnamon powder were 34.29±0.99, 32.58±0.93, 31.55±0.45, 26.15±0.94, 31.43±0.34, 36.55±1.41 mg GAE/g, respectively. Figure 1 shows that the combination of cinnamon from 1-3% decreased the total phenol content in seaweed tea (34.29±0.99 to 26.15±0.94 mg GAE/g), but the combination of cinnamon at 4-5% increased the total phenolic content (36.55±1.41 mg GAE/g). A similar phenomenon was also reported by Wijaya (2019), where the addition of 5% cinnamon to parboiled rice soaked at 60°C could increase the total phenolic content (31.55±0.45 to 36.55±1.41 mg GAE/g). A similar phenomenon was also reported by Wijaya (2019), where the addition of 5% cinnamon to parboiled rice soaked at 60°C could increase the total phenolic content (357.2±1.99 to 1586.6±7.53 mg GAE/g), then the addition of cinnamon 10% there was a decrease in the total phenolic content (369.31±7.72 mg GAE/g), but the addition of 15% cinnamon increased the total phenol content (916.75±3.89 mg GAE/g).

3.2 Antioxidant activity

3.2.1 Hydroxyl radical (·OH) scavenging activity

The hydroxyl radical scavenging activity of S. hystrix seaweed tea combined with 0 ~ 5% cinnamon...
The combination of adding ground cinnamon to *S. hystrix* seaweed tea has a significant effect (P<0.05) on hydroxyl radical scavenging activity. Furthermore, the seaweed tea without cinnamon powder had the highest hydroxyl radical scavenging activity of 46.90±0.92%, while the lowest was in combination with the addition of 5% cinnamon powder, 39.53±0.11%. These results were in accordance with the research carried out by Lailatussifa *et al.* (2017), which reported that *S. hystrix* powder has nutritional and bioactive components that are potent antioxidants. However, addition of cinnamon to *S. hystrix* seaweed tea reduced the activity of hydroxyl radical scavenging. The cinnamon powder was known to have antioxidant activity (Gulcin *et al.*, 2019), although in this study was relatively low (Figure 2). The combination of *S. hystrix* and cinnamon powder in this study decreased antioxidant activity. This can occur not only because the antioxidant activity of cinnamon powder in this study was relatively low but also possibly because of its presence of antioxidant compounds that are antagonistic after mixing the two ingredients. The results of the research by Hastuti and Ninik (2014) showed that the addition of cinnamon to secang drinks was not able to increase the antioxidant activity, due to the presence of catechins in cinnamon which are antagonistic to brazilin in secang. The phenomenon of decreasing antioxidant activity due to the mixing of two ingredients also occurs in the mixing of the ethanol extract of temu giring rhizome (*Curcuma heynaeana*) and pugun tanoh leaves (*Curanga fel-terrae*) (Marianne *et al.*, 2018). The antagonistic properties after mixing two or more ingredients were also widely explained by Olszowy-Tomczyk (2020), and Nunes *et al.* (2021). Furthermore, the results of the hydroxyl radical scavenging activity test in this study were higher than Lim *et al.* (2017) research on *S. binderi* seaweed tea (30.80%) with the same concentration.

### 3.2.2 Superoxide anion scavenging activity

The superoxide anion scavenging activity using *S. hystrix* seaweed tea with a cinnamon combination of 0 ~ 5% is shown in Figure 3. The combination has a significant effect (P<0.05) on the decreased activity of anion superoxide scavenging, where 0% and 5% showed the highest (82.00±1.19%), and lowest (73.56±1.23%) activities. This shows that an increase in cinnamon powder led to a decrease in the activity of anion superoxide scavenging. This phenomenon can occur as described in the hydroxyl radical scavenging activity. However, the anion superoxide trapping activity in this research (82.00±1.19%) was higher when compared to *S. binderi* seaweed tea (16.46±2.83%) that was reported by Lim *et al.* (2017) in the same concentration. Superoxide anion scavenging is essential for the human body because it occurs naturally and continues to form in the human body through cell oxidation reactions. However, superoxide anions are relatively weak oxidants and can be broken into stronger radicals, such as hydroxyl and hydrogen peroxide which are formed through mutation reactions (Sarikurkcu *et al.*, 2010).

![Figure 1](image1.png)

Figure 1. Effect of combination of cinnamon powder on total phenol levels of *S. hystrix* seaweed tea (2 g/200 mL). Different notations above the bars indicate significant difference between groups (P<0.05).

![Figure 2](image2.png)

Figure 2. Effect of combination of cinnamon powder on hydroxyl radical scavenging activity of *S. hystrix* seaweed tea (2 g/200 mL). Different notations above the bars indicate significant difference between groups (P<0.05).

![Figure 3](image3.png)

Figure 3. Effect of combination of cinnamon powder on superoxide anion scavenging activity of *S. hystrix* seaweed tea (2 g/200 mL). Different notations above the bars indicate significant difference between groups (P<0.05).
3.3 Antidiabetic activity

3.3.1 Inhibitory activity of α-glucosidase

The combination effect of cinnamon powder in producing S. hystrix seaweed tea on α-glucosidase is shown in Figure 4. The combination of adding ground cinnamon to S. hystrix seaweed tea has a significant effect (P<0.05) on α-glucosidase inhibitory activity. Furthermore, the seaweed tea without cinnamon powder had the lowest α-glucosidase inhibitory activity of 22.27±1.74%, while the highest was the addition of 5% cinnamon powder, 83.98±2.37%. Meanwhile, the use of acarbose as a standard antidiabetic drug produced an inhibition value of 88.79±0.59% with a similar concentration (2 g/200 mL). The cinnamon extract contains many phenolic compounds, especially p-hydroxybenzoic acid, p-coumaric acid, pyrogallol, vanillin, ferulic acid, caffeic acid which contribute to inhibiting α-glucosidase (Gulcin et al., 2019), while S. hystrix contains compounds that can inhibit α-glucosidases such as 9-octadecenoic acid, 1-hexadecanecarboxylic acid, 9,12-octadecadienoic acid (Z,Z), and octadecanoic acid methyl ester (Azizi et al., 2019), 2-hexadecene-1-ol, 3,7,11, 15-tetramethyl-, hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester, phytol, stigmasta-5,24(28)-dien-3-ol, (3.beta,24Z)-, and 6-hydroxy-4,4,7a-trimethyl-5,6,7a-tetrahydro benzofuran-2(4H)-one (Azizah et al., 2019), 1,2-benzenedicarboxylic acid, 1,3,5-benzenetriol, flamenol, and eicosanoic acid (Husni et al., 2020), pentadecanoic acid, benzenedicarboxylic acid, and hexadecanoic acid (Nurkhanifah et al., 2020). Therefore, the use of cinnamon powder in making S. hystrix seaweed tea significantly affects the α-glucosidase inhibitory activity.

Figure 4. Effect of combination of cinnamon powder on the α-glucosidase activity of S. hystrix seaweed tea (2 g/200 mL) and acarbose (2 g/200 mL). Different notations above the bars indicate significant difference between groups (P<0.05).

3.3.2 Inhibitory activity of α-amylase

The effect of combining cinnamon powder in the manufacture of S. hystrix seaweed tea on α-amylase inhibitory activity is shown in Figure 5. The combination of adding ground cinnamon to S. hystrix seaweed tea has a significant effect (P<0.05) on α-amylase inhibitory activity. Furthermore, the seaweed tea without cinnamon powder had the lowest α-amylase inhibitory activity of 74.94±1.55%, while the highest was with the addition of 5% cinnamon powder, 95.83±1.06%. Meanwhile, the use of acarbose as a standard antidiabetic drug produced an inhibition value of 86.93±0.53%. The analysis showed that the increase in the amount of cinnamon in S. hystrix seaweed tea had a significant value (P<0.05). This result occurs due to cinnamon's inhibitory activity against α-amylase (Darmawan et al., 2016). According to Gulcin et al. (2019), the cinnamon extract contains many phenolic compounds such as p-hydroxybenzoic acid, p-coumaric acid, pyrogallol, vanillin, ferulic acid, caffeic acid which contribute to inhibiting α-amylase, while S. hystrix contains compounds that can inhibit α-amylase such as 1,2-benzenedicarboxylic acid, 1,3,5-benzenetriol, flamenol, and eicosanoic acid (Husni et al., 2020), pentadecanoic acid, benzenedicarboxylic acid, and hexadecanoic acid (Nurkhanifah et al., 2020). Therefore, cinnamon powder combination in S. hystrix seaweed tea significantly affects the inhibition of α-amylase activity.

Figure 5. Effect of combination of cinnamon powder on the α-amylase inhibitory activity of S. hystrix seaweed tea (2 g/200 mL) and acarbose (2 g/200 mL). Different notations above the bars indicate significant difference between groups (P<0.05).

3.4 Sensory evaluation of seaweed tea

Sargassum hystrix has antioxidant (Lailatussifa et al., 2017) and anti-diabetes activities (Nurkhanifah et al., 2020) and has the potential as a functional food ingredient. The use of pure S. hystrix as a product is less acceptable because of the fishy smell. Cinnamon has been used to overcome the fishy smell in cookies, flakes, and dry noodles enriched with Spirulina platensis (Fitiyia and Alfionita, 2018), as well as S. platensis ice cream (Farhah and Ekantari, 2020). Therefore, in this study cinnamon was used to treat the fishy smell in S. hystrix seaweed tea. The effect of using cinnamon in making S. hystrix seaweed tea can be seen in Figure 6. Figure 6 shows that 59 out of 75 panellists expressed that the use of cinnamon 5% can overcome the smell. Subsequently, 54 out of 75 panellists expressed that the taste can be control by adding 2% of the powder. Thus the use of 5% cinnamon powder can overcome the fishy smell of S. hystrix seaweed tea.
The addition of cinnamon in the process of *S. hystrix* seaweed tea can increase antidiabetic activity, but on the other hand, can reduce antioxidant activity. In order to produce *S. hystrix* seaweed tea, which has high antioxidant and antidiabetic activities, it is necessary to look for alternative ingredients, such as lemon essence. Lim et al. (2017) reported that the use of lemon essence 3% was able to overcome the fishy smell of *S. binderi* seaweed tea without reducing levels of fucoidan and antioxidant activity.

4. Conclusion

In conclusion, the addition of cinnamon powder to *S. hystrix* seaweed tea tends to affect the phenol content, antioxidant and antidiabetic activities as well as consumer acceptance. This reduces the levels of antioxidants and increases antidiabetic activities. The use of 5% cinnamon powder can overcome the fishy smell of *S. hystrix* seaweed tea, with 2% added to eliminate its taste.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors are grateful to the Directorate of Research and Community Service, Deputy for Strengthening Research and Development, Ministry of Research and Technology/National Research and Innovation Agency for funding this research through the 2020 Higher Education Leading Research Consortium Scheme with contract number 3846/UN1/DITLIT/DITLIT/PT/2020. This manuscript is part of the first author's Bachelor's thesis.

References


Setiyawan and Husni / Food Research 6 (2) (2022) 159 - 165

https://doi.org/10.1080/10942912.2019.1656232


