Yield and Extraction Rate Analysis of Phytochemical Compounds from *Eucheuma cottonii*, *Ganoderma lucidum*, and *Gracilaria* sp. using Subcritical Water Extraction

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**Abstract.** *Eucheuma cottonii* (*E. cottonii*), *Ganoderma lucidum* (*G. lucidum*), and *Gracilaria* sp. are plants that contain high phytochemicals, such as flavonoids, polyphenols, saponins, and tannins. In this work, the phytochemicals were obtained using the subcritical water extraction (SWE) process. The SWE method uses water as a solvent in subcritical conditions. Therefore, the SWE process is an environmentally friendly process for extraction. In order to run the SWE process optimally, measurement of the extraction rate of SWE is needed. Calculation of the extraction rate of SWE process used first and second-order models according to Lagergren equation. SWE process was started by setting temperatures from 140 to 180°C at a pressure of 7 MPa and solvent flow rate of 1 ml/min. Before starting the extraction, the raw material was loaded into the extractor. The raw materials used were *E. cottonii*, *G. lucidum*, and *Gracilaria* sp. The extraction process was carried out for 3 hours, and the product was collected every 30 minutes. The collected product was put into a sample bottle and dried using a freeze dryer. After that, the products obtained were balanced by an analytical scale. Based on the result, the optimum temperature for the SWE process was 180°C for *E. cottonii* and *G. lucidum* and 160°C for *Gracilaria* sp. The yields of the SWE process under the optimum temperature were 85.37%, 58.42%, and 75.73% for *E. cottonii*, *G. lucidum*, and *Gracilaria* sp, respectively. The extract contained phytochemical compounds detected by high-performance liquid chromatography analysis. The kinetics model of extraction rate for all variables exhibited a second-order kinetics model that indicated that the extraction process was influenced by more than one factor.

**Keywords:** *Eucheuma cottonii*; *Ganoderma lucidum*; *Gracilaria* sp; Kinetics Model; Subcritical Water Extraction

**INTRODUCTION**

Phytochemicals are chemical compounds derived from plants. Nowadays, nearly 30,000 phytochemicals have been found. The types of phytochemicals can be combined to produce active compounds that are useful for human health. Some phytochemical
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compounds used for human health are flavonoids as antioxidants, polyphenols for controlling pathogenic infections in humans, saponins for anti-cancer, and tannins for antiseptic and antibiotics. Seaweed and mushrooms are plants that have the highest phytochemical content due to their high antioxidant activity. Antioxidant activity indicates that the plant contains high phytochemical active compounds, especially flavonoids, polyphenols, saponins, and tannins (Indria et al., 2017).

Subcritical water extraction (SWE) is an environmentally friendly and highly selective extraction technology for phytochemical compounds from plants. The advantages of SWE are 1) safety because it uses harmless extraction solvents; 2) efficiency due to short extraction time required; 3) quality due to the good extracts obtained; and 4) environmentally friendly because the solvent used do not pollute the environment (Zheng et al., 2020). Subcritical water is water at a temperature between 100°C and 374°C and pressure between 0.1 and 22.4 MPa. Subcritical water has physical properties that remain liquid and have a low dielectric constant. So that subcritical water has almost the same properties as organic solvents. Finally, subcritical water can be used to extract non-polar compounds, especially phytochemical compounds. Previous SWE research has been published by Setyorini et al. (2018), however the extraction kinetics of *E. cottonii* and *Gracilaria* sp. extract at various conditions has not been further investigated. In the previous studies, they examined the types of phytochemicals obtained and their antioxidant activity.

The knowledge of extraction kinetic is necessary for the design and scale-up of the extraction process. The kinetic model for subcritical water extraction of essential oil has been proposed by Khajenoori et al. (2009). They proposed one-side and two-side kinetic desorption models to describe the extraction kinetic. The one-site and two-site kinetic desorption models describe the extraction data reasonably at lower volumetric flow rates. A kinetic model was also developed for the hydrolysis of soybean oil in subcritical water at the temperature range of 250–300 °C (Milliren et al., 2013). The kinetic model was the empirical model that includes reversible hydrolysis reactions to produce fatty acids from soybean oil and other reactions for autocatalysis by fatty acid reaction products. The model predicted the increase in the fatty acid yields experimentally observed with acid addition.

This work aims to investigate the effect of extraction temperature on the yield of the SWE process from *E. cottonii*, *G. lucidum*, and *Gracilaria* sp. The extraction rate of the process was also analyzed by first and second-order kinetic models.

**MATERIALS AND METHODS**

**Materials**

*E. cottonii* and *Gracilaria* sp. were found from the coast of Pamekasan, Madura Island. *G. lucidum* was purchased from distributor in Rungkut, Surabaya. Distilled water was obtained from U.D. Sumber Ilmiah Persada Surabaya.

**Experimental Apparatus**

SWE was conducted in a semi-batch extraction system. The main equipment of SWE included an extractor (10 ml volume, Thar Design Inc., USA), back pressure regulator (BPR, AKICO, Japan), high-pressure pump (200 LC Pump, Perkin Elmer, Germany), heater (Memmert UN 55), a filter made of aluminum (Swagelok, 0.5 µm), and sample
bottle (10 ml volume, Duran). SWE equipment scheme can be seen in Figure 1.

SWE process was started by inserting 1 gram of sample in the extractor. Glass beads were placed on both sides of the inlet and outlet to prevent channeling at the center of the extractor. Then the extractor was installed inside the heater. The heater was turned on. The temperature was set to 140°C-180°C. Distilled water (aquadest) was flowed by a high-pressure pump into the extractor at 1 ml/min of flow rate. The pressure was maintained at 7 MPa by turning the BPR into the right-side. Then the extract was cooled in the cooler and passed through the filter. Finally, the product was collected in a sample bottle every 30 minutes for a total extraction time of 3 hours. The product was stored in the refrigerator until further analysis.

Analytical Method
Phytochemical compounds in the extract were analyzed using high-performance liquid chromatography (HPLC). HPLC instrument used was a diode array detector SPD-M10A VP (Shimadzu, Kyoto, Japan) equipped with an STR ODS-II column (silica gel diameter, 5 μm; 250 × 4.6 mm (i.d.)) and was operated at 40°C. The mobile phase flow rate was 1 ml/min and consisted of acetonitrile and distilled water (70/30, v/v). The sample solution was injected in 10 ml volume. The extract was analyzed at a wavelength of 254 nm.

Calculation of SWE Process Yield
The yield of extract was determined by weighing the extract after removing water using freeze-drying (Nanbei Company, China). Each extraction product was obtained every 30 minutes, and there were 6 extracts for 3 hours for each temperature and raw material. The yield of extract was calculated with Eq. (1).

\[
\text{Yield} = \frac{\text{Total Product Weight Obtained}}{\text{Total Initial Sample Weight}} \times 100\% \quad (1)
\]

Calculation of SWE Process Kinetics Model
The kinetic extraction model was calculated using the Lagergren equation developed in 1898 (Ho, 2004). The calculation used first and second-order kinetic models. For the first-order model, the equation, according to Ho (2004), can be written in the differential forms in Eq. (2).

\[
\frac{dC_t}{dt} = k_1(C_s - C_t) \quad (2)
\]
Then integrated using the boundary conditions $C_t = 1$ at $t = 0$ and $C_t = C_s$ at $t = t$, and become Eq. (3).

$$\ln\left(\frac{C_s}{C_s - C_t}\right) = k_1 t$$  \hspace{1cm} (3)

The linearization of Eq. (3) becomes Eq. (4).

$$\ln(C_s - C_t) = \log(C_s) - \frac{k_1}{2.303} t$$  \hspace{1cm} (4)

Then plot between $\log(C_s - C_t)$ with $t$ to get the slope and intercept, which can be used to determine the value of the extraction rate constant for first order ($k_1$) and extraction capacity value ($C_s$).

The second-order kinetic equation for the extraction kinetic model of the SWE process is described by Eq. (5) (Jovanovic et al., 2012).

$$\frac{dC_t}{dt} = k_2 (C_s - C_t)^2$$  \hspace{1cm} (5)

by grouping the variables in Eq. (5), we can get Eq. (6).

$$\frac{dC_t}{(C_s - C_t)^2} = k_2 dt$$  \hspace{1cm} (6)

Equation (7) was obtained by integrating Eq. (6) using the boundary conditions $C_t = 0$ at $t = 0$ and $C_t = C_s$ at $t = t$ and by rearranging as:

$$C_t = \frac{C_s^2 k_2 t}{1 + C_s k_2 t}$$  \hspace{1cm} (7)

Equation (7) is the law of integrated extraction kinetic model for second-order and can be linearized as Eq. (8).

$$\frac{t}{C_t} = \frac{1}{k_2 C_s^2} + \frac{t}{C_s}$$  \hspace{1cm} (8)

The extraction rate ($\frac{C_t}{t}$) can be obtained from Eq. (8) as Eq. (9).

$$\frac{C_t}{t} = \frac{1}{\left(\frac{1}{k_2 C_s^2}\right) + \left(\frac{t}{C_s}\right)}$$  \hspace{1cm} (9)

The initial rate of extraction $h$, with $C_t = t$ when $t$ approaches 0, can be defined as Eq. (10).

$$h = k_2 C_s^2$$  \hspace{1cm} (10)

Equation (10) can be changed again so that it finally gets Eq. (11).

$$\frac{t}{C_t} = \frac{t}{C_s} + \frac{1}{h}$$  \hspace{1cm} (11)

$C_t$ is extraction capacity (mg/g) at time $t$. The initial extraction rate $h$, the extraction capacity (mg/g) at equilibrium $C_s$ and the extraction rate constant for second-order ($k_2$) can be determined experimentally from the slope and intercept by making a plot between $\left(\frac{t}{C_t}\right)$ and $t$. After that, the model is fitted to approach the SWE process. The error between the model and the experimental data are expressed as absolute average relative deviation (AARD) determined by Eq. (12), where $Q_{exp}$ is the experimental value, $Q_{mod}$ is the model value, and $N$ is the amount of data.

$$\text{AARD} = \frac{1}{N} \left( \sum_{i=1}^{N} \frac{|Q_{mod} - Q_{exp}|}{Q_{exp}} \right) \times 100\%$$  \hspace{1cm} (12)

**Statistical Data Analysis**

For statistical analysis of data experiment and the kinetics model, analysis of variance (ANOVA) was used to analyze the significance of experimental parameter. The parameter was significant if $P < 0.05$.

**RESULTS AND DISCUSSIONS**

In this research, experiments were carried out at various temperatures 140°C, 160°C, and 180°C with pressure 7 MPa, solvent flow rate 1 ml/min, and various raw materials G. lucidum, Gracilaria sp, and E. cottonii. From all variables, the yield of the SWE process was obtained.
Figure 2 shows that the optimum operating temperature with the highest yield for *E. cottonii* and *G. lucidum* are 180°C. The yield of extract for *E. cottonii* and *G. lucidum* increased with increasing operating temperatures in the range of 140°C-180°C. It occurs because the increasing operating temperature will result in decreasing surface tension, solute viscosity, and dielectric constant. It causes the increasing extraction efficiency and decreasing polarity that results in dissolving non-polar compounds (Min-Jung et al., 2011). But the yield of the SWE process decreased if the temperature was operated upper than the degradation temperature and loss of desirable compounds (Kubatova et al., 2001). The degradation temperature of *E. cottonii* and *G. lucidum* is 211°C (Jumaidin et al., 2017) and 229.68°C (Yu X. et al., 2019). For *Gracilaria sp* the highest yield was at temperature 160°C. According to the result reported by Min-Jung et al. (2011), the yield of *Gracilaria sp* extract would be lowered due to the hydrolysis reaction of the Glacilaria sp-skin cell-wall matrix. The hydrolysis reaction was caused by an increasing ionization constant of water and occurs at a temperature of 165°C (Bundrat and Shotipruk, 2008). For *Gracilaria sp*, the hydrolysis reaction might cause degradation of water-soluble extract and char formation, decreasing the yield. This result agrees with previous work by Asl and Khajenoori (2013) to extract thymol and carvacrol from *Z. multiflora*. They reported that the yield of thymol decreased at 175°C due to the formation of char with a burning smell of extract. From the three raw materials, the highest yield of the SWE process was found in *E. cottonii* because temperature 180°C is still under and near the degradation temperature. Therefore, the non-polarity of solvent is maximum and *E. cottonii* has not been degraded.

The evaluation of extraction rate using kinetics models was carried out by fitting the models with the experimental data. The experimental result approached the second-order kinetics model from all raw materials, and the values of AARD were less than 6%, as shown in Table 1. The first-order kinetics model does not fit the experimental data with the AARD more than 100% for all materials at 140°C. Figures 3, 4, and 5 show the comparison of extraction yield obtained from the experimental result and the calculation using the second-order kinetics model for the SWE process of *G. lucidum*, *Gracilaria sp*, and *E. cottonii* at various temperatures.

![Fig. 2: Yield of subcritical water extraction](image)

![Fig. 3: Extraction kinetics model for *G. lucidum* at various temperatures](image)
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fitting the models with the experimental data. The experimental result approached the second-order kinetics model from all raw materials, and the values of AARD were less than 6%, as shown in Table 1. The first-order kinetics model does not fit the experimental data with the AARD more than 100% for all materials at 140°C. Figures 3, 4, and 5 show the comparison of extraction yield obtained from the experimental result and the calculation using the second-order kinetics model for the SWE process of *G. lucidum*, *Gracilaria* sp, and *E. cottonii* at various temperatures.

![Fig. 4: Extraction kinetics model for *Gracilaria* sp at various temperatures.](image)

![Fig. 5: Extraction kinetics model for *E. Cottonii* at various temperatures.](image)

The calculated extraction yield of the kinetics model was determined from $C_t$ as extraction capacity at time $t$ in g extract/g sample loaded in the extractor. The result shows that the extraction process was influenced by more than one factor. The extraction was not only controlled by limitation solubility, but also desorption and intramolecular interaction. This result corresponds to Kubatova et al. (2001), who explained that for the SWE process with solvent flow rate 1 to 2 ml/min, the extraction process was not completely dependent on flowrate but also was controlled by desorption kinetic. Since the driving force of desorption from the matrix into the fluid is the concentration gradient from the solid to the extraction fluid, and then it affects the extraction kinetic rate to make two regions in the curve of kinetic rate “fast region” ($k_1$) and “slow region” ($k_2$). Kinetics rate constants resulted from the fitting of first and second-order kinetic models with the experimental data for *G. lucidum*, *Gracilaria* sp, and *E. cottonii* are listed in Table 1. Temperature significantly affected the extraction rate of all materials. The kinetics rate constant significantly increased with the increasing temperature. It also can be observed in Table 2 for the result of statistical analysis using ANOVA. The extraction yield and kinetics rate constants for all materials have $P$-value $< 0.05$. It means that temperature has a significant effect on the yield and kinetic or extraction rate.

The result of high-performance liquid chromatography (HPLC) analysis, the chromatogram of phytochemical compounds extracted from *E. cottonii*, *Gracilaria* sp, and *G. lucidum*, was demonstrated in Figure 6. Retention time and intensity for HPLC analysis are based on Su et al. (2001) and Suriyayathana et al. (2016), and Macmudah et al. (2017). The type of phytochemical compounds for *G. lucidum*, *Gracilaria* sp, and *E. cottonii* can be seen in Table 3.
### Table 1. Kinetics rate constants for first and second-order kinetic models

<table>
<thead>
<tr>
<th>Raw Material</th>
<th>Temperature (°C)</th>
<th>Kinetics Model</th>
<th>First Order</th>
<th>Second Order</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$k_1$ (min$^{-1}$)</td>
<td>$k_2$ (g/mg.min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$C_s$ (mg/g)</td>
<td>$C_s$ (mg/g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AARD (%)</td>
<td>AARD (%)</td>
</tr>
<tr>
<td><em>G. Lucidium</em></td>
<td>140</td>
<td></td>
<td>0.0131</td>
<td>2.179x10$^{-4}$</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td></td>
<td>0.01532</td>
<td>4.0128</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td></td>
<td>0.2708</td>
<td>4.49</td>
</tr>
<tr>
<td><em>Gracilaria sp</em></td>
<td>140</td>
<td></td>
<td>0.0113</td>
<td>1.9497</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td></td>
<td>0.12788</td>
<td>15.5280</td>
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<tr>
<td></td>
<td>180</td>
<td></td>
<td>186.53</td>
<td>5.46</td>
</tr>
<tr>
<td><em>E. Cottonii</em></td>
<td>140</td>
<td></td>
<td>0.0101</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>160</td>
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<td>4.0128</td>
<td>1.9497</td>
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<td></td>
<td></td>
<td></td>
<td>0.182x10$^{-4}$</td>
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<td>4.4248</td>
<td>1.9497</td>
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<td></td>
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<td>147.43</td>
<td>5.46</td>
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### Table 2. Analysis of variance (ANOVA) for all materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Yield</th>
<th>$k_1$</th>
<th>$k_2$</th>
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<tr>
<td></td>
<td>F</td>
<td>P value</td>
<td>F</td>
</tr>
<tr>
<td><em>G. lucidum</em></td>
<td>190.691</td>
<td>0.000159</td>
<td>191.962</td>
</tr>
<tr>
<td><em>Gracilaria sp</em></td>
<td>190.365</td>
<td>0.000160</td>
<td>191.972</td>
</tr>
<tr>
<td><em>E. cottonii</em></td>
<td>190.178</td>
<td>0.000160</td>
<td>191.971</td>
</tr>
</tbody>
</table>
Yield and Extraction Rate Analysis of Phytochemical Compounds from *Eucheuma cottonii*, *Ganoderma lucidum*, and *Gracilaria* sp. using Subcritical Water Extraction

**Table 3.** Types of Phytochemical Compound (Su et al. (2001), Suriyayathana et al. (2016), Macmudah et al. (2017))

<table>
<thead>
<tr>
<th>Raw Material</th>
<th>Phytochemical</th>
<th>Molecular Formula</th>
<th>Molecular Weight (g/mol)</th>
<th>Type of Phytochemical</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. lucidum</em></td>
<td>Ganodermic Acid Am₂</td>
<td>C₃₀H₄₄O₇</td>
<td>516</td>
<td>Triterpenoid</td>
</tr>
<tr>
<td></td>
<td>Ganodermic Acid B</td>
<td>C₃₀H₄₄O₇</td>
<td>516</td>
<td>Triterpenoid</td>
</tr>
<tr>
<td></td>
<td>Ganodermic Acid C₂</td>
<td>C₃₀H₄₆O₇</td>
<td>518</td>
<td>Triterpenoid</td>
</tr>
<tr>
<td></td>
<td>Gallic Acid</td>
<td>C₇H₆O₅</td>
<td>170</td>
<td>Phenolic</td>
</tr>
<tr>
<td></td>
<td>Caffeic Acid</td>
<td>C₉H₈O₄</td>
<td>180</td>
<td>Phenolic</td>
</tr>
<tr>
<td></td>
<td>Rutin</td>
<td>C₂₇H₃₀O₁₆</td>
<td>611</td>
<td>Flavonoid</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>C₁₅H₁₀O₇</td>
<td>302</td>
<td>Flavonoid</td>
</tr>
<tr>
<td></td>
<td>2-cyclooxygenase</td>
<td>COX-2</td>
<td>267</td>
<td>Triterpenoid</td>
</tr>
<tr>
<td></td>
<td>β-carotene</td>
<td>C₄₀H₅₆</td>
<td>537</td>
<td>β-carotene</td>
</tr>
<tr>
<td></td>
<td>Ferulic Acid</td>
<td>C₁₀H₁₉O₄</td>
<td>194</td>
<td>Phenolic</td>
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<tr>
<td></td>
<td>Quercetin</td>
<td>C₂₇H₃₀O₁₆</td>
<td>611</td>
<td>Flavonoid</td>
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<td></td>
<td>Rutin</td>
<td>C₁₅H₁₀O₇</td>
<td>302</td>
<td>Flavonoid</td>
</tr>
<tr>
<td><em>Gracilaria</em> sp</td>
<td>Carrageenan</td>
<td>High molecular weight polysaccharides made up of repeating galactose units and 3,6-anhydrogalactose (3,6-AG), both sulfated and nonsulfated.</td>
<td>&gt; 100.000</td>
<td>Carrageenan</td>
</tr>
<tr>
<td><em>E. cottonii</em></td>
<td>Quercetin</td>
<td>C₂₇H₃₀O₁₆</td>
<td>611</td>
<td>Flavonoid</td>
</tr>
<tr>
<td></td>
<td>Rutin</td>
<td>C₁₅H₁₀O₇</td>
<td>302</td>
<td>Flavonoid</td>
</tr>
<tr>
<td></td>
<td>Ecdysteroid</td>
<td>20E</td>
<td>481</td>
<td>Steroid</td>
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<tr>
<td></td>
<td>Linoleic Acid</td>
<td>C₁₈H₃₂O₂</td>
<td>280</td>
<td>Phenolic</td>
</tr>
<tr>
<td></td>
<td>Carrageenan</td>
<td>High molecular weight polysaccharides made up of repeating galactose units and 3,6-anhydrogalactose (3,6-AG), both sulfated and nonsulfated.</td>
<td>&gt; 100.000</td>
<td>Carrageenan</td>
</tr>
</tbody>
</table>
From Figure 6 and Table 3 can be seen that all materials contain phenolic and flavonoid compounds. *G. lucidum* contains triterpenoid, phenolic, and flavonoid compounds. *Gracilaria* sp. contains carotene, phenolic, flavonoid, and carrageenan. *E. cottonii* contains phenolic, steroid, flavonoid, and carrageenan. The Ganoderic acids are the most desirable compounds from *G. lucidum* due to their ability to protect against liver injury caused by the Hepatitis B virus (Li and Wang, 2006). For *Gracilaria* sp and *E. cottonii*, the antioxidant compounds, such as phenolic (gallic acid and caffeic acid) and flavonoid (quercetin and rutin), are the essential components due to their biological activities (Machmudah et al., 2017). *Gracilaria* sp and *E. cottonii* also contain polysaccharide carrageenan with high antioxidant efficiency.

**CONCLUSIONS**

This study aimed to determine the extraction rate using the kinetics model and analyze the extracted compounds by HPLC. The result showed that subcritical water could replace organic solvent for the extraction process. The following optimal temperatures were found in this research: the optimum extraction temperature for *E. cottonii* and *G. lucidum* was 180°C, and for *Gracilaria* sp was 160°C at pressure 7 MPa and flowrate 1 ml/min. The yield of the SWE process under the optimum temperature was 85.37%, 58.42%, and 75.73% for *E. cottonii*, *G. lucidum*, and *Gracilaria* sp, respectively. Kinetic model for SWE process of *E. cottonii*, *Gracilaria* sp, and *G. lucidum* followed the second-order kinetics models with AARD less than 6%. The second-order kinetics constants increased with an increasing temperature with the range from $0.182 \times 10^{-4}$ to $12.915 \times 10^{-4}$. Phytochemicals were successfully extracted with SWE and have been confirmed with HPLC analysis. The type of extracted phytochemicals compounds for *G. lucidum* was triterpenoid, phenolic, and flavonoid.
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Glacilaria sp. were triterpenoid, β-carotene, phenolic, flavonoid, and carrageenan. At last for \textit{E. cottonii} were flavonoid, steroid, phenolic, and carrageenan.

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