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## Effect of Harvest Age and Solvents on the Phenolic Content of *Eucheuma cottonii* Extract

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### Abstract

Bioactive compounds in *Eucheuma cottonii* include phenols or polyphenols with antioxidant, antibacterial, and antiviral properties. Seaweed quality, including phenolic content, is highly determined by several factors, including the growth location, cultivation technique, and harvest age. This study aimed to determine the effect of harvest age and solvent type on the phenolic content of *E. cottonii*. The effects of harvest age on water content and of simplicia on the extraction process were also determined. Phenolic active ingredients were extracted at three harvest ages (35, 40, and 45 days) and using four solvents (ethanol, ethyl acetate, hexane, and water). Analysis of variance was performed to determine the effects of both factors and their interactions. The older the seaweed, the lesser the water content. The powder produced at all ages is a good simplicia for materials extraction. Results showed that high phenolic content was obtained from the *E. cottonii* extract at the age of 35 days with ethyl acetate as the solvent.

*Keywords:* harvest age, active compound, *Eucheuma cottonii*, solvent

### Introduction

Seaweed is the main commodity in fishery exports, and its export volume increased by 6.02% from 2012 to 2017 [1]. Indonesia is the largest exporter of seaweed worldwide, dominated by seaweeds such as *Eucheuma cottonii* and *Gracillaria*. Given their extensive nutritional content and bioactivity, seaweeds are an important commodity with the potential to be continuously developed. They contain metabolites or active compounds, including polyphenols with antimicrobial, antioxidant, and anticancer properties [2,3].

Phenol or polyphenols are found in land and aquatic plants. Phenol (C<sub>6</sub>H<sub>6</sub>OH) is an organic compound with a hydroxyl group attached to the benzene ring [4]. Some types of seaweed contain phenols, which serve as a functional food [5]. Phenolic compounds in various types of seaweed reportedly exhibit antioxidant activity [6]. In fact, the antioxidant activity and other bioactivities of seaweed are conferred by phenolic compounds as secondary metabolites [7]. Specifically, *E. cottonii* has been proven to contain phenols as antioxidants [8,9].

The phenolic content in seaweed extract is determined by the extraction method and conditions [10]. It is also determined by the seaweed type, physiological conditions, climate, and cultivation environment [5]. Solvent type is a crucial factor in the extraction process. Selection of solvent is based on its polarity during extraction. Polar compounds only dissolve in polar solvents, such as ethanol, methanol, butanol, and water. Non to polar compounds only dissolve in non-polar solvents, such as ether, chloroform, and n-hexane [11]. Hence, the selection of suitable solvents is a crucial factor in extraction. The type and quality of the solvent determines the success of extraction [12].

Among different solvents, methanol produces the highest extraction yield of phenolic compounds [11]. Thus, methanol was used to extract polyphenols from brown seaweed [13]. Meanwhile, ethanol combined with water in a ratio of 1:1 yields the highest polyphenol contents [14]. A previous study reported that methanol extracts active ingredients with the best activity [15], but another study reported that ethyl acetate produces the highest yields of active ingredients [16].

Therefore, identifying the type of solvent that yields the highest phenolic content is important.

Seaweed quality, including the contents of phenols and other secondary metabolites, is largely determined by several factors, such as growth location, cultivation method, and harvest time [17]. Similarly, Kim [18] explained that the main components of seaweed, such as polysaccharides, minerals, proteins, fucoidan, lipids, and phenols, vary depending on the harvest time. However, little is known about the influence of harvest age on phenolic content. Some studies linked the effect of harvest age on the contents of primary metabolites, such as carrageenan and alginate. Carrageenan level increases with increasing harvest age [19]. The longer the harvest, the lower the nutritional value [20]. In addition, alginate amount decreases with increasing harvest age [21]. The phenolic content in seaweed is influenced by harvest age [22]. However, the effects of harvest age and solvent type on the phenolic content of seaweed, especially *E. cottonii*, are still limited. Therefore, the present study investigated the optimum harvest age and solvent type to produce phenolic compounds. In addition, the interaction between the harvest age of *E. cottonii* and the solvent type that can produce the highest phenolic content can be obtained.

## Methods

Seaweed used was *E. cottonii* originating from the village of South Lampung Hanura. The solvents used were hexane (Merck), ethyl acetate (Merck), ethanol (Merck), and water, which are non-polar, semi-polar, and polar, respectively. Each extraction was carried out on *E. cottonii* with 3 harvest ages, namely, 35, 40, and 45 days. Each treatment was repeated twice. Folin–Ciocalteu reagents, gallic acid, and  $\text{Na}_2\text{CO}_3$  were used in the total phenol analysis.

The equipment used included a shaking water bath (Lab Companion BS-21) for maceration, a Buchner vacuum for filtering, a shaker (type 37600 mixer, Barnstead Thermolyne), an oven, and a Ultraviolet-Visible (UV-Vis) HP spectrophotometer. Glass tools such as test tubes, beaker cups, scales, and Erlenmeyer flasks were also used.

**Simplicia preparation.** Fresh red seaweed (*E. cottonii*) at 3 harvest ages of 35, 40, and 45 days were washed and then oven dried at 50 °C–60 °C for 9–12 h in 2 days. The dried seaweed was smoothed and sifted to a size of 100 mesh [16].

**Extraction.** The maceration extraction of *E. cottonii* with various harvest ages was carried out using hexane (96%), ethyl acetate (96%), ethanol (96%), or water as a solvent at a 1:10 (w/v) ratio of *E. cottonii* powder and solvent [16]. *E. cottonii* samples at every harvest age

were extracted with ethanol, hexane, ethyl acetate, or water with two replications each.

Maceration was performed using a water shaker (lab Companion BS-21) at 120 rpm at room temperature for 24 h. Total phenolic content was measured with gallic acid as a reference standard, and the results were expressed as a percent of gallic acid equivalent (GAE) [8,23]. The extract with the highest phenolic content in a certain solvent and harvest age was determined.

**Total phenol level test.** Total phenolic content was determined using gallic acid as a reference standard, and the results were expressed as percent of GAE. The extract with the highest phenolic value in a certain solvent and harvest age was identified. A 0.5 mL aliquot of the extract solution was added to 5 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent 50% (v/v). The mixture was left to stand for 5 min then added with 1 mL of  $\text{Na}_2\text{CO}_3$  7.5% (b v). The mixture was homogenized and incubated in dark conditions for 1 h. The resulting absorption was obtained with a UV-Visible spectrophotometer at a wavelength of 730 nm. Gallic acid was used as a standard with concentrations of 0, 10, 30, 50, 70, and 100 mg/L. The total phenol of *E. cottonii* extract was expressed in mg GAE/g extract [24].

**Statistical analysis.** The experiment was carried out with two factors, namely, the three harvest ages (35, 40, and 45 days) and four types of solvents (ethanol, ethyl acetate, hexane, and water), using a completely randomized design with two replications for each harvest age and solvent type. The effect of factors and their interactions was determined through ANOVA. Significant difference between the treatments was determined using least significant difference test. Data analysis was performed using SPSS software version 19 and MS Excel.

## Results and Discussion

**Effect of harvest age and solvent on phenol produced** ANOVA showed that the factors of harvest age, solvent type, and their interaction significantly affected the phenols produced. Harvest age, solvent type, and their interactions significantly influenced the total phenol extracted. The phenolic amount in the *E. cottonii* extract with various types of solvents and harvest age is presented in Table 1.

**Effect of harvest age on phenol produced.** The phenolic contents of *E. cottonii* extracts aged 35, 40, and 45 days were 1.86–6.41, 0.79–2.09, and 0.77–1.89 mg GAE/g extract, respectively. The highest phenolic content of  $6.41 \pm 1.75$  mg GAE/g extract was produced from the *E. cottonii* extract with the harvest age of  $35 \pm$  days. Experimental results proved that the harvest age affects the phenolic content. The highest phenolic value at 35 days of harvest significantly differed from the phenolic

**Table 1. Total Phenolic *E. cottonii* Extract with Various Types of Solvents and Harvest Age**

Solvent	Harvest age (days)		
	35	40	45
Ethanol	3.54 ± 1.77 <sup>a</sup>	0.79 ± 0.19 <sup>a</sup>	0.77 ± 0.32 <sup>a</sup>
Ethyl acetate	6.41 ± 1.75 <sup>b</sup>	2.09 ± 0.61 <sup>a</sup>	1.16 ± 0.66 <sup>a</sup>
Hexane	2.62 ± 0.55 <sup>a</sup>	1.98 ± 1.01 <sup>a</sup>	1.31 ± 0.09 <sup>a</sup>
Water	1.86 ± 0.39 <sup>a</sup>	2.04 ± 1.12 <sup>a</sup>	1.89 ± 0.03 <sup>a</sup>

\*The numbers in the same column followed by different letters show significant differences ( $\alpha=0.05$ )

values at 40 and 45 days, whereas no significant difference in phenolic value was determined between the ages of 40 and 45 days. This result indicates that the *E. cottonii* extract with the harvest age of 35 days produces the highest phenolic content. Thus, the phenolic content reaches its peak at the harvest age of 35 days and decreases over time.

A previous study reported the same result that *E. cottonii* with a harvest age of 35 days produces the highest phenolic content [22]. The limited analysis of the effect of seaweed harvest age on phenolic content was caused by the lack of information about factors influencing seaweed harvest age. A previous research [25] also found abundant research related to the content of phenols, especially flavonoids, in terrestrial plants but not in seaweeds. Most researchers only explored the effects of harvest age and solvent type on the primary metabolites produced, namely, carrageenan. In a previous work, terrestrial plant *Talinum triangulare* at 30 and 60 days of harvest yields phenols at  $0.19 \pm 0.02$  and  $0.16 \pm 0.03$  mg GAE/g extract, respectively [26].

In the present experiment, phenol represented the active ingredient in *E. cottonii* extract. Other active ingredients in *E. cottonii* are carrageenan and fucoidan. All active compounds produced by seaweed are influenced by several factors, including seaweed type, age, collection site, climate, and temperature [23, 27]. Consequently, a difference between one of them could have produced different active ingredients. The growth rate and growing quality of seaweed also affect the active ingredients produced. The rate and quality of seaweed growth may differ even in the same habitat because of differences in nutrition and weather. The age of seaweed influences the fucoidan produced [28]. In one study, the best carrageenan is produced by 45-day-old *E. cottonii* [17]. In another study, the best carrageenan is produced by 20-day-old *E. cottonii* [29]. In general, the carrageenan content increases with increasing harvest age, reaches the peak at 45 days, and decreases over time [30].

**Effect of solvent type on the phenol produced.** Table 1 illustrates that the phenol in *E. cottonii* extract is the most stable in ethyl acetate solvent. The highest phenolic content in the ethyl acetate extract was  $6.41 \pm 1.75$  mg GAE/g extract. This value is higher than that obtained in

the *E. cottonii* ethyl acetate extract (i.e., 0.024387 mg GAE/g extract) from Talango village, Sumenep Madura, East Java Province [31]. In this experiment, ethyl acetate was able to dissolve the highest phenol present in the *E. cottonii* extract. A similar result was reported in Ref. [8], where ethyl acetate was also proven the best solvent in the extraction of *E. cottonii* to produce sunscreen. Among different solvents, ethyl acetate provided the best toxicity in *E. cottonii* extraction [32]. By contrast, several studies reported that the best solvent for the bioactive extraction of *E. cottonii* is methanol [8,33] or ethanol [34,35]. Meanwhile, the use of different solvents, including hexane, ethanol, methanol, acetone, acetone–methanol mixture (7:3), and hexane–ethanol mixture (9:1), exerts no significant influence on the total phenolic content from *Sargassum* maceration [36]. Phenolic compounds are generally easily extracted by semi-polar and polar organic solvents [37], considering that most phenolic compounds are polar in nature [38]. The polarity degrees of ethyl acetate, ethanol, hexane, and water are 4.4, 4.3, 0.1, and 10.2, respectively.

The polarity of phenolic compounds in this experiment cannot be determined because phenol represents a group of chemical compounds with different degrees of polarity. The difference in phenol solubility in solvents is influenced not only by polarity but also by the molecular structure and material solubility in the solvent. Thus, despite the similar polarities of ethanol and ethyl acetate, the maximum solubility of an ingredient in the two solvents can still be different. This phenomenon can explain the different extraction results in various solvents.

**Effect of interaction between harvest age and solvent type on the phenol produced.** ANOVA results showed that the interaction between harvest age and solvent type affected the phenolic content. The ANOVA results are presented in Table 2.

At 35 days of harvest, the total value of phenol with ethyl acetate solvent was  $6.41 \pm 1.75$  mg GAE/g extract (Figure 1). The total phenol produced with ethyl acetate as a solvent significantly differed from those produced when other solvents (ethanol, hexane, and water) were used, but no significant difference was found among those produced with ethanol, hexane, and water. These results suggest that the phenolic compound in the *E.*

*cottonii* extract at 35 days showed the highest solubility in ethyl acetate. In other words, the 35-day harvest is the peak production of phenol with ethyl acetate as a solvent.

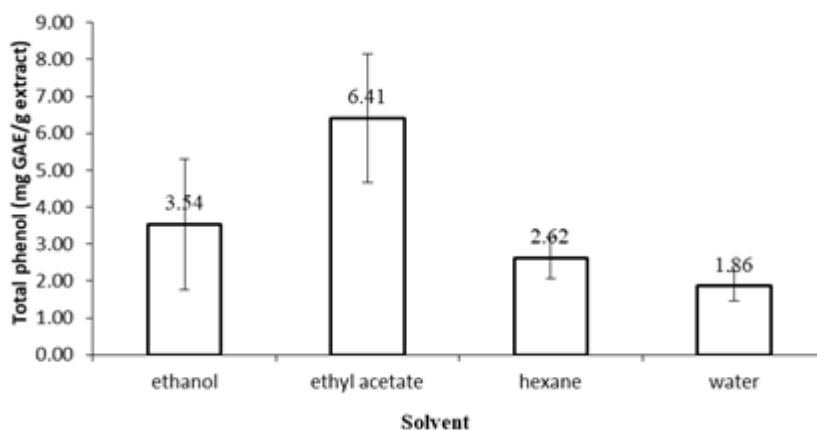
The phenolic content at the age of 40 days was 0.79–2.09 mg GAE/g extract with the highest value of  $2.09 \pm 0.61$  mg GAE/g extract (Figure 2). The analysis of the least significant difference showed that the harvest age of 40 days was not significantly different in all the solvents used. This result indicates that all phenols contained in *E. cottonii* extract at 40 days of harvest

have the same solubility in ethanol, ethyl acetate, hexane, and water. At a harvest age of 40 days, water is selected as a solvent for *E. cottonii* extraction for economic reasons.

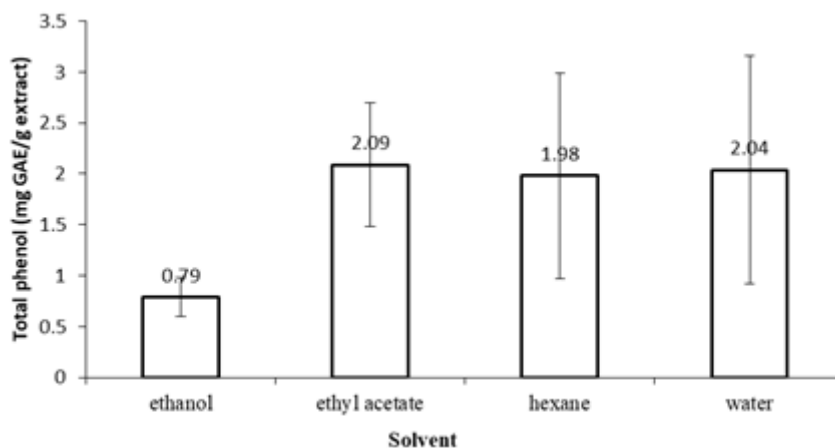
The highest phenol value at 45 days was  $1.89 \pm 0.03$  mg in water. However, ANOVA results revealed no significant difference among the solvents used (Figure 3). This result indicates that the phenolic compound in the 45-day extract has the same solubility in all solvents. Thus, water was chosen to extract 45-day-old *E. cottonii* seaweed because of economical reasons.

**Table 2. Analysis of Variance for Solvent and Harvest Age**

Source of Variation	SS	df	MS	F	P-value	F crit
Solvent	8.50995	3	2.836649964	3.93812	0.036133	3.490295
Harvest age	24.38537	2	12.19268331	16.9271	0.000321	3.885294
Interaction	18.94273	6	3.157121442	4.38303	0.014215	2.99612
Within	8.643668	12	0.720305627			
Total	60.48171	23				



**Figure 1. Total Phenolic *E. cottonii* Extract at 35 Days of Harvest**



**Figure 2. Total Phenolic *E. cottonii* Extract at 40 Days of Harvest**

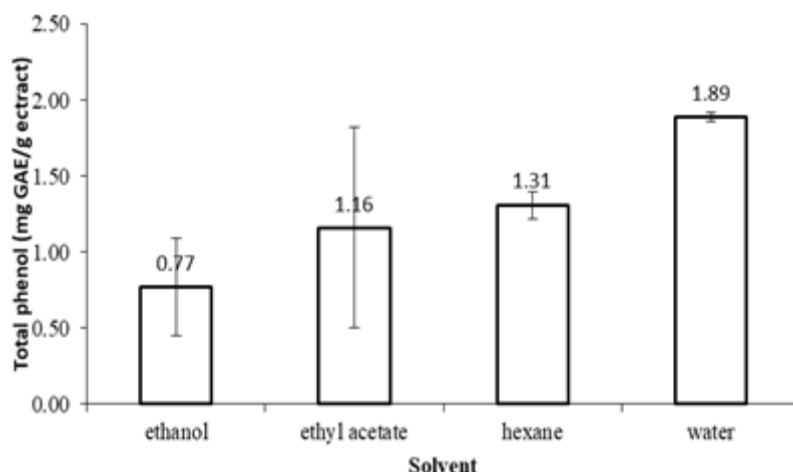


Figure 3. Total Phenolic *E. cottonii* Extract at 45 Days of Harvest

On the basis of these results, *E. cottonii* extraction at the highest harvest age of 35, 40, and 45 days produced the highest phenol solubility successively in ethyl acetate, water, and water solvents. However, when considering the total phenolic amount, the best conditions with the highest phenol solubility was obtained at the harvest age of 35 days and with the solvent of ethyl acetate.

In general, this study provided information about the phenolic content as an active ingredient at various harvest ages and solvents. This information is used as preliminary data for the exploration of phenols as active ingredients and their bioactivity. The research is expected to be an effort to utilize *E. cottonii* in the study area by increasing the added value of the product through its bioactivity content. Therefore, different growing regions of *E. cottonii* that affect phenolic content as active ingredients can be categorized as the research novelty.

## Conclusion

The harvest age and solvent type significantly affect the phenols produced by *E. cottonii* extract. The best solvent and harvest age to produce the highest phenol in the extraction of *E. cottonii* were obtained. The highest phenolic content was  $6.41 \pm 1.75$  mg GAE/g extract produced by *E. cottonii* extract aged 35 days in ethyl acetate solvent.

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