Phytochemical Screening of Seaweed Flour (*Eucheumacottonii*) Using Various Organic Solvents and Its Application in Tilapia Feed (*Oreochromisniloticus*)

ABSTRACT

This study aims to qualitatively analyze the bioactive compounds in Eucheumacottonii seaweed flour extract and its application in commercial feed for tilapia (*Oreochromisniloticus*). The methodology employed is experimental and consists of two phases. Phase 1 involves the extraction of *E. cottonii* flour using three different organic solvents with varying polarity (ethanol, ethyl acetate, and a combination of ethanol + ethyl acetate). The testing parameters include qualitative phytochemical tests for alkaloids, steroids/triterpenoids, saponins, flavonoids, and tannins. Phase 2 consists of the application of the extract to tilapia over a 30-day rearing period with four treatments: control/commercial feed (P0), commercial feed + ethanol extract (P1), commercial feed + ethyl acetate extract (P2), and commercial feed + combined ethanol and ethyl acetate extract (P3). The parameters tested include absolute weight, specific growth rate, survival rate, and water quality. Phytochemical and water quality data are presented in table format. Meanwhile, growth and survival data are analyzed using ANOVA, followed by Duncan's test for significant differences. The results of the researches carried out, it can be concluded that the phytochemical analysis of *E. cottonii* extract in ethanol and the ethanol + ethyl acetate combination yielded similar compounds: alkaloids, steroids/triterpenoids, and flavonoids. The ethyl acetate extract contained triterpenoids and flavonoids. Both saponins and tannins returned negative results across all organic solvent treatments. All treatments with the addition of *E. cottonii* seaweed extract in various organic solvents demonstrated better growth in tilapia compared to the control treatment

*Keywords: Eucheuma**cottonii*;extract; phytochemistry; tilapia; organic solvents

1. INTRODUCTION

Seaweed contains secondary metabolites that have the potential to produce diverse bioactive metabolites with a wide range of activities, including antibacterial [1], antiviral [2], antifungal [3[, and cytotoxic [4] properties. According to Hashimoto[5], the chemical compounds resulting from the secondary metabolism of seaweed that act as antibacterial agents include fatty acids, terpenoids, bromophenols, and tannins. Darusman et al.[6] also explained that marine-derived natural products are a result of secondary metabolites from various groups, including alkaloids, terpenoids, and flavonoids, and can also directly originate from primary metabolite compounds such as dipeptides.

The presence of bioactive compounds in seaweed can be assessed using methods that provide information about these compounds. One such method is phytochemical screening [7]. Phytochemical analysis involves a series of processes or techniques to identify and

characterize the types of bioactive compounds in seaweed, such as alkaloids, flavonoids, and tannins, qualitatively [8]. Bioactive compounds resulting from secondary metabolism can be obtained through extraction processes. The solvents used in extraction can be polar, semi-polar, or non-polar [9].

Previous studies have shown that the ethanol fraction of *E. spinosum* contains triterpenoids, alkaloids, and flavonoids [10]. Research byHanapi et al.[11] demonstrated that the active compounds in the methanol extract of *E. spinosum* include flavonoids, triterpenoids, and ascorbic acid. Similar findings were reported by [12], indicating that phytochemical screening of both fresh and dried *E. spinosum* extracts contained flavonoids, alkaloids, terpenoids, saponins, and tannins. Meanwhile, phytochemical tests of the methanol extract from *E. cottonii* qualitatively revealed the presence of alkaloids, flavonoids, steroids/terpenoids, and saponins. The n-hexane fraction contained steroids/terpenoids and saponins, while the ethyl acetate fraction contained alkaloids, flavonoids, and steroids/terpenoids, and the butanol fraction contained alkaloids [9].The addition of *K. alvarezii* ethanol extract 1:5 to the feed showed the best results because it could increase the growth and utilization of tilapia feed with a survival rate of tilapia of 83% [13]. Meanwhile, the addition of 1:5 ethyl acetate extract can increase growth and better feed utilization in tilapia with a survival rate of tilapia of 90%. [14].

Based on the description above, this study aims to qualitatively analyze the bioactive compounds in Eucheumacottonii seaweed flour extract and its application in commercial feed for tilapia (*Oreochromisniloticus*)

2. MATERIAL AND METHODS

2.1 Materials and Methodology

The materials used in the extraction process include *E. cottonii* flour, ethanol, and ethyl acetate. The materials for phytochemical testing include *E. cottonii* flour extract, Mg-HCl (magnesium chloride), NaOH (sodium hydroxide), H_2SO_4 (sulfuric acid), Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Liebermann-Burchard reagent, distilled water, and $FeCl_3$. All chemicals used are classified as PA (Pro-analysis). The materials for the feed test include Hi Pro Vite 783 commercial feed and tilapia.

This research was carried out in October – December at the Laboratory of Fish Production and Reproduction, University of Mataram. This study employs an experimental method consisting of two phases :
Phase 1.Preparation of crude extract and identification of phytochemical compounds: the treatments involve organic solvents with varying polarity (ethanol, ethyl acetate, and a combination of ethanol + ethyl acetate). The parameter tested is the qualitative identification of phytochemical compounds in *E. cottonii* flour extract.

Phase 2. Application testing of the crude seaweed extract in feed: This phase evaluates the growth and survival of tilapia cultivated for 60 days. The treatments tested are the effects of adding *E. cottonii*extract with different solvents to commercial tilapia feed, as follows:

P1: Commercial feed (control)
P2: Commercial feed + ethanol extract
P3: Commercial feed + ethyl acetate extract
P4: Commercial feed + combination of ethanol and ethyl acetate extracts

The experimental design used in this phase was a Completely Randomized Design (CRD) consisting of four treatments, each repeated three times, resulting in a total of 12 treatments.

2.2Preparation of Seaweed

The seaweed used was fresh seaweed obtained from cultivators. A total of 2 kg of fresh seaweed was air-dried for 14 days, depending on weather conditions, until fully dried. The dried seaweed was then cut into small pieces using scissors. The cut seaweed was ground using a blender and sieved to obtain flour.

2.3Preparation of Seaweed Extraction

The extraction process of *E. cottonii* flour was conducted using a maceration method. Ground seaweed was weighed at 100 g and placed into three Erlenmeyer flasks, to which 96% ethanol, ethyl acetate, and a combination of ethanol + ethyl acetate was added according to the treatment used. The ratio of extract to solvent used was 1:5 (m/v). The maceration time followed the guidelines from Purba et al. [15], lasting 30 hours at 30°C. During maceration, the solution was stirred and shaken every 5 hours. The macerated flour was then filtered using Whatman No. 42 filter paper to produce a filtrate. The resulting filtrate was evaporated at 40°C using a vacuum rotary evaporator [16]

2.4Qualitative Analysis of Bioactive Compounds (Followed The Guidelines FromHarborne[17])

2.4.1 Flavonoids

An amount of 15 mg of extract was added to 10 mL of ethanol and homogenized. It was then boiled for 5 minutes and filtered. Then, 5 mL of the filtrate was mixed with 0.05 mg of powdered Mg and 1 mL of concentrated HCl and shaken vigorously. A positive test is indicated by the formation of red, yellow, or orange color.

2.4.2 Alkaloids

An amount of 50 mg of extract was mixed with 2 mL of chloroform and 2 mL of ammonia and then filtered. The filtrate was treated with 3-5 drops of concentrated H_2SO_4 and shaken to form two layers. The acidic fraction is collected. Mayer's and Dragendorff's reagents (4-5 drops each) were added. The formation of a precipitate indicates the presence of alkaloids, with Mayer's reagent producing a white precipitate and Dragendorff's reagent yielding a reddish-orange precipitate.

2.4.3 Steroids and Terpenoids

A 50 mg extract was treated with 10 drops of concentrated CH_3COOH and 2 drops of concentrated H_2SO_4. The solution was gently mixed and allowed to stand for a few minutes. Steroids produce a blue or green color, while terpenoids yield a red or purple color.

2.4.4 Saponins

An amount of 50 mg extract was mixed with 10 mL of water and shaken for 1 minute, then 2 drops of 1N HCl were added. If stable foam forms for approximately 7 minutes, the extract is considered positive for saponins.

2.4.5 Tannins

An amount of 0.5 g of *E. spinosum* extract was infused with 10 mL of distilled water and then filtered. The resulting filtrate was diluted with water until colorless. Then, 2 mL of this solution was taken, and 1-2 drops of 1% ferric (III) chloride reagent were added. The development of blue or dark green color indicates the presence of tannins.

2.5Testing of the*E. cottonii*Extract in Tilapia Commercial Feed

The application of the extract to tilapia feed involved spraying the seaweed extract at a dosage of 2 g/kg of feed according to the treatment. Before spraying, the extract was diluted with distilled water, and after even application, the feed was air-dried. The feed was then provided to the tilapia in a 45 L container for 30 days, with a stocking density of 10 fish per container. Throughout the cultivation period, growth and water quality were measured every 10 days, along with monitoring fish mortality and performing siphoning. The parameters analyzed include absolute weight, specific growth rate (SGR), survival rate (SR), and water quality parameters such as temperature, pH, and dissolved oxygen (DO).

2.6Data Analysis

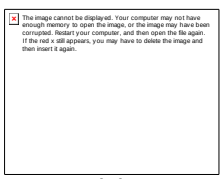
The results of the phytochemical tests and water quality are presented in table format. Meanwhile, the fish growth and survival rate data were analyzed using ANOVA, followed by Duncan's test if significant differences were observed

3. RESULTS AND DISCUSSION

3.1 Phytochemical Testing

The results of the phytochemical testing to identify secondary metabolite compounds qualitatively in *E. cottonii* seaweed using ethanol, ethyl acetate, and a combination of ethanol and ethyl acetate are presented in Table 1. A positive result (+) indicates the presence of specific compound groups, while a negative result (-) indicates their absence.

Table 1. Phytochemical Test Results of Eucheumacottonii Extract with Various Solvents

Phytochemical Testing	Standard Color (+)	Solvents		
		Ethanol	Ethyl Acetate	Ethanol + Ethyl Acetate
A. Alkaloids				
Dragendrof's Reagent	Orange-red precipitate	 (+)	 (-)	 (+)

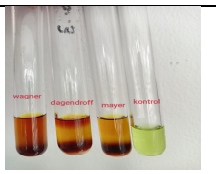
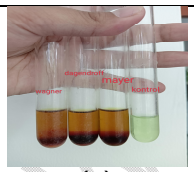
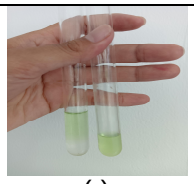
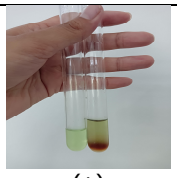
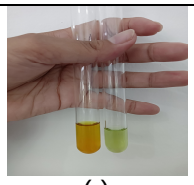
Meyer's Reagent	A white or yellowish precipitate	(+)	(-)	 (+)
:.Wanger's Reagent	Brown precipitate	(+)	(-)	 (+)
B. Triterpenoids and steroid	Greenish-blue and blue color (**steroid**)	(+)	(-)	 (+)
	Brown, red, orange, or purple ring (**triterpenoids**)	(+)	(+)	 (+)
C. Saponins	Stable foam for 3-5 minutes	(-)	(-)	 (-)
D. Flavonoids	Color change to red or yellow	(+)	(+)	 (+)
E. Tannins	Color change to bluish-black or green	(-)	(-)	 (-)

Table 1 shows that the extracts of *E. cottonii* in both ethanol and the combination of ethanol + ethyl acetate yielded the same positive results for the same compound groups, namely alkaloids, steroids/triterpenoids, and flavonoids. In contrast, the ethyl acetate extract contained triterpenoids and flavonoids. The groups of compounds saponins and tannins showed negative results for all treatments with the organic solvents.

The alkaloid test for *E. cottonii* in ethyl acetate showed no precipitate after reacting with Mayer's and Dragendorff's reagents. In contrast, both ethanol and the combination of ethanol + ethyl acetate resulted in precipitates when treated with these reagents. According toPurba[18], alkaloids contain nitrogen in their cyclic structure and have varying functional groups, such as amines, amides, phenols, and methoxy groups, which makes alkaloids semi-polar. This semi-polar characteristic allows alkaloids to be more soluble in semi-polar solvents and dissolve in both semi-polar and polar solvents [17]

The steroid and terpenoid tests using the Liebermann-Bouchard method showed positive results, with color changes observed in the ethanol and combination of ethanol + ethyl acetate extracts of *E. cottonii*. The ethyl acetate extract showed a positive result for steroids but negative for terpenoids due to the absence of color change. Saidi et al.[19] state that terpenoid and steroid compounds are soluble in non-polar to semi-polar solvents. Some triterpenoid compounds may have cyclic alcohol structures, contributing to their semi-polar nature [20]. Steroids can exist in glycoside forms. Glycosides, which consist of sugar and aglycone, have a polar sugar component that allows them to dissolve in polar solvents. Hence, steroids were detected in the methanol extract.

The bioactive compound test for saponins yielded negative results across all organic solvent treatments. The saponin test involves the hydrolysis of saponins in water. Saponins are triterpenoid glycosides that tend to be polar due to their glycosidic bonds [21]. According to Robinson[22], saponin compounds possess both polar and non-polar groups that exhibit surface-active properties. When saponins are shaken with water, hydrolysis occurs, forming micelles. The structure of these micelles causes the polar groups to face outward while the non-polar groups face inward, resulting in a foam-like appearance.

The identification of flavonoids in the *E. cottonii* flour extract, treated with magnesium powder (Mg) and 2 N hydrochloric acid (HCl), showed positive results across all organic solvent treatments due to the color change to red. This is attributed to the reduction reaction of flavonoids induced by hydrochloric acid and magnesium [23]. According toSjahid[24], flavonoids belong to the phenolic group and are polar compounds due to the presence of several untraceable hydroxyl groups or sugars, allowing them to dissolve in polar solvents such as methanol, ethanol, butanol, acetone, and dimethyl sulfoxide. The presence of flavonoids in all solvent treatments indicates that flavonoid compounds share similar polarity with ethanol and ethyl acetate, thus allowing the formation of flavonoids in these solutions.

3.2Testing of the*E. cottonii*Extract in Tilapia Commercial Feed

The ANOVA results for the application of the extract in commercial feed for tilapia during the 30-day cultivation period indicated that the addition of commercial feed with extracts of the seaweed *E. cottonii* using various organic solvents significantly affected the absolute weight and specific growth rate of tilapia, but it did not have a significant effect on the survival rate of the fish. Duncan's post hoc test revealed that all treatment groups that received commercial feed supplemented with extracts of *E. cottonii* (P1, P2, and P3) resulted in higher absolute weight and specific growth rates compared to the control group (P0) (Figures 1–3).

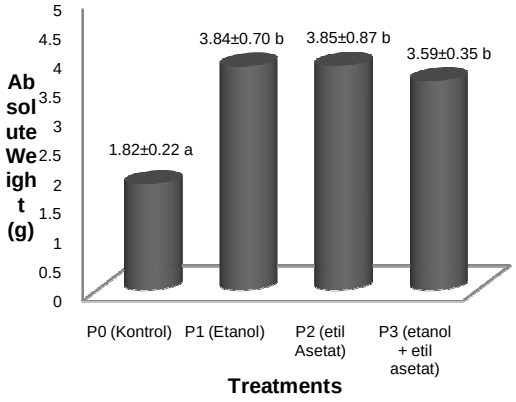


Fig 1. Absolute Weight of Tilapia (*O. niloticus)*

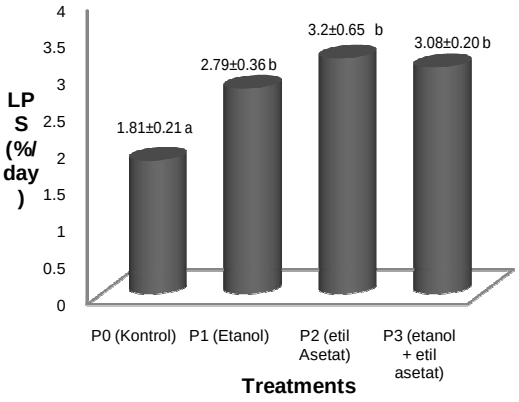


Fig 2. Specific Growth Rate of Tilapia (*O. niloticus*)

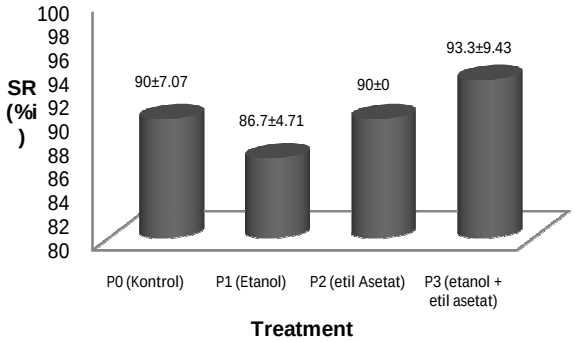


Fig 3. Survival Rate of Tilapia (*O. niloticus)*

The better growth of tilapia in all feed treatments supplemented with *E. cottonii* extracts using various solvents (P1, P2, and P3) is believed to be related to the presence of phytochemical compounds, specifically flavonoids and terpenoids. According to Munglue[25], flavonoids and terpenoids have the potential to enhance growth and feed utilization in Nile tilapia. Phytochemical compounds such as flavonoids and terpenoids can stimulate specific enzymes in the digestive tract, such as trypsin, amylase, and cytochrome c oxidase, which help improve digestion and nutrient absorption in fish. Mansour et al. [26] note that feed rich in flavonoids can significantly enhance weight gain, specific growth rate, and feed ratio in tilapia up to a level of 5 g/kg. These bioactive compounds directly increase digestive enzyme activity, positively affecting fish growth performance. Furthermore, Zhai andLiu[27] state that flavonoid bioactive compounds can enhance animal growth.

Meanwhile, all feed treatments in this study did not affect the survival rate of the tilapia. This indicates that the addition of *E. cottonii* extracts using various solvents in commercial feed is not harmful and does not have toxic effects compared to the control treatment. This is supported by temperature, pH, and dissolved oxygen (DO) measurements in the cultivation water, which remained within the optimal range for the survival of tilapia (Table 2).

Table 2. Water Quality Parameters

Parameter	Treatments				Source[28]
	P0	P1	P2	P3	
Suhu (°C)	26.9 – 28.6	26.6 - 29.4	26.9 - 28.3	26.9 - 28.5	25 - 30°C
DO (mg/L)	4.9 - 7.9	6 - 6.5	6 - 8	5.7 – 7.5	> 5 mg/L
pH	6.1 - 7.8	6.5 - 7.5	6.8 - 7.4	6.5 - 7.4	5 - 11

4. CONCLUSION

The phytochemical test results of *E. cottonii* extract using ethanol and a combination of ethanol + ethyl acetate produced the same compounds: alkaloids, steroids/triterpenoids, and flavonoids. Meanwhile, the ethyl acetate solvent contained triterpenoids and flavonoids. The groups of compounds saponins and tannins yielded negative results across all organic solvent treatments. All treatments involving the addition of commercial feed with extracts of the seaweed *E. cottonii* using various organic solvents significantly improved the growth of tilapia compared to the control treatment

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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