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Original Article

Crude Extract Preparation of Sea Grapes (*Caulerpa racemosa*) Using the Maceration Method

Pembuatan Ekstrak Kasar Anggur Laut (*Caulerpa racemosa*) Menggunakan Metode Maserasi

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Abstract

Sea grapes (*Caulerpa racemosa*) are a species of seaweed with considerable potential as a source of bioactive compounds; however, their utilization in Indonesia remains limited. This study aimed to describe the preparation process of crude extract from sea grapes using the maceration method and to determine its yield. The procedure involved drying the seaweed in a drying cabinet at 45 °C for 23 hours, grinding the dried seaweed material into fine powder using a chopper, macerating the powder with 96% ethanol at a ratio of 1:5 for three consecutive 24-hour periods, and evaporating the solvent with a rotary evaporator at 40 °C. The obtained extract was further concentrated using a water bath until a paste form was achieved. From 3 kg of fresh material, 100 g of dried material was produced, yielding 5.595 g of crude extract with a yield value of 5.595%. The maceration method was selected due to its efficiency, simplicity, and minimal requirement for specialized equipment. Ethanol 96% was chosen as the solvent because of its ability to dissolve both polar and non-polar active compounds and its safety for consumable products.

Abstrak

Anggur laut (*Caulerpa racemosa*) merupakan salah satu jenis rumput laut yang memiliki potensi besar sebagai sumber senyawa bioaktif, namun pemanfaatannya di Indonesia masih terbatas. Penelitian ini bertujuan untuk mendeskripsikan proses pembuatan ekstrak kasar anggur laut menggunakan metode maserasi serta menentukan nilai rendemennya. Prosedur penelitian meliputi pengeringan rumput laut menggunakan lemari pengering pada suhu 45 °C selama 23 jam, penghalusan bahan kering menjadi serbuk menggunakan chopper, maserasi serbuk dengan etanol 96% pada perbandingan 1:5 selama tiga kali periode berturut-turut masing-masing 24 jam, serta penguapan pelarut menggunakan rotary evaporator pada suhu 40 °C. Ekstrak yang diperoleh kemudian diperkental menggunakan water bath hingga berbentuk pasta. Dari 3 kg bahan segar diperoleh 100 g bahan kering, yang menghasilkan 5,595 g ekstrak kasar dengan nilai rendemen sebesar 5,595%. Metode maserasi dipilih karena efisien, sederhana, dan tidak memerlukan peralatan khusus. Etanol 96% digunakan sebagai pelarut karena mampu melarutkan senyawa aktif polar maupun nonpolar serta aman digunakan untuk produk yang dapat dikonsumsi.

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INTRODUCTION

Indonesia, as an archipelagic country, has a rich marine biodiversity, including the presence of seaweed [1]. One type of seaweed with great potential yet still underutilized is *Caulerpa racemosa* [2]. This species belongs to the green algae group (Chlorophyta) and is recognized for its shape that resembles small grape-like clusters. In several Asian countries such as Japan, the Philippines, and Vietnam, *C. racemosa* has long been used as food and traditional medicine due to its high nutritional content and various bioactive compounds [3].

Several studies have shown that *C. racemosa* contains various secondary metabolites such as flavonoids, tannins, phenols, and alkaloids [4], which have significant biological activities, including antibacterial [5], antioxidant [6], and anticancer [7]. This potential makes *C. racemosa* one of the most promising natural sources to be developed in the pharmaceutical field, such as facial creams [8], functional foods, and health. However, the utilization of *C. racemosa* in Indonesia is still limited to fresh consumption or as processed food products without extracting its active compounds. The extraction process is an important step to isolate and concentrate bioactive compounds so that they can be used more specifically and applicably [9].

The issue in developing the utilization of *C. racemosa* lies in the lack of data and standard methods regarding the effective and efficient production of crude extracts. Variables such as solvent type, material-to-solvent ratio, temperature, extraction time, and methods used (e.g., maceration, reflux, soxhlet extraction, etc.) greatly influence the quality and content of the resulting extract [10]. Without the appropriate method, the bioactive potential of *C. racemosa* cannot be fully optimized, which becomes one of the obstacles in the downstream processing of high-value marine products.

Among various extraction techniques, maceration is a simple and cost-effective method that requires minimal equipment and low energy input. This method is suitable for preserving thermolabile bioactive compounds because it is conducted at room temperature without heat application. Moreover, maceration is easily reproducible and applicable at a small scale, making it relevant for developing countries such as Indonesia. However, systematic studies on the preparation of crude extracts from *C. racemosa* using maceration as a preliminary step for bioactivity screening remain limited. Therefore, this study highlights maceration as a practical approach for optimizing the utilization of bioactive compounds from *C. racemosa*.

Theoretically, extraction is the process of separating chemical compounds from natural materials using specific solvents, based on the solubility of active compounds. In the initial stage, crude extracts are obtained from the process of dissolving and filtering plant materials without further purification, so they still contain a mixture of various compounds. Although crude, this extract can still be used for preliminary activity tests (prescreening), such as antibacterial, antioxidant, or phytochemical tests. Therefore, the preparation of crude extracts is an important first step in natural product research before the isolation and further characterization of specific compounds.

Based on the above background, this study aims to describe the preparation of crude extracts from C. racemosa using the maceration method, including detailed information on extraction procedures, equipment, solvent selection, and extraction outcomes. This study is expected to serve as a technical reference for future research and as an initial step toward the development of bioactive compounds from C. racemosa for pharmaceutical and aquaculture-related applications.

METHODS

Tools and Materials

Tools. The tools used in this study include a drying cabinet, drying cabinet rack, rotary evaporator (Stuart), chopper, filter paper, glass jars, basin, stirrer, porcelain crucible, water bath HH-6, spatula, digital balance, tissue, and documentation tools.

Materials. The materials used in this study include clean fresh water, *C. racemosa* (sea grape) from the *Balai Besar Perikanan Budidaya Air Payau* (BBPBAP) Jepara (3 kg), according to the information provided at the time of purchase, and 96% ethanol (500 mL).

Study Duration and Location

This study was conducted from July 26 to August 2, 2023, at the Food Technology Laboratory, Universitas Jenderal Soedirman.

Research Procedure

Stage 1: Drying of *C. racemosa* (Sea Grape)

C. racemosa was first washed thoroughly with fresh water to reduce surface salt content. Before washing, the sea grape was cut into small pieces (approximately 1 cm) to facilitate the removal of adhering salts and debris [11]. The cleaned sea grape is then drained until no water drips from it. Next, the sea grape is arranged on the drying cabinet tray with spacing to accelerate drying. The trays are then placed in the drying cabinet in a slightly zigzag pattern. The top tray is placed slightly to the right, while the tray below it is placed slightly to the left, and so on. Afterward, the drying cabinet is closed, and the temperature is set to 45°C for 23 hours.

Stage 2: Maceration of *C. racemosa* (Sea Grape)

The dried sea grape is crushed using a chopper until it becomes a fine powder, then weighed. After weighing, the powder is placed in a glass jar and ethanol 96% (pro analysis grade) is added in a 1:5 ratio. The maceration of the sea grape is done by tightly sealing the jar and storing it at room temperature for 3x24 hours. Every 6 hours, the sea grape is stirred for 5 minutes. The macerate obtained is then filtered to separate the residue from the extract to be used.

Stage 3: Evaporation

Evaporation is performed using a rotary evaporator. The first step is to place the macerate into a round-bottomed flask according to the specified volume, then fill the water bath with distilled water and set the temperature to 40°C. The water bath is turned on. The round-bottomed flask is lowered to be partially submerged in the chamber, and the rotation speed is set to 3, using a clockwise rotation. The vacuum is turned on to reduce the air pressure. The evaporation process is carried out until a thick extract is obtained. The evaporated extract is then transferred to a porcelain crucible and further evaporated using the water bath at 45°C until a crude extract in the form of paste is obtained. The crude extract is then transferred to another container. The container is weighed before use, and the extract is placed into the container. The container with the extract is weighed again to determine the mass of the extract obtained.

Yield Determination

The yield was determined at two stages: during the preparation of the dried material and the final extract. The yield of the dried material is calculated based on the ratio between the dry weight of the dried material and the initial fresh weight of the material, while the yield of the extract is calculated using the following formula:

$$\%\ \text{Extract Yield} = \frac{Weight\ of\ Crude\ Extract\ (gram)}{Initial\ Weight\ of\ Dried\ Material\ (gram)}\ x\ 100\%$$

RESULT AND DISCUSSION

Sea Grape (*Caulerpa racemosa*) is a type of green algae found in various regions of Indonesia [12]. Traditionally, *C. racemosa* is used as a salad by coastal communities due to its high content of carbohydrates, proteins, minerals, and dietary fiber [13,14]. However, *C. racemosa* is also used in cosmetics and medicine due to its antibacterial properties [4,11,15] and antioxidant effects [6]. In order to be used as a pharmaceutical ingredient that can be scientifically validated, extraction is necessary to obtain an extract with compounds that can be stored for long periods.

The extraction process in this study consists of three stages: drying, maceration, and evaporation, as shown in Figure 1. Drying of C. racemosa using fresh water washing followed by cabinet and air-drying resulted in a substantial reduction in sample weight, indicating the high moisture content of fresh sea grapes. In addition to using a drying cabinet, drying can also be done under sunlight [16] and air-dried [17]. Drying is an important factor as bioactive compounds dissolve more easily when dry. Maceration begins by grinding *C. racemosa*; in addition to using a chopper, it can also be ground using a mortar [18]. Next, the sea grape undergoes maceration, a process also performed by Assagaf *et al.*[19], who used powdered nutmeg and 96% ethanol in a 1:5 ratio, and by Suharli *et al.*[18], who used a 1:5 ratio of betel leaf powder and 70% ethanol as the solvent. In addition to using analytical-grade ethanol, *C. racemosa* can also be macerated with other solvents. In Wiyanto's study[20], *C. racemosa* was macerated using methanol in a 1:1 ratio, while in Luhulima *et al.*[21] study, ethanol 70% was used with a 1:5 ratio. Maceration of *C. racemosa* can also be performed using distilled water as the solvent [22]. The time required for evaporation depends on the sample, solvent, and temperature used. In Assagaf *et al.'s* study [19], for the evaporation of nutmeg using 96% ethanol with a temperature of 40°C, it took 3 hours. Evaporation aims to concentrate the solution before further processing, reduce the solution volume, and decrease the water activity [23].

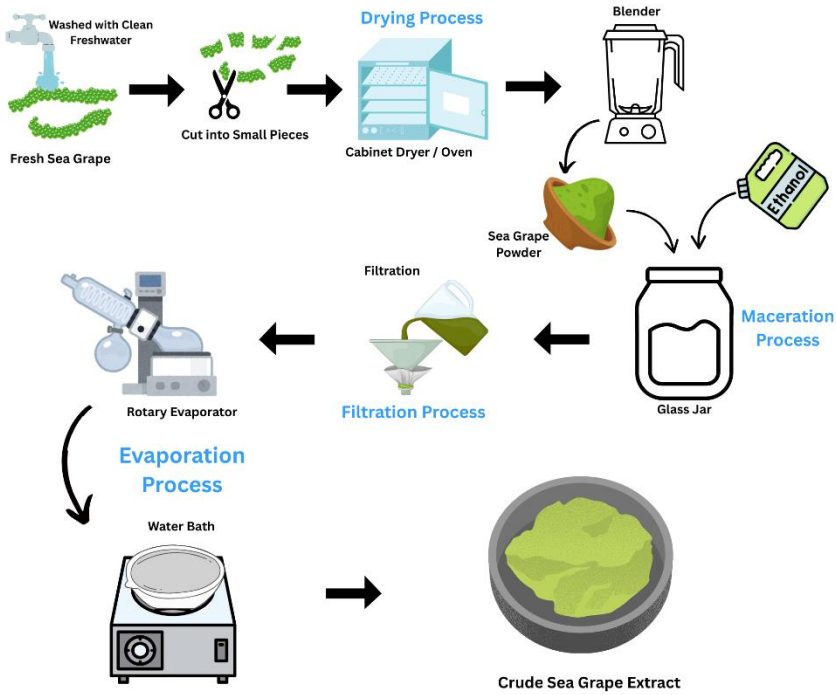


Figure 1. Extraction Process of Sea Grape

Extraction methods are the processes of drawing specific compounds from their source material using appropriate solvents. The extraction method used in this study is maceration. Maceration is a process of soaking the

dried material in a solvent at room temperature to obtain the extract [24]. In addition to maceration, extraction can also be performed using methods such as sonication, Soxhlet extraction, reflux, and Microwave Assisted Extraction (MAE) [25]. The advantage of the maceration method over other extraction methods is its simplicity, low cost, ease of implementation, and the fact that it does not require specialized equipment [26]. The use of 96% ethanol as a solvent in the maceration process is due to its excellent extractive ability for both polar and nonpolar active compounds. Ethanol is safe, non-toxic, easily evaporates, which facilitates the concentration of the extract without damaging the bioactive compounds, and is safe for consumable products [27].

The extract yield obtained in this study (5.595%) was higher than that reported by Soltif *et al.* [28], who obtained a yield of 1.521% from *C. racemosa* using the maceration method with 70% ethanol as the solvent. The difference in extract yield may be influenced by solvent concentration, as higher ethanol concentrations can enhance the solubility of both polar and semi-polar compounds. In contrast, Indriani *et al.* [29] reported a substantially higher extract yield (29.9029%) using the sonication method. Sonication is known to enhance mass transfer and disrupt cell structures more effectively than conventional maceration, thereby increasing extraction efficiency. However, despite producing a lower yield compared to sonication, maceration remains a practical and economical method due to its simplicity, lower energy requirements, and suitability for preliminary extraction and small-scale applications [30].

Nevertheless, higher extraction yields from sonication are not consistently observed across studies. Triyanti *et al.* [24] found that sonication of *C. racemosa* resulted in a lower extract yield (3.74%) compared to maceration (16.29%). These findings suggest that extraction efficiency is not solely determined by the extraction method, but is also strongly influenced by factors such as solvent type and concentration, extraction time, temperature, and the physical characteristics of the sample. Although sonication may yield higher extract recovery under certain conditions, maceration remains a practical and economical extraction technique due to its operational simplicity, lower energy consumption, and suitability for preliminary extraction and small-scale applications

Table 1. Extraction Results and Yield of Sea Grape

Description	Result
Wet Sample Weight	3 Kg
Dried Material Weight	100 gram
Total Solvent Volume	500 mL
Thick Extract Weight	5,595 gram
Extract Yield	5,595%

LIMITATION

This study has several limitations that should be considered when interpreting the results. First, the sample size of *C. racemosa* was limited to 3 kg, which may not fully represent the variability of the sea grape species from different regions. The extraction process, while standardized, may also have been influenced by variables such as the drying temperature and the type of solvent used, which could lead to variations in the yield and bioactive compounds. Moreover, the method of maceration using ethanol 96% as a solvent might not have extracted all possible bioactive compounds present in *C. racemosa*, and the use of different solvents could yield different results.

Future research could address these limitations by using larger sample sizes and exploring different extraction methods, such as sonication or microwave-assisted extraction, to compare the yield and effectiveness of different solvents. Additionally, studies could examine the stability of the extracted compounds over extended periods and in various environmental conditions to better understand their potential for pharmaceutical or functional food applications.

CONCLUSION

This study demonstrated the potential of *C. racemosa* as a source of bioactive compounds through an effective extraction process using 96% ethanol. The crude extract, with a yield of 5.595%, highlights its applications in pharmaceuticals and functional foods. However, the study's limitations, including small sample size and short duration, suggest the need for further research to optimize extraction methods, explore alternative solvents, and assess the stability of the compounds for long-term applications in the industry.

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AUTHOR CONTRIBUTION STATEMENT

AAG: Conceptualization, Methodology, Data Collection, Analysis, Writing—Original Draft Preparation, Writing—Final Editing. **AE**: Supervision, Review, and Correction of Manuscript.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

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