Characteristics of Nanoparticle *Caulerpa Racemosa* as an Anti-Breast Cancer Agent

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Abstract. *Caulerpa racemosa* is a marine algae rich in folic acid, thiamine, and caulerpenyne, characterized by potential anticancer, antitumor and antiproliferative properties. Despite the promising potential, the clinical application of natural ingredients often faces challenges due to low bioavailability. To address this limitation, nanotechnology provides a promising avenue for enhancing bioavailability. Therefore, this research aimed to investigate the physical characteristics, including particle size and zeta potential, of nanoencapsulated *Caulerpa racemosa* extract at three dosage levels of 100 mg/mL, 125 mg/mL, and 150 mg/mL. The extraction process entailed maceration for 3x24 hours, resulting in nanoparticle formed through the combination of Caulerpa racemosa extract with tween 80, a 0.3% chitosan solution in acetate buffer (pH 4), and a tripolyphosphate (TPP) solution in distilled water. Particle size analysis using Particle Size Analyzer showed that the nanoencapsulated extract had larger particle sizes in the nano range, while phytochemical screening confirmed the presence of flavonoids, alkaloids, terpenoids, and phenolics. Zeta potential and polydispersity index measurements showed that higher dosage levels led to smaller zeta potential values, although remaining below +30 mV. Specifically, the polydispersity index was highest at a dosage level of 125 mg/mL.

Keywords: *Caulerpa Racemose* Extract, Nanoparticle, Physical Characteristics, Anticancer

1 Introduction

Cancer is a significant health problem globally, accounting for 7.9 million deaths recorded in 2007. This number is predicted to continue rising, reaching an estimated 12 million deaths in 2030 [1], with breast cancer being the most common type [2].

As an archipelagic country, Indonesia has approximately 70% rich marine resources, including various plant sources with medicinal properties [3]. Among these resources, Caulerpa racemosa is a green algae that spreads in several Indonesian waters. Previous research showed that this seaweed produced secondary metabolites functioning as antioxidants. Chew et al. (2008) stated that Caulerpa racemosa was capable of neutralizing free radicals due to the content of folate, thiamine, and ascorbic acid. Furthermore,

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it has caulerpenin content, which shows bioactivity against human cell lines, with anticancer, antitumor, and antiproliferative properties [4].

The use of natural materials has limitations, often experiencing failures in the clinical phase due to low bioavailability [5]. To address these limitations, nanotechnology offers advantages in improving the bioavailability of active ingredients, controlling the release of active substances, and improving sensory properties. In nano-sized particles (50-500 nm), active ingredients are more easily absorbed by the small intestine wall, increasing bioavailability [6]. Therefore, this research aimed to determine the physical characteristics of particle size and zeta potential of nanoencapsulated Caulerpa racemosa extract at three dosage levels, namely 100 mg/mL, 125 mg/mL, and 150 mg/mL.

2 Methods

2.1 Plant determination

The determination was carried in the Ecology and Biosystematics Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang in January 2024.

2.2 Extraction of Caulerpa racemosa Leaves

This research used fresh Caulerpa racemosa with a green color, resembling grass with small, slightly flat circles. The sample was washed with seawater (saltwater), rinsed using freshwater until clean to remove adhering impurities, cut into small pieces, and pounded. Subsequently, 1 kg of fresh seaweed was cut into small pieces, pounded, placed in a maceration container, and added with 5 L methanol solvent. The mixture was homogeneously blended while occasionally stirred, maceration was carried out for 3 x 24 hours. The result was filtered using a cloth filter and the extract was concentrated using a rotary evaporator at 40°C and 50 rpm.

2.3 Preparation of Nanoparticle from Caulerpa racemosa Extract

Table 1. Formula nanopartikel *Caulerpa racemose*

Material	F1	F2	F3
Ekstrak *Caulerpa racemosa*	100 mg/mL	125 mg/mL	150 mg/mL
Tween 80	2,5 mL	2,5 mL	2,5 mL
Chitosan solution 0,3 %	1 part	1 part	1 part
TPP solution 0,1 %	5 part	5 part	5 part

A total of 2.5 mL of Caulerpa racemosa extract solution (concentrations: 100 mg/mL; 125 mg/mL; 150 mg/mL) was added to 2.5 mL of tween 80 (a), followed by mixing using a magnetic stirrer for 5 minutes at a speed of 1200 rpm. A 0.3% chitosan solution in acetate buffer at pH 4 was added to TPP solution in distilled water (concentration 0.1%) at a ratio of 1:5. This was followed by mixing using a magnetic stirrer for 5

minutes at a speed of 1200 rpm (b). Subsequently, a total of 3 mL mixture (a) and (b) each were combined and homogenized using a magnetic stirrer for 5 minutes at a speed of 1200 rpm. The complete formula can be seen in Table 1.

2.4 Particle Size Determination

Particle size and zeta potential were determined to ascertain the obtained nanoparticle size and surface charge. Subsequently, particle size measurement was performed using Particle Size Analyzer based on dynamic light scattering (DLS) principle. This method allowed the measurement of the particle size and molecules dispersed or dissolved in a solution. A 2-drop of nanoparticle samples were placed in a cuvette and added to 5 mL distilled water. The particle size was determined using the Delsa TM Nano Submicron Particle Size Analyzer [7]. Zeta potential examination was measured using a Particle Size Analyzer. A 5 mL sample was taken and placed in a cuvette to measure the sample over 15 minutes [8].

2.5 Polydispersity Index Determination

The polydispersity index was determined using Particle Size Analyzer. A 5 mL sample was taken and placed in a cuvette for measurement over 15 minutes [8].

3 Results and Discussion

The determination carried out obtained results from the Ecology and Biosystematics Laboratory of the Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang in January 2024, which showed that the plant tested was the Caulerpa racemosa species. Determination data can be seen in Table 2.

Table 2. Results of determination of *Caulerpa racemosa* plants

Classification	Results
Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Chlorophyta
Divisi	Chlorophyta
Subdivisi	Chlorophytina
Kelas	Ulvophyceae
Ordo	Bryopsidales
Famili	Caulerpaceae
Genus	Caulerpa
Spesies	Caulerpa racemosa

The nanoencapsulation formulation obtained showed distinctive characteristics, including green color, clarity, and absence of odor, as shown in Fig. 1. Based on the measurement results, the particle size is in the nano range, with sizes ranging from

approximately 10–1000 nm [9]. The polydispersity index served as a measure of molecular mass distribution in a sample. The results showed that formulas 1 to 3 were homogeneous, as values closer to zero showed better distribution [7]. Generally, zeta potential measurement is based on the electrokinetic movement of the drug in the medium. A high zeta potential suspension prevents particles from flocculating and aggregation, with stable values being above +30 mV or -30 mV [3]. Based on the results presented in Table 2, the zeta potential values were below +30 mV, showing that the nanoparticle solution in all formulations was a colloidal solution insufficiently stable against negative charges [7].

Fig. 1. Nanoencapsulation of *Caulerpa racemosa* Extract

The mechanism of chitosan nanoparticle formation in this method was based on the electrostatic interaction between the amino groups of chitosan and the negative charge groups of polyanions such as TPP [10]. The ionic gelation method entails the cross-linking process between polyelectrolytes with their multivalent ion pairs. This is followed by the complexation of polyelectrolytes with oppositely charged polyelectrolytes (Table 3). The formation of these cross-linking bonds strengthens the mechanical strength of the resulting particles. Subsequently, the positive charge of chitosan amino groups interacts with the negative charge of TPP to form complexes in nanoparticle size range. During formulation, polymers such as chitosan and TPP [7] are combined with ionic gelation to create a nanoparticle delivery system with good stability [11].

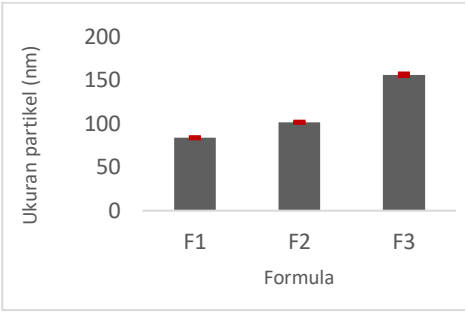
Table 3. Characteristics of Nanoencapsulation from *Caulerpa racemosa* Extract

Formula	Nano Particle Size (nm)	Zeta Potential (mV)	Polydispersity Index
F1	83.7 ± 0.65	29.15 ±0.55	0.37 ± 0.01
F2	101.47 ± 0.68	-2.34 ± 0.17	0.78 ± 0.03
F3	156 ± 1.42	-6.83 ± 0.43	0.57 ± 0.03

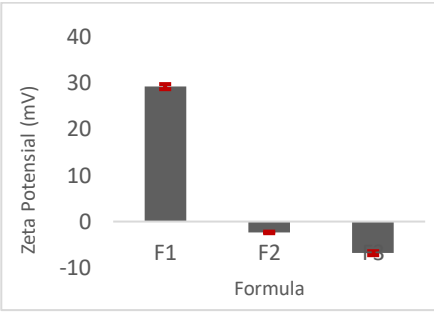
Description: F1 = nanoencapsulation formulation with a dosage of 100 mg/mL;
F2 = nanoencapsulation formulation with a dosage of 125 mg/mL;
F3 = nanoencapsulation formulation with a dosage of 150 mg/mL

Based on the resulting data (Fig. 2), it shows that the higher the extract concentration, the lower the zeta potential. According to Gao et al (2008), a lower polydispersity index value indicates that the dispersion system formed becomes more stable over time. Of

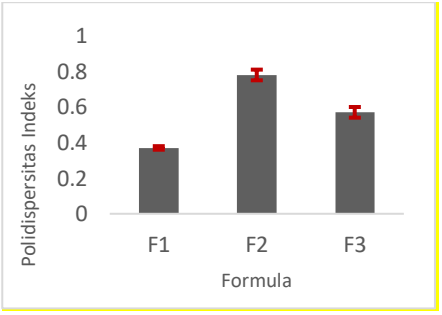
the three formulas, F1 with an extract dose of 100 mg/mL is a formula that can form nanoparticles with a particle size of less than 100 nm and a polydispersity index value of less than 0.5, so this formula has good characteristics compared to other formulas.



a



b



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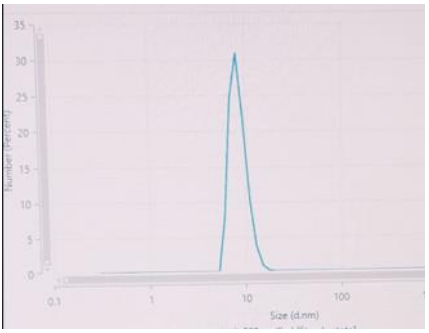

d

Name	Mean
Zeta Potential (mV)	-2.54
Conductivity (mS/cm)	0.0468
Wall Zeta Potential (mV)	2.261
Quality Factor	0.4075
Zeta Peak 1 Mean (mV)	-2.54

e

Fig. 2. Graph of test results for the physical characteristics of Caulerpa recemosa nanoparticles. Description: a. particle size; b. zeta potential; c. polydispersity index; d. graphic image of particle size; e. zeta potential distribution image.

4 Conclusion

In conclusion, this research indicated that the nanoparticle formulation of Caulerpa racemosa extract showed good stability with the combination of chitosan and TPP polymers.

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