Trop J Nat Prod Res, January 2026; 10(1): 6514 - 6519
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Optimization and Antioxidant Activity of Seagrass (*Enhalus acoroides*) Leaf Extract Capsule Formula with Porang (*Amorphophallus mueller*) Tuber as Binding Agent

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ARTICLE INFO

Article history:
Received 28 March 2025
Revised 02 October 2025
Accepted 11 October 2025
Published online 01 February 2026

ABSTRACT

Seagrass (*Enhalus acoroides* (L.f.) Royle) is one of the marine plants with significant medicinal potential. The plant contains flavonoids, alkaloids, phenols, and steroids which have demonstrated antidiabetic, antiviral, and antioxidant properties. The direct consumption of seagrass leaves is, however, considered inefficient, necessitating their processing into capsule dosage forms to enhance their applicability. Due to the relatively high moisture content of seagrass leaf extract, selecting appropriate excipients is crucial for achieving an optimal and stable formulation. This study aimed to assess the synergistic potential of seagrass leaf extract as an active antioxidant agent with Porang tuber starch as a binder excipient. The capsules were prepared using seagrass extract obtained through the ultrasound-assisted extraction method with distilled water as the solvent. The antioxidant activity was evaluated using the DPPH free radical scavenging assay. Eight formulations were developed, incorporating various Aerosil and Porang tuber starch concentrations derived from the simplex lattice design (SLD). The responses measured were moisture content, angle of repose, flow rate, and bulk density. The extract exhibited potent antioxidant activity with an IC_{50} value of 12.234 µg/mL. The SLD results indicated that the optimal formulation consisted of 3% Aerosil and 12% Porang tuber starch, although no statistically significant differences ($p > 0.05$) were observed in the physical parameters among the various formulations. Therefore, the combination of Aerosil and Porang tuber starch as excipients in seagrass extract capsule formulation has great potential in enhancing the stability and applicability of seagrass extract capsules.

Keywords: Seagrass, Antioxidant, Capsule, Porang Tuber Starch, Simplex Lattice Design

Introduction

As an archipelagic nation endowed with abundant marine biodiversity, Indonesia holds significant potential for developing natural resources as raw materials for pharmaceutical and healthcare applications.[1] Traditionally, Indonesian communities have extensively utilized plants as herbal medicines, whether in fresh or dried forms, or processed into traditional remedies.[2] One of the marine plants with significant potential medicinal value is seagrass (*Enhalus acoroides* (L.f.) *Royle*).[3] Green plants that thrive abundantly in the ocean are enriched with natural compounds, which have proven to be effective in promoting health. According to a previous study,[4] seagrass leaves have been reported to contain secondary metabolites, such as flavonoids,[5] alkaloids, phenols, and steroids,[6] with antioxidant[7] as well as antidiabetic, and antiviral properties.[8–10] The extraction of various beneficial bioactive components from seagrass, without causing damage, can be achieved using the Ultrasound-Assisted Extraction (UAE) method. This technique is environmentally friendly, more efficient, and requires minimal solvent use, resulting in higher-quality extracts.[11]

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Citation: Widyaningrum N, Wulansari E, Arief TA. Optimization and Antioxidant Activity of Seagrass (*Enhalus acoroides*) Leaf Extract Capsule Formula with Porang (*Amorphophallus mueller*) Tuber as Binding Agent. Trop J Nat Prod Res. 2026; 10(1): 6514 – 6519 https://doi.org/10.26538/tjnpr/v10i1.12

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

In addition, the direct consumption of seagrass leaves is considered less effective, making it necessary to process them into capsule formulations for enhanced applicability. Capsules are solid pharmaceutical preparations encased in easily dissolvable hard or soft shells, effectively masking unpleasant tastes and odours.[12]

Since the extract of seagrass leaves contains relatively high moisture content,[13] it requires the selection of appropriate excipients to produce an optimal formulation.[2] Aerosil and Porang tuber starch were used as a binder to improve the flowability of the seagrass leaf granules. Furthermore, the advancement of formulation technology requires the selection of excipients that meet pharmaceutical standards, and are environmentally friendly, economical, and readily available. Porang tuber (*Amorphophallus mueller*) is a rich source of glucomannan, which possesses excellent binding properties for granule preparation. Glucomannan can form a stable matrix, thus supporting a successful capsule granule formulation. The combination of these two excipients enhances their ability to absorb water. Additionally, the glucomannan content in Porang tubers can effectively bind extracts with high moisture content.[14]

The Simplex Lattice Design method is an efficient and effective experimental design used to determine the optimal combination of formulation ingredients, thereby avoiding the trial-and-error process in formula selection.[15] This method produces a formulation that meets the expected physicochemical parameters.[16] The combination of *Enhalus acoroides* leaf extract with the binding agent from Porang tuber has the potential to produce stable, effective, and high-quality capsule granules. The present study aimed to optimize the formulation of *Enhalus acoroides* leaf extract capsules using Porang tuber (*Amorphophallus muelleri*) as a natural binding material through a Simplex Lattice Design (SLD) approach, and to evaluate the antioxidant activity of the optimized formulation. Porang tuber, an abundant yet underutilized natural excipient in the region, was combined with seagrass extract, known for its potent antioxidant properties, to create a standardized capsule dosage form, thus highlighting the novelty of the study. To the

best of the authors' knowledge, this is the first investigation to incorporate Porang tuber as an environmentally friendly and sustainable binder in capsule technology while simultaneously examining the antioxidant properties of *E. acoroides* in a pharmaceutical formulation.

Materials and Methods

Chemicals and equipment

The materials used included distilled water (Merck), 96% ethanol (Merck), Aerosil (CV Pancaran Sinar, Indonesia), Porang tuber starch (CV Pancaran Sinar Indonesia), microcrystalline cellulose (Avicel®), DPPH (2,2-diphenyl-1-picrylhydrazyl, ≥95% purity, Sigma-Aldrich, St. Louis, MO, USA), Ascorbic acid (≥99%, analytical grade, Merck, Darmstadt, Germany), and corn starch (Brataco Chemika, Indonesia). The equipment used were Branson C 3800 ultrasonic extraction apparatus, rotary evaporator (Heidolph, Germany), moisture analyzer (Ohaus MB25 Moisture Analyzers), Flowmeter Granule Tester (Fujiwara, Scientific Co., Japan), stopwatch (Alba, Japan), UV-Vis spectrophotometer (Shimadzu UV-1800, Japan), and pycnometer.

Plant collection and identification

Seagrass (*Enhalus acoroides* (L.f.) Royle leaves were collected in July 2024 from Rembang Regency, Central Java, Indonesia (GPS coordinates: 6°42'48.0"S 111°20'15.0" E). The plant was taxonomically identified at the Herbal Pharmacognosy Section, Faculty of Pharmacy, Universitas Islam Sultan Agung, Semarang, Indonesia. A voucher specimen (No: 009/B.1./SA-F//II/2024) was deposited at the herbarium of the Faculty of Pharmacy, Universitas Islam Sultan Agung for future reference.

Preparation of seagrass leaf simplicia

Fresh seagrass leaves, harvested from the sea, were rinsed with running water to remove impurities, such as sand and other unwanted plants. Once cleaned, the leaves were sorted to separate those suitable for use from those that were not. After sorting, the leaves were cut into the desired size to facilitate extraction.[17]

Preparation of seagrass leaf extract

The seagrass leaf extract was prepared using Ultrasound-Assisted Extraction (UAE) method. Briefly, blended seagrass leaves (100 g) were placed into a 600 mL beaker, and 500 mL of distilled water was added, resulting in a sample-to-solvent ratio of 1:5. The beaker was then placed into the ultrasonic device set to a frequency of 40 kHz for 120 minutes at a temperature of 40°C. After the extraction process was completed, the extract was filtered, and the filtrate was concentrated using a rotary evaporator at 40°C.[18]

Determination of antioxidant activity of seagrass extract

The antioxidant activity of seagrass extract was determined using the DPPH radical scavenging assay. The seagrass extract (10 mg) was placed in a 10 mL volumetric flask. A small amount of ethanol was added and stirred until a homogeneous mixture was obtained, then ethanol was added up to the mark, resulting in a concentration of 1000 ppm. Five serial concentrations were then prepared: 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. Each test solution (2 mL) with varying concentrations was mixed with 2 mL of 40 ppm DPPH solution in methanol, and the mixture was incubated in the dark at room temperature for 30 minutes. Finally, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer.[19]

The percentage inhibition of DPPH radical was calculated using the following equation (1):

$$\% \text{ Inhibition} = \frac{Blank\ Absorbance - Sample\ Absorbance}{Blank\ Absorbance} x\ 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The extract or standard concentrations, along with their respective inhibition percentages, were plotted according to the following linear regression equation (2):

$$y = a + bx \quad \ldots\ldots\ldots\ldots\ldots (2)$$

The linear regression equation was used to determine the IC_{50} value for each sample, where the value of y was 50 (representing 50% inhibition), and x was the IC_{50}. The IC_{50} value indicated the sample concentration required to reduce 50% of the DPPH free radicals.[7]

Formulation of seagrass leaf extract capsule

Eight capsule formulas containing seagrass leaf extract, as shown in Table 1, were selected based on the results obtained from the Design Expert software version 13, with two variables and three replications. The range for each variable, Aerosil and Porang Tuber Starch was between 0 and 15%. The dry extract was mixed with excipients until homogeneous. Porang tuber starch was dissolved in 96% ethanol and then blended into the starch containing the extract. The wet granules were sieved using a mesh 14 sieve and dried at 40-60°C for 1 hour. The dried granules were then sieved using a mesh 16 sieve and filled into No. 1 capsule shells.

Table 1: Seagrass (*Enhalus acoroides (L.f.)* leaf extract capsule formulation

Ingredient	Concentration	Run							
		I	II	III	IV	V	VI	VII	VIII
Extract (mg)	250	250	250	250	250	250	250	250	250
Aerosil (%)	0 – 15	7,5	11.25	15	0	0	7.5	3.75	15
Porang Tuber Starch (%)	0 – 15	7.5	3.75	0	15	15	7.5	11.25	0
Avicel (%)	10	10	10	10	10	10	10	10	10
Corn Starch (mg)	500	500	500	500	500	500	500	500	500

Physical evaluation of the capsule formulation

Determination of moisture content of the granules

Approximately 5 g of granules were accurately weighed and placed in a moisture analyzer. The setting was adjusted to 105°C for 3 minutes. The required moisture content for the granules was between 2% and 4%.[11]

Determination of flowability

The flow rate of the granules was tested using a flow tester. Twenty-five grams of granules were weighed and placed into the flow tester, and allowed to flow, with the time recorded using a stopwatch (Casio HS 80TW). The ideal flow rate for granules is < 2.5 seconds or should not exceed 10 grams per second.[11]

Determination of angle of repose

The angle of repose of the granules was determined using a flow tester to measure the height and diameter of the pile formed.

The ideal condition for the angle of repose of granules is as follows:[11]

<25: Very Good
25-30: Good
30-40: Fair
> 40: Very Poor

Determination of bulk and tapped density

The actual bulk density was determined by weighing 15 grams of granules. Then, the granules were placed in a 50 mL graduated cylinder (Iwaki), and the volume was recorded. Tapped density was determined by tapping the granules 100 times using a tap density tester. The final volume was recorded after tapping, and the tapped density was calculated as follows (equation 3):[11]

$$\text{Tapped Density (TD)} = \frac{Weight\ of\ Granules}{Tapped\ Volume} \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Verification of the optimal formula

The physical evaluation results of the seagrass leaf extract capsule formulation were compared with the predictions from the Design

Expert software using the Simplex Lattice Design (SLD) method. The purpose was to determine whether there was no significant difference between the predicted values displayed by the SLD approach and the actual experimental results. This comparison ensured the accuracy and reliability of the formulated product based on the software's predictions.[20,21]

Statistical analysis
The data obtained from the physical evaluation tests was analyzed using the Shapiro-Wilk test (normality) and the Levene test (homogeneity). Subsequently, a one-sample T-test was conducted to validate the optimal formula against the predicted values from the SLD approach. All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). This statistical analysis ensured that the experimental results were consistent with the predictions, providing a reliable evaluation of the formulation's accuracy.

Results and Discussion

Seagrass leaf extract yield
Enhalus acoroides leaves were extracted using fresh simplicia and the Ultrasonic-Assisted Extraction (UAE) method. The extraction process using distilled water as a solvent resulted in a yield of 0.48%. The highest yield of seagrass leaf extract was obtained when using polar solvents.[15] This finding aligns with the bioactive compound profile of seagrass leaves, as most of these compounds are more effectively extracted using polar solvents.[20]

Antioxidant activity of seagrass leaf extract
The antioxidant activity was assessed using the DPPH radical scavenging activity. The linear regression equation obtained from the plot of extract concentration versus percentage inhibition of DPPH radical was $Y = 1.48x - 31.893$ with an r^2 value of 0.9924. A regression equation is considered linear when r^2 approaches 1, indicating a strong correlation between concentration and absorbance. Based on the antioxidant activity results presented in Table 2, the IC_{50} value was determined to be 12.234 µg/mL, indicating a powerful antioxidant potential. This finding aligns with previous studies confirming the antioxidant activity of seagrass leaves. Additionally, a comparative study using different solvents; methanol, ethyl acetate, and n-hexane reported varying IC_{50} values. The methanol extract showed no significant results, while ethyl acetate yielded an IC_{50} of 25.98 µg/mL, and n-hexane showed a weaker antioxidant activity with an IC_{50} of 139.50 µg/mL. These results suggested that ethyl acetate was the most effective solvent for extracting antioxidant compounds from *Enhalus acoroides*.[22,23]

Table 2: Antioxidant activity of seagrass *(Enhalus acoroides* (L.f.) *l*eaf *e*xtract

Concentration (ppm)	Absorbance (Mean ± SD)	% Inhibition	IC_{50} (µg/mL)	Antioxidant Potential
5	0.588 ± 0.000	38.333		
10	0.507 ± 0.005	46.901		
15	0.424 ± 0.000	55.604	12.234	Very strong
20	0.365 ± 0.002	61.691		
25	0.306 ± 0.005	67.939		

The antioxidant activity of plant extracts can be effectively evaluated using radical scavenging and reducing power assays, which provide insight into their potential to neutralize reactive oxygen species. Studies have consistently proven the effectiveness of these methods in assessing the antioxidant capacity of phytochemicals. For example, in a previous study by Egharevba *et al*. (2019), the methanol and ethyl acetate leaf extracts of *Stachytarpheta jamaicensis* demonstrated significant free radical scavenging activity using the DPPH assay, with IC_{50} values of 16.95 µg/mL and 33.12 µg/mL, respectively.[24]
Similarly, the stem bark extracts of *Anacardium occidentale* using both DPPH and ferric reducing antioxidant power (FRAP) assays found that the 90% methanol–10% water fraction exhibited strong radical scavenging capacity with an exceptionally low IC_{50} of 0.43 µg/mL.[25] In another study, the ethanol root extract of *Dennettia tripetala was* evaluated for its antioxidant activity, and a notable hydrogen peroxide (H_2O_2), nitric oxide (NO) scavenging abilities, as well as ferric ion-reducing power was reported.[26] These findings collectively highlight the value of integrating multiple antioxidant assays to comprehensively characterize the free radical-quenching capacity of plant-derived preparations.

Physical properties of seagrass leaf extract formulation
Moisture content
The moisture content test was conducted to determine the water content within the granules. Based on the Simplex Lattice Design (SLD) approach (Table 3), the optimization equation for moisture content was obtained as follows: $Y = 9.38\ (A) + 8.50\ (B) - 0.6600\ (AB) - 3.33\ AB(A-B) + 8.79\ AB(A-B)^2$. Aerosil (A) and Porang Tuber (B), when used individually, exhibited a positive effect on increasing granule moisture. The AB coefficient displayed a negative value (-0.6600), indicating that the Porang tuber and Aerosil combination reduced moisture content. In this equation, the individual contribution of Aerosil was the highest (+9.38), signifying its strong influence on granule moisture and its role in increasing humidity. Conversely, the combination of both components (AB) had a negative impact on moisture content. The coefficient AB(A-B) showed a negative value (-3.33), implying a decrease in granule moisture content. Meanwhile, the coefficient $AB(A-B)^2$ was positive (+8.79), suggesting that a twofold increase in the AB combination would lead to higher moisture content. This finding highlights Aerosil's high water absorption capacity, which could bind approximately 40% of its mass in water due to silanol groups capable of absorbing moisture. Consequently, Aerosil contributed to the production of granules with well-regulated water content.[11,27]

Table 3: Physical properties of the seagrass extract formulation using the Simplex Lattice Design (SLD) Approach

Run	Moisture Content (%)	Angle of Repose (°)	Flow Rate (g/sec)	Specific Gravity
1	8.65	26.89	8.30	11.06
2	9.13	30.90	5.01	11.41
3	9.53	31.39	6.33	17.57
4	8.50	28.48	13.84	21.13
5	8.50	27.18	14.66	19.68
6	8.90	27.14	7.82	11.14
7	9.32	28.42	5.35	27.28
8	9.22	31.52	6.15	17.35

The contour plot (Figure 1a) demonstrated an increase in moisture content corresponding to the increased concentration of Aerosil and the decreased concentration of Porang tuber. Conversely, when Aerosil and Porang tuber were combined in equal concentrations, the graph trends downward, indicating a reduction in moisture content. The lack-of-fit test yielded a non-significant value of 0.1126 ($p > 0.05$), indicating that the model adequately fits the data. Additionally, the model demonstrated statistical significance, with an adequate precision value of 6.8277 (greater than 4), confirming its reliability. Based on these results, the moisture content optimization equation for granules could be considered valid, with no significant deviations in the optimization process.

Flow rate
Flow time refers to the duration required for granules to flow, a crucial parameter used to ensure that granules adequately fill the capsule

space.[27] The results of the flow rate analysis are presented in Table 3. The equation derived from the Simplex Lattice Design (SLD) approach was as follows: Y = 6.24 (A) + 14.25 (B) – 875 (AB) + 19.56 AB(A-B) – 73.09 AB(A-B)^2, where Aerosil (A) and Porang tuber (B), when used individually, had a positive coefficient, indicating an increase in flow rate. However, the combination of these components produced a negative coefficient, suggesting that the mixture of Aerosil and Porang tuber reduced the granules' flow properties. Notably, the coefficient AB(A−B)^2 was -73.09, signifying that doubling the combination of these components further decreased flowability.

The contour plot in Figure 1b, generated from the SLD analysis, revealed that an increase in the Porang tuber concentration enhanced the flow rate. In contrast, a combination of Aerosil and Porang tuber resulted in a decrease in flowability. The lack-of-fit test yielded a significant value of 0.0033 ($p < 0.05$), indicating a statistically significant model. Moreover, the adequate precision value of 29.6608 (>4) confirmed the reliability of the model. Based on these findings, it could be concluded that no deviations occurred in the obtained equation.

Angle of repose

The repose angle of the granules was influenced by moisture content, where higher moisture contents resulted in a lower repose angle. This phenomenon occurred because moisture strengthened interparticle bonds, increasing cohesion and allowing the granules to flow more easily. Based on the results obtained, the angle of repose of the granules met the required standards, as the values recorded were below 30°, indicating good flow properties.[28]

The results of the angle of repose are presented in Table 3. Based on the optimization of the formula using the Simplex Lattice Design, the equation obtained was Y = 31.46 (A) + 27.83 (B) – 10.50 (AB) + 3.51 AB(A-B) + 42.35 AB(A-B)^2, where the individual values for Aerosil (A) and Porang tuber (B) had a positive impact on the repose angle of the granules. In contrast, the combination of these components showed a negative result (-10.50), indicating an adverse effect or a reduction in the repose angle of the granules. The coefficient AB(A-B)^2, with a positive value (42.35), implied that doubling the combination of components AB (Aerosil and Porang tuber) would positively affect the repose angle, leading to an increase in the repose angle during granule evaluation.

The contour plot (Figure 1c) illustrated an upward trend, which resulted from an increase in the concentration of the Aerosil component and a decrease in the concentration of the Porang tuber component. The graph showed a downward curve, indicating decreased repose angles when Aerosil and Porang tuber were combined in equal concentrations. An increase in the repose angle occurred again when the combination of Aerosil and Porang tuber was doubled, as shown by the graph, which curves upward. The size of the angle formed was influenced by particle size and was also related to the flow rate of the granules.

The lack of fit value showed a non-significant result with a value of 0.0557 ($p > 0.05$), and the model was found to be significant. The difference between adjusted R^2 and predicted R^2 was 0.1658, indicating a value smaller than 0.2. The adequacy of the precision value was obtained at 10.3828 (> 4), suggesting an adequate signal. Based on these results, it can be concluded that the equation in the repose angle evaluation test was appropriate, with no deviation or error occurring in the model.

Bulk density

The result of the bulk density is shown in Table 3. Using the SLD approach, the equation obtained was Y = 17.46 (A) + 20.41 (B) – 31.33 (AB) – 76.74 (A-B) + 134.17 AB(A-B)^2, where the individual values for Aerosil (A) and Porang tuber (B) had a positive effect on the capsule's bulk density. In contrast, the combination of these components showed a negative result (-31.33), indicating an adverse effect or a reduction in the bulk density of the granules. The coefficient AB(A-B)^2, with a positive value (134.17), suggested that doubling the combination of components AB (Aerosil and Porang tuber) would positively affect and increase the bulk density of the capsules. The contour plot graph is presented in Figure 1d.

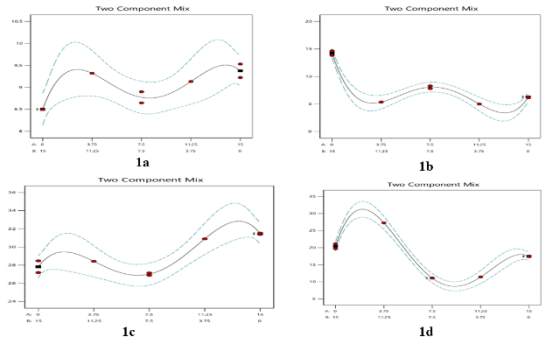

Figure 1: Contour plot of the physical evaluation of Seagrass leaf extract capsule formulation. Description**:** Moisture Content Analysis (1a), Flow Rate Analysis (1b), Angle of Repose Analysis (1c), Bulk and Tapped Density Analysis (1d)

Verification of the optimum formula

The verification was conducted to determine the ideal proportion of the combination of the excipients Aerosil and Porang tuber starch (Table 4). The actual test responses were carried out with three replications, and the average results for each test response were compared with the predictions from the Simplex Lattice Design (SLD). The physical evaluation data for the formulations showed normal and homogeneous distribution with $p > 0.05$. The results of the one-sample t-test indicated no significant difference ($p > 0.05$) between the predicted optimum formula from the Simplex Lattice Design and the experimental response values. Therefore, the Simplex Lattice Design approach was considered valid and could be used to determine the most ideal and appropriate formula for the capsule formulation of extract from *Enhalus acoroides* leaf, combined with Aerosil and Porang tuber starch.[20]

Table 4: Verification of the Optimum Formula

Parameter	Prediction	Optimum Formula	Sig 2-tailed
Moisture Content (%)	9.320	9.0766	0.115
Flow Rate (g/sec)	5.347	5.3466	0.990
Angle of Repose (°)	28.424	28.3333	0.412
Bulk Density (g/mL)	27.279	27.47	0.080

In summary, the Simplex Lattice Design (SLD) has shown that the interaction between Porang tuber starch and Aerosil significantly influenced the moisture content, flow time, and angle of repose of the granules ($p < 0.05$). For all responses, the lack-of-fit tests were not significant ($p > 0.05$), indicating that the fitted polynomial models adequately described the data. Importantly, one-sample *t*-test showed no significant differences between the model-predicted and experimental values ($p > 0.05$), confirming the reliability of the SLD optimization. These results demonstrated that Porang starch acts as an effective natural binder, while its synergy with Aerosil is critical to simultaneously achieve mechanical integrity and acceptable flow. Relative to the non-optimal trials, the optimized formula yielded a statistically lower flow time and repose angle ($p < 0.05$) while maintaining moisture within the targeted range. This pattern suggests that Porang starch provides cohesive strength without imposing excessive inter-particle friction; Aerosil likely reduces liquid bridging and improves surface glidants, enhancing flow. The statistically validated convergence of these attributes explains the favorable capsule characteristics observed after granulation and encapsulation.

Statistically, the study confirms two key points: (i) Porang tuber starch can significantly and reproducibly improve critical quality attributes of capsules when optimized with SLD, and (ii) the antioxidant potency of *E. acoroides* is maintained post-formulation, supporting its suitability for standardized nutraceutical products.

Conclusion

The optimum formula, determined using the Simplex Lattice Design (SLD) with $p > 0.05$, consists of 3% Aerosil and 12% Porang tuber starch. This combination effectively maintains the stability of the seagrass extract capsule formulation, which exhibits extreme antioxidant activity, with an IC_{50} value of 12.234 µg/mL. Future work should include *in vivo* evaluations, stability testing, and scale-up experiments to support industrial application. Integrating seagrass bioactives with sustainable local excipients offers promising prospects for developing eco-friendly nutraceuticals and preventive healthcare products.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors thank the Faculty of Pharmacy, Universitas Islam Sultan Agung, Semarang, for the financial support through Research Grant No.40a/B.1/SA-LPPM/VII/2024. The authors also gratefully acknowledge Dhea Alvionita Utami for her assistance.

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