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Optimization and Characterization of *Syzygium cumini* Leaves Extract Alginate–Kappa-Carrageenan Microspheres

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ABSTRACT

Syzygium cumini leaves contain flavonoids and other phytochemicals with promising pharmaceutical potential; however, the direct use of the extract is limited by poor stability and low bioavailability. This study aimed to formulate and characterize alginate–kappa-carrageenan microspheres containing the ethanol extract of *S. cumini* leaves prepared by ionic gelation using $CaCl_2$ as a crosslinking agent. The extract was obtained by maceration with 96% ethanol, yielding 19.6%. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, and polyphenols. Three formulations containing 0.2%, 0.3%, and 0.4% extract were prepared and evaluated for organoleptic properties, microsphere yield, morphology, particle size, moisture content, and entrapment efficiency (EE). All formulations produced fine light-green powders with a characteristic herbal odor. Microsphere yield ranged from 92.45 ± 0.52% to 93.96 ± 0.52% and differed significantly among formulations ($p < 0.05$). Particle size ranged from 3.16 ± 0.25 to 3.30 ± 0.00 µm, with polydispersity index values of 0.55–0.58, indicating moderately polydisperse systems. Moisture content remained low, ranging from 3.43 ± 0.86% to 3.81 ± 0.45%. EE increased with extract concentration, and F3 showed the highest value (69.91 ± 1.18%), which differed significantly from the other formulations ($p < 0.05$). These findings indicate that alginate–kappa-carrageenan microspheres are a promising carrier system for *S. cumini* leaf extract and may improve flavonoid protection. Further studies on release behavior and storage stability are needed to confirm their performance.

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ABSTRAK

Daun *Syzygium cumini* mengandung flavonoid dan fitokimia lain yang memiliki potensi farmasetik, namun penggunaan langsung ekstraknya masih dibatasi oleh stabilitas yang rendah dan bioavailabilitas yang kurang baik. Penelitian ini bertujuan untuk memformulasi dan mengkarakterisasi mikrosfer alginat–kappa-karagenan yang mengandung ekstrak etanol daun *S. cumini* yang dibuat dengan metode gelasi ionik menggunakan $CaCl_2$ sebagai agen pengikat silang. Ekstrak diperoleh melalui maserasi dengan etanol 96% dan menghasilkan rendemen sebesar 19,6%. Skrining fitokimia mengonfirmasi adanya alkaloid, flavonoid, saponin, tanin, dan polifenol. Tiga formulasi dengan konsentrasi ekstrak 0,2%, 0,3%, dan 0,4% dibuat dan dievaluasi meliputi sifat organoleptik, rendemen mikrosfer, morfologi, ukuran partikel, kadar air, dan efisiensi penjerapan (EE). Seluruh formulasi menghasilkan serbuk hijau muda halus dengan aroma herbal khas. Rendemen mikrosfer berkisar antara 92,45 ± 0,52% hingga 93,96 ± 0,52% dan menunjukkan perbedaan bermakna antarformulasi ($p < 0,05$). Ukuran partikel berada pada rentang 3,16 ± 0,25 hingga 3,30 ± 0,00 µm, dengan nilai indeks polidispersitas 0,55–0,58 yang menunjukkan sistem polidispersi sedang. Kadar air tetap rendah, yaitu 3,43 ± 0,86% hingga 3,81 ± 0,45%. Nilai EE meningkat seiring kenaikan konsentrasi ekstrak, dan F3 menunjukkan nilai tertinggi sebesar 69,91 ± 1,18% yang berbeda bermakna dibandingkan formulasi lainnya ($p < 0,05$). Temuan ini menunjukkan bahwa mikrosfer alginat–kappa-karagenan merupakan sistem pembawa yang menjanjikan untuk ekstrak daun *S. cumini* dan berpotensi meningkatkan perlindungan flavonoid. Penelitian lanjutan mengenai profil pelepasan dan stabilitas penyimpanan masih diperlukan untuk mengonfirmasi kinerjanya.

Kata Kunci: *Syzygium cumini*; Alginat; Kappa-karagenan; Gelasi ionik; Mikrosfer; Flavonoid; Efisiensi penjerapan

1. Introduction

Syzygium cumini is a medicinal plant widely recognized for its rich phytochemical composition, particularly flavonoids, tannins, and other polyphenolic compounds that contribute to its biological activity [1]. Previous studies have reported that extracts of *S. cumini* possess antioxidant and antidiabetic potential, indicating that this plant may serve as a promising natural source for pharmaceutical development [1],[2],[3]. Nevertheless, the direct use of plant extracts remains challenging because many phytoconstituents are vulnerable to physicochemical degradation and may exhibit poor stability and limited bioavailability during formulation and administration [3],[4]. These limitations highlight the need for an appropriate carrier system capable of protecting the active constituents and improving their pharmaceutical performance.

Microsphere-based delivery systems have attracted considerable attention as one of the promising strategies for the encapsulation of natural products. Microspheres can entrap bioactive compounds within a polymeric matrix, thereby improving protection against environmental stress, enhancing handling properties, and supporting controlled or prolonged release behavior [5]. In addition, polysaccharide-based carriers are particularly advantageous because of their biocompatibility, biodegradability, and ability to form hydrogel networks suitable for encapsulation applications [6],[7]. Among these materials, sodium alginate is widely used in drug delivery because it readily forms gels in the presence of divalent cations and provides a mild encapsulation environment for sensitive compounds [7]. Kappa-carrageenan is also a useful hydrocolloid polymer with good gel-forming properties, and its combination with alginate may improve the integrity and performance of the resulting microsphere matrix [6].

Previous studies have shown that alginate-based and alginate–kappa-carrageenan microspheres can be successfully developed as carriers for active

compounds, with polymer composition significantly affecting microsphere characteristics, release behavior, and stability [8]. Similar encapsulation approaches have also been applied to *Syzygium cumini*-derived polyphenols, demonstrating that alginate-based microcapsules can enhance entrapment and support controlled release of plant bioactives [9]. These findings suggest that the combination of alginate and kappa-carrageenan has considerable potential for the development of extract-loaded microspheres.

Although the pharmacological relevance of *S. cumini* has been increasingly documented, studies on alginate–kappa-carrageenan microspheres containing *S. cumini* leaf extract remain limited. Therefore, this study aimed to formulate and characterize alginate–kappa-carrageenan microspheres containing ethanol extract of *S. cumini* leaves prepared by ionic gelation. The characterization included organoleptic properties, microsphere yield, morphology, particle size, moisture content, and entrapment efficiency. This study is expected to provide a scientific basis for the development of a more stable microsphere-based delivery system for *S. cumini* leaf extract.

2. Methods

Materials

The materials used in this study were *Syzygium cumini* leaf simplicia, sodium alginate pharmaceutical grade (Sigma-Aldrich, USA), kappa-carrageenan pharmaceutical grade (Danisco-Cultor), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) pharmaceutical grade (Solvay Chemicals International), maltodextrin pharmaceutical grade (PT Bratachem), ethanol 96%, and demineralized water. The selection of alginate and kappa-carrageenan as the polymer matrix was based on their suitability for ionic gelation systems and their established use in microsphere preparation [10],[11].

Preparation of *Syzygium cumini* Leaf Extract

Dried *S. cumini* leaves were extracted by maceration using 96% ethanol. A total of 350 g of powdered simplicia was immersed in ethanol for 24 h at room temperature. The filtrate was separated and concentrated using a rotary evaporator to obtain a viscous extract. The extract yield was calculated using Eq. [1]:

$$\text{Extract yield (\%)} = \frac{\text{weight of concentrated extract}}{\text{weight of simplicia}} \times 100 \qquad [1]$$

This extraction approach was selected to minimize thermal degradation of flavonoid-containing constituents and to obtain an ethanol-soluble phytochemical fraction from *S. cumini* leaves [12].

Preparation of Alginate–Kappa-Carrageenan Microspheres

Microspheres containing *S. cumini* leaf extract were prepared using the ionic gelation method adapted from previous alginate–kappa-carrageenan microsphere formulations [10]. Sodium alginate and kappa-carrageenan were each prepared at a concentration of 0.9% (w/v) in demineralized water and stirred until homogeneous. The extract was then added into the polymer mixture according to the formulation shown in **Table 1**. Three formulations were prepared with different extract concentrations, namely 0.2%, 0.3%, and 0.4%, while the polymer concentration was kept constant.

A 0.5 M $CaCl_2$ solution was prepared separately as a crosslinking medium. The extract–polymer dispersion was sprayed into the $CaCl_2$ solution using an aerosol sprayer from a distance of 8 cm at a pressure of 40 psi while the crosslinking solution was stirred continuously at 1000 rpm. The system was maintained at 25°C, and the dispersion was allowed to crosslink for 2 h. The formed microspheres were collected by

centrifugation at 2500 rpm for 6 min. The supernatant was discarded, and the microspheres were washed twice with demineralized water followed by repeated centrifugation under the same conditions. The washed microspheres were filtered and resuspended in 5% maltodextrin solution as a lyoprotectant, then freeze-dried at –80°C for 120 h to obtain dry microsphere powder [10],11].

Table 1. Formulation of *Syzygium cumini* Leaf Extract Microspheres

Ingredient	Function	F1	F2	F3
S. cumini leaf extract	Active ingredient	0.2%	0.3%	0.4%
Sodium alginate	Polymer	0.9%	0.9%	0.9%
Kappa-carrageenan	Polymer	0.9%	0.9%	0.9%
$CaCl_2$	Crosslinker	0.5 M	0.5 M	0.5 M
Maltodextrin	Lyoprotectant	5%	5%	5%

Phytochemical Screening of the Extract

Phytochemical screening was performed on the ethanol extract of *S. cumini* leaves to qualitatively identify the presence of major secondary metabolite groups, namely alkaloids, flavonoids, saponins, tannins, and polyphenols. The screening procedure was carried out using standard phytochemical identification methods described by Harborne [13]. The results were interpreted based on characteristic color changes, precipitate formation, or stable foam formation according to each corresponding test.

Characterization of Microspheres

Organoleptic Evaluation

The dried microspheres were evaluated visually for their physical appearance, including dosage form, color, and odor.

Microsphere Yield

Microsphere yield was calculated by comparing the weight of dried microspheres obtained after freeze-drying with the total weight of formulation materials used in microsphere preparation. Yield was calculated using Eq. [2]:

$$\text{Microsphere yield (\%)} = \frac{\text{weight of dried microspheres}}{\text{total weight of initial formulation solids}} \times 100 \qquad [2]$$

Morphological Analysis

Surface morphology of the microspheres was examined using Scanning Electron Microscopy (SEM). Dried samples were mounted on an aluminum stub and observed under appropriate magnification to evaluate particle shape and surface characteristics.

Particle Size and Polydispersity Index

Particle size and polydispersity index (PDI) were determined by Dynamic Light Scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, UK) at 25°C.

Moisture Content

Moisture content of the dried microspheres was determined using a Mettler Toledo HB43 S Moisture Analyzer.

Entrapment Efficiency

Entrapment efficiency (EE) was determined indirectly using total flavonoid content as the marker of the encapsulated extract. Quercetin was used as the reference standard for flavonoid quantification. The amount of flavonoid present in the microspheres was compared with the total flavonoid content of the extract initially used in the formulation process. EE was calculated using Eq. [3]:

$$\text{EE (\%)} = \frac{\text{total flavonoid content in microspheres}}{\text{total flavonoid content in initial extract}} \times 100 \qquad [3]$$

The use of total flavonoid content as a surrogate marker for the encapsulated phytochemical fraction was chosen to avoid misclassification of the extract as a single pure drug entity and to align the analytical basis with the actual composition of the botanical payload [14].

Data Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD) ($n = 3$). Statistical analysis was carried out using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Differences among formulations were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test when the ANOVA result was significant. If normality assumptions were not fulfilled, the Kruskal–Wallis test was used. A p-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

Extraction Yield and Characteristics of the Extract

The extraction of *Syzygium cumini* leaves was carried out by maceration using 96% ethanol as the solvent. This solvent was selected because ethanol is widely used for the extraction of flavonoid- and phenolic-rich fractions from *Syzygium* leaves, while the maceration technique is considered suitable for minimizing thermal degradation of heat-sensitive phytoconstituents during the extraction process [4],[6]. From 350 g of dried leaf simplicia, 68.6 g of concentrated extract was obtained, corresponding to an extraction yield of 19.6% (**Table 2**). The resulting extract was thick and dark green in appearance, indicating that the extraction process successfully recovered a substantial ethanolic fraction of leaf constituents.

Table 2. Extraction Yield of *Syzygium cumini* Leaf Extract

Test	Initial Weight (g)	Final Weight (g)	Extract Yield (%)
Yield	350	68.6	19.6

The obtained yield of 19.6% should not be interpreted merely on the basis of a general cutoff value, but rather as an indication that the selected extraction conditions were effective for isolating ethanol-soluble constituents from *S. cumini* leaves. Extraction yield is influenced by several factors, including solvent polarity, extraction duration, plant matrix characteristics, and the physicochemical properties of the target compounds [4]. In the present study, the use of 96% ethanol likely facilitated the extraction of flavonoids and other phenolic constituents, which are known to be present in *Syzygium* leaves and are relevant to their pharmaceutical potential [4],[6].

The dark green color and viscous consistency of the extract also suggest that the extraction process concentrated both polar and semi-polar constituents from the plant material. Previous studies have shown that ethanol extracts of *S. cumini* leaves contain measurable flavonoid content and exhibit biologically relevant phytochemical activity, supporting their suitability for further formulation development [4]. Moreover, flavonoids derived from Indonesian medicinal plants have been widely recognized for their pharmacological relevance, including antioxidant and glucose-modulating potential, which strengthens the rationale for using *S. cumini* leaf extract as the bioactive material in the present microsphere system [6]. Therefore, the extract obtained in this

study was considered appropriate for subsequent encapsulation into alginate–kappa-carrageenan microspheres.

Phytochemical Screening of *S. cumini* Leaf Extract

Phytochemical screening was performed to identify the major secondary metabolite groups present in the ethanol extract of *Syzygium cumini* leaves. As shown in **Table 3**, the extract gave positive results for alkaloids, flavonoids, saponins, tannins, and polyphenols. The positive reactions were indicated by the formation of characteristic visual changes, including precipitate formation in the alkaloid test, red coloration in the flavonoid test, persistent foam in the saponin test, dark blue to blackish-green coloration in the tannin test, and green to dark coloration in the polyphenol test, in accordance with standard phytochemical identification principles described by Harborne [7].

Table 3. Phytochemical Screening Results of *Syzygium cumini* Leaf Extract

Metabolite group	Result	Positive indication
Alkaloids	Positive (+)	Orange-yellow or white precipitate
Flavonoids	Positive (+)	Red color formation
Saponins	Positive (+)	Stable foam for approximately 10 min
Tannins	Positive (+)	Dark blue or blackish-green color
Polyphenols	Positive (+)	Green, blue, purple, red, or dark black color

The detection of these metabolite groups indicates that the ethanolic extract of *S. cumini* leaves contains a chemically diverse phytoconstituent profile that is relevant to pharmaceutical formulation. Among the identified compounds, flavonoids and polyphenols are particularly important because they are widely recognized as major bioactive constituents in medicinal plants and are often associated with antioxidant and other protective biological activities. In the context of the present study, the presence of flavonoids and polyphenols provides a strong rationale for encapsulation, since these compounds are susceptible to environmental degradation and may benefit from incorporation into a polymeric carrier system designed to improve physicochemical protection and formulation stability [6].

Rather than overextending the interpretation toward direct antidiabetic efficacy, the phytochemical findings in this study should be understood as confirming that the extract contains bioactive groups with promising pharmaceutical relevance. Therefore, the phytochemical profile obtained supports the selection of *S. cumini* leaf extract as a suitable candidate for further formulation into alginate–kappa-carrageenan microspheres.

Organoleptic Characteristics of Microspheres

The organoleptic evaluation showed that all microsphere formulations exhibited similar macroscopic characteristics, as presented in **Figure 1**. Formulations F1, F2, and F3 were obtained as dry powder preparations with a light green appearance and a characteristic extract odor. The powder form indicates that the ionic gelation followed by freeze-drying process successfully produced a dry microsphere system, while the greenish color suggests the presence of encapsulated *Syzygium cumini* leaf extract within the polymeric matrix. The persistence of the characteristic extract odor further indicates that the extract-related sensory properties were retained after formulation. Overall, no visually observable organoleptic differences were found among the three formulations, indicating that variation in extract concentration did not markedly affect the macroscopic appearance of the microspheres [8].

F1 **F2** **F3**

Figure 1. Macroscopic appearance of microspheres containing *Syzygium cumini* leaf extract in formulations F1, F2, and F3

Microsphere Yield

Microsphere yield was evaluated to determine the efficiency of the ionic gelation and freeze-drying processes in producing dry microsphere preparations. As presented in **Table 4**, the yield values ranged from 92.45 ± 0.52% to 93.96 ± 0.52%, indicating that all formulations produced high recovery of microsphere mass after processing. Among the tested formulations, F2 showed the highest yield (93.96 ± 0.52%), followed by F3 (92.97 ± 0.31%) and F1 (92.45 ± 0.52%).

Table 4. Microsphere Yield of *Syzygium cumini* Leaf Extract Microspheres

Formula	Rep 1	Rep 2	Rep 3	Mean ± SD
F1	92.01	93.04	92.31	92.45 ± 0.52[a]
F2	93.49	93.87	94.52	93.96 ± 0.52[b]
F3	92.62	93.06	93.23	92.97 ± 0.31[ab]

Note: Data are presented as mean ± SD (n = 3). Values with different superscript letters are significantly different at p < 0.05. One-way ANOVA showed a significant difference among formulations (p = 0.020). Tukey HSD indicated a significant difference between F1 and F2 (p = 0.017).

The high yield values obtained in all formulations suggest that the alginate–kappa-carrageenan system was able to form microspheres efficiently under the selected preparation conditions. In microsphere formulations, yield is an important practical parameter because it reflects the extent to which the initial formulation components are successfully converted into recoverable solid particles. The relatively narrow range of values observed in this study also indicates that the preparation process was reproducible across formulations. Similar observations have been reported for alginate-based microsphere systems, in which polymer composition and formulation ratio can influence the total mass recovery and integrity of the resulting particles [1], [10].

Statistical analysis using one-way ANOVA showed a significant difference in yield among formulations ($p = 0.020$), indicating that variation in extract concentration affected microsphere yield. Further analysis using Tukey HSD demonstrated that the significant difference was found only between F1 and F2 ($p = 0.017$), whereas no significant differences were observed between F1 and F3 ($p = 0.418$) or between F2 and F3 ($p = 0.090$). This finding suggests that increasing extract concentration may influence the mass recovery of microspheres, although the effect was not consistently observed across all pairwise comparisons. Overall, the results indicate that all three formulations provided high microsphere yield, with F2 showing the most favorable recovery under the present experimental conditions.

Morphology of Microspheres

The surface morphology of the microspheres was evaluated using Scanning Electron Microscopy (SEM), and the representative images are presented in **Figure 2**.

SEM observations showed that the microspheres were generally spherical, indicating that the ionic gelation process was able to produce discrete particulate structures. Nevertheless, several particles exhibited surface concavities and wrinkles, suggesting that the formed microspheres were not completely uniform in external texture.

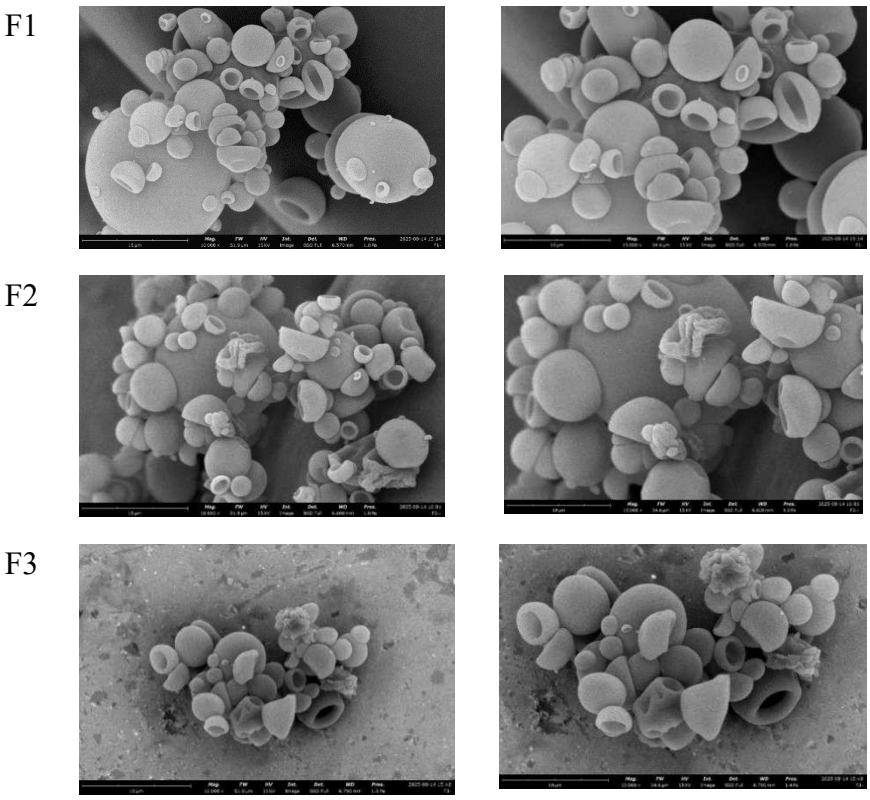

Figure 2. SEM morphology of alginate–kappa-carrageenan microspheres containing *Syzygium cumini* leaf extract (F1–F3)

These morphological features are consistent with polymeric microspheres produced through aqueous ionic gelation, in which electrostatic interactions between calcium ions and the alginate–kappa-carrageenan matrix generate a three-dimensional gel network [10], [11]. In such systems, differences in crosslinking density between the outer layer and the inner core may create internal stress during particle solidification, which can subsequently appear as slight depressions or surface irregularities after drying. In addition, the freeze-drying process may contribute to shrinkage and structural rearrangement as frozen water is sublimated from the hydrogel matrix, thereby promoting the formation of wrinkled or partially collapsed surfaces [11],[12]. Variations in polymer organization and matrix rigidity may also influence the degree of smoothness of the resulting particles, with more homogeneous gel networks generally producing more regular microspheres [13].

Overall, the SEM findings indicate that the prepared microspheres possessed an acceptable spherical morphology, although minor surface imperfections were still observed. These surface characteristics are important because they may affect the protective capacity of the polymer matrix and influence the encapsulation behavior of flavonoid-containing extract within the microsphere system [13].

Particle Size and Polydispersity Index

Particle size and polydispersity index (PDI) were evaluated to assess the dimensional characteristics and size distribution of the prepared microspheres. As presented in **Table 5**, the average particle size ranged from 3.16 ± 0.25 µm to 3.30 ± 0.00 µm. Among the tested formulations, F2 showed the largest mean particle size (3.30 ± 0.00 µm), followed by F3 (3.26 ± 0.00 µm) and F1 (3.16 ± 0.25 µm). These findings indicate that all formulations produced microspheres within a relatively narrow micrometer range, suggesting that the ionic gelation process generated particles with generally comparable dimensions.

Table 5. Particle Size and Polydispersity Index of *Syzygium cumini* Leaf Extract Microspheres

Formula	Rep 1	Rep 2	Rep 3	Mean ± SD	PDI
F1	2.869	3.292	3.306	3.16 ± 0.25[a]	0.557 ± 0.01
F2	3.297	3.295	3.299	3.30 ± 0.00[a]	0.572 ± 0.00
F3	3.258	3.259	3.260	3.26 ± 0.00[a]	0.581 ± 0.00

Note: Data are presented as mean ± SD (n = 3). Values with the same superscript letter are not significantly different at p > 0.05. One-way ANOVA showed no significant difference among formulations (p = 0.500). PDI values are presented descriptively

The PDI values of F1, F2, and F3 were 0.557 ± 0.01, 0.572 ± 0.00, and 0.581 ± 0.00, respectively. These values indicate that the microsphere dispersions were moderately polydisperse, meaning that the particle populations were not completely uniform in size. In polymeric microsphere systems, such a distribution may arise from slight variations during droplet formation, crosslinking, and matrix solidification during ionic gelation [10], [11]. The presence of moderate polydispersity is also compatible with the SEM observations, which showed particles with generally spherical morphology but with some surface irregularities.

Statistical analysis using one-way ANOVA demonstrated that the differences in particle size among formulations were not significant (**p = 0.500**). This result suggests that increasing extract concentration from F1 to F3 did not significantly influence the average particle size of the microspheres under the present preparation conditions. Overall, the data indicate that the alginate–kappa-carrageenan system was able to produce microspheres with relatively consistent particle size across formulations, although the PDI values show that a certain degree of heterogeneity in particle distribution was still present.

Moisture Content

Moisture content was evaluated to determine the residual water remaining in the freeze-dried microspheres and to assess its implication for the physical stability of the formulation. As presented in **Table 6**, the moisture content values of F1, F2, and F3 were 3.43 ± 0.86%, 3.75 ± 0.17%, and 3.81 ± 0.45%, respectively. All formulations showed relatively low moisture content, indicating that the freeze-drying process was effective in reducing residual water in the microsphere system. Low moisture content is desirable in polymeric microspheres because excessive residual water may reduce storage stability, promote structural changes in the matrix, and increase the risk of degradation of encapsulated phytoconstituents [12], [16].

Table 6. Moisture Content of *Syzygium cumini* Leaf Extract Microspheres

Formula	Rep 1	Rep 2	Rep 3	Mean ± SD
F1	3.15	2.75	4.40	3.43 ± 0.86[a]
F2	3.75	3.92	3.58	3.75 ± 0.17[a]
F3	3.56	4.33	3.55	3.81 ± 0.45[a]

Note: Data are presented as mean ± SD (n = 3). Values with the same superscript letter are not significantly different (one-way ANOVA, p > 0.05)

Among the tested formulations, F3 showed the highest mean moisture content, followed by F2 and F1. However, the differences among formulations were relatively small, and all values remained below 10%, suggesting satisfactory physical dryness of the microspheres. This finding indicates that variation in extract concentration did not substantially affect the final water content of the alginate–kappa-carrageenan matrix under the selected preparation conditions. In freeze-dried polysaccharide-based systems, such behavior is consistent with the role of lyophilization in removing water while preserving the structural integrity of the hydrogel-derived particles [12].

Statistical analysis using one-way ANOVA showed that the differences in moisture content among formulations were not significant (p = 0.689). Therefore, all formulations can be considered to have comparable moisture characteristics. Overall, these results demonstrate that the prepared microspheres possessed sufficiently low moisture content, which is favorable for maintaining the stability of flavonoid-containing encapsulated systems during storage [16].

Entrapment Efficiency Based on Total Flavonoid Content

Entrapment efficiency (EE) was determined indirectly using total flavonoid content as the analytical marker of the encapsulated *Syzygium cumini* leaf extract, with quercetin used as the reference standard. In this approach, the total flavonoid content measured in the microspheres was compared with the total flavonoid content initially present in the extract used during the formulation process. This method is appropriate for botanical encapsulation systems because it reflects the retention of the flavonoid-rich phytochemical fraction within the polymeric matrix rather than implying the presence of a single pure drug compound [16]. The raw EE-related calculations in the draft are currently distributed across multiple tables, where the total flavonoid content of the extract and microspheres was used as the basis for %EE calculation.

Table 7. Entrapment Efficiency of *Syzygium cumini* Leaf Extract Microspheres Based on Total Flavonoid Content

Formula	EE Rep 1	EE Rep 2	EE Rep 3	Mean ± SD
F1	52.07	54.11	55.82	54.00 ± 1.88[a]
F2	66.91	64.00	64.35	65.09 ± 1.59[b]
F3	68.63	70.16	70.95	69.91 ± 1.18[c]

Note: Data are presented as mean ± SD (n = 3). Values with different superscript letters are significantly different at p < 0.05. One-way ANOVA showed a significant difference among formulations (p = 0.000). Tukey HSD indicated significant differences between all formulation pairs.

As presented in **Table 7**, the EE values increased with increasing extract concentration. Formula F1 showed EE values of 52.07%, 54.11%, and 55.82%, giving a mean of 54.00 ± 1.88%. Formula F2 showed values of 66.91%, 64.00%, and 64.35%, with a mean of 65.09 ± 1.59%, whereas Formula F3 showed the highest EE values, namely 68.63%, 70.16%, and 70.95%, with a mean of 69.91 ± 1.18%. These results indicate a clear

upward trend in flavonoid entrapment as the extract concentration increased from F1 to F3.

The increase in EE suggests that the alginate–kappa-carrageenan matrix remained capable of retaining a greater proportion of the flavonoid-containing extract as the concentration of extract increased. This finding is consistent with previous reports on *Syzygium cumini* polyphenol encapsulation, in which alginate-based systems were able to improve retention of plant polyphenols within the microcapsule structure [16]. In the present study, the highest EE observed in F3 indicates that this formulation provided the most favorable entrapment performance among the three tested formulations. Because entrapment efficiency reflects the proportion of flavonoid material successfully incorporated into the microsphere matrix, higher EE values suggest more efficient encapsulation of the phytochemical fraction under the selected formulation conditions [1],[16],[17].

Statistical analysis using one-way ANOVA demonstrated a significant difference in EE among formulations ($p = 0.000$). Post-hoc Tukey HSD analysis further showed that all formulation pairs differed significantly, namely F1 versus F2, F1 versus F3, and F2 versus F3. These results confirm that variation in formulation composition significantly influenced the efficiency of flavonoid entrapment in the microspheres. Overall, the EE findings identify F3 as the best-performing formulation in terms of flavonoid encapsulation efficiency.

Limitation of the Study

This study primarily evaluated the physicochemical characteristics of *Syzygium cumini* leaf extract microspheres, with particular emphasis on entrapment efficiency based on total flavonoid content. Although the formulation showed promising encapsulation performance, several important aspects were not investigated in the present work. The study did not evaluate release kinetics, long-term storage stability, or in vitro and in vivo biological activity of the microspheres. In addition, the morphological assessment was limited to qualitative SEM observation, and quantitative structural parameters such as porosity, density, and detailed particle size distribution were not determined. Therefore, the present findings should be interpreted as preliminary formulation data, and further studies are required to confirm the functional performance, stability, and practical applicability of the developed microsphere system.

4. Conclusion

Alginate–kappa-carrageenan microspheres containing *Syzygium cumini* leaf extract were successfully prepared by ionic gelation and showed acceptable physicochemical characteristics. All formulations produced dry microspheres with similar organoleptic appearance, generally spherical morphology, particle sizes in the micrometer range, and low moisture content. Microsphere yield remained high across formulations, while particle size and moisture content did not differ significantly among formulations. In contrast, entrapment efficiency increased with increasing extract concentration and differed significantly among all formulations, with F3 showing the highest value (69.91 ± 1.18%). These findings indicate that formulation composition influenced flavonoid entrapment performance, and that F3 was the most favorable formulation in terms of encapsulation efficiency. Overall, the developed alginate–kappa-carrageenan microspheres show potential as a carrier system for *S. cumini* leaf extract and provide a basis for further studies on release behavior, storage stability, and biological activity.

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Conflict of Interest:
The authors declare no conflict of interest related to this study.

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