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Optimization of red algae (*Kappaphycus alvarezii*) lozenges: formulation design and antioxidant evaluation

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ABSTRACT: Red algae (*Kappaphycus alvarezii*) are a marine resource containing bioactive compounds, such as flavonoids and carotenoids, with potential antioxidant properties. This study aimed to develop lozenge formulations of red algae powder using direct compression, optimize the excipient composition, and evaluate the physicochemical properties and antioxidant activity. Red algae powder was characterized microscopically and by LC–MS, confirming the presence of quercetin, rutin, kaempferol, and astaxanthin in the powder. Five lozenge formulations (F1–F5) were prepared using varying concentrations of hydroxypropyl methylcellulose (HPMC) and starch. In-process evaluation included flow rate, angle of repose, loss on drying, and particle size distribution, while post-compression testing covered organoleptic properties, weight variation, hardness, friability, surface abrasion, and disintegration time. Antioxidant activity was determined using the DPPH radical scavenging assay, with quercetin as the standard. All formulations met the pharmacopeia standards, with hardness ranging from 74.3–111.1 N, friability 0.61–0.98%, and disintegration times 6.61–15.88 min. Optimization using a Simplex Lattice Design identified Formula 3 (3.5% HPMC and 10.5% starch) as the most desirable composition. The red algae powder and lozenge exhibited weak antioxidant activity, with IC_{50} values of 5,969 ppm and 6,854 ppm, respectively. These findings demonstrate that lozenges containing red algae powder can be successfully produced with acceptable physical properties; however, improvement of raw material standardization or enrichment is required to enhance antioxidant efficacy.

KEYWORDS: Antioxidant activity; formulation; *Kappaphycus alvarezii;* lozenges; simplex lattice design.

INTRODUCTION

Oxidative stress arises when the production of reactive oxygen species exceeds the capacity of the body's antioxidant defense system, resulting in damage to essential biomolecules, including lipids, proteins, and DNA. This imbalance is closely associated with the pathogenesis of various chronic and degenerative conditions, including cancer, inflammation-related disorders, and premature aging [1]. Although endogenous antioxidants help maintain redox homeostasis under normal physiological conditions, continuous exposure to external factors such as smoking, alcohol consumption, environmental pollution, and ultraviolet radiation can overwhelm these defence mechanisms. Therefore, exogenous antioxidants are important supportive agents that reduce oxidative damage and help prevent the progression of oxidative stress-related disorders [1],[2].

Natural antioxidants have attracted increasing attention because, in addition to their free radical scavenging activity, they often exhibit broader biological effects, such as anti-inflammatory, anticancer, and anti-aging properties [4]. Compounds such as polyphenols, flavonoids, carotenoids, and certain vitamins have been widely reported to contribute to these activities [4],[5]. Compared with synthetic antioxidants, natural sources are generally considered more favorable because of their multifunctional bioactivity, renewable availability, and better public acceptance for long-term use [6].

Marine algae are a particularly promising source of antioxidants. In contrast to terrestrial plants, marine algae grow under distinctive environmental conditions, including high salinity, intense light exposure, tidal fluctuations, and constant oxidative stress [5]. These ecological pressures are known to stimulate the production of diverse protective secondary metabolites, providing marine organisms with a phytochemical

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profile that may differ from that of terrestrial plants. Consequently, marine algae have garnered increasing interest as sources of unique bioactive compounds with potential pharmaceutical and nutraceutical applications [4],[5].

One marine species with considerable potential is red algae (*Kappaphycus alvarezii*), a seaweed abundantly cultivated in Indonesia and widely recognized as an important raw material for carrageenan production [7]. In addition to its economic value and broad availability, *K. alvarezii* contains antioxidant-related metabolites, including flavonoids and carotenoids, which may contribute to its biological activity [7],[8]. These characteristics make *K. alvarezii* an attractive test material because it combines local availability, renewable production, and bioactivity. Previous studies on red algae, including *K. alvarezii*, have mainly focused on extraction, phytochemical characterization, and antioxidant activity, whereas its incorporation into patient-friendly pharmaceutical dosage forms has been explored to a lesser extent. This gap highlights the need for formulation-oriented studies that can translate marine bioactive resources into practical applications.

To convert a promising antioxidant source into a usable preparation, an appropriate dosage form must be selected. Lozenges were chosen in this study because they are relatively simple to manufacture, convenient to administer without water, and suitable for populations that may have difficulty swallowing conventional tablets, such as children and older adults [9,10]. In addition, their slow dissolution in the oral cavity may allow the gradual release of the active material while improving the acceptability of functional or natural health products [10]. From a formulation perspective, the development of *K. alvarezii* into lozenges is relevant not only for dosage convenience but also for expanding the pharmaceutical applications of marine-derived antioxidants.

The DPPH radical scavenging assay was uses for antioxidant evaluation. Its a simpel, rapid, and widely used screening method for assessing the free radical scavenging capacity of natural materials and formulated products [11]. This method is suitable for preliminary antioxidant evaluation because it requires only a small sample volume, provides a clear spectrophotometric response, and enables antioxidant potency to be expressed quantitatively through the IC_{50} value in comparison with a reference standard. Based on these considerations, this study aimed to formulate *K. alvarezii* into lozenges, optimize the excipient composition, evaluate the physicochemical properties of the resulting formulation, and assess its antioxidant activity using the DPPH method.

▪ MATERIALS AND METHODS

Materials

Red algae powder (*Kappaphycus alvarezii*) was purchased from MSH Rempah (South Jakarta, Indonesia) in a 50 g package. The product was labeled as 100% pure algae powder and had a valid PIRT license (No. 2123171150185-25) and a halal certification. The pharmaceutical-grade excipients used in this study were lactose, starch, hydroxypropyl methylcellulose (HPMC), sucrose, saccharin, magnesium stearate, talc, grape flavoring, and coloring. The excipients were donated by Dexa Medica (Tangerang, Indonesia) and Pharos Indonesia (Jakarta, Indonesia). Analytical-grade reagents included distilled water (aquadest), methanol, dichloromethane, glycerin, iodine, chloral hydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, and ascorbic acid.

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Instruments

The instruments employed were a sieve and shaker (AS 200, RETSCH®, Haan, Germany), a moisture content analyzer (HE73, METTLER TOLEDO®, Greifensee, Switzerland), a flowability tester (GTL, ERWEKA®, Langen, Germany), a laboratory oven, a single punch tablet press (equipped with a VFD007S21A

drive, DELTA®, Neihu, Taiwan), a digital caliper, a hardness tester (PTB 111E, PHARMATEST®, Hainburg, Germany), a friability tester (PTF20ER, PHARMATEST®, Hainburg, Germany), a disintegration tester (Dist 3, PHARMATEST®, Hainburg, Germany), a UV–Vis spectrophotometer (UV-1900i, SHIMADZU®, Kyoto, Japan), a microplate reader with a 96-well plate (AU–Microplate Reader, AZURE BIOSYSTEMS®, Dublin, CA, USA), an LC–MS (QTRAP 4500, SCIEX®, Framingham, MA, USA), a light microscope (CX31, OLYMPUS®, Tokyo, Japan), and micropipettes.

Microscopic and phytochemical characterization

Microscopic evaluation of the red algae powder was conducted using light microscopy with glycerin, iodine, and chloral hydrate as staining reagents at 40× magnification. Flavonoid and carotenoid contents were analyzed using LC–MS (SCIEX® QTRAP 4500) with methanol and dichloromethane. The mass spectra results were compared with those of the ChemSpider and NIST databases.

Tablet formulation and optimization

The lozenges were prepared using the direct compression method according to the formulations presented in Table 1.

Table 1. Composition of red algae powder lozenge formulations (F1–F5) expressed as a percentage (%) of the total tablet weight (800 mg).

Material	Function	F1	F2	F3	F4	F5
Red algae powder	API	20.0	20.0	20.0	20.0	20.0
HPMC	Binder	2.00	2.75	3.50	4.25	5.00
Starch	Disintegrant	12.0	11.25	10.5	9.7	9.00
Talc	Glidant	1.00	1.00	1.00	1.00	1.00
Magnesium stearate	Lubricant	1.00	1.00	1.00	1.00	1.00
Lactose	Filler	53.4	53.4	53.4	53.4	53.4
Sucrose	Sweetener	10.0	10.0	10.0	10	10.0
Saccharin	Sweetener	0.10	0.10	0.10	0.1	0.10
Grape flavor and coloring	Flavoring	0.50	0.50	0.50	0.5	0.50

Evaluation of powder and lozenges

Powder blends were assessed for flow properties (direct flow rate, angle of repose, and loss on drying) and particle size distribution using sieve analysis (850–150 µm). The resulting lozenges were further evaluated in accordance with the Indonesian Pharmacopoeia, 6th Edition, including organoleptic properties (color, odor, taste, and texture), weight variation, thickness, hardness, friability, surface abrasion, and disintegration time.

Antioxidant activity analysis

The antioxidant activities of red algae powder, lozenges, and blank tablets were assessed using the DPPH radical scavenging method in a 96-well plate. Quercetin was used as the reference standard. Serial dilutions were prepared from stock solutions (red algae powder 4000 ppm, lozenges 8000 ppm, and blank tablets 8000 ppm) and mixed with 100 ppm DPPH. Absorbance was measured at 516 nm using a UV-Vis spectrophotometer with a microplate reader. The percentage inhibition was determined, and IC_{50} values were calculated using regression analysis.

Data analysis

Data were analyzed using Design-Expert® software version 13 (Stat-Ease Inc., Minneapolis, MN, USA), applying a Simplex Lattice Design (SLD) model to determine the optimal concentrations of the binder and disintegrant.

▪ RESULTS

Microscopic and phytochemical characterization

The red algae powder appeared as a coarse-textured, light-brown material with a characteristic herbal aroma (Figure 1a). Microscopic examination with glycerin revealed circular white structures (Figure 1b), iodine

produced a dark purple coloration (Figure 1c), and chloral hydrate showed compact, aggregated fragments (Figure 1d). LC–MS analysis confirmed the presence of quercetin (~9.9 min, m/z = 301.0/150.9), rutin (~29 min, m/z = 609.2/300.0), kaempferol (~15 min, m/z = 284.9/93.0), and astaxanthin (~5.3–5.4 min, m/z = 597.3/147.1) (Figures 2–5).

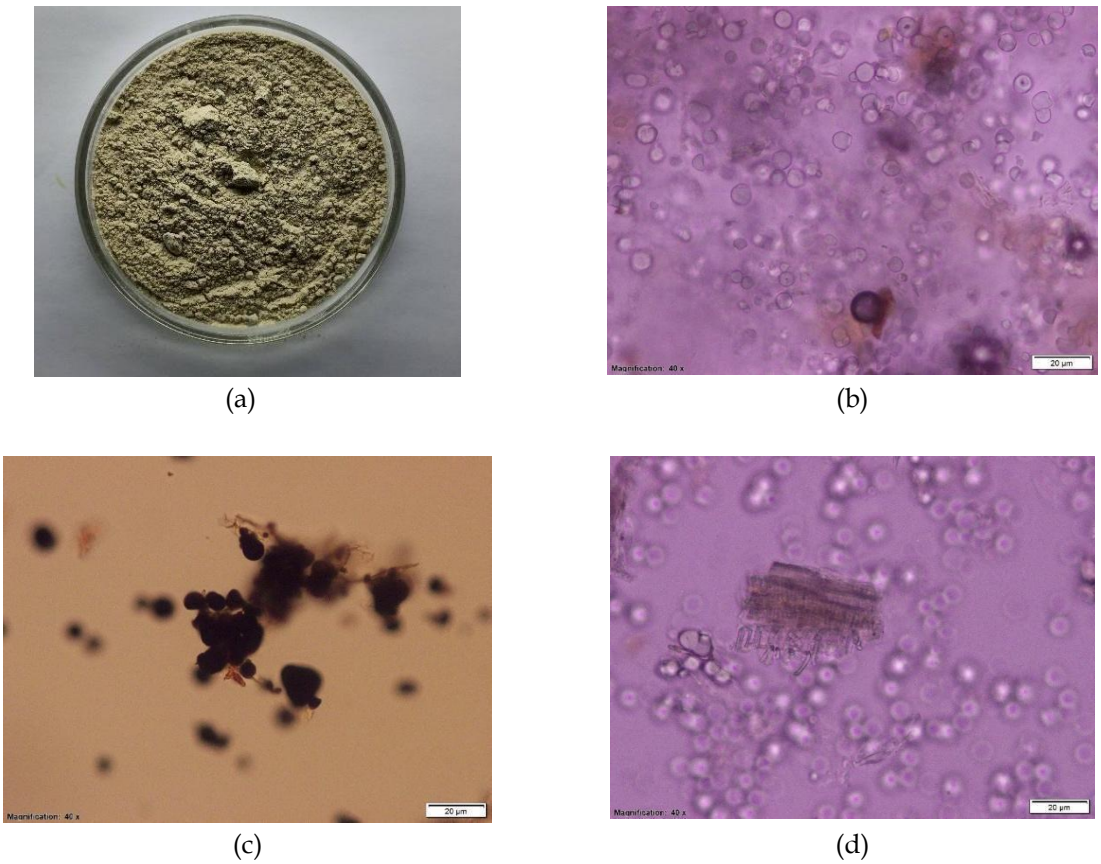


Figure 1. Microscopic characterization of the red algae powder. (a) Macroscopic appearance of the powder; (b) microscopic profile after treatment with glycerin reagent (40×; scale bar = 20 µm); (c) microscopic profile after treatment with iodine reagent (40×; scale bar = 20 µm); and (d) microscopic profile showing cell fragments after treatment with chloral hydrate reagent (40×; scale bar = 20 µm).

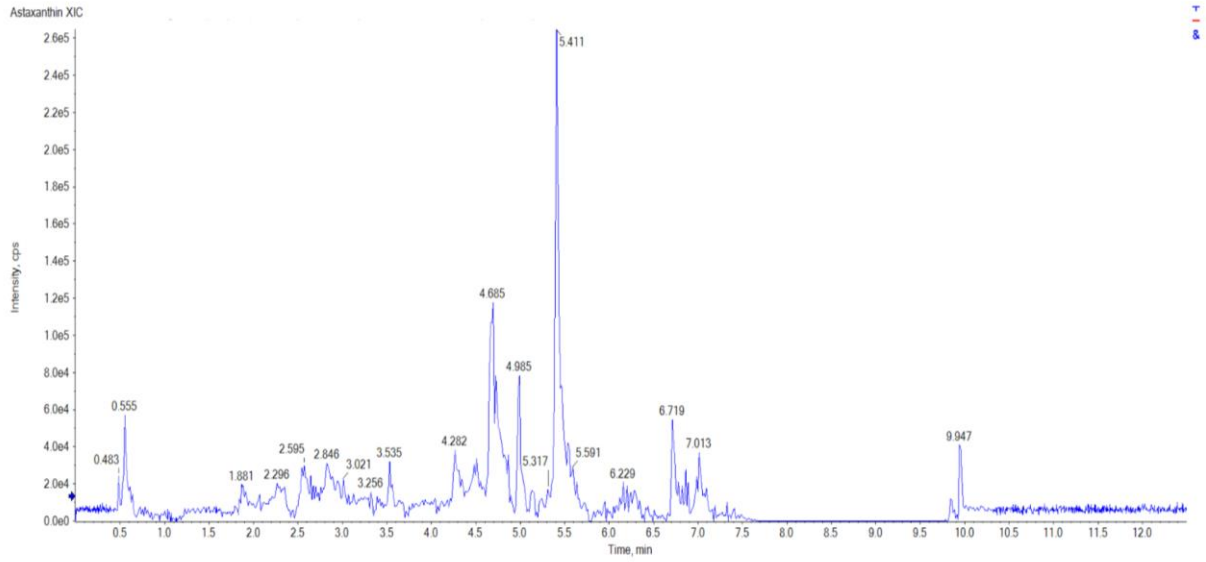


Figure 2. Extracted ion chromatogram of the red algae powder in dichloromethane, showing the astaxanthin peak at ~5.3–5.4 min with m/z 597.3/147.1.

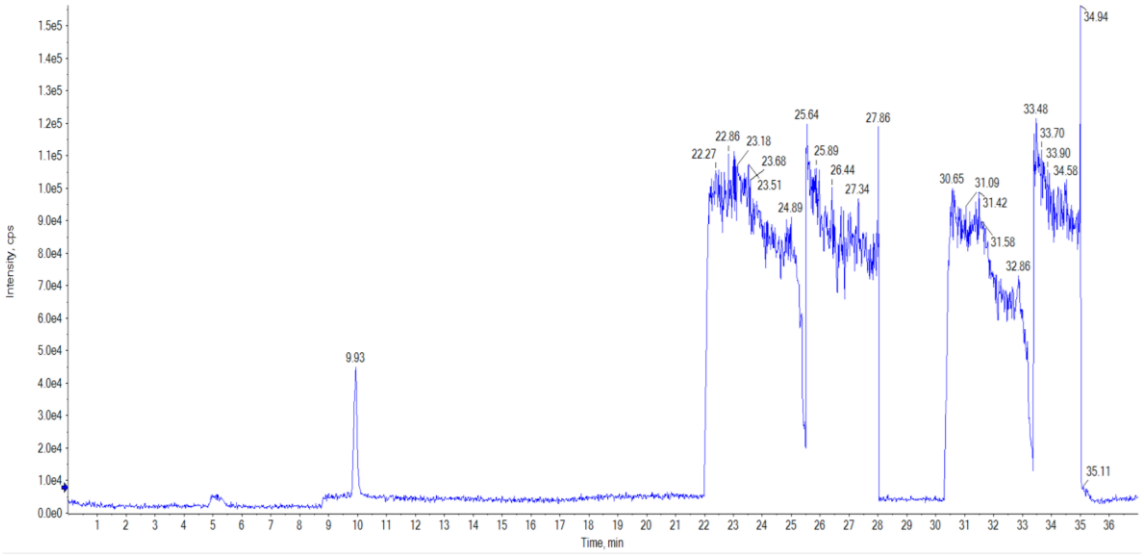


Figure 3. Extracted ion chromatogram of the red algae powder in methanol, showing the quercetin peak at ~9.9 min with m/z 301.0/150.9.

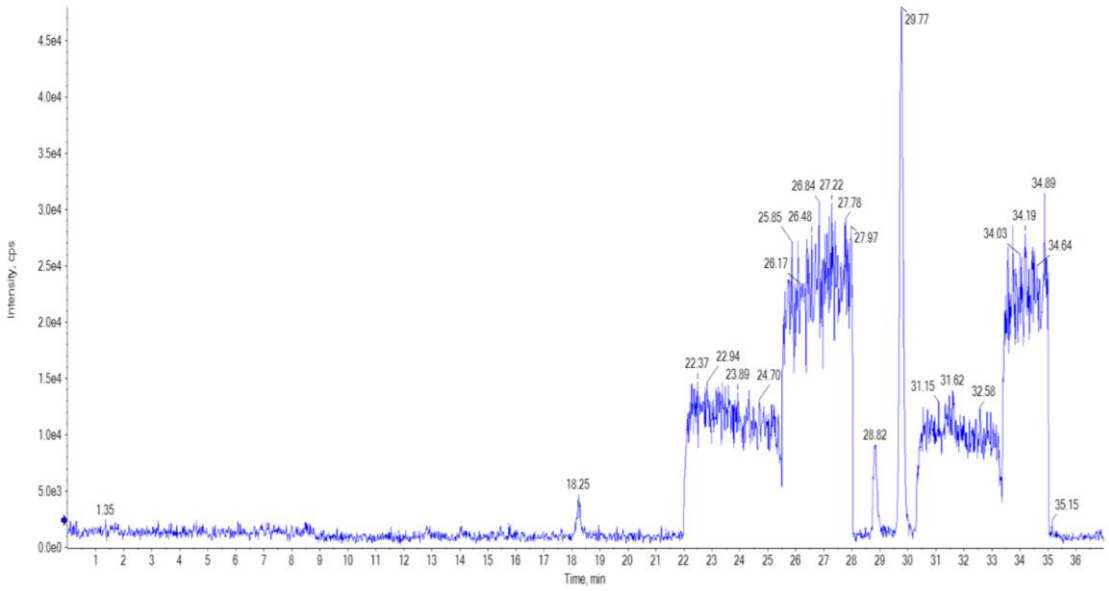


Figure 4. Extracted ion chromatogram of the red algae powder in methanol, showing the rutin peak at ~29 min with m/z 609.2/300.0.

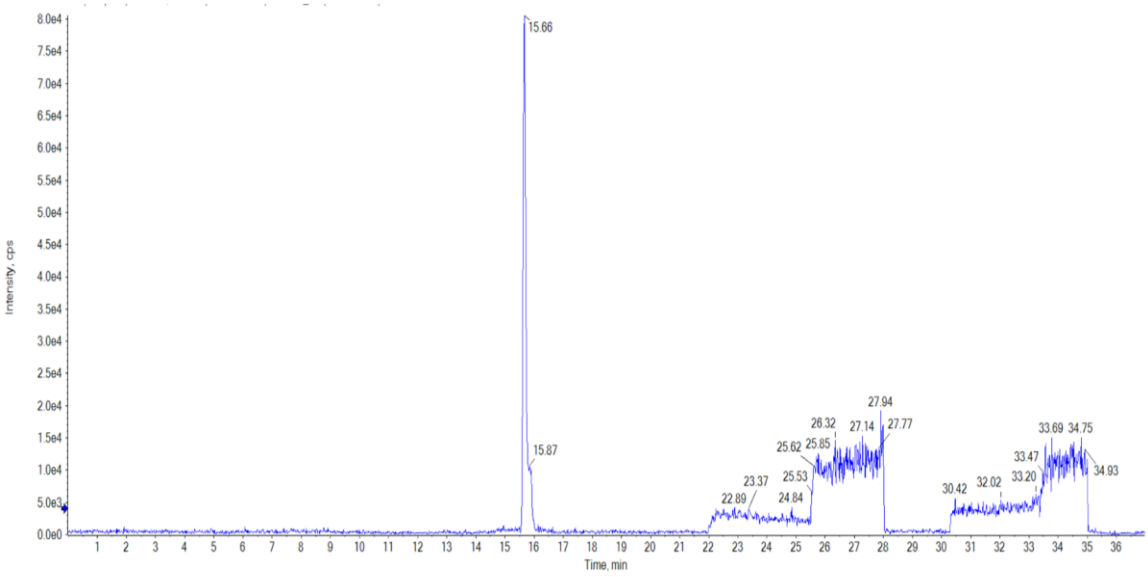


Figure 5. Extracted ion chromatogram of the red algae powder in methanol, showing the kaempferol peak at ~15 min with m/z 284.9/93.0.

In-process control (IPC)

Table 2 summarizes the IPC results for formulations F1–F5, including the flow rate, angle of repose, and loss on drying. The particle size distribution profile (Figure 6) showed that most of the powder was retained below 150 µm, with smaller fractions between 150 and 425 µm and negligible amounts above 425 µm.

Table 2. IPC evaluation of the powder from red algae powder lozenge formulations (F1–F5).

Parameters	F1	F2	F3	F4	F5
Mean flow rate (g/s) ± SD	17.6±0.75	16.7±0.55	19.0±4.95	19.4±2.04	14.2±0.31
Angle of repose (°)	14.6±1.0	12.7±2.1	12.0±1.1	13.3±2.1	15.0±3.6
Loss on drying (%)	3.3	3.2	3.3	3.3	3.2

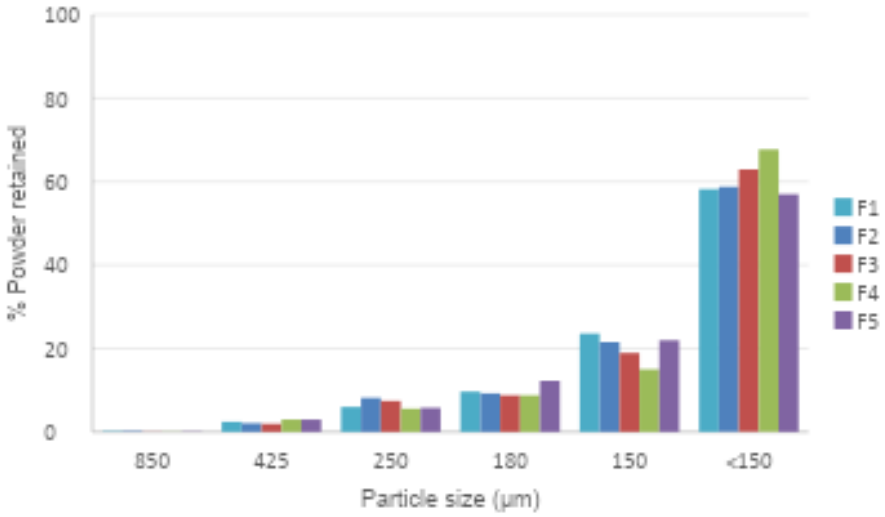


Figure 6. Particle size distribution of red algae powder lozenge formulations (F1–F5).

Evaluation of lozenge formulations

Table 3 summarizes the evaluations of the lozenge formulations. All formulations produced flat tablets with a break line on one side and a smooth surface. The lozenges had a grape aroma, light brownish-white color, and sweet taste profile. The dimensions were uniform, and the tablet weights were within the pharmacopeial limits. The hardness, friability, surface abrasion, and disintegration times for each formulation are presented in Table 3.

Table 3. Evaluation of red algae powder lozenge formulations (F1–F5). Values are presented as mean ± SD (n = 10 for hardness and dimensions; n = 6 for disintegration; and n = 20 for weight variation).

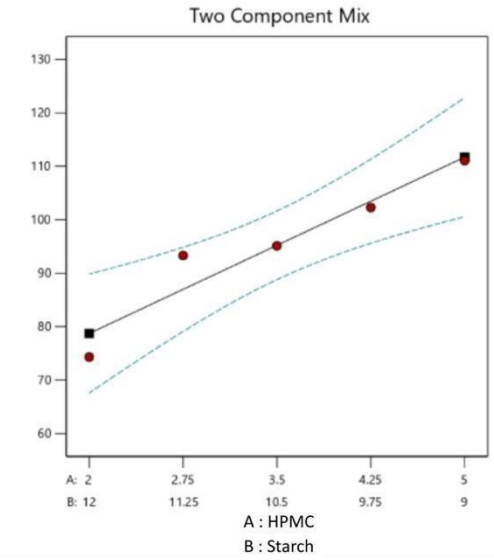
Parameters	F1	F2	F3	F4	F5
Dimension (diameter; thickness, in cm)	0.445; 1.32	0.445; 1.32	0.445; 1.32	0.445; 1.32	0.445; 1.32
Weight (mg)	799 (780 – 820)	807 (790 – 820)	802 (780 – 830)	823 (810 – 830)	815 (790 – 830)
Hardness (N)	74.3±4.47	93.3±8.9	95.1±2.4	102.3±5.8	111.1±6.9
Friability (%)	0.98	0.98	0.86	0.73	0.61
Surface abrasion (%)	0.99	0.93	0.86	0.55	0.43
Disintegration time (minutes)	6.61±0.66	7.87±3.27	9.64±3.31	13.41±3.58	15.88±3.21

Response surface analysis

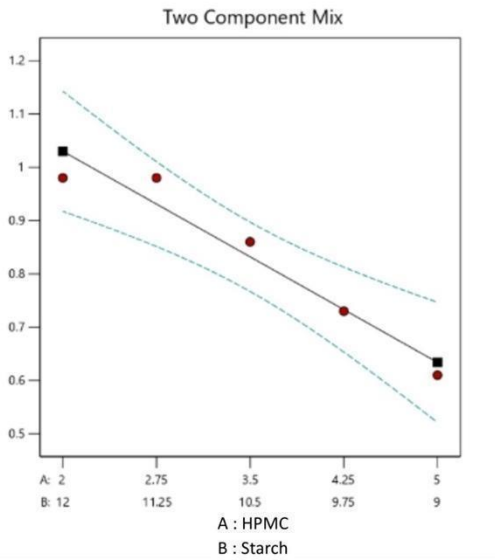
Regression analysis using the SLD model showed significant effects of HPMC and starch concentrations on hardness, friability, surface abrasion, and disintegration ($p<0.05$), with R^2 values of 0.9175–0.9653 (Table 4). The hardness values increased from F1 to F5, whereas the friability and abrasion decreased, and the disintegration times increased. The optimization plot identified F3 (3.5% HPMC, 10.5% starch) as the most desirable formulation, with a desirability value of 1 (Figure 7a–e).

Table 4. Regression results of the SLD model for tablet evaluation parameters ($p<0.05$, considered statistically significant).

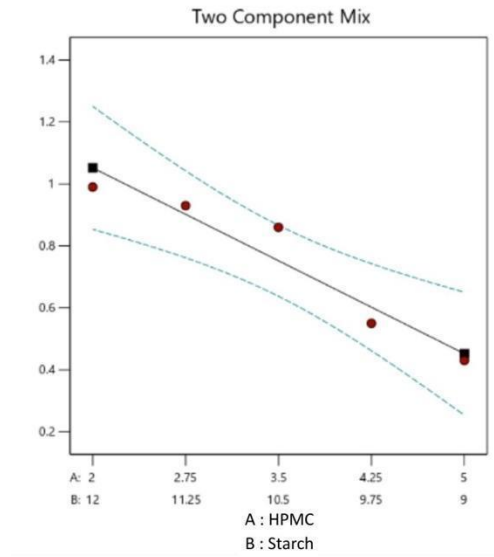
Response	Sum of squares	df	Mean square	*p*-value	R^2
Hardness (N)	680.13	1	680.13	0.0103	0.9175
Friability (%)	0.0980	1	0.0980	0.0064	0.9399
Surface abrasion (%)	0.2250	1	0.2250	0.0098	0.920
Disintegration time (minutes)	58.32	1	58.32	0.0028	0.9653


Two Component Mix
130
120
110
100
90
80
70
60
A: 2 2.75 3.5 4.25 5
B: 12 11.25 10.5 9.75 9
A : HPMC
B : Starch

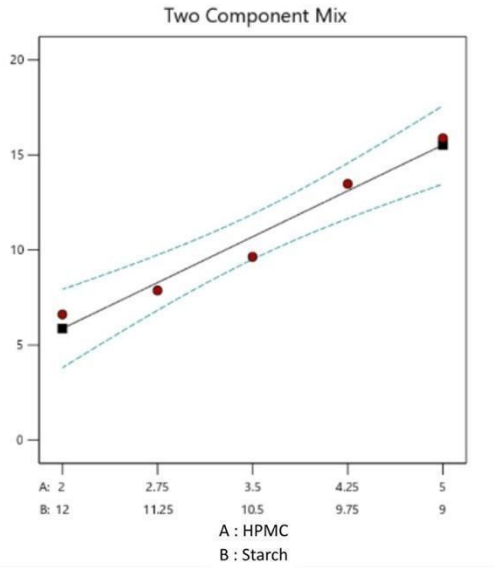
(a)


Two Component Mix
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
A: 2 2.75 3.5 4.25 5
B: 12 11.25 10.5 9.75 9
A : HPMC
B : Starch

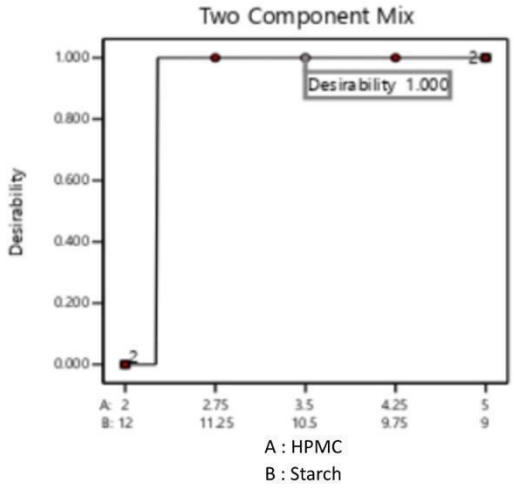
(b)


Two Component Mix
1.4
1.2
1
0.8
0.6
0.4
0.2
A: 2 2.75 3.5 4.25 5
B: 12 11.25 10.5 9.75 9
A : HPMC
B : Starch

(c)


Two Component Mix
20
15
10
5
0
A: 2 2.75 3.5 4.25 5
B: 12 11.25 10.5 9.75 9
A : HPMC
B : Starch

(d)



(e)

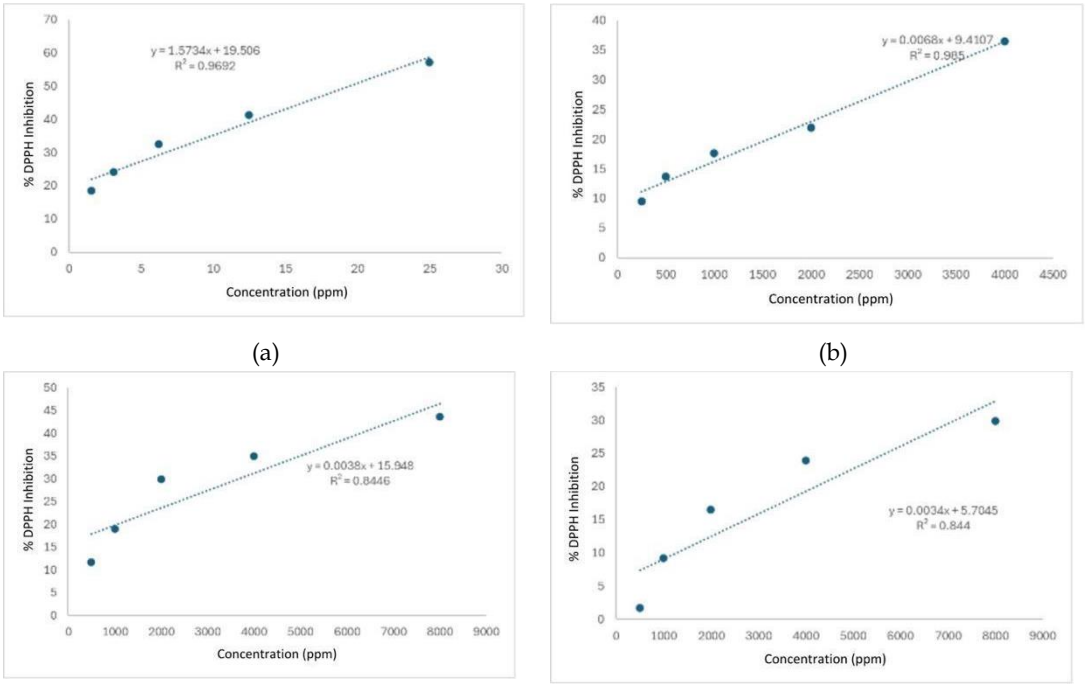
Figure 7. Response surface analysis of lozenge formulations using the SLD method: (a) tablet hardness; (b) friability; (c) surface abrasion; (d) disintegration time; (e) optimization plot showing the desirability of the optimal formulation.

Antioxidant activity

Table 5 presents the IC_{50} values for quercetin, red algae powder, lozenge formulations, and the blank lozenge. The corresponding linear regression curves are presented in Figure 8.

Table 5. Antioxidant activity of the samples was expressed as IC_{50} values from the DPPH assay.

Sample	IC_{50} (ppm)
Quercetin	19.38±0.97
Red algae powder	5.969.02±123.14
Red algae powder lozenge	6.854.34±126.36
Blank lozenge	13.028.09±153.93



(c) (d)

Figure 8. Linear regression curves for DPPH radical scavenging activity: (a) quercetin standard; (b) red algae powder; (c) red algae powder lozenge; (d) blank lozenge.

DISCUSSION

Microscopy and LC–MS characterization of *Kappaphycus alvarezii* powder

Microscopic examination of the red algae powder using iodine reagent revealed the presence of additional compounds in the form of starch granules (Figure 1c). This finding indicates that the red algae powder was not entirely pure, despite its labeling claim of 100% *K. alvarezii*. A similar observation was reported by Yang et al., who described microscopic profiles of corn starch resembling those observed in red algae powder when analyzed with glycerin reagent [12]. The presence of starch in the red algae powder suggests that it was added to increase the bulk weight of the material.

Furthermore, microscopic analysis with chloral hydrate revealed compact cellular fragments (Figure 1d), which closely resembled the thallus microstructure of red algae reported by Tsiresy et al. [13]. This morphological similarity indicates that the powder retained the characteristic structural elements of *K. alvarezii* tissue despite being in a processed powder form. Therefore, the microscopic findings reinforce that the tested material contained genuine red algae components, specifically *K. alvarezii*, although the concurrent presence of starch suggested that the sample was not entirely pure.

LC–MS/MS was chosen because it offers high sensitivity and selectivity for the analysis of complex natural matrices, such as red algae powder. By combining chromatographic separation with mass spectral detection, the SCIEX QTRAP 4500 allows the tentative identification of compounds based on m/z values, fragmentation behavior, and retention characteristics. The analysis further supported the phytochemical richness of the material, identifying quercetin, rutin, kaempferol, and astaxanthin (Figure 3-5).

Quercetin, rutin, and kaempferol are flavonoids with diverse pharmacological activities, particularly antioxidant and anti-inflammatory properties. Their presence in red algae powder is consistent with previous studies on marine-derived materials, where these compounds have been reported as the principal flavonoids contributing to antioxidant potential [14]. Interestingly, the retention times for these flavonoids in this study differed from those reported by Satheeshkumar et al., who observed significantly shorter retention times (5.971, 5.925, and 6.345 min, respectively) [15]. Such discrepancies are commonly attributed to methodological variations, including differences in the solvent systems, mobile phases, and stationary phases used in chromatographic analyses. Despite these differences, the confirmation of compound identity in this study was supported by m/z values that corresponded with database references, ensuring the reliability of the findings of this study.

Astaxanthin was detected at a retention time of 5.3–5.4 min (Figure 2), consistent with the results reported by Kingkaew et al. [16]. This carotenoid is recognized for its high antioxidant activity compared to other carotenoids, and its presence in red algae powder adds further value to the material. The detection of astaxanthin corroborates earlier findings and reinforces the role of red algae as a natural source of potent antioxidant compounds.

Powder evaluation and IPC

IPC results showed that all formulations exhibited suitable flow characteristics for direct compression (DC). The mean flow rate values ranged from 14.2±0.31 g/s (F5) to 19.4±2.04 g/s (F4), while the angle of repose values ranged between 12.0±1.1° and 15.0±3.6°, indicating good flowability across all formulations. The LOD values remained consistent at approximately 3.2–3.3%, suggesting a uniform moisture content within the acceptable range for compression.

Particle size distribution analysis (Figure 6) showed that most of the powder in all formulations was retained in the fraction smaller than 150 µm, indicating the predominance of fine particles. Smaller proportions were distributed within the 150–425 µm range, whereas only negligible amounts were retained above 425 µm. This profile suggests that the powder blends were dominated by fine particles, which favored compactibility, whereas the small proportion of coarser particles may have contributed to maintaining an acceptable flow during processing. As no granulation step was applied, the blends were expected to contain a relatively high

proportion of fines. The selected HPMC concentration range (2.0–5.0%) was chosen to provide sufficient binding capacity for direct compression while still allowing the effect of starch to be evaluated as a disintegrant. At lower concentrations, HPMC is expected to provide basic interparticle adhesion without excessively prolonging tablet disintegration, whereas at higher concentrations, it is expected to increase cohesiveness, hardness, and resistance to friability. Thus, the range was designed to capture the balance between the mechanical strength and disintegration behavior of the lozenge formulations. Variations in the loss on drying (LOD) among the formulations may be related to the hygroscopic nature of HPMC and non-uniform particle distribution during testing [17].

Direct compression with a 1.32 cm die yielded tablets of uniform diameter, while in-process sampling confirmed the consistency of tablet weight and dimensions in accordance with pharmacopeial standards [18]. The favorable flow properties of the powders in formulations F1–F5 supported the decision to employ direct compression, and the presence of HPMC further enhanced the powder flow and tablet compaction. Mechanical properties and disintegration behavior of the lozenges. The evaluation of the red algae powder lozenges demonstrated that the formulations met the pharmacopeial requirements for organoleptic properties, uniformity, hardness, friability, surface abrasion, and disintegration time. The organoleptic assessment showed that all formulas (F1–F5) produced flat tablets with a break line on one side and a plain surface on the other side, consistent with the die design. The use of grape flavor and coloring successfully masked the herbal odor of the active ingredient, while the combination of sucrose and saccharin provided a sweet taste that was acceptable to children. No observable differences were found among the five formulations in terms of their organoleptic characteristics. Uniformity testing confirmed that the tableting machine consistently produced tablets with uniform thickness, diameter, and weight. These results indicate the good reproducibility of the direct compression process, supported by the excellent flowability of the powder observed during in-process control. The hardness test revealed values ranging from 74.3 N in F1 to 111.1 N in F5, all of which complied with the specified limits for lozenges (38.65–137.29 N). A clear trend was observed in which increasing the concentration of HPMC from 2% (F1) to 5% (F5) resulted in higher tablet hardness. The SLD regression analysis confirmed that HPMC was the most significant factor influencing tablet hardness ($p = 0.0103$). These findings align with those of Vlad et al., who reported that higher HPMC concentrations increase hardness through strong adhesive properties activated by water during the formulation [19]. In this study, HPMC was combined with liquid grape flavor and coloring, which further enhanced adhesion, contributing to increased hardness. The SLD regression modeling confirmed that variations in HPMC and starch concentrations significantly influenced the hardness ($p = 0.0103$), friability ($p = 0.0064$), surface abrasion ($p = 0.0098$), and disintegration time ($p = 0.0028$), as presented in Table 4.

Friability and surface abrasion testing also demonstrated compliance, with all formulations achieving values within the acceptable limits (<1%). However, F1 and F2 required repeated testing because the initial friability values exceeded 1%, although the mean of the three trials fell within the permissible range. Regression analysis revealed that starch concentration had the greatest influence on friability and surface abrasion, indicating that higher starch concentrations increased fragility. This trend was consistent with that of Wu et al., who noted that elevated levels of starch, acting as a disintegrant, increased the propensity for friability and surface abrasion due to hydrogen bonding and swelling behavior upon hydration [20]. Consequently, lower starch concentrations resulted in higher mechanical resistance values.

Disintegration testing confirmed that all formulations exceeded the pharmacopeial minimum requirement for lozenges (>30 min). The disintegration times increased from 6.61 ± 0.66 min in F1 to 15.88 ± 3.20 min in F5, reflecting the inverse relationship between starch concentration and disintegration time. Regression analysis further confirmed that HPMC was the most dominant factor ($p = 0.0028$). This observation supports the findings of Allenspach et al., who reported that higher concentrations of HPMC prolonged disintegration due to increased interparticle adhesion and tablet density [21]. In this study, formulations with higher hardness and lower friability also exhibited slower disintegration, indicating that stronger and more mechanically stable tablets were less readily penetrated by the disintegration medium. This can be explained by the formation of a more compact tablet matrix, in which stronger particle bonding reduces the pore space and limits water ingress into the tablet structure. Consequently, the wetting, swelling, and breakup of the matrix occurred more slowly. Conversely, formulations with lower hardness and higher friability are likely to have a less compact structure, allowing easier liquid penetration and faster tablet disintegration. These findings indicate that the mechanical strength imparted by HPMC improved tablet integrity but simultaneously

delayed disintegration, highlighting the need to balance binder and disintegrant concentrations in lozenge formulations.

Taken together, these findings demonstrate the importance of balancing the HPMC and starch concentrations in lozenge formulations. HPMC contributes to greater hardness, reduced friability, and longer disintegration times, whereas starch enhances disintegration but increases fragility when present in excess. From a functional perspective, this balance is critical for ensuring that lozenges maintain sufficient hardness to resist handling and storage stress while providing a prolonged sucking time, which allows the gradual release of bioactive compounds such as quercetin, rutin, kaempferol, and astaxanthin. This controlled release profile not only enhances consumer acceptability but also maximizes the therapeutic and antioxidant benefits of red algae powder.

Formulation optimization using SLD

Formulation optimization was performed using the SLD method with Design Expert 13 software, incorporating hardness, friability, surface abrasion, and disintegration time as the primary responses. Formula 3 (HPMC 3.5% and starch 10.5%) was identified as the most suitable formulation, with a desirability value of 1.0, indicating excellent alignment with the targeted response. The optimization plot (Figure 7) shows that the desirability values of Formula 3 were close to or equal to, 1 reflecting the strong predictive capability of the model in identifying the desired outcome. In this study, Formula 3 not only fulfilled the statistical optimization requirements (tablet hardness, friability, surface abrasion, and disintegration time) but also satisfied the non-statistical quality parameters (organoleptic properties, uniformity of size, and uniformity of weight). Thus, Formula 3 was established as the optimal red algae powder lozenge.

Antioxidant activity (DPPH assay)

The antioxidant activity test using the DPPH method revealed that quercetin, used as a reference standard, exhibited the lowest IC_{50} value of 19.38 ppm (Table 5), classifying it as a very strong antioxidant (<50 ppm) [21]. The DPPH method was used because it is a simple and rapid assay that provides results readily expressed as IC_{50} values. In contrast, the IC_{50} value of the red algae powder was 5,969.02 ppm, indicating very weak antioxidant activity. This observation is consistent with that of Souza et al., who reported an IC_{50} value of 4,280 ppm for methanolic extracts of *K. Alvarezii* [22]. The higher IC_{50} observed in the present study may be attributed to differences in raw material quality, as the red algae powder was sourced from a small-scale household industry and was not verified for purity or antioxidant content. Microscopic analysis confirmed the presence of starch, further supporting that the powder was not composed of 100% pure *K. alvarezii* species.

For the lozenge formulations containing red algae powder, the IC_{50} value was determined to be 6,854.34 ppm, which also classifies it as very weak (>500 ppm). This low antioxidant activity can be explained by the relatively small proportion of red algae powder (20%) incorporated into the lozenges, in addition to the already high IC_{50} value of the powder. Meanwhile, the blank lozenge, lacking any red algae powder, exhibited an IC_{50} value of 13,028.09 ppm, confirming that the excipients used in the formulation made no measurable contribution to the antioxidant activity.

Taken together, these findings suggest that while red algae powder was successfully incorporated into the lozenges, its contribution to antioxidant activity remained minimal. The weak performance is likely due to the limited proportion of active material and the lack of phytochemical standardization in the raw powder. This highlights the importance of improving raw material quality, such as by ensuring the use of standardized extracts with verified phytochemical content and increasing the proportion of active material in formulations. Future studies should consider employing extraction and enrichment techniques to enhance the antioxidant capacity of *K. alvarezii*, thereby enabling the development of lozenges with acceptable physical characteristics and improved functional efficacy.

▪ CONCLUSION

All lozenge formulations of red algae (*Kappaphycus alvarezii*) prepared by direct compression met the pharmacopeial standards for physical quality, with Formula 3 (3.5% HPMC and 10.5% starch) identified as the optimal composition based on hardness, friability, surface abrasion, and disintegration time. Phytochemical analysis confirmed the presence of quercetin, rutin, kaempferol, and astaxanthin in the raw

powder; however, both the powder and its lozenge formulation exhibited weak antioxidant activity (IC_{50} values of 5,969 ppm and 6,854 ppm, respectively). These findings indicate that although acceptable lozenges can be produced, their functional efficacy remains limited owing to the low antioxidant capacity of the starting material.

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