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Antioxidant activity and formulation development of cucumber (*Cucumis sativus*) extract for an under-eye hydrogel mask

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Abstract

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The periorbital region is susceptible to oxidative stress and visible signs of skin aging. Cucumber (*Cucumis sativus*) is a natural source of antioxidant-related constituents. This study compared ultrasound-assisted extraction (UAE) and shaking extraction (SKE) of cucumber and developed an antioxidant-oriented under-eye hydrogel mask. Total phenolic, flavonoid, and polysaccharide contents were determined, and antioxidant activity was assessed using DPPH and FRAP assays. Antibacterial activity against *Cutibacterium acnes* was included as an exploratory screening assay. UAE produced extracts with higher phenolic (38.14 mg GAE/g extract), flavonoid (51.33 mg QE/g extract), and polysaccharide contents than SKE and showed stronger antioxidant activity. No inhibition zone or definable MIC against *C. acnes* was observed under the tested conditions. The selected F3 hydrogel formulation containing 1.5% w/w cucumber extract maintained a pH of 5.51–5.56 and showed no visible phase separation, cracking, or changes in color and transparency after eight heating–cooling cycles. Cucumber extract obtained by UAE showed favorable antioxidant-related characteristics and was successfully incorporated into an under-eye hydrogel mask. The formulation may be considered an antioxidant-oriented natural cosmetic prototype for periorbital skincare and is not presented as an anti-acne treatment.

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1. Introduction

The periorbital region is one of the thinnest and most delicate areas of facial skin and is particularly susceptible to dryness, fine lines, dark circles, and puffiness. These visible changes are associated with oxidative stress, in which excessive reactive oxygen species can contribute to premature skin aging. Therefore, plant-derived antioxidant ingredients are of interest for eye-contour formulations [1–5].

Plant-derived bioactive ingredients are increasingly incorporated into cosmetic formulations as multifunctional agents with antioxidant and soothing properties, including in under-eye creams and eye masks. Among these, cucumber (*Cucumis sativus*) has a long history of use in traditional and contemporary skincare and is recognized as a rich source of phenolic and flavonoid compounds that contribute to its antioxidant and anti-inflammatory activities. Cucumber polysaccharides are hydrophilic constituents that may be suitable for water-based cosmetic formulations. Hydrogel eye patches provide close contact with the periorbital area and offer a convenient format for incorporating natural extracts. Previous studies have quantified total phenolic and flavonoid contents of cucumber extracts and demonstrated significant antioxidant capacity using assays such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant

power (FRAP), supporting its potential as a natural antioxidant resource. These phytochemicals and biofunctional properties suggest that cucumber extract is a promising active ingredient for periorbital formulations aimed at mitigating oxidative stress, puffiness, and irritation, while potentially complementing strategies to reduce inflammation associated with acne-prone skin [6–9].

Although cucumber-based cosmetic products are widely used, comparative information on how extraction conditions affect the recovery of bioactive constituents and their incorporation into under-eye hydrogel masks remains limited. This study compared ultrasound-assisted extraction (UAE) and shaking extraction (SKE) of cucumber and evaluated total phenolic, flavonoid, and polysaccharide contents together with antioxidant activity. We hypothesized that UAE would produce cucumber extracts with higher bioactive contents and stronger antioxidant activity than SKE, supporting the development of an under-eye hydrogel mask. Antibacterial testing against *Cutibacterium acnes* was included as exploratory screening of the extract and was not intended to establish an anti-acne indication for the periorbital product.

2. Materials and Methods

Cucumber was purchased from a local market in Thailand. For the extraction and biochemical analysis, various chemical reagents were utilized, including ethanol (Merck, Germany), gallic acid (Sigma-Aldrich, Germany), Sodium Carbonate (Sigma-Aldrich, Germany), Folin-Ciocalteu reagent (Sigma-Aldrich, Germany), quercetin (Sigma-Aldrich, Germany), aluminium chloride (KemAus, Australia), potassium acetate (QReC, New Zealand), D-glucose (QReC, New Zealand), phenol (QReC, New Zealand), and sulfuric acid (QReC, New Zealand). For the anti-acne activity testing, the bacterial strain *Cutibacterium acnes* was obtained from the Faculty of Medicine, Chiang Mai University, Thailand. The standard drugs used were Clindamycin from PRC International Co., Ltd., Thailand and Clindamycin 0.002 mg discs from Oxide Ltd., UK. The microbiological assays also utilized Brain Heart Infusion (BHI) medium from HiMedia Laboratories Pvt. Ltd., India, Anaerocult® A from Merck KGaA, Darmstadt, Germany, and 6 mm filter paper discs from Macherey-Nagel GmbH & Co. KG, Germany. Subsequently, for the preparation of the under-eye mask, the formulation included gelatin, glycerin, sodium chloride, and the preservative Microcare PHC from Chemipan of Thailand. The principal equipment used in this study included an ultrasonic bath, shaking water bath, centrifuge, rotary evaporator, microplate reader, anaerobic jar, biosafety cabinet, and incubator. General laboratory consumables, including pipette tips and Petri dishes, were used as required.

Extraction of Polysaccharides from Cucumber Extracts Ultrasound-Assisted Extraction (UAE)

Dried cucumber powder was dispersed in distilled water at a fixed liquid-to-solid ratio of 20 mL/g and extracted by UAE at 60 °C for 30 min. The suspension will then be subjected to ultrasound using an ultrasonic bath or probe operating at a fixed frequency 20 kHz with an applied power of 200 W, at a controlled temperature of 60 °C for 30 min, to enhance cell disruption and polysaccharide release. After extraction, the mixture will be cooled to room temperature and centrifuged at approximately 5000 g for 20 min to remove insoluble residues. The supernatant will be concentrated under reduced pressure and polysaccharides were precipitated by the addition of three volumes of ethanol and storage at 4 °C for 12 h The resulting precipitate will be collected by centrifugation, washed with ethanol, and dried to obtain the UKE polysaccharide fraction [10–12].

Shaking Extraction (SKE)

Shaking extraction (SKE), dried cucumber powder was mixed with distilled water at a fixed liquid-to-solid ratio of 80 mL/g and extracted at 80 °C for 2 h, and the extraction may be repeated two to three times to maximize polysaccharide recovery. The combined extracts will be filtered to remove plant residues and then concentrated under reduced pressure. Crude polysaccharides will be obtained by adding three volumes of ethanol to the concentrate, followed by incubation at 4 °C for 12 h to allow complete precipitation. The precipitated polysaccharides will be separated by centrifugation, washed with ethanol to remove low-molecular-weight impurities, and dried to yield the SKE polysaccharide extract [13–15]. The UAE and SKE procedures were compared as practical extraction protocols performed under their respective fixed operating conditions. They were not designed as a fully standardized comparison with identical temperature, extraction time, and liquid-to-solid ratio; therefore, differences between the extracts should be interpreted as the outcomes of the two complete extraction conditions rather than the isolated effect of extraction technique.

Determination of Phytochemical Activity Total Phenolic Content (TPC)

The total phenolic content of extract was analyzed by the Folin–Ciocalteu colorimetric assay as previously described. Briefly, deionized water, extract solution, and Folin–Ciocalteu reagent were combined in a 130:10:10 volume ratio. Next, 100 µL of 7% w/v sodium carbonate solution was added to neutralize the reaction mixture. After incubation in the dark at room temperature for 30 min, the absorbance was recorded at 734 nm using a microplate spectrophotometer (Sunrise, Tecan, Männedorf, Switzerland). The phenolic content was calculated from a gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) [16].

Total Flavonoid Content (TFC)

The total flavonoid content of the extracts was assessed using an aluminum chloride-based colorimetric method as previously described. In brief, 30 µL of each sample solution was dispensed into individual wells of a 96-well microplate,

followed by the addition of 50 µL of 5% w/v sodium nitrite solution and 35 µL of 10% w/v aluminum chloride solution. The reaction mixtures were then kept in the dark at room temperature for 5 min, after which 85 µL of 1 N sodium hydroxide was added to each well. Following a further 6-min incubation under the same conditions, the absorbance was recorded at 520 nm using a microplate reader (CLARIOstar®, BMG LABTECH, Ortenberg, Germany). A quercetin calibration curve (5–100 µg/mL) was constructed, and flavonoid content was calculated and reported as milligrams of quercetin equivalents per gram of extract (mg QE/g extract) [17].

Evaluation of Antioxidant Activity DPPH Radical Scavenging

The antioxidant activity of the cucumber extracts was evaluated using the DPPH free-radical scavenging assay. Stock solutions of the UAE and SKE extracts were prepared in ethanol and serially diluted to obtain extract concentrations of 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 µg/mL. These concentrations were selected to generate concentration–response curves encompassing approximately 50% DPPH radical scavenging activity. A 0.2 mM DPPH solution was freshly prepared in ethanol. Subsequently, 75 µL of each extract solution was mixed with 150 µL of DPPH solution in a 96-well microplate and incubated in the dark at ambient temperature for 30 min. The absorbance was measured at 517 nm using a microplate spectrophotometer. Ethanol was used as the blank, while L-ascorbic acid was used as the reference standard. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ DPPH radical scavenging activity} = [1 - (A/B)] \times 100$$

where A is the absorbance of the DPPH solution mixed with the extract or L-ascorbic acid, and B is the absorbance of the DPPH solution mixed with ethanol (blank) [16].

Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) of the samples was evaluated following a previously described protocol. In brief, the FRAP working reagent was freshly prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution, and 20 mM $FeCl_3$ at a volumetric ratio of 10:1:1. A 200 µL aliquot of this reagent was then added to 20 µL of the sample, and the mixture was incubated at 37 °C for 30 min. The absorbance of the resulting solution was recorded at 570 nm, using L-ascorbic acid as the reference antioxidant. FRAP values were calculated using the same approach employed for the DPPH assay [16, 17].

Quantification of Polysaccharide Content

Total polysaccharide content was determined using a microplate-adapted phenol–sulfuric acid colorimetric assay based on the method of Yue *et al.* [18] with minor modifications. Briefly, sample solutions will be prepared in distilled water at appropriate dilutions, and 20 µL of each sample will be pipetted into the wells of a 96-well microplate. An equal volume of 5 % w/v phenol solution (20 µL) will then be added to each well and mixed gently. Immediately thereafter, 100 µL of concentrated sulfuric acid will be rapidly dispensed into each well, ensuring that the total volume of sulfuric acid is 3 times the combined aqueous volume. The plate was incubated at 90 °C for 10 min in a preheated oven or water-bath-compatible microplate holder to allow color development, then cooled to room temperature. Absorbance will be measured at 490 nm using a microplate reader. A calibration curve will be constructed using glucose standard solutions (20–100 µg/mL), treated in the same way as the samples, and total polysaccharide content will be expressed as milligrams of glucose equivalent per gram of extract.

Antibacterial Activity against C. acnes

The antibacterial activity of cucumber extract against *C. acnes* was evaluated by agar disc diffusion [19, 20]. Crude extract was dissolved in ethanol to prepare test solutions of 5, 50, and 500 mg/mL. Ten microliters of each solution were applied to sterile 6-mm filter-paper discs, providing 0.05, 0.5, and 5 mg extract per disc, respectively, and dried aseptically. Clindamycin (0.002 mg per disc) and ethanol-loaded discs served as positive and negative controls, respectively. A *C. acnes* suspension in Brain Heart Infusion (BHI) broth was adjusted to 0.5 McFarland and spread uniformly onto BHI agar plates. The extract, control, and antibiotic discs were placed on the inoculated agar and incubated anaerobically at 37±1°C for 48 h. Antibacterial activity was determined from the diameter of the inhibition zone, including the disc, in millimeters. As poorly diffusing high-molecular-weight or polysaccharide-rich compounds may yield small inhibition zones, disc diffusion results were interpreted alongside broth microdilution results to evaluate bacterial growth inhibition in liquid medium.

The minimum inhibitory concentration (MIC)

The MIC of cucumber extract and clindamycin against *C. acnes* was determined by broth microdilution in 96-well plates. Filter-sterilized cucumber extract (500 mg/mL in ethanol) and clindamycin (10 µg/mL in sterile water) were serially two-fold diluted in growth medium, followed by addition of standardized *C. acnes* suspension (approximately 0.5 McFarland). Plates were incubated anaerobically at 37±1°C for 48 h. The MIC was the lowest concentration producing a clear well without visible turbidity or sediment [21, 22].

Cucumber-Based Under-Eye Hydrogel Mask
Polysaccharide Under-Eye Hydrogel Mask base

The compositions of the polysaccharide under-eye hydrogel mask base were first optimized by varying the levels of gelling agent, humectant, and water while keeping the isotonicity-adjusting agent and preservative system constant. Accordingly, four preliminary formulations (F1–F4) were prepared using different concentrations of gelatin and glycerin, as summarized in Table 1.

Table 1 Formulation of polysaccharide under-eye hydrogel mask base.

Phase	Ingredient	%w/w			
		F1	**F2**	**F3**	**F 4**
A	gelatin	8	12	14	16
	glycerin	38	36	36	34
	DI water	Qs.100	Qs.100	Qs.100	Qs.100
B	sodium chloride	0.3	0.3	0.3	0.3
C	phenoxyethanol, chlorphenesin, and glycerin	1	1	1	1

Note: Qs.100 = Quantity sufficient to 100% w/w. Phase A = gelling and humectant phase (gelatin, glycerin, water). Phase B = isotonicity-adjusting agent (sodium chloride). Phase C = preservative system (phenoxyethanol, chlorphenesin, and glycerin).

Procedure

Phase A ingredients (gelatin and glycerin) are weighed and mixed, then DI water is added to make the batch up to 100% w/w and the dispersion is heated with gentle stirring until the gelatin is completely dissolved and a clear solution is obtained. The solution is allowed to cool to about 45 °C, after which sodium chloride (Phase B) is added and mixed until fully dissolved. Finally, the preservative blend containing phenoxyethanol, chlorphenesin, and glycerin (Phase C) is incorporated under continuous stirring until a homogeneous under-eye mask base is formed; the formulation is then degassed if necessary and poured into suitable containers or molds for further evaluation.

Optimization of Hydrogel Mask Base and Active Loading

The hydrogel under-eye mask base was optimized by varying gelatin and glycerin concentrations in 50 g batches to obtain clear, flexible, and easily handled patches. Cucumber extract was then incorporated at 0.5, 1.0, and 1.5% w/w, with water adjusted to maintain a total composition of 100% w/w. Formulations were evaluated for homogeneity, color, transparency, phase separation, and structural integrity before further testing.

Evaluation of Under-Eye Hydrogel Mask Properties

Physicochemical Characterization and Stability

The physicochemical stability of the under-eye mask formulations was evaluated using pH measurement and qualitative physical observations, including appearance, color, transparency, phase separation, surface condition, odor, and manual handling characteristics. Instrumental measurements of viscosity, rheology, tensile strength, swelling ratio, water retention, moisture content, and adhesiveness were not performed in the present study. Therefore, the formulation results are limited to qualitative physical evaluation and should be confirmed by quantitative mechanical and performance testing in future studies.

Physical Stability Test

The physical stability of the under-eye masks will be assessed by visual inspection and simple handling tests at predefined time points during storage. The overall appearance, color, transparency or opacity, presence of phase separation or syneresis, surface smoothness, and any evidence of cracking or deformation of the hydrogel will be recorded. In addition, the texture and mechanical behavior (flexibility, elasticity, softness, and firmness) will be qualitatively evaluated by manual stretching and compression to ensure that the patches maintain adequate integrity and user-acceptable sensory properties throughout the study period [23, 24].

Conditional Stability Test (Heating–Cooling Cycle)

Conditional stability was investigated using a heating–cooling cycle test adapted from cosmetic stability guidelines and hydrogel mask literature. Briefly, samples of each formulation will be stored alternately at elevated and reduced temperatures by placing them at 45 °C for 8 h in a temperature-controlled oven, followed by transfer to a cooled chamber at 15 °C for 8 h, constituting one full cycle. This heating–cooling cycle will be repeated for eight consecutive cycles, and after each cycle the formulations will be examined for changes in appearance, color, odor, pH, viscosity, and integrity of the hydrogel structure, as well as any visible interactions with the packaging, to confirm that the under-eye masks remain stable under thermal stress conditions representative of transport and variable storage environments [23, 25].

Statistical analysis

All experiments were performed independently in triplicate, and data are presented as mean ± standard deviation. Differences between UAE and SKE extracts were analyzed using an independent-samples t-test. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was applied. Statistical analysis was performed using SPSS software, and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

Extraction of Polysaccharides from Cucumber Extracts

SKE produced a higher total extract yield (3.40 ± 0.25%) than UAE (2.75 ± 0.80%; $p < 0.05$), whereas UAE showed higher total phenolic, flavonoid, and polysaccharide contents by calculating the percentage yield of dried extracts relative to the initial dry weight of plant material. The mean %yield values for each extraction method are summarized in Table 2.

Table 2 Yield of cucumber extracts obtained by ultrasound-assisted extraction (UAE) and shaking extraction (SKE).

Sample	Extraction method	%Yield (Mean ± S.D.)
Cucumber extract	UAE	2.75 ± 0.80^{a}
	SKE	3.40 ± 0.25^{b}

Note: %Yield represents the percentage (w/w) of dried extract obtained relative to the initial dry weight of cucumber material used in each extraction. Different superscript letters (a–b) within the column indicate a statistically significant difference between extraction methods at $p < 0.05$.

The cucumber extract obtained by SKE showed a slightly higher extraction yield (3.40 ± 0.25 %) than that obtained by UAE (2.75 ± 0.80 %), suggesting that, under the present experimental conditions, prolonged mechanical agitation with sufficient contact time between solvent and plant matrix favored solubilization of solid constituents. This result contrasts with several reports in which ultrasound-assisted extraction generally provides equal or higher polysaccharide yields than conventional hot-water and shaking extraction, mainly due to acoustic cavitation–induced cell wall disruption and enhanced mass transfer. The higher total yield obtained by SKE should be interpreted cautiously because UAE and SKE were performed under different liquid-to-solid ratios, temperatures, and extraction times. Therefore, the observed difference reflects the overall outcomes of the two extraction conditions rather than the isolated effect of extraction technique (Fig.1). Nevertheless, literature comparing optimized UAE and hot-water protocols indicates that UAE can still offer advantages such as shorter extraction time, reduced energy consumption, and, in some cases, improved functional properties of the recovered polysaccharides, suggesting that further process optimization might increase the UAE yield for cucumber to a level comparable to or higher than that of SKE [26–31].

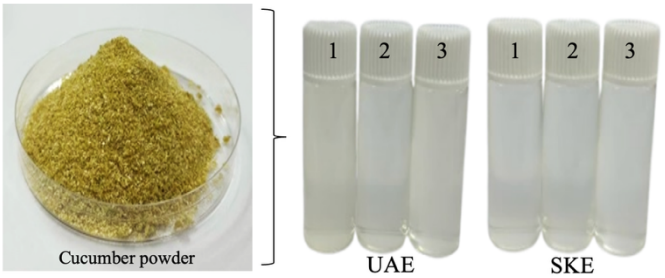


Fig. 1 Cucumber powder and aqueous extracts obtained by ultrasound-assisted extraction (UAE) and shaking extraction (SKE).

Determination of Phytochemical Activity

The total phenolic and flavonoid contents of the cucumber extracts obtained by ultrasound-assisted extraction (UAE) and shaking extraction (SKE) were quantified to compare the efficiency of both techniques in recovering polyphenolic constituents. The mean values of total phenolic content (TPC) and total flavonoid content (TFC) for each extraction method are summarized in Table 3.

Table 3 Total phenolic and flavonoid contents of cucumber extracts obtained by ultrasound-assisted extraction (UAE) and shaking extraction (SKE).

Sample	Extraction Method	Total Phenolic Content (mg GAE/g extract)	Total Flavonoids Content (mg QE/g extract)
Cucumber extract	UAE	38.14 ± 0.28^{a}	51.33 ± 0.11^{a}
	SKE	28.54 ± 0.97^{b}	38.09 ± 0.09^{b}

Note: Data are expressed as mean ± standard deviation (n = 3). GAE = gallic acid equivalent; QE = quercetin equivalent. Different superscript letters (a–b) within the same column indicate a statistically significant difference between extraction methods at $p < 0.05$.

The cucumber extract obtained by UAE showed a significantly higher total phenolic content (38.14 ± 0.28 mg GAE/g extract) than that produced by SKE (28.54 ± 0.97 mg GAE/g extract), indicating that ultrasound treatment enhanced the recovery of phenolic constituents from the cucumber matrix. This observation agrees with previous work demonstrating that ultrasonic-assisted extraction improves cell wall disruption and solvent penetration, thereby increasing phenolic extraction efficiency compared with conventional techniques [32–34].

A similar pattern was observed for total flavonoids, where UAE yielded 51.33 ± 0.11 mg QE/g extract, markedly higher than the 38.09 ± 0.09 mg QE/g extract obtained with SKE, suggesting that flavonoid compounds in cucumber are also more efficiently solubilized under ultrasonic conditions. Studies in other plant materials have reported comparable enhancements in flavonoid recovery and associated antioxidant activity when ultrasound is applied, likely due to cavitation-induced disruption of plant tissues and shortened diffusion distances [35–37].

Taken together, these results indicate that UAE is a more effective method than SKE for producing cucumber extracts with higher total phenolic and total flavonoid contents, as estimated by the respective colorimetric assays. These assays

do not identify individual phytochemical compounds, which are key contributors to antioxidant capacity and potential anti-inflammatory effects relevant for cosmeceutical applications such as under-eye mask formulations. The magnitude of TPC and TFC obtained here falls within the range reported for *Cucumis sativus* and related cucurbit species, supporting the suitability of cucumber as a valuable source of bioactive phytochemicals for topical products [38].

Evaluation of Antioxidant Activity

The antioxidant capacity of the cucumber extracts obtained by ultrasound-assisted extraction (UAE) and shaking extraction (SKE) was further assessed using two complementary in vitro assays, namely DPPH radical-scavenging and ferric reducing antioxidant power (FRAP). The mean DPPH IC_{50} values and FRAP activities for each extract are presented in Table 4.

The DPPH IC_{50} values of the UAE and SKE extracts were 8.39 ± 0.32 and 9.20 ± 0.40 μg/mL, respectively. The UAE extract showed a significantly lower IC_{50} value than the SKE extract, indicating stronger free-radical scavenging activity under the tested conditions. This modest improvement is consistent with previous studies reporting that ultrasound-assisted extraction can enhance the accessibility of antioxidant phytochemicals through cavitation-driven cell-wall disruption and improved solvent penetration, thereby lowering IC_{50} values in DPPH assays compared with conventional extraction techniques [39–41]. A more pronounced difference was observed in the FRAP assay, where the UAE extract showed a markedly higher ferric-reducing capacity (53.71 mmol AAE/g extract) relative to the SKE extract (45.51 mmol AAE/g extract), suggesting that ultrasound treatment generated a cucumber extract richer in electron-donating constituents. The superior FRAP value of the UAE extract is consistent with its higher phenolic and flavonoid contents and with literature indicating positive correlations between total phenolics, total flavonoids, and reducing power in plant extracts, including *Cucumis* species [42–44]. Overall, these findings demonstrate that ultrasound-assisted extraction not only increases the polyphenolic content of cucumber extracts but also translates into significantly enhanced antioxidant performance in both radical-scavenging (DPPH) and ferric-reducing (FRAP) assays, supporting the selection of UAE-derived extracts as preferred actives for antioxidant-enriched under-eye mask formulations.

Quantification of Polysaccharide Content

The total polysaccharide content of the cucumber extracts obtained by ultrasound-assisted extraction (UAE) and shaking extraction (SKE) was quantified using the phenol–sulfuric acid colorimetric assay to compare the efficiency of both techniques in recovering carbohydrate polymers from the plant material. The mean values of D-glucose equivalent per gram of extract for each extraction method are summarized in Table 5.

SKE provided a higher total extract yield, whereas UAE produced extracts with higher phenolic, flavonoid, and polysaccharide contents per gram of extract. Notably, UAE significantly increased total polysaccharides (38.33 vs. 22.45 mg D-glucose/g extract for SKE), likely because ultrasonic cavitation improves cell-wall disruption, solvent penetration, and mass transfer. These polysaccharides may contribute to functional properties such as viscosity and gel-forming ability in cosmetic formulations [45–50].

Table 4 Antioxidant activity of cucumber extracts determined by DPPH and FRAP assays.

Sample	Extraction Method	DPPH IC_{50} (μg/mL) (Mean ± S.D.)	FRAP (mmol AAE/g extract) (Mean ± S.D.)
Cucumber extract	UAE	8.39 ± 0.32^{a}	53.71 ± 0.41^{a}
	SKE	9.20 ± 0.40^{b}	45.51 ± 0.04^{b}

Note: Lower IC_{50} values indicate stronger free-radical scavenging activity in the DPPH assay. AAE = ascorbic acid equivalent. Data are expressed as mean ± standard deviation (n = 3). Different superscript letters (a–b) within the same column denote statistically significant differences between extraction methods at $p < 0.05$

Table 5 Total polysaccharide content of cucumber extracts determined by the phenol–sulfuric acid method.

Sample	Extraction Method	Total polysaccharide content (mg D-glucose/g extract) (Mean ± S.D.)
Cucumber extract	UAE	38.33 ± 0.03^{a}
	SKE	22.45 ± 0.30^{b}

Note: Data are expressed as mean ± standard deviation (n = 3). Total polysaccharide content is reported as milligrams of D-glucose equivalent per gram of extract (mg D-glucose/g extract) determined using the phenol–sulfuric acid colorimetric assay. Different superscript letters (a–b) within the same column indicate a statistically significant difference between extraction methods at $p < 0.05$.

Table 6 Antibacterial activity of cucumber extract against *C. acnes* by disc diffusion.

Sample	Amount per disc	Inhibition zone against *C. acnes* (mm)			
		Plate 1	Plate 2	Plate 3	Mean ± SD
Cucumber extract	5 mg	ND	ND	ND	ND
	0.5 mg	ND	ND	ND	ND
	0.05 mg	ND	ND	ND	ND
Ethanol (Negative Control)	10 mg	ND	ND	ND	ND
Clindamycin (Positive Control)	0.002 mg	46.85	47.45	47.95	47.42 ± 0.55

Note: ND = not detected (no visible inhibition zone)

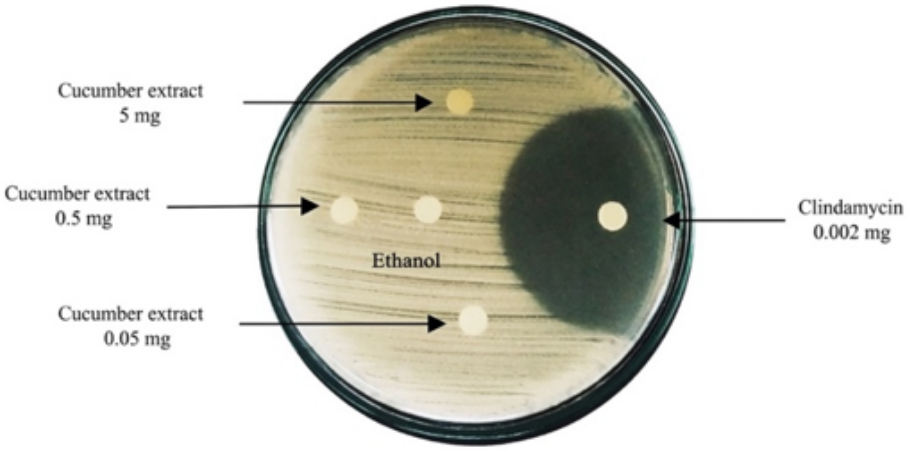


Fig. 2 Antibacterial activity of cucumber extract against *C. acnes* compared with the reference drug clindamycin using the disc diffusion method.

Antibacterial Activity against C. acnes

No inhibition zones were observed for cucumber extract at 0.05–5 mg per disc, and no MIC was detected at 0.06–125 mg/mL, indicating no direct anti-*C. acnes* activity under the tested conditions. In contrast, clindamycin (0.002 mg per disc) produced an inhibition zone of 47.42 ± 0.55 mm, whereas ethanol showed no activity (Table 6). As the periorbital area is not a typical acne site, this antibacterial test was exploratory and does not support an anti-acne claim for the under-eye mask [51–53]. A representative agar plate illustrating the antibacterial activity of cucumber extract against *C. acnes* compared with the reference drug clindamycin in the disc diffusion assay is shown in Fig. 2.

The minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of cucumber extract and the reference antibiotic clindamycin against *C. acnes* was further evaluated using a standard broth microdilution assay in 96-well plates, based on a 2-fold serial dilution of each stock solution. The final concentrations of cucumber extract and clindamycin present in wells 1–12, which were used to determine the MIC values, are summarized in Table 7.

The broth microdilution assay revealed that cucumber extract did not achieve a definable minimum inhibitory concentration against *C. acnes* within the tested concentration range (62.5–0.03 mg/mL), as visible bacterial growth was observed in all wells containing the extract. This lack of MIC agrees with several reports in which crude plant extracts rich in polysaccharides or non-polar constituents showed weak or no direct bacteriostatic effects on *C. acnes* when evaluated by standardized microdilution protocols, despite possessing antioxidant or anti-inflammatory activities that may still be beneficial in acne management [54, 55].

In contrast, the reference antibiotic clindamycin exhibited clear inhibitory activity, with a MIC value of 0.02 µg/mL, defined as the lowest concentration that completely prevented visible growth of *C. acnes* after anaerobic incubation. This MIC lies within the susceptibility range reported for clinical *C. acnes* isolates in previous studies that used broth microdilution or E-test methods, confirming that the experimental conditions were appropriate and that the test strain was clindamycin-susceptible. The pronounced difference between the inactive cucumber extract and the potent clindamycin control underscores that, in this formulation, cucumber polysaccharides do not act as classical antibiotics against *C. acnes* and that any anti-acne benefit in a topical product would likely derive from indirect mechanisms such as reduction of oxidative stress, modulation of inflammation, or improvement of skin barrier function rather than direct bacteriostatic effects [56–59]. A representative 96-well microplate from the broth microdilution assay used to determine the minimum inhibitory concentration of clindamycin and cucumber extract against *C. acnes* is shown in Fig. 3.

Table 7 Minimum inhibitory concentration (MIC) of cucumber extract and clindamycin against *C. acnes* determined by broth microdilution.

Sample	Concentration of Well no.1-12											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Cucumber extract (mg/mL)*	125	62.5	31.25	15.62	7.81	3.9	1.95	0.97	0.48	0.24	0.12	0.06
Clindamycin (µg/mL)**	2.5	1.25	0.62	0.31	0.16	0.08	0.04	0.02	0.01	0.005	0.002	0.001

Note: *Final test concentrations obtained from a 2-fold serial dilution of a 500 mg/mL^{-1} cucumber extract stock solution (starting concentration in the first well 125 mg/mL). **Final test concentrations obtained from a 2-fold serial dilution of a 10 µg/mL clindamycin stock solution (starting concentration in the first well 2.5 µg/mL).

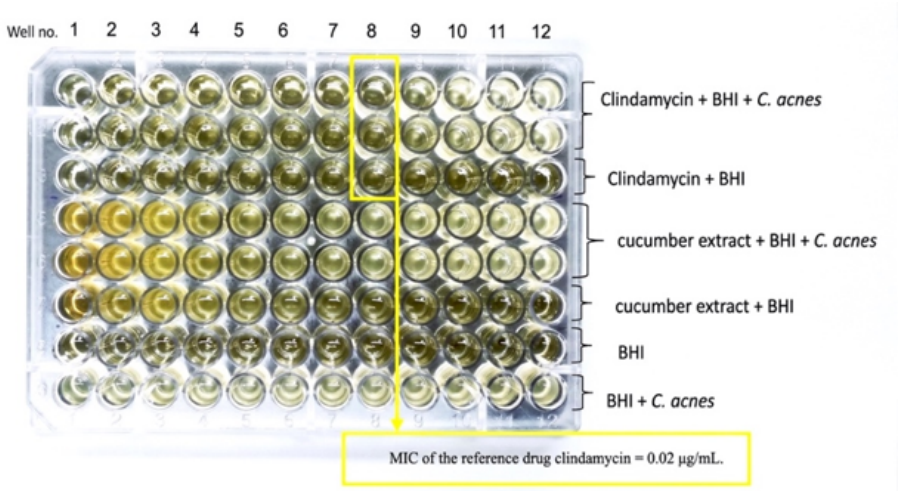


Fig. 3 Results of the minimum inhibitory concentration (MIC) test for *C. acnes* using the broth microdilution method for the reference drug clindamycin and cucumber extract.

Cucumber-Based Under-Eye Hydrogel Mask

The hydrogel base was optimized by varying gelatin and glycerin levels (F1–F4). F3 was selected based on its clear appearance, flexibility, handling, and suitable skin adhesion [60]. Cucumber extract (0.5–1.5% w/w) was incorporated into F3. All masks remained homogeneous and transparent, with a slight increase in yellow color as extract concentration increased. The 1.5% w/w formulation provided the best smoothness, elasticity, and skin feel and was selected as the optimal under-eye hydrogel mask [61, 62]. As summarized in Table 8, the base hydrogel mask was clear and uniform, indicating good film formation. Incorporation of 0.5–1.5% w/w cucumber extract produced a progressively yellowish color while maintaining transparency and homogeneity, with no visible phase separation or precipitation.

Table 8 Visual appearance of under-eye hydrogel mask containing cucumber extract.

Sample	Base (0%)	F1 extract (0.5%w/w)	F2 extract (1 %w/w)	F3 extract (1.5%w/w)
Under-eye Hydrogel mask				
Visual Appearance	Clear, uniform film	Slight yellowish, clear and homogeneous	Light yellow, clear and homogeneous	More intense yellow, clear and homogeneous

Note: The visual characteristics of the under-eye hydrogel mask developed from the base and from formulas containing 0.5, 1.0, and 1.5% w/w cucumber extract were evaluated in terms of color, transparency, and uniformity of the gel matrix.

Table 9 Physicochemical and stability evaluation of cucumber-based under-eye hydrogel mask.

Parameter	Initial (Day 0)	After Heating–Cooling Cycle 4	After Heating–Cooling Cycle 8
pH (mean ± SD)	5.53 ± 0.53	5.56 ± 0.32	5.51 ± 0.94
Appearance	Clear, smooth, no defects	Clear, smooth, no defects	Clear, smooth, no defects
Color	Pale yellow, unchanged	Pale yellow, unchanged	Pale yellow, unchanged
Transparency	Transparent	Transparent	Transparent
Phase separation	None observed	None observed	None observed
Surface condition	No cracking	No cracking	No cracking
Odor	Characteristic, acceptable	Characteristic, acceptable	Characteristic, acceptable
Texture(elasticity/softness)	Soft, elastic, intact gel	Soft, elastic, intact gel	Soft, elastic, intact gel
Mechanical integrity	No tearing on stretching	No tearing on stretching	No tearing on stretching

Note: pH values at each time point were not significantly different ($p < 0.05$)

Evaluation of Under-Eye Hydrogel Mask Properties Physicochemical Characterization and Stability

The physicochemical properties and accelerated stability of the optimized cucumber-based under-eye hydrogel mask was evaluated under heating–cooling conditions, and the results are summarized in Table 9. The selected formulation-maintained pH and qualitative physical appearance, including color, transparency, absence of phase separation, and absence of cracking, during eight heating–cooling cycles. under the heating–cooling protocol. The pH values remained within a narrow, statistically unchanged range and all visual attributes including color, transparency, surface condition, and odor were preserved throughout eight cycles. The texture also stayed soft and elastic without tearing, instrumental rheological properties, tensile strength, adhesiveness, active-content stability, antioxidant retention, and microbial stability were not evaluated; therefore, long-term and comprehensive product stability cannot be concluded.

4. Conclusion

Although SKE produced a slightly higher total extract yield, UAE recovered higher total phenolic, flavonoid, and polysaccharide contents from cucumber. Consequently, the extracts obtained via UAE exhibited superior antioxidant capacities in both DPPH radical scavenging and FRAP assays. Microbiological evaluation showed that the cucumber extract had no direct antibacterial activity against *C. acnes* under the tested conditions. Nevertheless, an under-eye hydrogel mask containing cucumber extract was successfully developed using an optimized gelatin-glycerin base containing 1.5% w/w cucumber extract. This formulation displayed high transparency, homogeneity, and favorable sensory properties. Furthermore, accelerated stability testing via heating–cooling cycles confirmed that the mask maintained its mechanical integrity, pH, and visual appearance. Therefore, while the mechanism is not directly antimicrobial, the developed cucumber-based hydrogel mask represents a natural cosmetic prototype for antioxidant-oriented periorbital skincare. Moreover, the developed under-eye hydrogel mask should not be considered an anti-acne treatment. Future studies should evaluate anti-inflammatory activity, instrumental hydrogel properties, long-term stability, and skin compatibility before further cosmetic development.

5. Suggestions

Given that the cucumber extract exhibited no direct antibacterial activity against *C. acnes* in both disc diffusion and broth microdilution assays, future studies should focus on evaluating the indirect anti-acne potential of the formulation by investigating its in vitro anti-inflammatory effects, such as the inhibition of pro-inflammatory cytokines, to validate its use for soothing acne-prone periorbital skin.

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7. Declaration of Generative AI in Scientific Writing

The authors affirm that, in drafting the manuscript, any generative AI systems, including language models, were employed solely to improve the clarity and fluency of the English writing. The study itself was designed, conducted, and interpreted by the authors, who take full responsibility for the originality and authenticity of the content.

8. CRediT Author Statement

Warongporn Rattanabun contributed to conceptualization, methodology, investigation, formal analysis, and writing original draft. **Pattima Duangthong** was involved in methodology, validation, investigation, data curation, and writing review and editing. **Kanyarat Thiinto** contributed to investigation, resources, and data curation **Kittikawin Buasaart** was responsible for software, formal analysis, and visualization. **Pattarawat Kraipiyaset** contributed to visualization and writing review and editing. **Wongnapa Nakyai** provided resources and supervision. **Natnicha Phungsara and Daraporn Panyasang** contributed to methodology, supervision, and project administration **Wannisa Keawbankrud** was responsible for conceptualization, supervision, funding acquisition, and writing review and editing.

9. Research Involving Human and Animals Rights

This research does not involve experiments on animals and human.

10. Ethics Approval and Consent to Participate

Ethics approval was not required for this study because no experiments involving human subjects were conducted.

11. Declaration of Competing Interest

The authors declare that they have no competing interests.

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