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Citrus Hystrix Oil Incorporated into Cosmetic Formulation: Physicochemical, Stability and Antimicrobial Properties

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ABSTRACT

This study explores the incorporation of *Citrus hystrix* oil (CHO) into cosmetic formulation as natural alternative to synthetic additives due to its antioxidant, antimicrobial and anti-inflammatory properties. The peel of *Citrus hystrix* fruit was extracted by using hydrodistillation method and the bioactive components were identified by GC-MS. The CHO was incorporated into cosmetic formulation at different amounts (0.1, 0.2, 0.3 and 0.4%). FTIR analysis revealed the successful incorporation of CHO into the cosmetic formulation. The formulation containing 0.4% CHO exhibited the highest total phenolic content which was 163.63 mg GAE/mL, confirming its superior antioxidant potential. The cosmetic formulation incorporating CHO shows improved physicochemical properties. The shear stress trends demonstrate shear-thinning properties, confirming the desirability for cosmetic application. The increase of spreadability over time reflects that the formulation can easily spread which offers smooth coverage and fast absorption on skin. In addition, stability studies for 8 weeks show that the formulations containing CHO maintained the pH, weight and moisture content with minimal variation, suggesting enhanced stability. The antimicrobial activity against *Escherichia coli* shows the inhibition zone increased with the increasing of CHO, ranging from 6.0 mm to 15.7 mm, validating the efficacy of CHO. Overall, the findings of this study demonstrate that CHO enhances physicochemical, stability and antimicrobial activity of the cosmetic formulation, confirming its viability as a safer, effective and sustainable additive for natural skincare products.

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INTRODUCTION

The rapid growth experienced by the cosmetic industry in the past decades has been driven by the increasing consumer demand for beauty enhancement, improving skin health, and promoting well-being. However, the widespread use of synthetic chemicals in cosmetic products has raised concern among consumers as it could trigger the potential risk that is associated with long-term exposure to these substances (Kamaruzaman & Yusop, 2021). This is because most of the cosmetic formulations contain synthetic ingredients that act as preservatives, fragrance, and other chemical additives to enhance product stability, scent, and overall performance (Hartmann & Klaschka, 2017). These attract the cosmetic industry's attention to emphasize transparency and the use of natural ingredients in their products. Therefore, *Citrus hystrix* oil (CHO), also known as kaffir lime oil, is taken as one such plant-based ingredient that has garnered significant interest which can be derived from the leaves and peels of the lime plant. Additionally, with its rich in bioactive compounds, including antioxidants, antimicrobials, anti-inflammatory agents, and anti-enzymatic properties making it a suitable component for use in cosmetic formulations (Long & Quoc, 2023). Essential oils which rich in phenolic compounds demonstrate strong antioxidant activity by neutralizing reactive oxygen species, and they also inhibit melanogenesis through anti-tyrosinase effects, making them promising agents for skin-brightening and anti-aging formulations (Yohannis et al., 2026). Thus, CHO is now gaining attention for its potential role in cosmetics, particularly in improving skin health and delivering fragrance naturally without the need for harmful synthetic ingredients (Zhao et al., 2023).

CHO has long been used in a traditional way in Southeast Asian cuisine and medicinal practices due to its ability to provide a wide range of health benefits (Kulig et al., 2022). Research has shown that plant-based ingredients like CHO offer significant benefits, including protection against oxidative stress and reduction of inflammation of microbial growth (Long & Quoc, 2023). Moreover, citronellal, citronellol, and terpinene-4-ol act as antimicrobial agents, are used in aromatherapy and function as repellents, while citronellal and citronellol also provide natural fragrance and preservative properties (Budiarto et al., 2024). Lubinska-Szczygeł et al. (2018) analyzed the main aromatic compounds in peels and pulps of *Citrus hystrix* were α-pinene, limonene, citronellal, linalool, and terpinen-4-ol along with bioactive properties evaluation such as total phenolic content, ferric-reducing or antioxidant power and binding to human serum albumin, indicating *Citrus hystrix* as a strong potential raw material in cosmetic applications.

Previous studies about CHO have focused on its bioactive aspects, such as antimicrobial and antioxidant activities. Less attention has been given to the application in cosmetic formulation. Its potential use as a plant-based alternative for synthetic preservatives and fragrances has not been proven to be effective and reliable (Guzm & Lucia, 2021). This shortfall leads to further investigation into whether the oil can effectively substitute such synthetic ingredients without affecting product quality, stability, or consumer safety. Besides, the formulation of cosmetic emulsions with the incorporation of CHO is more challenging. Cosmetic emulsions must reach a stable mix of oil and water, where careful choice and balance of ingredients, including emulsifiers and stabilizers, must be met (Rios et al., 2025). Thus, there is a need to study the potential of plant-based cosmetics ingredients for the cosmetics industry towards natural and sustainable products.

This study aims to determine the bioactive compounds in CHO that were incorporated into a cosmetic emulsion. The physicochemical characterization, stability, and antimicrobial properties of the cosmetic emulsion were assessed. The findings in this study could lead to the development of safer and sustainable cosmetic products with antioxidant, antimicrobial, and anti-inflammatory properties in cosmetic emulsions, including their ability in product stabilization and protection from microbial contamination to skin-benefiting properties.

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METHODOLOGY

Materials

Fresh *Citrus hystrix* fruits were purchased from a local market in Shah Alam, Selangor. Peels of fresh *Citrus hystrix* were used to extract the oil. Materials for cosmetic emulsion were purchased from e-commerce stores such as mineral oil, stearic acid, cetyl alcohol, distilled water, glycerin, triethanolamine, dimethylol dimethyl hydantoin (DMDM hydantoin). *Escherichia coli* was employed for antimicrobial activity purposes. Folin-Ciocalteu reagent, distilled water, anhydrous sodium carbonate and gallic acid were required for total phenolic content tests.

Extraction of CHO by hydrodistillation

The extraction of CHO using the hydrodistillation technique was adopted by Dinh Phuc et al. (2019) with slight modification. 100 g of *Citrus hystrix* peels were cut into squares (2 x 2 cm) to increase the surface area and enable higher rates of extraction. Then, it was transferred to a round-bottomed flask and 500 mL of distilled water was added in a ratio of peel to water at 1:5. The Clevenger apparatus was assembled by ensuring that the water inlet and outlet are well connected and the knob is closed to create a closed system. Heat was applied to the apparatus for 3 to 4 hours, keeping the temperature at 100 °C. After that, the oil was collected in an amber bottle by dripping it out slowly. The CHO was stored at 4 °C in a refrigerator to maintain its bioactive properties.

Analysis of CHO compounds by GC-MS

Gas Chromatography-Mass Spectrometry (GC-MS) (Varian 450-GC/Varian 240-MS) was used to analyze the volatile compounds of the extracted CHO. Table 1 shows the conditions for GC-MS that were programmed. The volatile compounds in the extracted CHO were analyzed by comparing their mass spectra with database libraries.

Table 1. GC-MS condition for CHO compounds detection

Parameter	Description
Head column pressure	9.31 psi
Column	Stationary phase HP-5 ms capillary column (30 m × 0.25 mm × 0.25 µm)
Flowrate of carrier gas (helium)	1.0 ml/min
Injector temperature	250 °C with a volume of 1 µL extract at a split mode ratio of 100:1
Detector temperature	280 °C with mass range recorded band of 45-450 mass ratio with electron energy of 70 eV
Oven temperature	60 °C for 3 min and gradually increased to 180 °C at a rate of 5 °C/min, followed by a ramp of 10 °C/min to 250 °C and held for 5 min

Formulation of cosmetic emulsion

The formulation involves preparing the oil phase and water phase separately according to the method by Shkreli et al. (2022). As shown in Table 2, the oil phase includes mineral oil, act as the base oil and stearic acid, used both as an emulsifier and a thickening agent, and cetyl alcohol provides a creamy and spreadable texture with a good sensory feel. Besides, the water phase includes distilled water and glycerin that provide the aqueous medium and retain moisture, while triethanolamine, a neutralizing agent that is used to stabilize the emulsions. Meanwhile, DMDM hydantoin and CHO act as a preservative to prevent microbial growth. The oil-in-water emulsion was preferred due to greater adherence, so that the active ingredients that are in

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the oily phase will be able to act on the skin, making it suitable for lotions, creams and moisturizers because of a less greasy texture (Cortes et al., 2024).

The oil phase ingredients were added into a beaker and were heated to 70-80 °C to ensure that solid ingredients such as stearic acid and cetyl alcohol were fully melted and homogenized. The aqueous phase was similarly prepared by dissolving distilled water and glycerin, then was heated to 70–80 °C. In this way, the temperature for both phases was almost the same when mixed. The oil phase was now added to the water phase gradually with constant agitation. After incorporating the oil and water phases, the emulsion was allowed to cool gradually while continuously stirred. During the cooldown phase, CHO and DMDM hydantoin were added as natural fragrance and preservative (Castro, 2018). These ingredients were added at this stage to avoid degradation or evaporation that may be caused by high temperatures in the earlier phases. The mixture was continuously stirred to ensure even distribution of these active ingredients within the emulsion. The amount of CHO varied from 0.05 g to 0.2 g (S1 to S5) while the amount of the other ingredients was constant as shown in Table 2. Upon cooling to room temperature, the texture of the emulsion becomes more defined and uniform.

Table 2. Formulation of cosmetic emulsion

Phase	Ingredients	Amount (g)				
		S1	S2	S3	S4	S5
Oil phase	Mineral oil	1.5	1.5	1.5	1.5	1.5
	Stearic acid	1.5	1.5	1.5	1.5	1.5
	Cetyl alcohol	1.2	1.2	1.2	1.2	1.2
Water phase	Distilled water	43.95	43.9	43.85	43.80	43.75
	Glycerin	1.0	1.0	1.0	1.0	1.0
	Triethanolamine	0.7	0.7	0.7	0.7	0.7
Preservative	CHO	0	0.05	0.10	0.15	0.20
	DMDM hydantoin	0.15	0.15	0.15	0.15	0.15
	Total	50 g				

Fourier Transformed Infrared Spectroscopy (FTIR) analysis

The functional group of the extracted CHO and cosmetic emulsion was characterized by using FTIR spectrophotometer (Perkin Elmer, Spectrum One) under wavelength ranges between 400 and 4,000 cm^{-1}.

Total Phenolic Content (TPC) determination

The TPC was determined by using the Folin-Ciocalteu method as reported by Fuloria et al. (2020). Briefly, a standard solution of gallic acid was prepared at different concentrations (50, 100, 250, 500 μg/mL) to produce a standard curve. For each standard solution, Folin-Ciocalteu reagent was added, followed by 0.6 mL of anhydrous sodium carbonate solution. Then, 40 μL of each standard solution was added to the corresponding test tube. The mixture was shaken vigorously and incubated in the dark at room temperature for 2 hours as gallic acid is light sensitive. After incubation, the absorbance was measured at 765 nm using a spectrophotometer. A linear equation of the standard curve was used to determine the total phenolic content of CHO and cosmetic emulsion. The same procedure was repeated using CHO and cosmetic emulsion samples instead of gallic acid to evaluate their total phenolic content.

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Rheology behavior

Oscillating Disc Rheometer (Haake RheoStress 6000) equipped with parallel plate geometry (diameter 35 mm) was used for rheological study following method from Maru & Lahoti (2018). A 2 g sample was placed between parallel plates with a 1 mm gap and tested at 25±1 °C. The shear rate varied from 0.1 to 100 s^{-1} to observe the flow behavior. Each measurement was preceded by a 5-minute rest period to allow structural relaxation. The test was done in triplicate.

Spreadability test

One gram of cosmetic emulsion was placed between two flat glass surfaces with a diameter of 9 cm. Then, a 150 g load was applied for 1 minute as reported by Mayangsari et al. (2023). After taking away the load, the diameter of the spread sample was assessed.

Stability study

Stability study was determined by measuring the pH, weight and moisture content of the samples once a week for 8 weeks. The sample was stored at room temperature to ensure the shelf life and quality. This stability study follows the guidelines for cosmetic product testing and involves several experimental techniques as reported by Cekić et al. (2023).

pH

The pH was determined by pH meter once a week for 8 weeks. This analysis will ensure the product remains within a skin-compatible pH range of 4 to 9 during storage. According to Kamaruzaman & Yusop (2021) any changes in pH will be monitored to detect potential hydrolysis or degradation of active ingredients and excipients.

Weight

The weight of the cosmetic emulsion was measured to detect possible evaporation losses or water absorption. Samples were placed in sealed containers, and the weight was measured using an analytical balance.

Moisture content

The moisture content of the samples was determined using a loss on drying (LOD) test. 1 g of cosmetic emulsion was subjected to heat at 105 °C for 15 minutes. The moisture content was calculated by expressing the weight loss upon drying as a fraction of the initial weight of the sample used using eq. (1) (Lagha-Benamrouche & Madani, 2013):

$$MC\ (\%) = \frac{W_o}{W_i} \quad (1)$$

where MC is the moisture content, W_o is the loss in weight on drying and W_i is the initial weight of the sample.

Antimicrobial activity

Escherichia coli (*E. coli*) was used because it is a standard test organism in antimicrobial testing and is frequently used as a microbial indicator in cosmetic microbiology to assess hygiene and contamination control. *E. coli* can also cause skin and wound infections under unhygienic conditions. Since cosmetic products come into direct contact with skin, it is relevant to test their inhibitory effect. In this study, the antimicrobial activity of CHO and cosmetic emulsion against *E. coli* was determined using the agar disc diffusion method according to the reported protocol by Fuloria et al. (2020) with slight modifications. Briefly, nutrient agar was prepared by dissolving 7.5 g of nutrient agar powder in 500 mL of distilled water.

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The medium was autoclaved at 121 °C for 20 minutes and then poured into sterile disposable petri dishes under a laminar air flow at room temperature. Once solidified, the plates were stored in a refrigerator until use.

Bacterial suspensions were prepared from overnight cultures and adjusted to an optical density of ~0.1 at 600 nm (≈ 10^8 CFU/mL) prior to inoculation, to ensure consistent bacterial concentration comparable to a 0.5 McFarland standard. *E. coli* was transferred into 10 mL of nutrient broth using a sterile loop and incubated at 37 °C for 3 hours to reach the exponential growth phase. The *E. coli* suspension was then spread uniformly over the surface of nutrient agar plates using a sterile cotton swab. Before application of samples labelled S1, S2, S3, S4 and S5, each sample was diluted with ethanol at a ratio of 1:10. After inoculating the agar with *E. coli,* small round filter paper discs with a diameter of 5 mm were impregnated with the diluted samples and CHO. All plates were incubated at 37 °C for 24 hours, after which zones of inhibition were observed and measured to assess the antimicrobial activity of each test sample.

RESULTS AND DISCUSSIONS

CHO compounds by GC-MS

GC-MS analysis identified 21 components in the CHO. The single run analysis of the CHO detected four major components which are linalool (9.689%), α-pinene (9.56%), D-limonene (9.067%) and citronellal (7.744%) eluted at retention times of 10.076, 9.847, 10.216 and 11.778 min, respectively. Other constituents had significant levels such as terpinen-4-ol (4.922%), β-phellandrene (4.855%), β-pinene (3.866%), β-terpineol (3.54%), citral (3.40%) and β-terpinene (3.149%). Fifteen compounds were found at trace levels with peak areas below 2.0% as shown in Fig. 1. The obtained compounds in this study were similar to Hien et al. (2020) who found β-pinene (45.206%), α-pinene (2.365%), D-limonene (18.358%), citronellal (17.745%), terpinen-4-ol (4.9964%), γ-Terpinene (1.034%), α-Terpineol (1.768%), β-Citronellol (1.241%) as major compounds in extraction of kaffir lime peel by solvent free microwave extraction method. In hydrodistillation extraction of kaffir lime peel by Thonglem et al. (2023), four major components were β-pinene (27.37%), β-phellandrene (22.69%), D-limonene (16.21%), and citronellal (12.43%). The compounds found were typically similar to this study. However, the variation in composition can be attributed to plant part, extraction method and parameters of extraction (Budiarto et al., 2024).

Bioactive compounds in essential oils are widely known for their therapeutic properties. Linalool, a terpene alcohol, contributes to antimicrobial and calming effects (Mayangsari et al., 2023). α-pinene and D-limonene, both monoterpenes have strong anti-inflammatory and bronchodilatory properties that have a potential use in treating respiratory conditions (Muhammad et al., 2025). D-limonene found in the essential oil of lime peel also possesses antimicrobial activity against *Staphylococcus aureus* (Thonglem et al., 2023). Citronellal is commonly used as an insect repellent and exhibits antimicrobial properties (Budiarto et al., 2024). Furthermore, the presence of terpinene-4-ol and a-terpineol enhances the oil's functional potential due to their known antibacterial activities (Long & Quoc, 2023). Blejan et al. (2021) highlighted the antimicrobial activity of oregano, basil and tea tree oil could be attributed to linalool, 1,8-cineole, terpinen-4-ol, α-pinene, p-cymene, α/γ-terpinene. The antioxidant effects of monoterpenes like limonene and pinene were also discussed by Miguel (2010) to serve as potential preservatives in cosmeceuticals or nutraceuticals industries.

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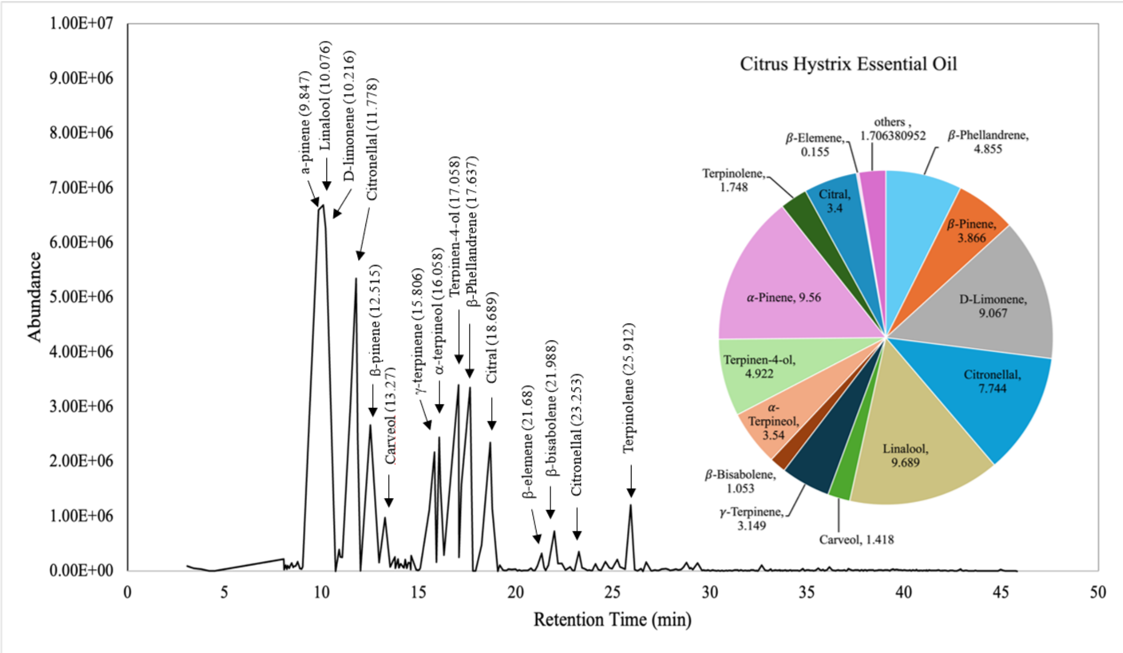

Fig. 1. Chromatogram of GC-MS analysis of CHO and percentage of CHO compounds

The incorporation of essential oils and their components in various cosmetic products have grown due to proven health and beauty benefits, enhancing the overall effectiveness of formulations. Guzm & Lucia (2021) reviewed that lemon and orange essential oil which are rich in terpenes such as limonene, exhibited strong antiseptic properties that are valuable as active ingredients in skin and hair care applications. Therefore, the presence of bioactive components of CHO reflects a potent role for natural antimicrobial and preservative in cosmetic products.

Functional group analysis by FTIR

The interpretation of FTIR peaks as shown in Fig. 2 was guided by Socrates (2001). For CHO, strong absorption bands at 3610.19 cm^{-1} caused by O–H stretching vibrations for the presence of free alcohol groups were attributed to compounds such as linalool. A band at 3390.59 cm^{-1} for hydrogen-bonded O–H stretching, typical of citronellol was noticed. The presence of C–H stretching at around 2918.43 cm^{-1} conforms to alkane groups, which are found in all terpenes. The peak at 1718.82 cm^{-1} would be indicative of C=O stretching of a carbonyl group, corresponding to citral (Istiqomah et al., 2022), as evidenced from the GC-MS chromatogram at retention time 18.689. Also visible is the C=C stretching (1644.71 cm^{-1}) characteristic of limonene, along with several alkane bending modes, such as those at 1444.31 cm^{-1} and 1372.94 cm^{-1}. Another indication of alcohol content, this time in agreement with linalool, is the fact that C–O stretching at 1024.31 cm^{-1} is present. Lastly, =C–H out-of-plane bending vibration at 870.59 cm^{-1} shows the alpha olefin structure in agreement with β-pinene.

FTIR spectra of cosmetic emulsions (S1 to S5) were analyzed to investigate the chemical stability and interaction of CHO in the formulations. Key regions of interest were identified, corresponding to O–H stretching (3256–3344 cm^{-1}) and C=C stretching (1630–1645 cm^{-1}) as these functional groups are characteristic of functionalities found in CHO. Previous studies on cosmetic formulations and oil emulsions also showed similar corresponding functional groups. In a work by Jarzębski et al. (2024), FTIR spectra of the soap nuts water solution containing hemp seed oil showed the dominant bands between 3400 and 3300 cm^{-1} (O-H, stretching), 2916 cm^{-1} and 2850 cm^{-1} (C-H, stretching), and 1634 cm^{-1} (C=O, stretching). The

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FTIR analysis of neem-based lotion shows the appearance of peaks of O-H stretch (3457 cm^{-1}), C-H stretch (2954 cm^{-1}), C=C stretch (1645 cm^{-1}), C-H bend (1459 cm^{-1}) and C=O stretch (1112 cm^{-1}) (Hashim et al., 2023).

An increase in CHO concentration resulted in the intensification and slight broadening of the O–H stretching bands, particularly in samples S4 and S5. This behavior indicates enhanced hydrogen bonding between the emulsion matrix and CHO alcoholic components such as citronellol and linalool, which promoted greater formulation stability and uniform distribution of the oil into the formulations. The C=C stretching (1630–1645 cm^{-1}) persisted in all formulations, confirming that the unsaturated bonds of terpenes were retained after formulation. Notably, no new absorption peaks appeared in the spectra of S1–S5, confirming that no formation of new functional groups. This observation confirms the stability of the active compounds, thus suggesting good compatibility between CHO and the formulations.

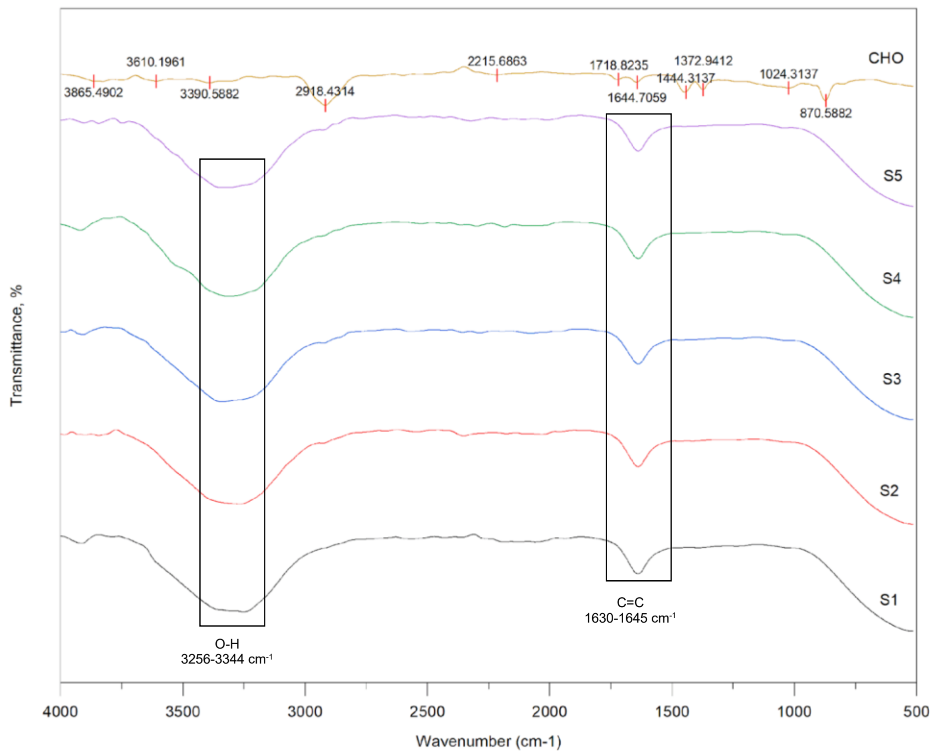

Fig. 2. FTIR spectroscopy of CHO and cosmetic emulsions

Total phenolic content

The total phenolic content (TPC) was determined by Folin-Ciocalteu method using a standard curve of gallic acid shown in Fig. 3. The linear equation of the standard curve is given by equation (2) with R^2 = 0.9984.

$$y = 0.0038x + 0.1273 \quad (2)$$

Where y represents the absorbance and x is the concentration of phenolic content in mg GAE/mL.

From Table 3, TPC was significantly different between the samples depending on the percentage of CHO incorporated into the cosmetic emulsion formulation. The CHO recorded the highest TPC value of

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167.58 mg GAE/mL, indicating the highest antioxidant activity that was exceptionally high. In contrast, S1 with no CHO had the lowest TPC of 65.24 mg GAE/mL, reflecting the absence of any phenolic-rich plant oil. With an incremental percentage of CHO from 0.1% to 0.4% (S2 to S5), there was a consistent rising trend in TPC, which are 76.58, 117.16, 161.55 and 163.63 mg GAE/mL. This suggests that the phenolic content of the cosmetic emulsion increases proportionally with the amount of CHO added. The observed plateau between S4 and S5 (161.55 to 163.63 mg GAE/mL) suggests a saturation point, where further increases in CHO concentration do not significantly enhance TPC. Such saturation may result from limited solubility or phenolic dispersion capacity within the emulsion matrix, consistent with findings in other essential oil–based systems (Aumeeruddy-Elalfi et al., 2015). This finding was significant for formulation optimization, as it highlights the concentration threshold beyond which further CHO addition yields minimal antioxidant improvement but may influence texture or stability.

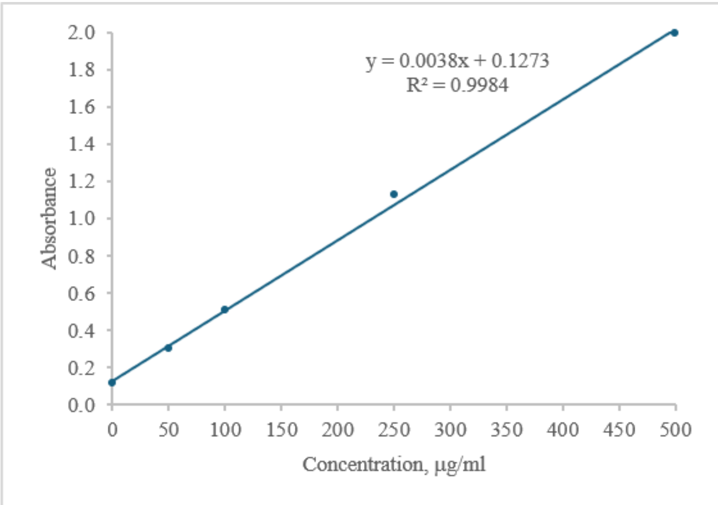

Fig. 3. Standard curve of gallic acid

Table 3. Total phenolic content of CHO and cosmetic emulsion

Sample	Absorbance	Total phenolic content (mg GAE/mL)
CHO	0.7641	167.58
S1	0.3752	65.24
S2	0.4183	76.58
S3	0.5745	117.16
S4	0.7412	161.55
S5	0.7491	163.63

TPC represents only an indirect measure of antioxidant capacity, as total phenolics contribute to reducing power and radical scavenging but do not replace direct antioxidant assays such as DPPH and FRAP (Abd Ghafar et al., 2010). Studies by Abd Ghafar et al. (2010) on citrus species including *Citrus hystrix* have shown that phenolic content correlates with antioxidant activity measured by both FRAP and DPPH assays, demonstrating that higher phenolic content generally corresponds to higher antioxidant capacity. Other studies also have shown that *Citrus hystrix* oil with high TPC demonstrates significant activity in DPPH and FRAP assays, confirming a positive relationship between phenolic content and antioxidant performance (Abdullah et al., 2023; Zhao et al., 2023).

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The results in this study confirm that the incorporation of CHO in cosmetic emulsion formulation enhances its total phenolic content, which can further improve the overall antioxidant activity. These results also show that higher concentrations of CHO, in turn, yield higher phenolic content, confirming its effectiveness as a bioactive ingredient. Although TPC of S5 (0.4% CHO) did not exceed that of the pure CHO, it was quite close, confirming effective phenolic retention even after being added into the formulation. This is in line with the findings by Wang et al. (2024) who suggested that *Citrus hystrix* leaf and peel extracts are rich in polyphenols and demonstrate high antioxidant activity. The work by Gordobil et al. (2023) showed that incorporating natural plant oils rich in phenolic compounds into emulsions or cream bases can improve in vitro sun protection factor (SPF). The phenolic compounds found in lemon can act as antioxidants, preventing oxidation and degradation of cosmetic formulations, which is crucial for maintaining the product's freshness (Magalhães et al., 2023). Hence, the results of this research support evidence for the perception that the development of cosmetic emulsions using CHO not only improves the antioxidant capacity but also the phenolic content in the formulations, providing protection to the skin and serving as a natural preservative.

Rheological behavior of cosmetic emulsion

All five samples demonstrated non-Newtonian or shear-thinning flow behavior, which is expected and desirable for cosmetic formulation. This means that shear stress increased with increasing shear rate, allowing the texture to maintain its form in the container while becoming spreadable during application. Such rheological behavior enhances user experience and formulation stability, ensuring that the product is easy to apply, non-dripping, and maintains structural integrity over time (Danila et al., 2019). Based on Fig. 4, it was observed that at day 0 and day 7, all samples show a moderate increase in shear stress. This is a good indication of product stability because a significant drop in viscosity or rise in shear stress over time could point to emulsion breakdown, phase separation and degradation of the ingredient (Shkreli et al., 2022).

As shown in Fig. 4(a), S1 and S2 exhibited the lowest shear stress across all shear rates, indicating lower viscosity and a weaker internal network. In contrast, S1 and S5 showed higher shear stress, suggesting that an increase in CHO enhanced intermolecular hydrogen bonding within the formulation. The differences became apparent at shear rate above 50 s^{-1}, which implies that the resistance to deformation was improved at higher CHO concentration. At day 7, all samples maintained non-Newtonian, shear-thinning behavior with increasing shear stress with shear rate as shown in Fig. 4(b). An overall rise in shear stress compared to day 0 was observed for all samples, which may reflect thickening or structural reinforcement of the emulsions over time. S1 (no CHO) showed moderate increases in shear stress, while S2 to S5 (with greater CHO concentrations) exhibited greater increases in shear stress, with a significant increase of shear stress by S5 (0.4% CHO) being the greatest at all shear rates. Notably, as opposed to day 0 when S4 (0.3% CHO) was the highest, at day 7, the increase of 0.4% CHO may provide a better structural reinforcement as it aged. This could be evidenced by the fact that CHO may act not only as a thickener but also as a structural stabilizer over time.

S5 reached the highest shear stress (160 Pa at 100 s^{-1}), which reflects the most viscous and stable internal structure. S3 and S4 exhibited moderate increases in shear stress (20-30 Pa at 100 s^{-1}), suggesting that incorporation of CHO promoted gradual strengthening of the emulsion matrix. Importantly, none of the formulations showed a drop in shear stress, confirming no phase separation or destabilization within 7 days. This shows that CHO contributes to long-term emulsion stability as well as thickening, and S5 also had a superior internal structure over time. The observed shear stress values (ranging from 100 to 160 Pa at 100 s^{-1}) are consistent with literature reports for stable oil-in-water emulsions containing essential oils or natural lipophilic actives. Similar shear stress ranges (80–160 Pa at 100 s^{-1}) were reported for rosemary oil-based creams (Zhou et al., 2024) and microemulsion gels incorporating argan and coconut oils (Agarwal et al., 2023). This similarity suggests that the CHO-based formulations fall within the typical rheological range for cosmetically stable emulsions.

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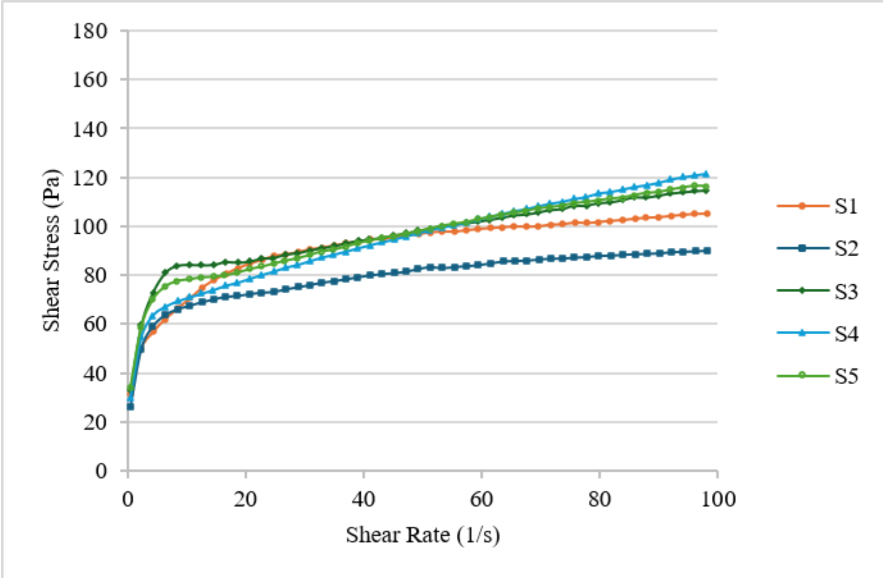
(a)



(b)

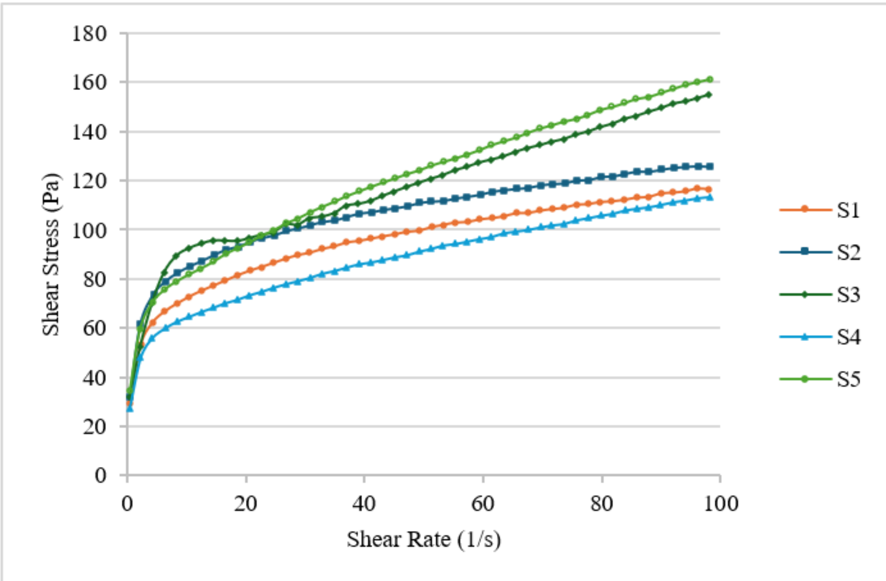


Fig. 4. Shear rate vs shear stress of cosmetic emulsion at (a) day 0 and (b) day 7

The flow behavior of all formulations was evaluated using the Power Law (Ostwald–de Waele) model, expressed as $\tau = k\gamma^n$. The flow behavior index (n) and consistency index (k) values derived from fitting the shear stress–shear rate data are presented in Table 4. All samples achieved excellent Power Law fitting (R^2 = 0.94–0.99) and exhibited $n < 1$, confirming non-Newtonian pseudoplastic behavior which is typical of cosmetic emulsions. At day 0, n values ranged from 0.141 to 0.219, while after 7 days, the n values increased slightly to 0.179–0.276, indicating improved structural uniformity and enhanced flow under shear. The k values ranged from 42.228 to 56.312 Pa.s^n initially and showed small changes after day 7, suggesting microstructural rearrangements or slight water distribution within the emulsion network.

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Table 4. Power law parameters at day 0 and day 7

Sample	Day 0			Day 7		
	n	k (Pa.s^n)	R^2	n	k (Pa.s^n)	R^2
S1	0.187	45.962	0.97	0.207	44.854	0.99
S2	0.141	47.435	0.99	0.179	55.271	0.99
S3	0.148	56.312	0.94	0.248	47.098	0.95
S4	0.219	42.228	0.96	0.240	36.669	0.96
S5	0.174	50.722	0.96	0.276	43.182	0.98

In summary, although there was a rise in shear stress in all samples after seven days, the shift was not drastic and is considered good for cream-type emulsion. The slight increase in shear stress observed after 7 days indicates strengthening of the emulsion structure, possibly due to microstructural rearrangements and water evaporation. This is considered favorable, as it enhances product stability without compromising spreadability, a desirable attribute in cosmetic emulsions (Franceschini et al., 2025). Therefore, the rheological behavior confirmed that all samples exhibited shear-thinning, non-Newtonian characteristics, as intended, and became more stable and functionally consistent over time. This functionality reflects their improved resistance to phase separation, retention of desirable shear thinning flow, and enhanced consumer usability, ensuring that the emulsions remain uniform, easily spreadable, and texturally stable during storage (Baptista et al., 2023).

Assessment of spreadability

A distinct change in the diameter of spreading was observed in all samples during seven days of storage as shown in Table 5. Under a uniform load of 150 g in 1 minute, most formulations exhibited a progressive increase in spread diameter over time. This indicates the formulations became easier to spread and improved mobility of the formulation matrix. S2 and S5 showed an increase from 3.6 cm and 3.8 cm on day 0 to 5.3 cm and 5.8 cm on day 7, respectively. In contrast, S4 maintained a consistent spread diameter throughout the storage, which reflects excellent structural stability and balanced rheological performance.

These observations were consistent with findings by Gore et al. (2018), who reported that essential oil-based moisturizers soften over time due to oil migration or mild hydrolysis of emulsifiers, resulting in greater spreadability and smoother application. Similarly, Savary et al. (2013) observed that the large spread diameter was due to the increase of oil phase proportion which enhances emulsion fluidity. This improved user satisfaction due to easier application and better sensory perception.

An increase in spreadability over time is desirable as it enhances skin coverage, absorption, and smoothness, thus improving consumer appeal. However, excessive softening could imply instability, such as coalescence or phase migration (Gore et al., 2018). In this context, S5, while showing excellent spreadability, may require monitoring for long term consistency, whereas S4 represents the most ideal balance between stability and usability, maintaining both structure and application comfort over time. Such behavior represents the functional integrity desired in a high-quality cosmetic emulsion.

The rheology and spreadability of the formulation proved a good correlation. Samples that exhibit lower viscosity and higher flow behavior index (n), also demonstrated greater spread diameters. This relationship aligns with shear thinning behavior, providing both stability during storage and effortless spread during use (Baptista et al., 2023). These findings confirm that CHO containing formulation retained their shear-thinning behavior and functional stability during storage.

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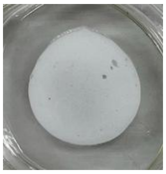
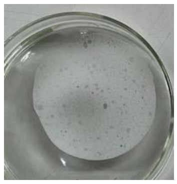
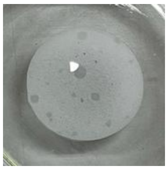
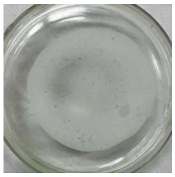
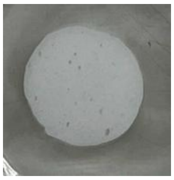
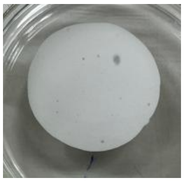
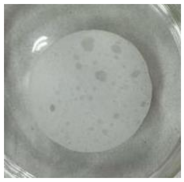
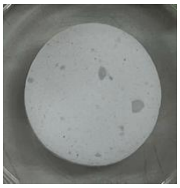
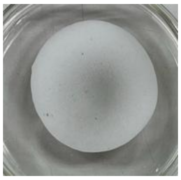
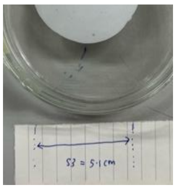
Table 5. Spreadability of cosmetic emulsion using 150 g load in one minute

Sample	Day 0	Day 4	Day 7	Observation
S1	 Diameter = 4.3 cm	 Diameter = 4.8 cm	 Diameter = 4.8 cm	Slight increase by Day 4, then stable. Moderate to easy spreadability overall.
S2	 Diameter = 3.6 cm	 Diameter = 4.1 cm	 Diameter = 5.3 cm	Large increase by Day 7. Initially thick, then become very easy to spread.
S3	 Diameter = 3.8 cm	 Diameter = 4.5 cm	 Diameter = 4.8 cm	Shows steady improvement in spread. Moderate at start, but then easy to spread.
S4	 Diameter = 4.6 cm	 Diameter = 5.1 cm	 Diameter = 5.1 cm	Remain consistent spreadability from Day 0. Easy to spread throughout.
S5	 Diameter = 3.8 cm	 Diameter = 5.4 cm	 Diameter = 5.8 cm	Very large increase where it started moderate and then become the easiest to spread over time.

Stability study

Based on Fig. 5, the pH values of all samples showed very minimal fluctuations over the eight-week observation period. S1 which contains no CHO recorded consistent pH values around 7.11±0.01 to 7.15±0.03. Samples S2 to S5 (0.1% to 0.4% CHO), showed slightly higher but consistent readings, generally within 7.20±0.02 to 7.31±0.02. This stable pH suggests that the base formulation itself had enough buffering capacity to resist small pH changes. The emulsifiers, stabilizers, and aqueous components likely helped maintain this balance, even with the addition of slightly acidic or volatile CHO compounds. Overall, this confirms that CHO is not only physically but also chemically compatible with cosmetic emulsion, making it a suitable bioactive addition without compromising product stability.

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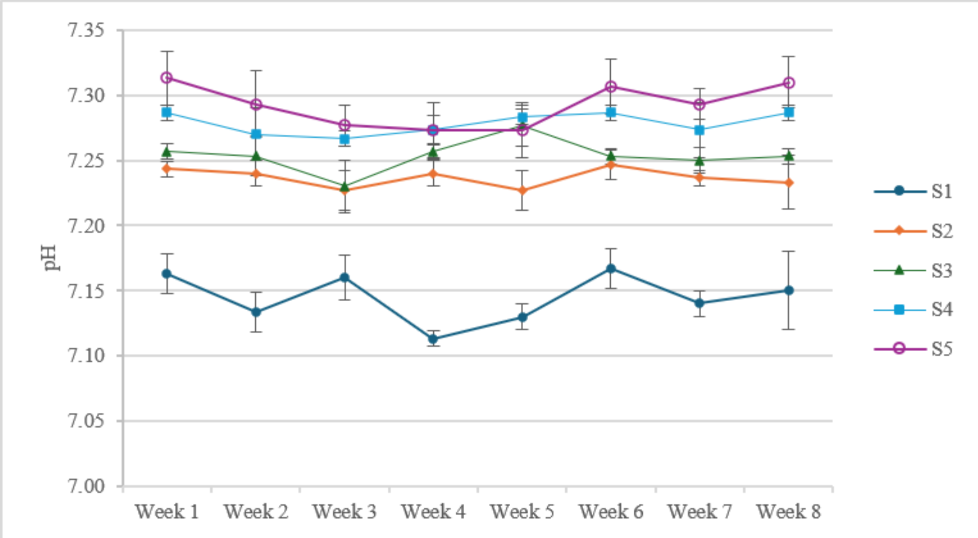

Fig. 5. pH measurement of cosmetic emulsion over 8 weeks

The pH values of all formulations containing varying CHO showed no statistically significant variation between Week 1 and Week 8 ($p > 0.05$). This confirms the formulations were chemically stable over time. The pH values recorded between 7.11±0.01 and 7.31±0.02 fall within the acceptable pH range for cosmetic products. According to Vrinda Rajwadi (2022), cosmetic formulations such as moisturizers, sunscreen and skin creams should have a pH range of 4 to 9 to ensure both skin safety and product stability. Thus, the pH values of the CHO-infused cosmetic formulation are not only functionally stable but also align with those guidelines.

In Fig. 6, all samples experienced consistent weight loss over the eight weeks of observation. However, the rate of weight loss is different among samples. S4 exhibited the highest weight loss (4.80%±0.011) by Week 8 compared to S1 (4.33%±0.009), possibly due to a slightly lower viscosity or less compact microstructure, which could facilitate minor water migration. In contrast, S5 showed the lowest weight loss (4.13±0.006), indicating better retention of moisture and volatile components. The steady increase in weight loss for CHO-containing formulations suggests that CHO is accountable for greater volatility or evaporation rates. The essential oils have volatile components with low molecular weight like limonene and citronellal, which are highly volatile at room temperature. In emulsions, concentrations higher than a certain amount of volatile components can lead to greater mass loss on evaporation in the long term. Therefore, the findings indicate that although CHO can enhance functional attributes like antimicrobial or antioxidant activity, the presence of CHO especially at high concentrations, can reduce weight stability during storage. This requires formulation adjustments to minimize evaporation, such as encapsulation or a stabilizer.

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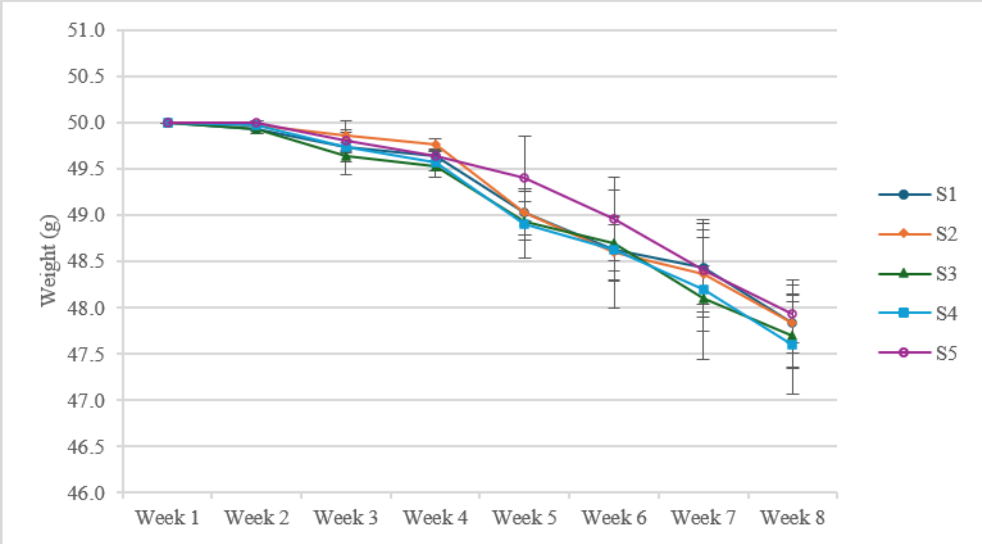

Fig. 6. Weight measurement of cosmetic emulsion over 8 weeks

Meanwhile, Fig. 7 shows the moisture content pattern for all samples over eight weeks. The CHO-containing formulation had a higher and more consistent moisture content than the formulation without CHO. S5 had the highest moisture content throughout the storage period, whereas S1 had more fluctuation and the sharpest decline, especially by Week 8. This indicates that the addition of CHO helps in stabilizing moisture content over a long period. CHO possesses polar functional groups such as hydroxyl and aldehyde, which can bind with water molecules and help in retaining moisture in the sample matrix. These interactions also strengthen the emulsion's internal structure, increasing viscosity and creating a tighter, semipermeable matrix that slows down water diffusion and evaporation. As a result, CHO helps reduce weight loss by stabilizing the emulsions and improving moisture stability. This is supported by Castro (2018) who identified that there are amphiphilic elements in essential oils that can interact with water and stabilize emulsions. These elements are the reason for holding moisture through reinforcing the structural matrix of the sample and by forming hydrogen bonds with water molecules. Therefore, the CHO not only stabilizes moisture but also gives physical structure to the sample, making it more resistant to drying over a period.

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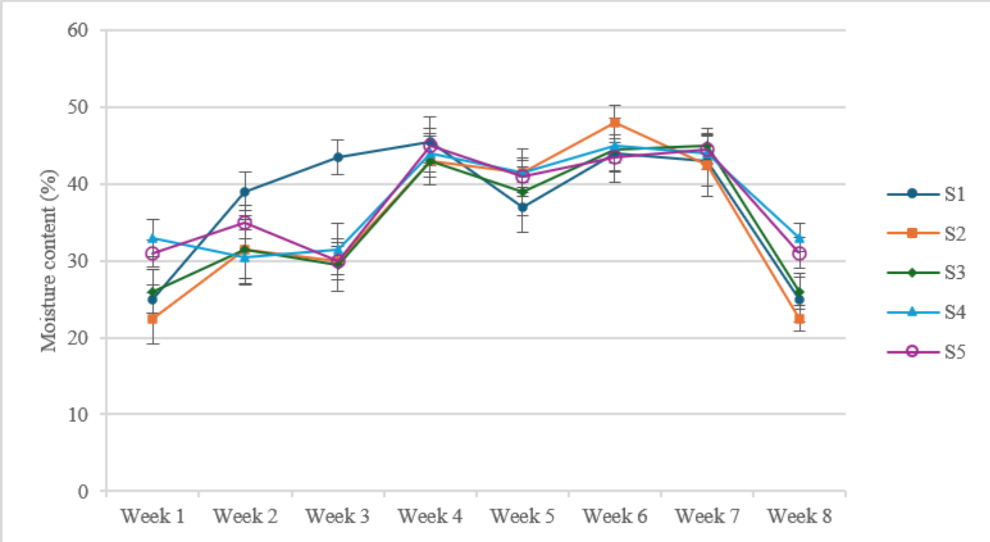

Fig. 7. Moisture content of cosmetic emulsion over 8 weeks

Evaluation of antimicrobial activity

The antimicrobial activity against *E. coli* showed a clear relationship between CHO concentration and the inhibition zone as indicated in Figs 8 and 9. S1 (no CHO), showed minimal inhibition of 6.0±2.65 mm, as expected. As CHO was introduced into the formulations, the inhibition zones increased to 6.7±0.58 mm (S2), 7.7±3.06 mm (S3), 9.0±2.0 mm (S4) and 9.0±2.0 mm (S5). Pure CHO showed a strong inhibition zone of 15.7±3.06 mm. These results indicate that higher concentrations of CHO lead to greater antimicrobial effects. Ng et al. (2011) found that emulsions containing *Citrus hystrix* oil of different formulations were able to strongly inhibit *E. coli*, where inhibition zone diameters are between 14.0 mm and 18.0 mm. The inhibitory effect against *E. coli* was observed in the emulsion formulations containing 0.5%, 1% and 2% *Thymus vulgaris* oil with inhibition zones of 27 mm, 30 mm and 33 mm, respectively (Yorgancioglu & Bayramoglu, 2013). The antimicrobial activity of virgin coconut oil lotion against *E. coli* showed an inhibition zone of 5 mm to 17 mm for formulation containing 20 to 80 µl oil (Patil et al., 2024). Thus, the cosmetic formulations in this study were effective in inhibiting the *E.coli* growth with an inhibition zone within the range reported in previous studies.

The consistent increase in inhibition zones of the samples supports the effectiveness of CHO as an antimicrobial agent. The slight difference between S4 and S5 may suggest that the antimicrobial effect reaches a maximum at a certain concentration. One-way ANOVA showed significant differences among inhibition zone diameters across formulations ($p < 0.05$). Post-hoc t-tests confirmed that pure CHO had significantly greater antibacterial activity than all cosmetic formulations ($p < 0.05$), while S1–S5 showed no significant differences ($p > 0.05$), indicating that increasing CHO beyond 0.2% did not further enhance antimicrobial activity. This suggests a plateau effect, where active compounds from CHO reach their maximum effective concentration within the formulation matrix.

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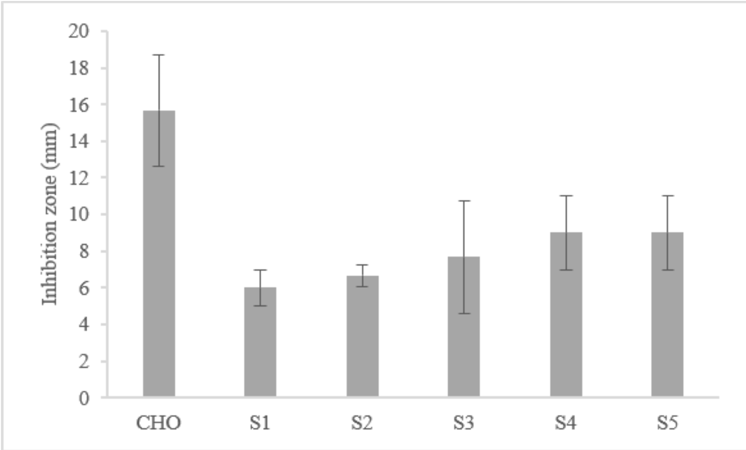

Fig. 8. Inhibition zone of CHO and cosmetic formulations against *Escherichia coli*

In a study by Shkreli et al. (2022), no growth of germs and fungi was seen after 48 hours of incubation in formulations of cosmetic emulsion incorporating lemongrass oil, showing great potential for the creation of stabilized micellar systems which is meaningful in regard to the utilization of essential oil in cosmetic formulations. CHO compounds, particularly citral and limonene, act primarily by disrupting bacterial membrane integrity, increasing permeability, inhibiting metabolic enzymes, and inducing oxidative stress, ultimately compromising *E. coli* viability and growth (Mohideen et al., 2022). Blejan et al. (2021) found that linalool might be responsible for the activity against *S. aureus*, whereas, for *E. coli*, the effect could be attributed to linalool, 1,8-cineole, terpinen-4-ol, α-pinene, p-cymene, and α/γ-terpinene. The effectiveness of essential oils as preservatives for cosmetic formulations against bacteria and fungi was correlated to the relative content of specific essential oils' components (Guzm & Lucia, 2021).

(a) (b)

Fig. 9. Antimicrobial activity against *Escherichia coli* (a) S1, S2 and S3, (b) S4, S5 and CHO

CONCLUSION

This study demonstrated the potential of *Citrus hystrix* oil (CHO) as a natural alternative to synthetic ingredients in cosmetic emulsions. The oil extracted via hydrodistillation was rich in bioactive compounds such as linalool (9.869%), β-pinene (9.56%), D-limonene (9.067%) and citronellal (7.744%), which contributed to its antioxidant and antimicrobial activities. FTIR confirmed successful incorporation through the characteristic of O–H and C=C stretching bands. The formulation containing 0.4% CHO exhibited the highest total phenolic content (163.63 mg GAE/mL), indicating strong antioxidant potential. Rheological analysis revealed desirable shear-thinning behavior and improved spreadability, while stability tests over

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eight weeks showed consistent pH, weight, and moisture values, confirming structural stability. Antimicrobial assays against *E. coli* demonstrated concentration-dependent inhibition zones (6.0–9.0 mm). Overall, CHO-enhanced emulsions showed promising functionality, stability, and bioactivity, supporting their use as sustainable cosmetic ingredients. Future work should include quantitative antioxidant and antimicrobial assays, sensory evaluation, and safety assessments to further validate CHO-based formulations.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHORS' CONTRIBUTIONS

Normasyitah Nabiha Sainon carried out research, analyzed data and wrote the draft article. Norasmah Mohammed Manshor conceptualized the research idea, supervised the research progress, provided feedback on the draft article and revised the final version of the article. Norashikin Ahmad Zamanhuri assisted with data interpretation, reviewed and proofread the article.

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