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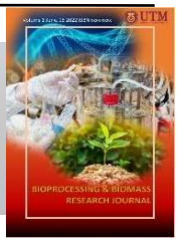

Research Article

FORMULATION AND CHARACTERIZATION OF HERBAL CLEANSING GEL FROM TURMERIC EXTRACT LOADED-NANOSTRUCTURED LIPID CARRIERS

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ABSTRACT

The herbal cleansing gel from turmeric extract is a natural skin care product with antioxidant, antimicrobial, and anti-inflammatory properties. Despite its advantages, turmeric extract has a higher metabolism, a low absorption rate, stability, and solubility. Encapsulation of turmeric extract had been developed to increase the bioavailability of turmeric extract for topical drug delivery. Hence, turmeric extract had been encapsulated via nanostructured lipid carriers (NLCs) to produce turmeric extract-loaded nanostructured lipid carriers (T-NLCs). This project highlights the formulation and characterization of herbal cleansing gel from encapsulated turmeric extract. To produce T-NLCs, medium-chain triglycerides (MCT) as liquid lipid and glycerol monostearate (GMS) as solid lipid generated an imperfect matrix incorporating turmeric extract. The T-NLCs were then characterized in terms of particle size, polydispersity index, zeta potential, encapsulation efficiency, and stability. The T-NLCs exhibited particle size 129.407 ± 1.278 nm, polydispersity index 15.067 ± 2.105%, zeta potential -44.7 ± 1.8 mV, encapsulation efficiency 98.04 ± 9.19% and remained stable over 30 days. The herbal cleansing gel was formulated with T-NLCs and characterized in terms of sensory test. In conclusion, developing turmeric in an encapsulated formulation can aid in developing herbal-based cosmetic products for a wide range of skin care systems.

INTRODUCTION

Nowadays, the cleanser is essential for skin care for everyone, regardless of gender, race, or age, in our daily lives. Throughout the day, the skin on the face is covered with sebum, dirt, bacteria, environmental impurities, and makeup residue. As we know, sebum and dirt can clog the pores on the skin and cause the formation of acne (Bhadra, 2020). Hence, proper skin cleansing is essential to remove all these impurities from our skin and maintain skin health at the same time (Mijaljica et al., 2022). People nowadays are more concerned about a product's safety and healthy ingredients and always go for natural products (Rubin & Brod, 2019). Essential ingredients in the formulation of a cleanser include a surfactant, humectant, preservative, active ingredients, fragrance, and so on. However, most chemical-based cleansers on the market may contain sodium lauryl sulphate, parabens, and other synthetic compounds that can cause skin irritation with prolonged use (Fathima & Hiremath, 2022).

In ancient times of Ayurveda, Indian medicine showed that the turmeric can improve digestion, reduce obesity, and reduce inflammation of the skin and gastrointestinal tract (Kim & Lio, 2020). For example, turmeric treats various skin diseases, including acne, eczema, pruritus, radiodermatitis, psoriasis, and vitiligo (Kim & Lio, 2020). Scientific studies conducted over the last three decades have validated ethnomedicinal uses, and turmeric has been shown to have antioxidant, antimicrobial, and anti-inflammation properties (Abd El-Hack et al., 2021). Many herbal extracts have medical properties and are suitable for

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a cleanser. In this research, turmeric extract was used to treat acne as it showed many pharmacological effects such as anti-inflammatory, antimicrobial, and antioxidant due to the presence of curcumin (Gopinath & Karthikeyan, 2018).

However, topical products containing turmeric extract can stain the skin as it is a solid yellow colour. Also, turmeric extract has a higher metabolism, a low absorption rate, stability, and solubility. Hence, these characteristics reduce turmeric extract's bioavailability and limit its therapeutic use. In order to improve the effectiveness of topical drug delivery of turmeric extract, researchers have developed nanostructured lipid carriers (NLCs). NLCs are a type of advanced drug delivery system that use a combination of liquid and solid lipids (medium-chain triglycerides and glycerol monostearate, respectively) to create an imperfect matrix that effectively protects and incorporates turmeric extract (Suryawati & Jawi, 2020).

Typically, topical treatment is the most recommended method for treating acne. However, the skin's stratum corneum protective layer can impede medication absorption. To address this issue, researchers have developed a successful topical drug delivery system using NLCs made with a combination of liquid and solid lipids, specifically MCT and GMS. This lipid mixture creates an imperfect matrix that encapsulates and protects turmeric extract. Additionally, NLCs have several benefits, such as high encapsulation efficiency, stability, and non-toxicity (Park et al., 2018). Hence, NLCs are suitable for topical delivery due to their small particle size and occlusive nature, which allows them to penetrate the stratum corneum layer (Rapalli et al., 2020).

Hence, this paper aims to encapsulate turmeric extract via NLCs and characterize it. Then, the encapsulated turmeric extract was used to formulate herbal cleansing gel.

MATERIALS AND METHOD

Raw Materials and Chemicals

The source of the turmeric used in the study was provided by Herbagus Trading in Penang, Malaysia. Before use, the raw turmeric underwent several pre-treatment stages, including cleaning, drying, and grinding to reduce its size in between 2-3 mm. All the chemicals and ingredients involved in this project were analytical grade.

Extraction and Phytochemicals Analysis

An amount of 10 kg of turmeric was extracted with 100 L of water for 2 h. Once the solid parts were filtered out, the resulting solution was subjected to spray-drying using a pilot spray dryer. (Niro A/S, GEA Group, Soeborg Denmark). The yield of the extract was then calculated using **Equation 1**.

$$\text{Yield of extract} = \frac{\text{Weight of dry extract}}{\text{Weight of raw material}} \times 100 \qquad (1)$$

The chemical marker curcumin (Sigma-Aldrich, 1151855) was used as standard and analyzed using high-performance liquid chromatography (HPLC). Then, turmeric extract was analyzed using an HPLC system of HP-1200 Agilent Technologies. A reverse-phase HPLC assay using an isocratic system, with a flow rate of 0.6 mL/min, and a column temperature of 40 °C. The mobile phase consisted of a mixture of acetonitrile and 3% acetic acid (49:51, v/v), and a UV wavelength of 425 nm was used. A 20 µL injection volume was used, and solutions were filtered using a 0.45 µm nylon membrane before HPLC injection. Each sample was subjected to a total chromatographic analysis time of 12 minutes. The percentage yield of curcumin was calculated using **Equation 2**.

$$\text{Yield of curcumin} = \frac{[C] \times DF \times V}{m} \times 100\% \qquad (2)$$

Where [C] is concentration of peak area, DF is dilution factor, V is volume of solvent for dilution and m is mass of the sample.

Antioxidant Analysis of Turmeric Extract

The turmeric extract's antioxidant activity can be analyzed using a modified diphenyl-2-picrylhydrazyl (DPPH) assay (Bhoopathy et al., 2021). Firstly, 0.5 mM DPPH solution was prepared in methanol. Then, turmeric extract was mixed with DPPH solution 1:1 volume ratio. The mixture was kept for 90 minutes at room temperature. The absorbance was measured at 515 nm using ELISA Microplate Reader (BIOBASE-EL 10A, USA). Ascorbic acid with a concentration from 0 to 10000 µg/mL was used as the standard. Methanol was used as a negative control. The percentage of radical scavenging inhibition was calculated using **Equation 3**.

$$\text{Radical scavenging} = \frac{C - S}{C} \times 100\% \qquad (3)$$

Where C is absorbance without the sample and S is absorbance with the sample.

Antimicrobial Analysis of Turmeric Extract

Disk diffusion assay was used to evaluate the antimicrobial activity of turmeric extract against *Propionibacterium acnes* and *Staphylococcus aureus* (Hadi et al., 2022). Approximately 1 to 5 single bacteria colonies were transferred using a sterile inoculating loop from a nutrient agar plate into 3 mL of 0.9% saline solution, following strict aseptic techniques. The turbidity of the bacterial solution was compared with a 0.5 McFarland standard, equivalent to 1×10^8 CFU/mL. Using a sterile cotton swab, the bacterial solution was swabbed onto the surface of Mueller Hinton Agar, slantwise at a 60° angle and rotated six times on all sides to ensure the even distribution on the agar surface. A sterile disk containing the sample was gently placed on the agar, and the plate was then incubated overnight at 37 °C to observe the appearance of an inhibition zone. The mean diameter (mm) ± standard deviation (SD) of the zone of inhibition was measured and recorded.

Formulation of T-NLCs

To encapsulate turmeric extract via NLCs which consisted of a lipid phase and an aqueous surfactant phase, the methodology from Park et al. (2018) was referenced and modified accordingly. For the lipid phase, turmeric extract was added to ethanol under magnetic stirring at 500 rpm and 40 °C for 1 h. MCT was then added to stir and heat at 500 rpm and 70 °C for 2 h. Next, GMS was added to the lipid mixture under magnetic stirring at 500 rpm and 70 °C for 2 h. For the aqueous surfactant phase, soy lecithin, Tween 80, and distilled water were mixed under magnetic stirring at 500 rpm and 70 °C for 1 h. The hot aqueous mixture was then added to the lipid mixture and homogenized at 11000 rpm for one minute using a high-speed homogenizer (IKA Ultra Turrax, German). To produce T-NLCs dispersion, the pre-emulsion mixture was ultrasonicated at 100 amplitudes

for 20 minutes using a probe sonicator (Q125 Sonicator®, USA).

Characterization of T-NLCs

Centrifugation Stability Test

To estimate the stability of T-NLCs over time, a centrifugation stability test (Hu et al., 2016) was used by centrifuging T-NLCs at 3000 rpm for 30 minutes (Eppendorf AG 5804, Hamburg, Germany). After centrifugation, T-NLCs were evaluated visually by predicting stability problems such as phase separation, creaming, flocculation, or sedimentation. The procedures were repeated until no sedimentation occurred by adjusting the composition (w/w) of MCT as liquid lipid, GMS as solid lipid, soy lecithin, and Tween 80 as surfactants.

Particle Size (PS), Polydispersity Index (PDI), and Zeta Potential (ZP)

The PS, PDI, and ZP of T-NLCs were determined by dynamic light scattering (DLS) using particle size analyzer (Litesizer 500, Anton Paar) at room temperature. Firstly, T-NLCs were dispersed in distilled water with a ratio of 1:9 and injected directly into the chamber of the Litesizer 500 instrument. Then, the ZP of T-NLCs was evaluated by measuring the direction and velocity of the droplet movement in a defined electric field. The PS, PDI, and ZP of T-NLCs were analyzed in triplicate and then recorded in nm ± SD and mV ± SD, respectively.

Encapsulation Efficiency (EE) of T-NLCs

The encapsulation efficiency (EE) of T-NLCs was determined according to Gonçalves et al. (2021) with some modifications. Firstly, a standard calibration curve (y = 4.8966x+0.0456, R^2= 0.999) of gallic acid from concentration 0 µg/mL to 500 µg/mL was constructed using the Folin-Ciocalteu method. The fabricated T-NLCs were centrifuged using a mini microcentrifuge at 10000 rpm for 2 minutes (Eppendorf, MiniSpin®). The supernatant collected and T-NLCs were diluted with a solvent in a ratio of 1:3 (v/v). The solvent used is mixed from ethanol and 2-propanol in a ratio of 6:4 (v/v). After diluted, 30 µL of the samples were mixed with 15 µL Folin reagent, 60 µL of 10% (w/v) aqueous solution of sodium carbonate, and 195 µL of distilled water in a 96-well plate, respectively. The 96-well plate was incubated for 60 minutes and the absorbance was measured at 750 nm using ELISA Microplate Reader (BIOBASE-EL 10A, USA). The readings were done in triplicate expressed in % ± SD using **Equation 4**.

$$\mathrm{EE} = \frac{\text{Total curcumin} - \text{Free curcumin}}{\text{Total curcumin}} \times 100\% \qquad (4)$$

Real-Time Stability Test

For the real-time stability test, the T-NLCs were stored at room temperature (25 ± 2 °C), oven (45 ± 2 °C), and also refrigerated (4 ± 2 °C) for 30 days, respectively. The physical appearance of PS and PDI of T-NLCs were evaluated on the 1st day and 30th day. Through this study, the separation of T-NLCs was considered an indicator of physical stability. The PS and PDI were measured using a particle size analyzer (Litesizer 500, Anton Paar). The PS and PDI of T-NLCs were analyzed in triplicate and then recorded in nm ± SD and mV ± SD, respectively.

Formulation of Herbal Cleansing Gel

The optimum formulation of T-NLCs was used to formulate the herbal cleansing gel. Firstly, glycerine and propylene glycol were added to distilled water, followed by potassium sorbate and allantoin, and heated at 40 °C until a light, clear solution formed. Next, PEG-150 distearate, Cocamidopropyl betaine, decyl glucoside, PEG-40 hydrogenated castor oil, and PEG-7 glyceryl cocoate were mixed in the second beaker. The mixture was then heated at 40 °C until fully dissolved to form a surfactant phase. After that, both mixtures were mixed and stirred well, then cooled to room temperature. Lastly, T-NLCs and tea tree essential oil were added as they are heat-sensitive ingredients.

Characterization of Herbal Cleansing Gel

Sensory Test

A sensory test was conducted by 30 panellists of different genders who were between 15-30 years old to compare between F2 and the commercial herbal cleansing gel in the market. The physical appearance, odour, clarity, viscosity, foaming ability, freshness, and overall acceptability of herbal cleansing gel were evaluated. A hedonic scale from 1 to 9 was used, in which 1 means dislike extremely and 9 means like extremely**.**

RESULTS AND DISCUSSION

Extraction and Phytochemicals Analysis

The total extract obtained from the extraction process was 840 g, with a total yield of 8.4%. HPLC analysis revealed that the yield of curcumin in the *Curcuma longa* extract used was 0.36% ± 0.016 (**Figure 1**).

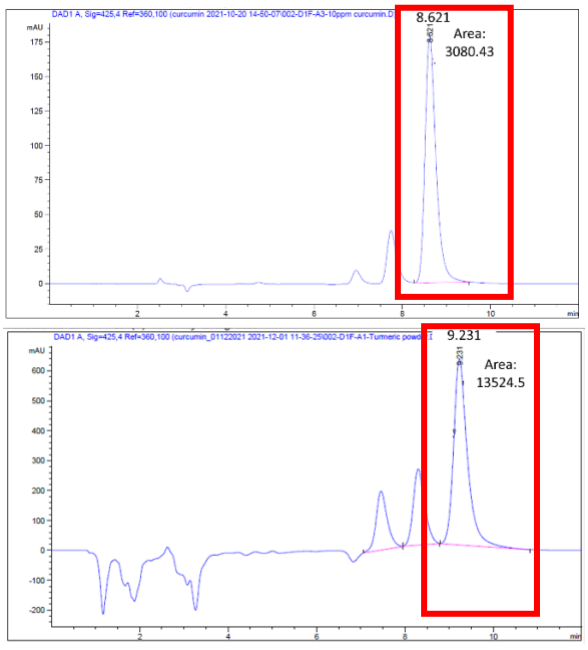

Figure 1 HPLC chromatograms of i) standard curcumin (ii) Turmeric extract monitored at a wavelength of 425 nm.

Antioxidant Analysis of Turmeric Extract

DPPH assay is a common method to determine turmeric extract's antioxidant activity. From **Figure 2**, turmeric extract and ascorbic acid showed maximum radical scavenging of 56.59% and 69.70% at 10000 µg/mL, respectively. Turmeric extract showed a lower free radical

scavenging activity than ascorbic acid at low concentrations. However, this potency was close to ascorbic acid at higher concentrations. The results showed that turmeric extract had a good antioxidant activity and this antioxidant potential increases when the concentration of turmeric extract increases. The results aligned with the findings reported by Abbas et al. (2022).

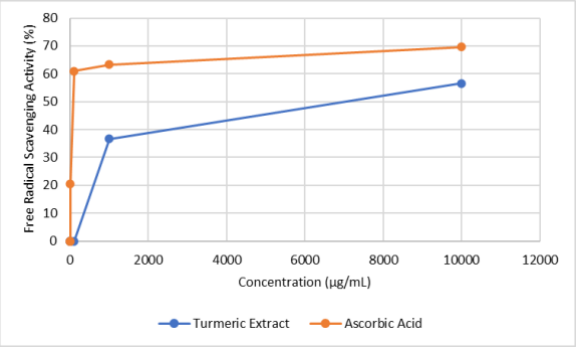

Figure 2 Free radical scavenging activity of turmeric extract

Antimicrobial Analysis of Turmeric Extract

The turmeric extract exhibited antimicrobial properties against all tested bacteria, which are *P. acnes* and *S. aureus*. It has been reported that turmeric extract was effective against *P. acnes* and *S. aureus by* Effiom and Abaye (2020) and Khatun et al. (2021).

Table 1 Treatment of turmeric extract on antimicrobial activity

Sample	Zone of Inhibition (mm)	Antimicrobial Activity
Chloramphenicol	30.0	Positive control
Water	0.0	Negative control
P. acnes	7.0 ± 1.5	Present
S. aureus	10.0 ± 1.4	Present

Formulation of T-NLCs

The composition of T-NLCs is shown in **Table 2**. After the centrifuge stability test, T-NLC1 and T-NLC2 were discarded due to separation problems. Thus, T-NLC3 was considered for further study, which is characterization.

Table 2 Composition of T-NLCs

Substances	Composition (w/w %)		
	T-NLC1	T-NLC2	T-NLC3
Turmeric Extract	0.1	0.1	0.1
MCT	15	20	20
GMS	0.5	0.5	0.5
Ethanol	5	5	5
Tween 80	5	5	6
Soy lecithin	5	5	6
Distilled water	69.4	64.4	62.4

Characterization of T-NLCs

Centrifugation Stability Test

After centrifuging at 3000 rpm for 30 minutes (Eppendorf AG 5804, Hamburg, Germany), all these formulations of T-NLCs in **Table 2** were evaluated visually. T-NLC1 shows much sediment formed at the bottom of the centrifuge tube. This result indicated that the T-NLC1 was unstable and needed to further adjust the composition of MCT and GMS in T-NLCs. Based on T-NLC2, sediment still formed at the bottom of the centrifuge tube but is lesser if compared with T-NLC1. Hence, a slight adjustment on the composition of Tween 80 and soy lecithin T-NLC2 was done to formulate T-NLC3. T-NLC3 showed no sediment after centrifuge, indicating that T-NLC3 is stable, according to Hu et al. (2016).

Particle Size (PS), Polydispersity Index (PDI), and Zeta Potential (ZP)

T-NLC3 was selected for further characterization regarding PS, PDI, and ZP as it remained stable after the centrifugation stability test. If the PS of T-NLCs is greater than 600 nm, T-NLCs could not reach the skin's stratum corneum. The PS of T-NLC3 was 129.407 ± 1.278 nm, which means delivering the encapsulated bioactive compound to deeper layers of the skin according to Danaei et al. (2018). PDI represents the distribution of size population in a given sample and PDI for T-NLC3 is 0.151 ± 0.211. Since the PDI of T-NLC3 is below 0.3, it is indicated that the PS of T-NLCs is homogenous and uniform. The result also aligned with the findings from Danaei et al. (2018). Besides, ZP was used to measure the particle charge and electrostatic repulsion. The ZP of T-NLC3 was -44.7 ± 1.8 mV and always exhibited in negative value due to OH^- in Tween 80. Tween 80 is a non-ionic surfactant and OH^- incorporated between the surface of the lipid phase and aqueous phase (Park et al., 2018). The ZP value also indicated that T-NLC3 had adequate stability and its particles would repel each other. They will not aggregate as this ZP value is greater than +30.0 mV or smaller than -30.0 mV according to Makoni et al. (2019).

Encapsulation Efficiency (EE) of T-NLCs

This study expressed EE as a percentage of total curcumin in turmeric extract that encapsulated in NLCs. Curcumin is a bioactive compound of turmeric extract, and the NLCs system should exhibit a high EE as it would represent a greater capacity for carrying the curcumin. For the chosen T-NLC3, the EE was 98.04 ± 9.19%, indicating that the formulation was effective for entrapping curcumin in turmeric extract, supported by Park et al. (2018).

Real-Time Stability Test

The real-time stability test is an important parameter to study as it provides us with a view of the stability of the NLCs under different media and temperatures. The stability of the T-NLC3 was studied for 30 days at room temperature (25 ± 2 °C), oven (45 ± 2 °C) and also refrigerated (4 ± 2 °C). The physical appearance of T-NLC3 had evaluated visually to anticipate its stability. Neither sediment nor creaming was observed during the storage (**Figure 3 and 4**), indicating T-NLC3 has remained stable over 30 days. These results were in alignment with the findings from Hu et al. (2016).

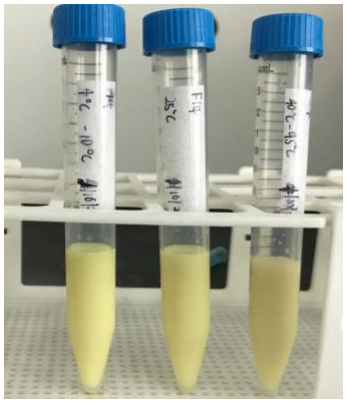

Figure 3 Stability results of T-NLC3 under different conditions on the 1st Day

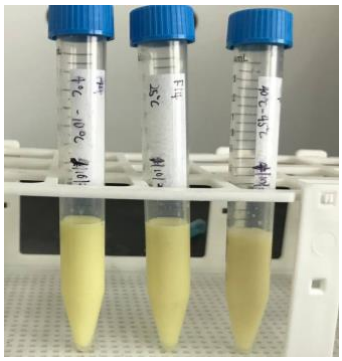

Figure 4 Stability results of T-NLC3 under different conditions on the 30th Day

Table 3 shows that the PS of T-NLCs increased over time depending on the conditions, and T-NLCs stored under 45 ± 2 °C experienced the largest change in PS. This had been reported by Makoni et al. (2019) that at a higher temperature, the collisions between particles increased because of the kinetic energy increase. Hence, the temperature is an important factor as particles destabilize and may agglomerate under higher temperatures. Based on these results, 4 ± 2 °C was the optimum storage temperature for T-NLCs because the particles grew slower at a lower temperature. Apart from that, all PDI values of T-NLCs under different conditions were below 0.3. These findings indicated that T-NLCs were homogenous and uniform in size in the samples after 30 days of storage, thus inferring that the T-NLCs formulated in this project were stable even after a specified period.

Table 3 Stability results of T-NLC3 under different conditions in terms of particle size and polydispersity index

Period and Condition	Particle Size (nm)	Polydispersity Index
Initial	129.407 ± 1.278	0.151 ± 0.211
25 ± 2 °C		
30th Day	142.233 ± 0.153	0.195 ± 0.002
45 ± 2 °C		
30th Day	143.333 ± 0.208	0.179 ± 0.015
4 ± 2 °C		
30th Day	141.800 ± 1.082	0.163 ± 0.009

Formulation of Herbal Cleansing Gel

The composition of the herbal cleansing gel is shown in **Table 4**. After trial and error, F1 was discarded due to being too viscous. Thus, F2 was considered for further study, which is characterization.

Table 4 Composition of herbal cleansing gel

Substances	Composition (w/w %)	
	F1	F2
Distilled water	77.60	82.60
Glycerine	2.00	2.00
Propylene glycol	1.00	1.00
Potassium sorbate	0.15	0.15
Allantoin	0.15	0.15
PEG-150 distearate	3.00	2.00
Cocamidopropyl betaine	8.00	4.00
Decyl glucoside	2.00	2.00
PEG-40 hydrogenated castor oil	2.00	2.00
PEG-7 glyceryl cocoate	2.00	2.00
T-NLCs	2.00	2.00
Geranium	0.10	0.10

Characterization of Herbal Cleansing Gel

Sensory Test

The sensory test on the herbal cleansing gel was conducted on 30 panellists using a hedonic scale. **Figure 5** shows that the panellists preferred F2 over commercial herbal cleansing gel regarding appearance, odour, clarity, viscosity, foaming ability, freshness, and overall acceptability. According to the feedback from panellists, they preferred F2 because F2 had a better physical appearance and clarity, while commercial herbal cleansing gel looked more turbid. In terms of odour, all of them prefer F2 as well because it smells like tea tree essential oil. Besides, the panellists commented that commercial herbal cleansing gel was too viscous and difficult to pour out. For foaming ability, F2 had a higher foaming ability as it created more foam and the panellists believed F2 had a better cleaning effect than commercial herbal cleansing gel. Moreover, they claimed that F2 had better freshness over commercial herbal cleansing gel after rinsing off with water. Generally, the panellists were more satisfied with F2 over commercial cleansing herbal gel regarding overall acceptability.

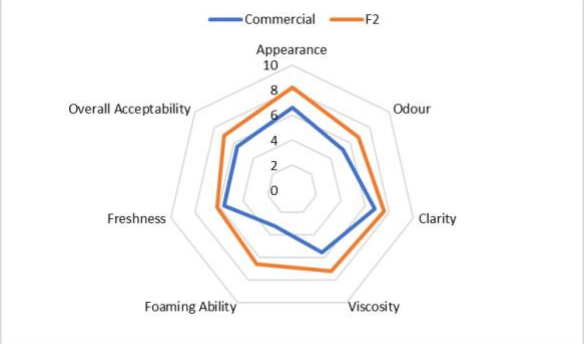

Figure 5 Sensory test for each formulation

Based on **Table 5**, there was no significant difference ($p < 0.05$) in appearance, odour, clarity, viscosity, freshness, and overall acceptability between F2 and commercial herbal cleansing gel except for foaming ability. However, the panellists still preferred the foaming ability of F2 according to the higher mean score obtained. Thus, the findings indicated that F2 could compete with the herbal cleansing gel on the market.

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Table 5 Sensory scores of panellists based on sensory attributes

Sensory Attributes	F2	Commercial
Appearance	8.2 ± 0.8[a]	6.6 ± 1.5 [a]
Odour	6.8 ± 1.8 [a]	5.2 ± 2.9 [a]
Clarity	7.6 ± 0.5 [a]	6.8 ± 1.3 [a]
Viscosity	7.2 ± 1.3 [a]	5.6 ± 1.7 [a]
Foaming Ability	6.6 ± 2.1 [a]	3.2 ± 1.8 [b]
Freshness	6.2 ± 1.9 [a]	5.6 ± 1.1 [a]
Overall Acceptability	7.0 ± 1.2 [a]	5.6 ± 1.1 [a]

* Values correspond to mean ± SD

** [(a-b)] Different letters in the column are significantly different ($p < 0.05$)

CONCLUSION

In conclusion, the characterization of turmeric extract-loaded nanostructured lipid carriers (T-NLCs) in terms of particle size, polydispersity index, zeta potential, encapsulation efficiency, and stability were all within acceptable limits. In this project, the bioavailability of turmeric extract was improved, and an herbal cleansing gel from turmeric extract was also developed.

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