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Development and characterization of a functional nanoemulsion using pomelo peel essential oil and curcumin

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Pomelo peel essential oil and curcumin possess potent antioxidant, anti-inflammatory, and antimicrobial properties. However, their poor solubility and instability limit their practical applications. To overcome these challenges, a nanoemulsion incorporating both bioactives was formulated and optimized using a Box–Behnken design. Essential oil, Tween 80, and curcumin were selected as independent variables, with DPPH radical scavenging activity, turbidity, and stability as response parameters. The optimized formulation was achieved at 15 mL essential oil, 5 mL Tween 80 and 0.10 g curcumin, with a desirability of 0.772. This desirability indicates that the optimized formulation fulfils the desired goals of selected independent variables and shows predicted *versus* experimental values with less than 10% deviation, confirming model adequacy. The nanoemulsion exhibited a droplet size of 12.98 nm ± 0.65 nm, a polydispersity index of 0.287 ± 0.008, and a zeta potential of −22.4 ± 0.20 mV, indicating good physical stability. Color measurements ($L^* = 75.59$, $a^* = 4.52$, $b^* = 79.78$) reflected uniform dispersion of components. The formulation showed strong antioxidant activity, elevated total phenolic and flavonoid contents, and effective antimicrobial action against common pathogens. The optimized nanoemulsion showed inhibition zones of 15, 18, 20, 16 and 19 mm against *Bacillus cereus*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, respectively. It remained stable at room temperature for one month without phase separation. These results highlight the potential of the developed nanoemulsion as a functional ingredient for food applications. This study highlights the sustainable utilization of pomelo peel, a by-product of the pomelo fruit after consumption, by harnessing it for essential oil extraction.

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Sustainability spotlight

This study contributes to sustainable scientific advancement by valorizing agricultural waste. The high degree of citrus fruit consumption results in an increase in processing waste. Pomelo peel, a by-product of pomelo processing with phytochemicals and bioactive compounds, is utilized as a source of essential oil, leading to the reduction of organic waste. Curcumin, a natural polyphenol with health benefits, is incorporated into a nanoemulsion system to increase its bioavailability. The development of such a nanoemulsion helps in sustainable delivery systems in the food, nutraceutical, and pharmaceutical industries. This work aligns with the United Nations Sustainable Development Goals (SDGs), Goal 12 (Responsible Consumption and Production) and Goal 3 (Good Health and Well-being), along with maximum resource utilization and promoting natural health products.

1 Introduction

Citrus maxima, also known as pomelo, is a citrus fruit native to Southern Asia and is widely found in countries such as China, Japan, Vietnam, Malaysia, India, and Thailand. It is considered the ancestral species of grapefruit and belongs to the Rutaceae family. It is extensively cultivated and consumed. It is the parent fruit of other citrus fruits such as lemon, orange, mandarin, and grapefruit.[1] While the fruit is typically eaten fresh or processed into juice, its peels, seeds, and other parts are often discarded as waste. However, various parts of the plant, including the leaves, pulp, and peels, have been traditionally used in medicine due to their therapeutic properties and safety for human use. Citrus species are recognized as key sources of essential oils, particularly from the peels, which are valued for their pleasant aroma and refreshing qualities. Growing interest in these oils has led to increasing commercial significance in recent years.[2]

Pomelo (*Citrus maxima*) peel essential oil (PPEO) is rich in bioactive compounds such as limonene, linalool, and other volatile components. These compounds are known for their antioxidant, antimicrobial, and anti-inflammatory properties.

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However, its application is often limited by its poor solubility, volatility, and susceptibility to degradation.[3] Similarly, curcumin, a polyphenolic compound derived from turmeric (*Curcuma longa*), exhibits remarkable therapeutic properties, including antioxidant, anti-inflammatory, and anticancer effects. Despite these advantages, curcumin faces challenges such as low aqueous solubility, instability, and poor bioavailability, which hinder its practical applications.[4] These challenges related to PPEO and curcumin demand an effective delivery system that can overcome these problems and enhance the usability of these bioactives. Nanoemulsions have emerged as a promising delivery system for enhancing the solubility, stability, and bioavailability of hydrophobic bioactives. They offer advantages such as small droplet size, high surface area, and improved encapsulation efficiency, making them suitable carriers for essential oils and bioactive compounds.[5]

This work explores the underutilized pomelo peel, a by-product of the food industry and a sustainable source of essential oil. The combination of PPEO and curcumin, each with its individual strong antioxidant and antimicrobial effects, and their incorporation into a single nanoemulsion system, not only addresses their individual limitations but also enables better delivery, increasing its overall efficacy. This kind of nanoemulsion system could provide synergistic health benefits of both bioactives, both functional and antimicrobial properties, and hence be referred to as a dual bioactive nanoemulsion. In addition, this dual bioactive nanoemulsion has scope to be utilised in food, nutraceuticals or packaging, and it can also be considered as a contribution toward value-added waste utilization with health-promoting qualities.

This study aims to optimize and characterize a nanoemulsion incorporating PPEO and curcumin, where droplet size distribution, zeta potential, encapsulation efficiency, antioxidant activity, stability, and antimicrobial activity of the optimized nanoemulsion are evaluated. The findings from this study could provide insights into the development of functional nanoemulsions for food. This study will lead to the valorization of waste such as pomelo peel, which can be an emerging sustainable strategy in food applications and promote a sustainable bioeconomy approach.

2 Materials and methods

2.1. Materials

Pomelo peel essential oil was extracted from the dried peels of mature pomelo (*Citrus maxima*) fruits. Curcumin and Tween 80 were obtained from Sisco Research Laboratories Pvt. Ltd (Maharashtra, India). Glycerol was obtained from Merck Specialities Pvt. Ltd (Mumbai, India). All other required reagents for analysis were of analytical grade and obtained from HiMedia Laboratories Pvt. Ltd, India.

2.2. Extraction of essential oil (EO)

About 30 g of dried pomelo peels in 400 mL of distilled water were placed into a round-bottom flask and subjected to hydrodistillation for 3 h at 40–50 °C using a Clevenger apparatus to extract essential oil. The obtained distillates of essential oils were dried over anhydrous sodium sulphate, filtered and stored at −4 °C for further analysis.[6] The yield of essential oil from dried pomelo peel was about 3.33%.

2.3. Development of pomelo peel essential oil loaded with curcumin nanoemulsion (NE)

2.3.1. Preparation of NE. The oil phase was prepared using a magnetic stirrer at 1000 rpm for 15 min by mixing pomelo peel essential oil, Tween 80 and curcumin, making 20% (20 mL) of the 100 mL NE. The aqueous phase, fixed at 80% (80 mL) of the 100 mL NE containing water (55%) and glycerol (25%), was added dropwise to the oil phase with the system being mechanically stirred at 1000 rpm. After complete addition of the aqueous phase to the oil phase, the mixture was stirred for 30 min at 1000 rpm. The coarse emulsion thus obtained was homogenized at 5000 rpm for 10 min and sonicated (20 min, 70% amplitude and 42 W power with 30 s ON and 15 s OFF pulsation cycle).[5]

2.3.2. Optimization of NE by response surface methodology (RSM). The optimization of NE was performed by RSM using Box–Behnken design (BBD) through Design Expert Software. A total of three independent variables for 100 mL formulations were taken, namely: (A) pomelo peel essential oil (PPEO) in the range 5–15 mL, (B) Tween 80 varied between 5 and 15 mL, and (C) curcumin ranged between 0.1 and 0.5 g. The levels were selected based on a preliminary trial, and Tween 80 was chosen based on its permissible levels in food systems. The independent variables were run at three levels for each of the individual coded values (−1, 0, and 1) shown in Table S1. The software generated a total of 17 tests with 5 center points, having different variations of the oil phase. The aqueous phase was adjusted at 80% of total NE according to the concentration of the oil phase to enable the calculation of pure error, and the experiments were conducted in a random order (Table 1). The effect of independent variables was studied on three responses, namely DPPH free radical scavenging activity (%), turbidity (cm^{-1}) and stability (%).

2.4. Characterization of NE

2.4.1. DPPH free radical scavenging activity of nanoemulsions. DPPH free radical scavenging activity of NE was estimated as per the method given by Yumnam *et al.*, (2023)[2] with slight modifications. To 100 µl of methanolic NE extract, methanol was added to make up the volume to 2 mL, then 2 mL of DPPH (0.1 mM in methanol) was added and the mixture was incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm. A mixture of equal volumes of methanol and DPPH reagent was taken as a control. It was calculated using eqn (1).

$$\text{DPPH free radical scavenging activity}(\%) = \frac{A_C - A_S}{A_C} \times 100 \quad (1)$$

where A_C is the absorbance of the control and A_S is the absorbance of the sample.

Table 1 Optimization of NE by response surface methodology (RSM)

Sl no.	Essential oil (mL)	Tween 80 (mL)	Curcumin (g)	DPPH free radical scavenging activity (%)	Turbidity (cm^{-1})	Stability (%)
1	5	15	0.3	54.44	2.32	76
2	5	10	0.1	33.45	1.92	68
3	5	10	0.5	57.52	3.53	61
4	5	5	0.3	42.08	4.98	58
5	10	10	0.3	62.61	5.31	82
6	10	10	0.3	62.23	5.51	79
7	10	10	0.1	59.84	5.76	81
8	10	15	0.1	61.77	1.03	87
9	10	10	0.3	60.56	6.2	76
10	10	5	0.1	55.59	1.19	73
11	10	15	0.5	72.22	3.75	84
12	10	5	0.5	70.56	6.92	60
13	10	10	0.3	59.07	5.43	80
14	15	5	0.3	76.06	6.66	66
15	15	15	0.3	79.52	5.84	90
16	15	10	0.1	74.39	2.38	84
17	15	10	0.5	82.55	7.15	79

The DPPH free radical scavenging activity of optimized NE was compared with that of standard ascorbic acid at the same concentration.

2.4.2. Turbidity and stability of NE. Turbidity of NE is measured at a wavelength of 600 nm using a UV-visible spectrophotometer (Model CARY 60 UV-VIS) at 25 °C. Deionized water was used as a blank. The turbidity was calculated using eqn (2).[7]

$$\text{Turbidity}(\text{cm}^{-1}) = \frac{2.303 \times \text{ABS}}{L} \quad (2)$$

where ABS is the absorbance and L (1 cm) is the path length of the cuvette.

For evaluating stability, 10 mL nanoemulsion samples were submerged in a hot water bath at 80 °C for 30 min, then placed in a freezer for 15 min, and then centrifuged at 5000 rpm for 30 min (REMI, R-8C PLUS). The nanoemulsion stability was calculated using eqn (3).[8]

$$\text{Nanoemulsion stability}(\%) = \frac{\text{Volume of nanoemulsion phase}}{\text{Total volume of nanoemulsion}} \times 100 \quad (3)$$

where volume of the nanoemulsion phase refers to the volume of the intact and homogeneous nanoemulsion layer. As the thermal-freeze-centrifugation test is a harsh protocol, absolute thresholds of stable *vs.* unstable are not typically applied. Nanoemulsion stability was used as a relative index for comparing formulations and taken as a response for BBD.

2.4.3. Measurement of droplet size, polydispersity index (PDI) and zeta potential of optimized NE. Dynamic light scattering was used to analyze the droplet size diameter, polydispersity index (PDI), and zeta potential of optimized NE utilizing a Zetasizer (Nano ZS, Malvern Panalytical, the United Kingdom). NE was diluted 100 times using double-distilled water and was further analysed for particle size and PDI.[9]

2.4.4. pH and color measurement of optimized NE. A pH meter (EUTECH ISTRUMENT, pH 700) calibrated at room temperature using standard buffer solutions of pH 4.0, 7.0 and 9.0 was used to measure the pH of the optimized NE. Measurements were taken in triplicate by immersing the electrode into the sample without dilution, and values were recorded after stabilization. Colour values (L^*, a^*, b^*) were measured using a Hunter Lab colorimeter (Ultrascan VIS, Hunter Lab Inc., USA). The instrument was calibrated with a standard white tile, and the sample was analysed in triplicate.[10]

2.4.5. Phytochemical properties of optimized NE. Total phenolic content (TPC) and total flavonoid content (TFC) of the optimized nanoemulsion were evaluated by the methods given by Phuyal *et al.*, (2020) with slight modifications.[11] TPC was determined by the Folin–Ciocalteu colorimetric assay and expressed as gallic acid equivalents (mg GAE per mL).To 1 mL of methanolic extract of NE or standard, 5 mL of 10% Folin–Ciocalteu reagent was added. After 5 min, 7% sodium carbonate was mixed, and the mixture was incubated at 40 °C for 30 min. Absorbance was measured at 760 nm against a blank, and calculations were carried out using a calibration curve (absorbance *vs.* gallic acid concentration).

TFC was measured by the aluminum chloride colorimetric assay and expressed as quercetin equivalents (mg QE per mL). To 1 mL of the methanolic extract of NE or standard, 4 mL of distilled water and 5% sodium nitrite were added. After 5 min, 0.3 mL of 10% aluminum chloride was added, and then after 6 min, 2 mL of 1 M sodium hydroxide was added. The volume was made up to 10 mL with distilled water and mixed well. After 15 min, absorbance was measured at 510 nm against a blank, and calculations were carried out using a calibration curve (absorbance *vs.* quercetin concentration).

2.4.6. Encapsulation efficiency (EE) of optimized NE. Encapsulation efficiency (EE) of NE was examined by centrifugation and ethanol extraction. Freshly prepared nanoemulsion

was subjected to centrifugation for 30 min at 5000 rpm using REMI, R-8C PLUS to separate excess curcumin. The resulting precipitate was collected and extracted using 1 mL of pure ethanol by vortexing for 5 min. The curcumin concentration (μg mL^{-1}) was quantified at 419 nm using a CARY 60 UV-VIS spectrophotometer. A standard calibration curve was prepared with known concentrations of curcumin dissolved in ethanol, and a calibration equation was obtained to calculate non-trapped curcumin content.[12] The EE of NE was obtained using eqn (4).

$$\mathrm{EE}(\%) = \frac{\text{Initial curcumin content} - \text{Nontrapped curcumin content}}{\text{Initial curcumin content}} \times 100 \quad (4)$$

2.5. Stability studies of optimized NE

The optimized NE was subjected to centrifugation, heating–cooling cycles, freezing–thawing cycles and storage to investigate the stability. NE was centrifuged at 5000 rpm for 30 min and evaluated for phase separation. For heating–cooling cycles, NE was treated with three cycles of heating (45 °C for 48 h) and cooling (4 °C for 48 h) alternatively and then assessed for phase separation. Three cycles of alternative freezing (−21 °C for 48 h) and thawing (25 °C for 48 h) were performed for NE and reviewed for phase separation. The optimized nanoemulsion was also kept at room temperature for one month and evaluated for phase separation and creaming index (CMI%) at an interval of 7 days.[10] The creaming index refers to the percentage of phase separation observed in the nanoemulsion after storage, calculated as the ratio of the total height of the cream layer to the total height of the nanoemulsion, expressed in percentage. CMI% was calculated using eqn (5).

$$\mathrm{CMI}\% = \frac{\mathrm{CC}}{\mathrm{CT}} \times 100 \quad (5)$$

where CT is the total height of the nanoemulsion layer and CC is the total height of the cream layer.

2.6. Morphological characterization of optimized NE

Optical imaging of the optimized NE was performed by placing a drop of the sample on a slide, which was then dried and checked at a magnification of 40×.[8] A field emission scanning electron microscope (JEOL JSM-7200F, Japan) was used to characterize the morphology of the optimized nanoemulsion at magnifications of 600 00× and 1500 00×.[13]

2.7. Fourier-transform infrared spectroscopy

Curcumin powder, pomelo peel essential oil and optimized NE were scanned in the range of 400–4000 cm^{-1} using a Fourier-transform infrared spectrometer (Impact 410 spectrometer, Nicolet, USA, Software: Omnic ESP50).[14]

2.8. Antimicrobial activity of optimized nanoemulsion

The antimicrobial activity of the optimized nanoemulsion was evaluated using the disc diffusion method with bacterial cultures, namely *Escherichia coli* (MTCC 40), *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 2297), and *Salmonella typhimurium* (MTCC 98). The plates were evaluated for antimicrobial activity, and the zone of inhibition (ZOI) was measured accordingly.

2.9. Statistical analysis

The experiments were conducted in triplicate, and the data are expressed as the mean values along with standard deviation. The statistical indices, such as mean and standard deviation values for all the properties, were calculated using Microsoft Office Excel 2007. Statistical significance was obtained by one-way analysis of variance (ANOVA) through Duncan's multiple range test ($p < 0.05$) using the package IBM SPSS Version 20.0, Armonk, NY: IBM Corporation software.

3 Results and discussion

3.1. Optimization of NE by response surface methodology (RSM)

A series of 17 experiments were performed to investigate the influence of formulation variables on DPPH free radical scavenging activity, turbidity and stability. The response data corresponding to each experiment are summarized in Table 1. The values of responses Y_1 (DPPH free radical scavenging activity), Y_2 (turbidity) and Y_3 (stability) varied from 33.45% to 82.55%, 1.03 cm^{-1} to 7.15 cm^{-1} and 58% to 90%, respectively.

Selection of the appropriate model was based on a non-significant lack of fit and satisfactory determination coefficients (R^2) for various responses (Tables S2–S4). The lack of fit values for DPPH free radical scavenging activity, turbidity and stability were non-significant with R^2 values of 0.98, 0.98 and 0.97, respectively. For all the responses, the predicted R^2 values were found to be in reasonable agreement with their corresponding adjusted R^2 values.

Optimization of the nanoemulsion was executed by keeping essential oil and curcumin within a range, whereas Tween 80 was kept at a minimum, and the responses, DPPH free radical scavenging activity and stability, were set at a maximum and turbidity at a minimum. The experimental values are given in Table S5.

3.1.1. Effect of formulation variables on DPPH free radical scavenging activity of NE. Essential oil, Tween 80 and curcumin showed significant effects on DPPH free radical scavenging activity of NE, along with significant effects of the interaction term (AC) and the quadratic term (B^2). The linear terms (A, B and C) had positive effects, while the interaction terms (AB, AC and BC) had negative effects (Fig. 1a–c & eqn (S1)). Pomelo peel essential oil is composed of different chemical compounds such as D-limonene, citral, and other terpenoids, which are responsible for efficient hydrogen or electron donor, resulting in its high natural antioxidant activity.[2] Li *et al.*, (2024)[15] demonstrated that the conjugated double bonds and reactive functional groups in citrus essential oils rich in D-limonene exhibited significant radical scavenging activity. In research studies, curcumin contributed to antioxidant activity by the

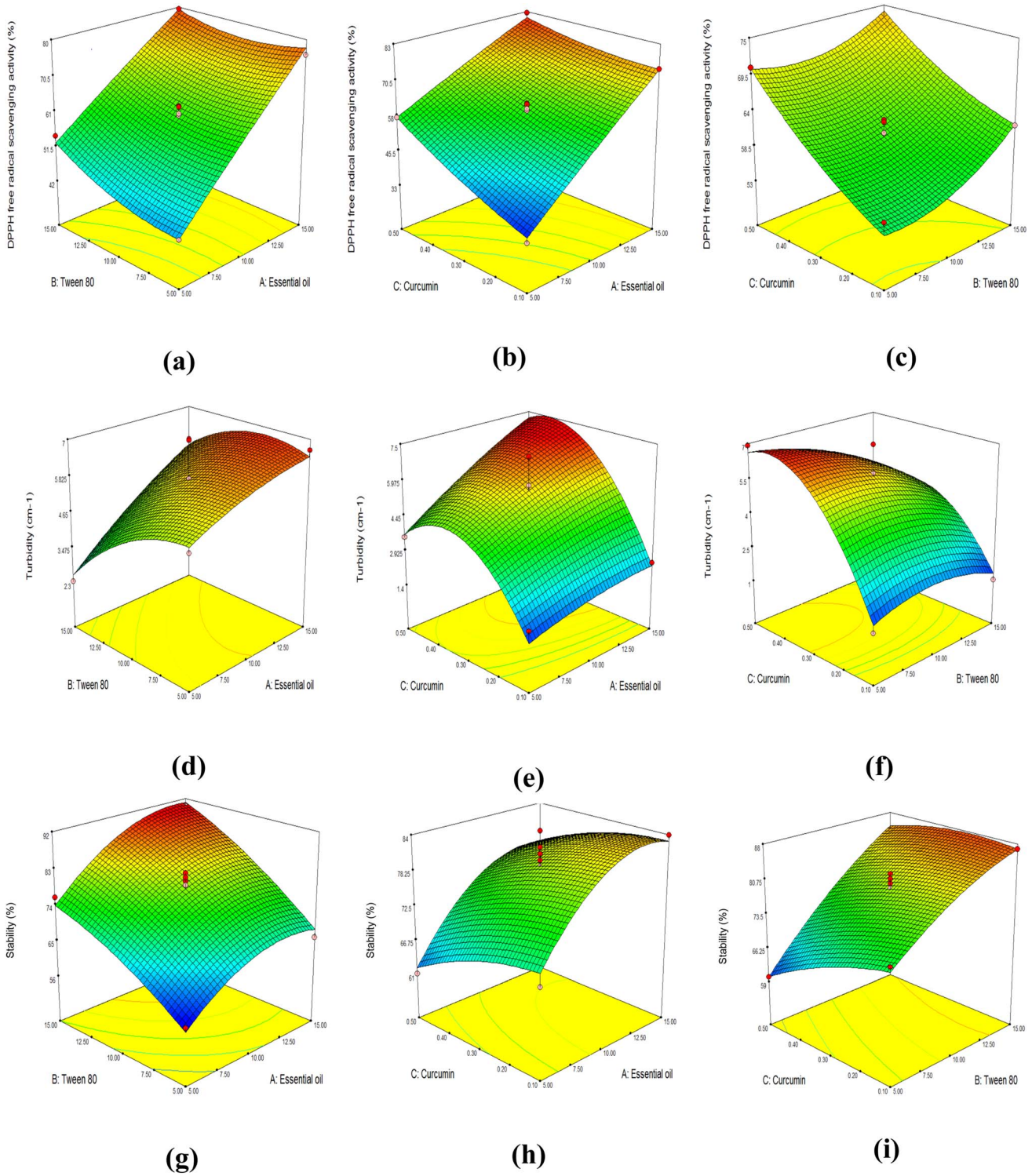

Fig. 1 3D surface plots for the effect of essential oil, Tween 80 and curcumin on DPPH free radical scavenging activity (a–c), turbidity (d–f) and stability (g–i).

formation of stabilized keto–enol structures during the free radical reaction by release of hydrogen from its phenolic –OH groups.[9] Tween 80, while not itself an antioxidant, favours efficient dispersion of essential oil and curcumin with stronger bonds and stabilized aqueous caging, enabling proficient interaction. Overall, the combined effect of these components indicates a synergistic improvement in radical scavenging activity. The DPPH free radical scavenging activity of the optimized NE was 79.53 ± 0.15%; however, the same concentration of ascorbic acid showed 96.35% of DPPH free radical scavenging activity.

3.1.2. Effect of formulation variables on turbidity of NE. Turbidity of NE had significant effects of essential oil, Tween 80 and curcumin along with quadratic terms (B^2 and C^2) and interaction terms (AC and BC). The interaction terms (AB and AC) had positive effects, while the other terms (BC, A^2, B^2 and C^2) had negative effects on the turbidity of NE (Fig. 1 d–f and eqn (S2)). Turbidity of NE decreased with the increase of Tween 80, and a similar fashion of negative correlation between the surfactant and turbidity of NE has been reported by Sharma *et al.*, (2021).[9] In the study, it was mentioned that the surfactant concentration and turbidity of curcumin root essential oil nanoemulsion were negatively correlated ($r = -0.613$). This can be explained by the ability of Tween 80 to reduce interfacial tension, forming a small droplet size that offers a path for transmission of light through it and hence delivers more clarity.[16] Pomelo peel essential oil shows a positive effect on the turbidity of NE, and it was observed in the same study as above[9] that at a fixed surfactant level, increasing essential oil concentration increased the turbidity of nanoemulsion. This may be due to the increase in oil concentration, which leads to larger droplet sizes or higher droplet intensity that hinders light transmission through it and increases turbidity. The effect of curcumin on the turbidity of NE depends on its concentration and the level of its dispersion into the system. At lower concentrations, curcumin may solubilize well within the system, causing minimal effect on turbidity. While at higher levels, curcumin has poor solubility, resulting in increased turbidity.

3.1.3. Effect of formulation variables on stability of NE. The essential oil, Tween 80 and curcumin had significantly affected the stability of NE, while the interaction terms (AB, AC and BC) and quadratic terms except A^2 (B^2 and C^2) had insignificant effects (Fig. g–i and eqn (S3)). Essential oil and tween 80 had positively affected the stability of NE; however, an increase in curcumin resulted in a decrease in stability. The application of ultrasonication and surfactant levels leads to the sustainability of the stability of NE under different conditions. Tween 80 played a critical role in the stability of NE by decreasing interfacial tension and forming a film around droplets, providing stearic and electrostatic stabilization. This layer also minimized droplet coalescence and aggregation.[16] The role of essential oil and curcumin in the stability of NE depends on how well they dissolve into the dispersion. Essential oil, when present in an optimal range, acts as a well-solubilized dispersed phase for surfactant-stabilized droplets. However, a greater quantity of essential oil may cause destabilization, leading to droplet coalescence and phase separation. In a study, it was found that the increase in essential oil concentration showed phase separation of curcumin root essential oil nanoemulsion during centrifugation, freezing and thawing.[9] Curcumin had a destabilizing effect at higher concentrations because of its limited solubility. It can self-associate within the system and disrupt the film around the droplets, causing an increase in interfacial tension and enhancing aggregation and creaming. Zou *et al.*, (2016)[17] reported that high curcumin loading increased instability because of low solubility and poor molecular compatibility. In another study, it was observed that higher curcumin loading (in the oil phase) led to instability in a formulation of ultrasound-assisted curcumin nanoemulsion with palm oil and tween-80.[18,19]

Table 2 Characterization of optimized nanoemulsion

Sl no.	Properties	Results
1	Average particle size (nm)	12.98 ± 0.65
2	Polydispersity index (PDI)	0.287 ± 0.008
3	Zeta potential (mV)	−22.4 ± 0.20
4	Encapsulation efficiency (%)	90.6 ± 0.84
5	L^* (lightness)	75.59 ± 0.38
6	a^* (red-green colour component)	4.52 ± 0.25
7	b^* (yellow-blue colour component)	79.78 ± 0.90
8	pH	4.74 ± 0.40
9	Total phenolic content (TPC) (mg GAE/mL)	1.84 ± 0.23
10	Total flavonoid content (TFC) (mg QE/mL)	0.69 ± 0.03

3.2. Characterization of optimized NE

The optimized nanoemulsion exhibited an average particle size of 15.24 + 3.20 nm, a polydispersity index (PDI) of 0.28 ± 0.008 and a zeta potential of −22.40 ± 0.20 mV (Table 2 and Fig. S1). The average particle size and PDI of the optimized nanoemulsion were within the range of values reported by Abdou *et al.*, (2018)[5] for curcumin–cinnamon essential oil (CCN) and curcumin–garlic essential oil (CGN) nanoemulsions, the average particle size was 9 nm for CCN and 40 nm for CGN, while the PDI values were 0.45 and 0.19, respectively.[5] The droplet size of a nanoemulsion is influenced by the viscosity of the oil phase; essential oils, having lower viscosities compared to triglyceride oils, tend to form nanoemulsions with smaller droplet sizes.[5] The encapsulation efficiency was found to be 90.6 ± 0.84%. The zeta potential and encapsulation efficiency of the optimized nanoemulsion were in agreement with curcumin root essential oil nanoemulsion, where the zeta potential varied between −20.80 and −27.60 mV, and encapsulation efficiency was as high as 93.60%.[9]

The high L^* value of the nanoemulsion indicates that the nanoemulsion is light-coloured due to the proper dispersion of essential oil and curcumin. There is a slight reddish hue to the optimized nanoemulsion with strong yellow colouration due to the presence of curcumin (Table 2). The pH of the optimized nanoemulsion was evaluated as 4.74 ± 0.40, which is in the nearby range of 5.55 to 5.69 of curcumin safflower oil.[8]

The SEM images reveal uniform distribution of spherical and smooth droplets with a mean droplet size of 37.48 ± 7.31 nm (Fig. 2a and b). The optical microscope image of the optimized nanoemulsion shows micelles, which represents encapsulation of curcumin by the oil layer without any aggregation in a stable form (Fig. 2c). The FTIR spectrum of pomelo peel essential oil (Fig. 3) showed a peak at 2925 cm^{-1}, which is associated with C–H stretching and a peak at 1640 cm^{-1} corresponding to C=C stretching. For the IR spectra of curcumin, C=O stretching, C–H bending and C–O stretching were observed at 1632 cm^{-1} 1426 cm^{-1} 1025 cm^{-1} peaks,

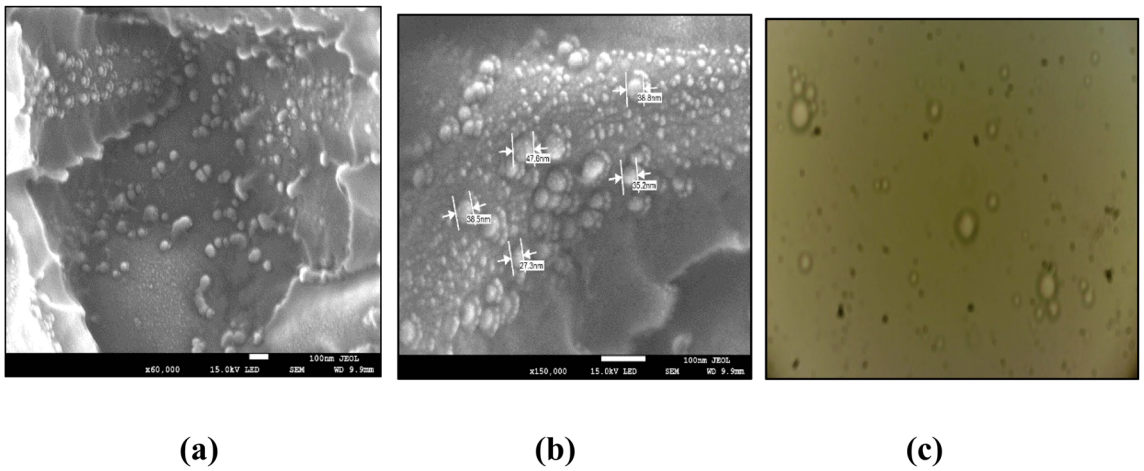

Fig. 2 (a and b) Scanning electron microscope images of the optimized nanoemulsion; (c) optical microscope image of the optimized nanoemulsion.

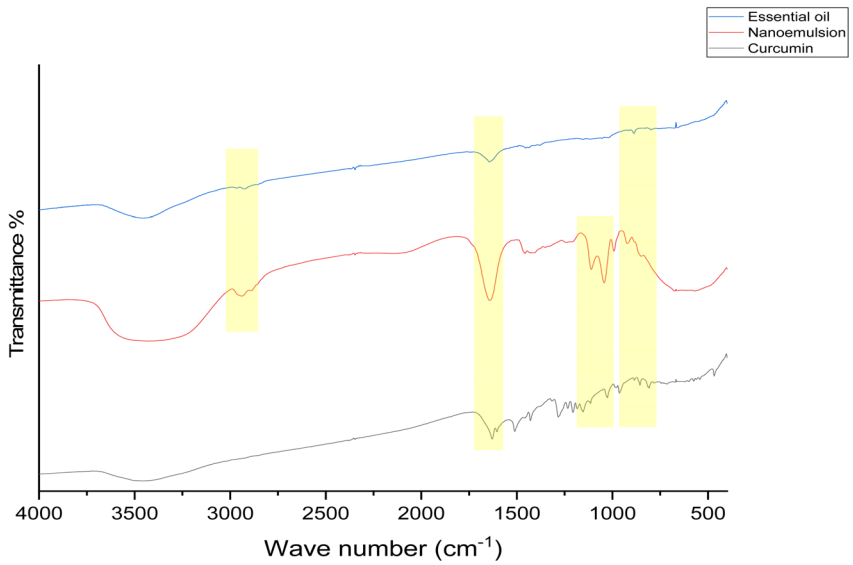

Fig. 3 FTIR spectra of pomelo peel essential oil, nanoemulsion and curcumin.

respectively. In the FTIR spectrum, a peak around 2900 cm^{-1} was observed in essential oil and nanoemulsion, suggesting C–H stretching. A peak around 1600–1500 cm^{-1}, corresponding to C=C stretching, was observed in curcumin and the nanoemulsion, indicating the presence of aromatic rings or conjugated alkenes. Peaks around 1200–1000 cm^{-1}, corresponding to C–O stretching, were observed, suggesting the presence of esters. In the regions 1600–1500 cm^{-1} and 1200–1000 cm^{-1}, some slight shifts, such as the C=O/C=C band observed at 1628 cm^{-1} (NE), were noted, showing a shift from 1632 cm^{-1} (curcumin) and 1640 cm^{-1} (PPEO). These shifts suggest possible hydrogen bonding and van der Waals interactions between the carbonyl and hydroxyl groups of curcumin and the oxygenated terpenes present in PPEO. The peaks of essential oil, curcumin and NE along with the shifts suggests that NE combines the features of both essential oil and curcumin indicating successful dispersion of essential oil and curcumin.

3.3. Antimicrobial activity of optimized nanoemulsion

The antimicrobial activity of the optimized nanoemulsion was studied against five well-known pathogenic microorganisms, with gentamicin taken as a positive control, as it is a well-established aminoglycoside antibiotic with a broad spectrum of activity and a standard reference in antimicrobial studies

Table 3 Zone of inhibition (ZOI)

Bacteria	Gentamicin (mm)	Optimized nanoemulsion (mm)
Bacillus cereus	24	15
Salmonella typhimurium	18	18
Staphylococcus aureus	25	20
Pseudomonas aeruginosa	17	16
Escherichia coli	28	19

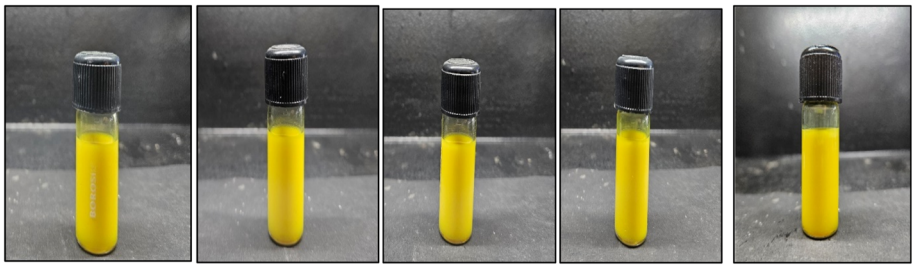

Fig. 4 Storage stability of optimized nanoemulsion for a period of one month: (a) 0 day, (b) 7 days, (c) 14 days, (d) 21 days, and (e) 28 days.

(Fig. S2). Maximum activity was observed against *Staphylococcus aureus* with a zone of inhibition of 20 nm, followed by *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Bacillus cereus* (Table 3). The antimicrobial activity of the optimized nanoemulsion is contributed by the collaborative function of curcumin and pomelo peel essential oil. According to the Clinical and Laboratory Standards Institute (CLSI), the inhibition zones above or near to 15–16 mm generally indicate susceptibility to the tested compound, which means that the optimized nanoemulsion exerted a relevant antimicrobial effect. The antibacterial activity of pomelo peel essential oil may be due to its chemical composition, primarily attributed to limonene. Yumnam *et al.*, 2023 (ref. 2) reported antimicrobial activity of pomelo peel essential oil against *Escherichia coli* with a ZOI of 14 nm. In another study, curcumin-loaded nanoparticles were prepared using poly(lactic-*co*-glycolic acid) (PLGA) and poly(vinyl alcohol) (PVA), which have shown antimicrobial activity against *Staphylococcus saprophyticus* subsp.*bovis* and *Escherichia coli*.[19] The optimized nanoemulsion inhibited the growth of all the tested microbial strains.

3.4. Stability studies of optimized nanoemulsion

The stability of the optimized nanoemulsion was confirmed by subjecting it to centrifugation, a heating–cooling cycle, a freezing–thawing cycle, and storage. There was no macroscopic instability such as creaming index or phase separation seen during centrifugation, the heating–cooling cycle and the freezing–thawing cycle. Towards the end of one-month storage study of optimized nanoemulsion at room temperature, a slightly visible cream layer was formed without phase separation, and the creaming index was calculated to be 6.66%, which indicates a stable nanoemulsion (Fig. 4). Generally, a creaming index below 10% is considered desirable for stable nanoemulsions, especially for long-term storage. The sustained stability of optimized nanoemulsion under these different conditions is due to the combined influence of ultrasonication and surfactant action that played a synergistic role. The steric hindrance provided by the surfactants led to the repulsive interactions between emulsion droplets, thereby preventing coalescence. Additionally, the application of ultrasonication effectively reduced droplet size and narrowed the size distribution.[9]

4 Conclusion

The nanoemulsion with pomelo peel essential oil and curcumin was successfully developed using ultrasonication and optimized using Box–Behnken design. It exhibited an excellent small droplet size of 12.98 ± 0.65 nm and superior stability and quality, with a PDI of 0.287 ± 0.008 and a zeta potential of −22.4 ± 0.20 mV. The nanoemulsion also displayed favourable turbidity, higher stability and encapsulation efficiency along with commendatory antioxidant activity, total phenolic and flavonoid content. The nanoemulsion was stable under different conditions and for up to one month at room temperature with an acceptable creaming index. The antimicrobial activity was also found to be optimal. Therefore, it could be concluded that the optimized nanoemulsion is suitable to be utilized in the food industry for new product development or in preservation or packaging. However, further research on production costs, scalability, and possible sensory properties after its incorporation into foods will be required to understand its industrial applicability. From a practical perspective, the optimized nanoemulsion can be added to food products based on the permissible limit of Tween 80 given by the food laws. This nanoemulsion could be used as a flavour enhancer or functional food in dairy products, bakery products and others. The cost is a consideration at the laboratory scale, but it is expected to reduce when the production process is scaled up to industrial levels.

In addition to the favourable properties, the optimized nanoemulsion also highlights the sustainable valorisation of pomelo peel, turning processing waste into a value-added resource for essential oil extraction. This approach contributes to waste reduction and sustainable food systems.

Author contributions

Parismita Koch: formal analysis; investigation; validation; writing – original draft. Sankar Chandra Deka: instrumentation. Munmi Borah: formal analysis; investigation. Poonam Mishra: supervision, visualization; writing – review & editing.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

All the data generated or analysed during this study are included in the manuscript.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d5fb00421g.

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