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Original Article

Effect of Tween 80 Concentration on the Physicochemical Characteristics of Strawberry (*Fragaria × ananassa*) Fruit Extract Nanoemulsion

Pengaruh Konsentrasi Tween 80 terhadap Karakteristik Fisikokimia Nanoemulsi Ekstrak Buah Stroberi (Fragaria × ananassa)

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Abstract

Strawberries (*Fragaria x ananassa* Duch.) contain non-polar flavonoids and function as antioxidants. Nanotechnology can increase drug bioavailability, has a kinetically stable system, has the ability to penetrate deeper into the skin, lowers viscosity, and delivers the active substance to the skin surface to form a thin layer. The purpose of this study was to observe the effect of Tween 80 as a surfactant on the fruit extract nanoemulsion formulation that can be used as a topical drug delivery system as an antioxidant. Nanoemulsion is made from Tween 80 surfactant, PEG 400 cosurfactant, and virgin coconut oil phase. The concentration of Tween 80 used by F1, F2, and F3 was 20%, 30%, and 40%. The preparations were tested for organoleptic properties, pH, viscosity, transmittance and *droplet* size on the formula with the smallest *droplet* size. The organoleptic of the resulting nanoemulsion is in the form of a clearer brown colour, a characteristic odour, and a liquid form, and there is no separation phase. The effect of varying the concentration of Tween 80 in the nanoemulsion formulation of strawberry fruit extract: the higher the concentration of Tween 80, the higher the pH value, % transmittance and viscosity of the preparation. The *droplet* sizes on F1, F2, and F3 were 5722.77 ± 1.53 nm, 3328.5 ± 0.00 nm, and 25.97 ± 0.90 nm with a polydispersity index of 16.312 ± 4.32, 9.927 ± 0.00, and 1.060 ± 0.11. Nanoemulsions of strawberry fruit extract at 40% concentration can achieved nano-sized droplets but with broad distribution. The antioxidant testing findings utilizing the ABTS method revealed that the strawberry extract nanoemulsion formulation exhibited an IC50 value of under 50, signifying exceptionally potent antioxidant activity.

Abstrak

Stroberi (Fragaria x ananassa Duch.) mengandung flavonoid nonpolar dan berfungsi sebagai antioksidan. Nanoteknologi dapat meningkatkan bioavailabilitas obat, memiliki sistem yang stabil secara kinetik, mampu menembus lebih dalam ke kulit, menurunkan viskositas, serta menghantarkan zat aktif ke permukaan kulit sehingga membentuk lapisan tipis. Penelitian ini bertujuan untuk mengamati pengaruh Tween 80 sebagai surfaktan terhadap formulasi nanoemulsi ekstrak buah stroberi yang dapat digunakan sebagai sistem penghantaran obat topikal sebagai antioksidan. Nanoemulsi dibuat menggunakan surfaktan Tween 80, kosurfaktan PEG 400, dan fase minyak virgin coconut oil (VCO). Konsentrasi Tween 80 yang digunakan pada formula F1, F2, dan F3 masing-masing sebesar 20%, 30%, dan 40%. Sediaan diuji meliputi uji organoleptik, pH, viskositas, transmitansi, serta ukuran droplet pada formula dengan ukuran droplet terkecil. Hasil uji organoleptik menunjukkan bahwa nanoemulsi yang dihasilkan berwarna cokelat lebih jernih, berbau khas, berbentuk cair, dan tidak mengalami pemisahan fase. Pengaruh variasi konsentrasi Tween 80 pada formulasi nanoemulsi ekstrak buah stroberi menunjukkan bahwa semakin tinggi konsentrasi Tween 80, maka semakin tinggi nilai pH, persentase transmitansi, dan viskositas sediaan. Ukuran droplet pada formula F1, F2, dan F3 masing-masing adalah 5722,77 ± 1,53 nm; 3328,5 ± 0,00 nm; dan 25,97 ± 0,90 nm dengan indeks polidispersitas sebesar 16,312 ± 4,32; 9,927 ± 0,00; dan 1,060 ± 0,11. Nanoemulsi ekstrak buah stroberi dengan konsentrasi Tween 80 sebesar 40% mampu menghasilkan ukuran droplet berskala nano, namun dengan distribusi ukuran yang masih cukup lebar. Hasil pengujian aktivitas antioksidan menggunakan metode ABTS menunjukkan bahwa formulasi nanoemulsi ekstrak buah stroberi memiliki nilai IC_{50} di bawah 50, yang menandakan aktivitas antioksidan yang sangat kuat.

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INTRODUCTION

Indonesia is geographically a significant reservoir of biodiversity, including the strawberry (*Fragaria x ananassa* Duch.) from the Rosaceae family. Indonesia is actively engaged in extensive strawberry production. Strawberry represents a viable fruit crop. Strawberries possess bioactive chemicals (flavonoids, phenols, and anthocyanins) and exhibit potential as natural antioxidants [1]. Flavonoids, particularly flavanols, are the predominant constituents, exhibiting both polar and non-polar characteristics [2]. Flavanols are non-polar flavonoids.

Antioxidants mitigate oxidative processes caused by reactive oxygen species and neutralise free radical molecules [3]. Molecules possessing one or more unpaired electrons are referred to as free radicals. These electrons disrupt cellular structure in the human body, potentially leading to diseases such as cataracts, cancer, accelerated ageing, coronary heart disease, and other degenerative conditions [1]. Premature skin ageing is a dermatological condition that can affect both health and appearance, requiring careful treatment and health maintenance.

Research by [4] employed the ABTS method to evaluate the antioxidant activity of a 96% ethanol extract of strawberries, yielding an IC50 of 21.79 ppm, indicating a robust antioxidant activity of the extract. The study by [5] on the manufacture of antioxidant extract lotions utilizing strawberries (Fragaria x ananassa) demonstrated an IC50 of 0.47 ppm at a concentration of 0.5% strawberry fruit extract, indicating significant antioxidant activity and categorising it as a compelling antioxidant. Many pharmaceuticals derived from natural compounds exhibit inadequate water solubility when synthesised directly. Drugs with low water solubility typically exhibit inadequate absorption and, as a result, diminished bioavailability. Consequently, it is formulated as nanoemulsion preparations anticipated to exhibit enhanced solubility and absorption characteristics, capable of protecting and delivering pharmaceuticals to the skin.

Nanoemulsion is a clear or translucent emulsion wherein oil and water are dispersed by a surfactant film with a size of less than 100 nm [6]. Nanoemulsion exhibits superior thermodynamic stability compared to conventional emulsions, penetrates more deeply into the dermis, possesses reduced viscosity, facilitates the delivery of active ingredients by creating a thin film on the skin's surface, and can be engineered to ensure skin comfort without imparting a sticky residue from concentrated oil, which is little [7]. The primary components of nanoemulsion formulations are surfactants and cosurfactants. Surfactants reduce the surface tension between immiscible liquids [8]. Cosurfactants facilitate the dissolution of the solute in the liquid by enhancing the flexibility of the coating around the droplet and diminishing the surface energy. This aids in maintaining the stability of the droplet and enhances the solubility of the liquid [9]. Furthermore, the oil phase is the most critical component in nanoemulsion formulations, since it influences the physical stability of the resulting preparations. Virgin Coconut Oil (VCO) is frequently utilized in the production of nanoemulsions. Moreover, it can inhibit Ostwald ripening and yield droplet sizes under 100 nm in formulations [10]. Approximately 53% lauric acid and 7% caprylic acid, both classified as Medium Chain Fatty Acids (MCFA), constitute the composition of VCO.

This research employed Tween 80 as a surfactant and PEG 400 as a cosurfactant. Tween 80 is a nonionic and nonirritating surfactant utilized in medicinal formulations. The cosurfactant commonly used in pharmaceutical formulations is PEG400, which is non-irritating (Rowe et al., 2009). An experiment was conducted to develop a

strawberry extract nanoemulsion formulation utilizing different amounts of Tween 80. The objective was to ascertain the quantity of Tween 80 that would yield an optimal nanoemulsion formulation of strawberry fruit extract and to evaluate antioxidant activity of the nanoemulsion.

METHODS

Tools and Materials

Tools

Maceration vessel, Rion viscometer VT 04, pH meter, particle size analyzer (Horiba Scientific, SZ-100), rotary evaporator (IKA), hotplate and magnetic stirrer, UV-Vis spectrophotometer (Shimadzu UV mini-1280), sonication (Branson Ultrasonic 5800), water bath (Memmert), digital scale, blender (Philips), 60-mesh sieve, glassware.

Materials

Strawberries (*Fragaria x ananassa* Duch.) sourced from the Tawangmangu plantation, Tween 80 (PT. Brataco Chemika; Surakarta, Indonesia), PEG 400 (PT. Brataco Chemika; Surakarta, Indonesia), VCO (Agung Jaya Surakarta, Indonesia), ethanol 96%(Agung Jaya; Surakarta, Indonesia), and distilled water (Agung Jaya Surakarta, Indonesia).

Research Procedure

Preparation of Strawberry Simplicia

Strawberry fruit approximately two months post-bloom, in fresh condition and exhibiting a red hue, is harvested, sorted, thoroughly washed with running water, and chopped with the skin intact. It is then sun-dried for approximately three days. The samples are subsequently blended, crushed, and sifted through a 60 mesh sieve to yield simplicia powder.

Preparation of Strawberry Fruit Extract

The maceration is conducted at a ratio of 1:10. Two hundred grammes of strawberry simplicia powder were utilized. Place it in the maceration vessel, immersed in 1500 ml of 96% ethanol extract. The maceration vessel was kept in a sealed chamber shielded from sunlight for four days, with regular agitation performed. The sample was filtered using a flannel cloth, yielding one filtrate and one residue. The second filtrate was macerated with dregs that had been treated with 500 ml of 96% ethanol and allowed to stand for one day. The first and second filtrates were amalgamated, the sample was vaporised, and concentrated via a rotary evaporator at 50°C, subsequently thickened using a waterbath to yield strawberry tofu extract. The extract is preserved in a securely sealed container.

Nanoemulsion Formula of Strawberry Fruit Extract

Table 1. Nanoemulsion Formula of Strawberry Fruit Extract (Fragaria x ananassa Duch.)

Material	F1	F2	F3
Strawberry Fruit Extract	0,5 %	0,5 %	0,5 %
VCO	2 %	2 %	2 %
Tween 80	20 %	30 %	40 %
PEG 400	10 %	10 %	10 %
Aquadest ad	100 ml	100 ml	100 ml

The preparation of strawberry fruit extract nanoemulsion commences with the precise measurement of all ingredients as specified in Table 1. Strawberry fruit extract, Tween 80, PEG 400, and VCO are combined in a beaker and agitated at 1000 rpm for 10 minutes utilizing a magnetic stirrer. After 10 minutes, distilled water was incrementally introduced until the total volume of the preparation reached 100 ml, and the stirring speed was elevated to 1250 rpm for an additional 10 minutes. The combined materials are thereafter sonicated for 40 minutes with intermittent stirring [10].

Evaluation of Strawberry Fruit Extract Nanoemulsion

Organoleptic Assessment Evaluations were conducted by examining the colour, odour, morphology, and phase separation of the nanoemulsion formulation.

pH Assessment

The instrument employed for this assessment is a pH meter. Calibration measurements are conducted using pH 4 and pH 7 standards. The pH reading on the meter aligns with the standard pH value of the buffer and stability, therefore indicating that calibration is complete. Submerge the electrode in a solution. The pH value seen on the screen is documented. Measurements were conducted at ambient temperature.

Viscosity Examination

This examination use the Rion VT 04F viscometer. The nanoemulsion was transferred into a 150 mL beaker and insert spindle number 2 until the spindle is fully immersed in the nanoemulsion. Activate the viscometer and monitor rotor spindle number 2 on the viscosity scale until it stabilises.

Transmittance test

The transmittance percentage was measured to assess the transparency of the nanoemulsion using a UV-Vis spectrophotometer at a wavelength of 650 nm, with distilled water serving as the blank.

Droplet Size Assessment

The droplet size was evaluated via a Particle Size Analyser (PSA) employing Dynamic Light Scattering (DLS) methodology. The necessary sample volume is 1 ml, which is to be placed in the cuvette. Insert the cuvette into the holder for droplet determination analysis.

Evaluation of Antioxidant Strawberry Fruit Extract and Its Nanoemulsion with ABTS Method

The antioxidant activity was evaluated by making stock solutions of quercetin and ABTS. The ABTS aqueous solution reacts with the $K_2S_2O_8$ aqueous solution to produce an ABTS radical cation solution ($ABTS^+$). The solution was allowed to stand at room temperature in a dark environment for 12-16 hours until a dark blue $ABTS^+$ solution was formed. The solution is subsequently analysed for absorption to ascertain the maximum wavelength and operational duration. The ABTS stock solution is assessed for absorbance at the peak wavelength. The quercetin solution and the sample solution were incorporated into the ABTS solution, and the volume was adjusted with distilled water to achieve varying concentrations of the solution. The solution was homogenized and allowed to react for the designated duration, after which the absorbance was measured at the maximum wavelength.

Data Analysis

The analysis of data utilizing SPSS 25.0 software (One-way ANOVA) seeks to ascertain the presence of significant variations in the outcomes of viscosity tests, pH tests, droplet size tests, and transmittance tests.

RESULT AND DISCUSSION (Calibri, 12 pt)

Fresh red strawberries from the Tawangmangu plantation undergo wet sorting undesirable foreign matter or dirt. The fruit is rinsed with running water to remove debris, then cut with the skin intact to expedite the sun-drying process. The samples were pulverised with a blender to reduce the size of the simplicia particles and subsequently sifted through a 60 mesh sieve to yield fine simplicia powder.

Strawberry fruit extract was procured by a maceration extraction procedure for a duration of 4 days, followed by a remaceration period of 1 day. The choice of this maceration technique is straightforward, as the flavonoid components in strawberries are not tolerant to temperatures over 50°C. Furthermore, this research

involved remaceration to extract the residual component content remaining from the initial maceration. The solvent employed is 96% ethanol; as noted by [11], ethanol is a universal solvent due to its ability to dissolve both nonpolar and polar molecules, exhibiting neutral characteristics. 96% ethanol was selected because of its selectivity, effective absorption, non-toxicity, and high solubility. Flavonoid are non-polar compound, making 96% ethanol more suited for their extraction than 70% ethanol due to their higher non-polarity. Evaporation utilizing a rotary evaporator and water bath at a temperature of 50 degrees Celsius to prevent degradation of the flavonoid compound as the active ingredient. Table 2 displays the yield of strawberry fruit extract.

Table 2. Yield results of thick strawberry extract

Simplicia powder weight (g)	Extract results (g)	Yield (%)
200	78,21	39,105

The organoleptic extract yields a thick substance characterised by a dark brown hue and a pronounced strawberry fragrance. This study's findings differ from those of [12], since the yield of strawberry fruit extract obtained was 4.717% using 70% solvent. This results from multiple variables, including solvent selection. This investigation utilized 96% ethanol. The lower boiling point of ethanol compared to water results in higher ethanol concentration yielding a superior extract relative to ethanol-free solvents.

Preparation of a nanoemulsion of strawberry fruit extract utilizing a hotplate, magnetic stirrer, and sonicator. The sonication technique can reduce size of the preparation by using ultrasonic waves. The components utilized comprise Tween 80, Virgin Coconut Oil (VCO), PEG 400, and strawberry fruit extract. A study demonstrating that the use of Tween 80 as a surfactant, PEG 400 as a cosurfactant, and VCO as the oil phase resulted in the formation of nanoemulsions with droplet sizes less than 100 nanometres, exhibiting commendable stability in pomegranate seed oil nanoemulsions. Virgin Coconut Oil (VCO) is a form of long-chain triglycerides (LCT) oil capable of generating small nanoemulsion sizes [10]. Moreover, it can inhibit Ostwald ripening. The application of VCO in topical formulations results in excellent spreading and emollient characteristics.

Surfactants operate by reducing the surface tension in nanoemulsions containing both lipophilic and hydrophilic groups. Tween 80 was selected as a surfactant due to its nonionic and nonirritating properties, commonly utilized in pharmaceutical formulations. Tween 80 exhibits stability in varying pH conditions and possesses an HLB value of 15 for M/A emulsion formulations. Cosurfactants assist surfactants in diminishing the surface tension of water and oil, enhancing drug solubility, and improving drug absorption[13]. PEG 400 is a cosurfactant utilized in nanoemulsion formulations that enhances the emulsification process and augments the flexibility of the coating around the droplet region, hence facilitating the solubility of solutes in the dispersion medium [14]. Figure 1 shows nanoemulsion of strawberry fruit extract.



Figure 1. Preparation of Nanoemulsion from Strawberry Fruit Extract

An effective and stable nanoemulsion formulation exhibits a transparent hue, a distinctive aroma, a liquid consistency, absence of phase separation, a pH within the skin's range of 4.5 to 6.5, a viscosity of 200 cps or 20 dpas, and a percent transmittance approaching 100%. Figure 1 illustrates the outcomes of producing strawberry extract nanoemulsion.The droplet size ranges from 20 to 200 nm, and the zeta potential values are either above +30 mV or below -30 mV. Consequently, physical quality assessments were conducted, encompassing organoleptic evaluation, pH measurement, viscosity analysis, percentage transmittance, droplet size determination, and zeta potential assessment. The outcomes of the nanoemulsion experiment are displayed in Table 3.

Table 3. Outcomes of nanoemulsion derived from strawberry fruit extract

Preparation	Organoleptic	pH	Viscosity (dPas)	Transmittance (%)
F1	Clear brown, characteristic odour, liquid, non-separating	4,07 ± 0.06	5,00 ± 0,00	96,77 ± 0,02
F2	Clear brown, characteristic odour, liquid, non-separating	4,53 ± 0,06	7,00 ± 0,50	97,26 ± 0,02
F3	Clear brown, characteristic odour, liquid, non-separating	5,57 ± 0,06	8,33 ± 0,29	99,27 ± 0,02

F1: Preparation of a nanoemulsion with a Tween 80 concentration of 20%.

F2: Preparation of a nanoemulsion with a Tween 80 concentration of 30%.

F3: Preparation of a nanoemulsion with a Tween 80 concentration of 40%.

Results of Organoleptic Testing

This test is conducted by examining the colour, odour, morphology, and phase separation that has occurred. An effective nanoemulsion is transparent, possesses a distinct aroma, exists in liquid form, and remains homogeneous. Table 3 demonstrates that the three formulations exhibit no differences and yield effective nanoemulsions. The organoleptic test results revealed clear brown nanoemulsion preparations, with a distinct odour, liquid form, and no separation. The variation in tween 80 concentration had no impact, as there was no alteration between the formulations. The brown hue and distinctive aroma are derived from the utilized extract, specifically strawberry fruit extract.

pH Test Outcomes

The pH test is conducted to ascertain the safety of the nanoemulsion formulation for dermal application. A pH range of 4 to 6 is optimal for skin health[15]. Topical formulations may cause discomfort if they are excessively acidic while the skin scales exhibit high alkalinity. Table 3 presents pH test results. Formula 1 has a pH value that falls below

the skin's pH range of 4.5 to 6.5, potentially causing in skin irritation. The pH values of formulas 2 and 3 align with the typical range of skin pH levels. The One-way ANOVA test yielded a p-value of 0.000 (<0.05), indicating a significant difference among the three formulations. The variation in Tween 80 concentration influenced the formulation of the strawberry fruit extract nanoemulsion. The pH of the strawberry fruit extract nanoemulsion increase with increasing of tween 80 concentration, indicating that Tween 80 elevates the pH. Tween 80 possesses a pH value of 6-8, which can render nanoemulsions slightly acidic due to the acidic nature of VCO and PEG 400[16]

Results of Viscosity Testing

The objective of this test is to ascertain the viscosity of the nanoemulsion formulation. Viscosity is another crucial consideration for the stability of nanoemulsions. The viscosity of nanoemulsion is a function of water, surfactant and co-surfactant concentration, and oil concentration[17]. The preparation's thickness is indicated by a high viscosity value. The anticipated viscosity is under 200 cps or 20 dPas [18]. The viscosity of nanoemulsion formulations is affected by various parameters, including stirring, particle size, and dispersed phase ratio. The viscosity test results are presented in Table 3. The three formulations exhibit anticipated viscosities below 20 dPas, with each showing a distinct viscosity. An elevated concentration of tween 80 reduced the globule diameter, leading to higher viscosity and greater in the surface area and viscosity of the nanoemulsion. The one-way ANOVA result is 0.000 (<0.05), indicating a significant difference among the three formulations, suggesting that Tween 80 influences the increase in viscosity of the preparation.

Transmittance Test Outcomes

The transmittance test quantifies and assesses the transparency of the nanoemulsion formulation. A high-quality nanoemulsion is approximated to consist of about 100% water, with droplets anticipated to attain nanometer-scale dimensions[19]. The outcomes of the % transmittance test are presented in Table 3. The outcomes of the three calculations exhibit varying % transmittance values, yet are near 100%. A higher concentration of Tween 80 results in an increased percent transmittance value of the nanoemulsion preparation and a reduced droplet size of the nanoemulsion. The outcomes of statistical analyses using SPSS one-way ANOVA indicated a significant difference among the three formulas, with a p-value of 0.000 (<0.05). This phenomenon may arise from the interplay between oil and surfactant composition, wherein strawberry fruit extract exhibits solubility in Virgin Coconut Oil and Tween 80, hence influencing the production of nanoemulsion sizes and leading to an enhancement in the transmittance percentage value[20]. Tween 80 enhances the clarity of nanoemulsion formulations and increase the transmittance percentage.

Results of Droplet Size Assessment

This test seeks to ascertain the droplet size in the nanoemulsion formulation. Sonicators utilizing ultrasonic waves facilitate the preparation of nano-sized samples, while particle-laden liquids subjected to ultrasonic energy can fragment larger particles into smaller ones, yielding nano-sized particles. Nanoemulsion possesses a particle size range of 20 to 200 nm [21]. The test outcomes are presented in Table 4.

Table 4. Outcomes of droplet size assessments of strawberry fruit extract mikroemulsion and nanoemulsion.

Preparation	Average *Droplet* size (nm)	PI
F1	5722,77 ± 1532,52	16,312 ± 4,32
F2	3329,50 ± 0,00	9,927 ± 0,00

F3	25,97 ± 0,90	1,060 ± 0,11

F1 and F2 : mikroemulsion

F3: nanoemulsion

Preparation 1 and 2 exceed the specified particle size criteria of 20 - 200 nm, so it was categorized as a microemulsion. The results of formula 3 conform to the nanoemulsion size criterion. The results of the one-way ANOVA statistical test yielded a p-value of 0.001 (<0.05), indicating a significant difference among the three formulas. This suggests that tween 80 influences the reduction of droplet size in the nanoemulsion preparation of strawberry fruit extract, as evidenced by the observation that higher concentrations of tween 80 correspond to smaller droplet sizes in the nanoemulsion of strawberry extract. An increased concentration of tween 80 correlates with larger particle sizes, whereas a higher concentration of tween 80 results in smaller particle sizes[22]. The increased concentration of tween 80 during dose preparation resulted in a clearer nanoemulsion compared to formulations 1 and 2. The increase in optical clarity observed in formulation F3, characterized by a transmittance value approaching 100%, further supports the formation of nano-sized droplets. High transmittance is commonly associated with droplet sizes smaller than the wavelength of visible light, leading to reduced light scattering and enhanced transparency. However, optical clarity alone does not guarantee long-term physical stability and should be interpreted in conjunction with droplet size distribution parameters. By reducing the size of drug with low solubility and bioavailability to the nanoscale, we can create stable formulations that have increased bioavailability[23].

The droplet size distribution is illustrated by the polydispersity index. A polydispersity index ranging from 0.01 to 0.5 signifies a monodisperse system, while values over 0.5 suggest polydispersity[15]. The monodispersity polydispersity index indicates a favourable level of particle uniformity, suggesting greater stability compared to polydispersity [24]. The polydispersity index (PI) values for F1, F2, and F3 were 16.312 ± 4.32, 9.927 ± 0.00, and 1.060 ± 0.11, respectively, all exceeding 0.7. The polydispersity of the strawberry fruit extract nanoemulsion indicates a non-uniform droplet size and a tendency towards instability, suggesting that Tween 80 influences the reduction of the polydispersity index (PI) value. The PI readings over 0.7 indicate instability, suggesting that the physical stability testing of the preparation may reveal instability. The extremely high PDI values observed in F1 and F2 suggest a highly heterogeneous system, rendering Dynamic Light Scattering (DLS) measurements unreliable for these formulations. These data indicate a highly polydisperse system and unreliable DLS data. F3 meets the nano-size criteria, but does not meet the optimal monodispersity and stability criteria.

Outcomes of Antioxidant Assessment of Strawberry Fruit Extract and Nanoemulsion Derived from Strawberry Fruit Extract Employing the ABTS Method

The antioxidant activity was assessed via UV-Visible spectrophotometry, commencing with the determination of the operating time (OT). The principle of operating time pertains to the stability of the resultant compound, considering that absorption commences at the onset of the reaction and continues until stable absorption is attained. The results were consistent at 6, 7, and 8 minutes, with an absorbance of 0.2589. A Research conducted by [6], the ABTS solution had a maximum wavelength (λ) of 740.2 nm with an absorbance of 0.709. The measurements of the ABTS solution produced a λmax of 743.2 nm and an absorbance of 0.2803. This test is conducted using the ABTS method, which relies on the capacity to decrease or absorb ABTS free radicals. This capability is indicated by a reduction in the

blue ABTS solution added to the sample which is quantified using the inhibitory concentration (IC_{50}) measure. An increase in ABTS radical scavenging activity in a sample correlates with a decrease in the IC_{50} value [25,26].

Table 5. Outcomes of the antioxidant assessment of strawberry fruit extract and its nanoemulsion.

Information	Average IC50 value (ppm)
Quercetin	13,77 ± 0,03
Strawberry Fruit Extract	45,83 ± 0,15
Formula 1	39,58 ± 0,05
Formula 2	36,55 ± 0,13
Formula 3	33,72 ± 0,10

The test outcomes are presented in Table 5. The results yielded an IC_{50} value of <50 ppm, indicating that both the strawberry fruit extract and its nanoemulsion possess flavonoid constituents with potent antioxidant activity. The quercetin comparator, which is classified as a potent antioxidant, measures 13.77 ± 0.03 ppm. The results in table 5 demonstrate that a reduction in particle size correlates with an increase in antioxidant activity, as evidenced by a lower IC_{50} value.

Limitation

A limitation of this research is the incomplete characterisation of the strawberry extract nanoemulsion. Consequently, additional research is required to comprehensively characterise the resultant nanoemulsion. Moreover, additional research is needed to assess the stability of the preparation, considering the current physical properties.

CONCLUSION

The strawberry fruit extract formulation containing 40% Tween 80 successfully achieved nano-sized droplets and exhibited improved physicochemical characteristics compared to formulations with lower surfactant concentrations. This formulation produced a clear, homogeneous liquid appearance with no visible phase separation, a skin-compatible pH, high transmittance, and a mean droplet size of approximately 26 nm. However, the relatively high polydispersity index indicates a broad droplet size distribution, suggesting that further formulation optimization is required to enhance uniformity and physical stability.

The antioxidant activity evaluated using the ABTS assay demonstrated strong radical scavenging potential of the strawberry fruit extract formulation, with an IC_{50} value below 50 µg/mL. Overall, increasing the concentration of Tween 80 played a critical role in achieving nano-sized droplets in the strawberry fruit extract system, although additional refinement of formulation parameters is necessary to obtain a more homogeneous and stable nanoemulsion suitable for topical application.

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AUTHOR CONTRIBUTION STATEMENT

DS: Conceptualization, Methodology, Supervision; writing-reviewing and editing **S**: Data curation,Supervision; **LM**: Writing—Original draft preparation, Visualization.

CONFLICT OF INTEREST DECLARATION

All authors declare no conflict of interest in this manuscript.

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