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Research Article

Phyto-Nanoemulsion Containing Tea Leaves Extract Incorporated into A Hydrogel-Based Peel-Off Mask Formulation: Development, Antioxidant Assay, and Ex-Vivo Transport Study

Dinar S.C. Wahyuni[1], Ratih P. Febrinasari[2], Sakinah[3], Ana Mardiyah[3], Syaiful Choiri[4*]

[1]*Pharmaceutical Biology, Department of Pharmacy, Universitas Sebelas Maret, Ir. Sutami 36A, Surakarta, Indonesia.*
[2]*Department of Pharmacology, Faculty of Medicine, Universitas Sebelas Maret, Ir. Sutami 36A, Surakarta, Indonesia.*
[3]*Department of Pharmacy, Universitas Sebelas Maret, Ir. Sutami 36A, Surakarta, Indonesia.*
[4]*Pharmaceutical Technology and Drug Delivery, Department of Pharmacy, Universitas Sebelas Maret. Ir. Sutami 36A, Surakarta, Indonesia.*

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Abstract

Background: Tea leaves contain remarkable antioxidant compounds suitable for cosmetics formulation development targeting anti-aging purposes. However, due to its hydrophilicity, it faced the main hurdle, namely the permeation of the potential antioxidant compounds. Nanoemulsion formulation offers the permeation improvement of water-soluble compounds Therefore, this work purposed to formulate and develop the nanoemulsion formulation containing tea leaf extract incorporated into a peel-off mask formulation and an *ex-vivo* transport study.

Methods: The nanoemulsion formulation was developed according to self-nano emulsification. The optimization process was performed using a design of experiment to define optimized formulation based on quality target product profiles. Dried green tea leaf extract (DGTE) was obtained by lyophilizing the boiled water-based extract. Antioxidant and phenolic content assays were also evaluated. The optimized formulation was incorporated into a hydrogel-based peel-off mask formulation. Permeated polyphenol through a rat skin membrane was carried out along with deposited polyphenols in the skin membrane.

Results: The results indicated that DGTE had powerful antioxidants with IC_{50} less than 15 mg/mL. The optimized nanoemulsion comprised 21.62% virgin coconut oil, 48.38% Tween 80, and 30% PEG 400, producing a droplet size of less than 50 nm. The peel-off formulation was successfully formulated along with PVA 10-11% and HPMC 0.25-1% with a drying time of around 30 min. The nanoemulgel peel-off mask formulation played significant roles in enhancing the permeation and deposition in the percutaneous transport using rat skin membrane for roughly 200% and 50%, respectively.

Conclusion: The nanoemulsion incorporated into a hydrogel-based peel-off mask enhanced the permeation of DGTE polyphenol compounds.

Introduction

Skin aging is one of the most problematic issues that requires particular consideration.[1] Several factors have been identified, for example, ultraviolet (UV) from sunlight, high temperature, lifestyle, pollution, and genetics, which induce skin aging.[2] Particularly in tropical urban cities, high intensity of sunlight will exacerbate this issue.[3,4] Furthermore, the UV generates free radicals and promotes reactive oxygen species (ROS) production. Our body can neutralize ROS through endogen antioxidants. However, an excessive amount of ROS increases the oxidative stress level; hence, it degrades collagen and reduces its production. This phenomenon is the leading cause of skin aging.[1,2] Therefore, the skin requires potent antioxidant compounds that could break up that phenomenon.

Antioxidant compounds, for instance, polyphenol bioactive compounds in tea leaves, have potent activity for radical scavenging.[5,6] They can inhibit ROS formation and maintain collagen and its production. Not only polyphenols but also flavonoids are considered to have the powerful capability for avoiding the formation of ROS.[7] Tea leaves contain polyphenols and catechin derivates, namely catechin, epigallocatechin gallate, gallocatechin, and epicatechin, which play a fundamental role in antioxidant

*Corresponding Author: Syaiful Choiri, E-mail: s.choiri@mipa.uns.ac.id

properties. In addition, they also contain flavonoid compounds such as quercetin glycoside and flavanols derivates.[6] The remarkable antioxidant is proven *in-vitro*, but it generally lacks activity when applied percutaneously.[8] Due to their size and hydrophilicity, they have challenges permeating the skin and reaching the collagen tissue. A sizeable molecular size is not easy to transport; meanwhile, the abundance of hydroxyl groups promotes a hurdle, i.e., pass through the skin membrane, particularly the stratum corneum, the hydrophobic first barrier in percutaneous transport.[9,10] As a result, the remarkable antioxidant compounds probably lack their potent activity in the targeted site. Hence, the appropriate formulation is required to enhance the permeation properties.[11]

Lipid-based formulation (LBF) was preferable for enhancing permeation, particularly percutaneous delivery. It helps the hydrophilic moieties to pass through the biological membrane and hinders the main obstacles in the transport system.[12,13] Among LBFs, nanoemulsion has feasibility and reliability for enhancing the permeation properties due to scale-up ability and manufacturability. Self and spontaneous emulsification processes are the advantages feature of nanoemulsion formulation.[14,15] It can produce nanodroplets by a self-emulsification mechanism; thus, it requires low energy to obtain nano-sized oily droplets.[16] The polyphenol compounds can be incorporated into the oily droplet. It provides better protection against environmental or oxidative agents.[17,18] Self-nanoemulsion comprises oil, surfactant, and co-surfactant in an isotropic form that can produce nanodroplets spontaneously during dilution with a medium. Each component has responsibilities to achieve an optimized formulation. Oil is a droplet core; the antioxidant compounds will be entrapped in the core. Surfactant governs the droplet size; co-surfactant helps the surfactant to stabilize the droplet and enhance the solubility in the oil phase.[17,19] Several research reported that nanoemulsion could increase antioxidant activity due to increasing the antioxidant compound in the target site.

An excellent delivery system is meaningless without a suitable device. Therefore, the potent antioxidant compounds incorporated into a nanoemulsion system should be in the appropriate formulation. In addition, the transport mechanism, particularly percutaneous, requires more contact time.[20] Moreover, the face is the weakest part of our body and has a high prevalence of skin aging due to less protection.[2] Peel-off mask formulation was selected in this study as a device model for delivering the antioxidant compounds in tea leaves extract. It is also beneficial due to prolonged use, and the formulation can increase skin moisture.[21] However, the polymer system affects the formulation performance. Polyvinyl alcohol (PVA) plays a fundamental role in drying properties; however, a plasticizer or elastic polymer should be added to enhance the formulation's elasticity.[20] This formulation's main quality target product profiles are the peel feature and the transport of antioxidant compounds. To the best of our knowledge study, there was no report regarding the formulation of nanoemulsion containing tea leave extract incorporated into a peel-off mask formulation. Hence, this work was purposed to formulate and develop the nanoemulsion formulation of tea leaf extract incorporated into peel-off mask formulation and *ex-vivo* transport study.

Methods

Material

Green tea (*Camellia sinensis* L.) leaf was obtained from Kemuning (Karanganyar, Indonesia) and identified in the Department of Biology, Universitas Sebelas Maret. Tween 80 and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Aldrich (St. Louis, MO). Polyvinyl alcohol (PVA) and polyethylene glycol 400 were purchased from Merck (Darmstadt, Germany). Propylene glycol (Dow Chemical Pacific, Singapore) and phenoxy ethanol (Galaxy Surfactant, India) were purchased from a local supplier. HPMC (4000 cps; 1% w/v) was obtained from Colorcon (West Point, PA). All other reagents were in analytical grade.

Dried leaves green tea extract preparation

The green tea leaves were dried in an oven at 60 °C until a predetermined loss on the drying level was achieved. The dried green tea was passed through a 60-mesh sieve. The extraction was carried out by brewing with boiled water. A 100 g of dried green tea was added to 1000 mL boiled water (95 °C) for 10 min. After that, it was filtered to separate the extract. Furthermore, it was lyophilized at -60 °C for two days until the dried green tea extract (DGTE) was obtained. The extract was stored in a desiccator until further evaluation.

Antioxidant assay of DGTE

In-vitro antioxidant activity was carried out to evaluate the antioxidant properties of DGTE using a DPPH assay. A part of 0.4 mM DPPH was added to a part of the sample solution and three parts of methanol. The mixture was incubated for 30 min, followed by scanning at 517 nm. The control solution was performed without containing a sample/standard concentration. Trolox was applied as the standard for antioxidant evaluation. The percentage between the gap of control and sample response and control response was calculated as an inhibition. The antioxidant activity was interpreted as the Trolox equivalent. The modeling-based approach using non-linear regression analysis was applied to estimate the concentration along with 50% inhibition (IC_{50}) using GraphPad Prism 8.0 software (San Diego, CA).

Total flavonoid content assay of DGTE

The total flavonoid content of DGTE was also evaluated according to Patle *et al.*[22] with modification.A 2.0 mL sample was mixed with 0.1 mL $AlCl_3$ and 0.1 mL sodium acetate 1 M, followed by dilution with 2.8 mL of water. The mixture was incubated in the ambient condition (25±1 °C ; RH 50±5%) for 30 min. The absorbance of the sample was

recorded at 439 nm using a Genesys-10 spectrophotometer. Quercetin was applied to quantify the total flavonoid content in the DGTE as a standard.

Preparation of nanoemulsion

Self-assembly nanoemulsion was applied in this work and formed by spontaneous formation during dilution. Nanoemulsion preparation consisted of two steps: screening of selected components and formulation development. The screening process was based on the DTGE solubility, while the formulation development was carried out based on the design of experiment model.[14,23]

Self-nanoemulsion's component screening

The screening process was performed by solubility study of DGTE in each component. Oleic acid and virgin coconut oil were selected as the oil phase in the nanoemulsion screening. Meanwhile, Transcutol CG, Propylene glycol, and PEG 400 were selected as co-surfactants. Tween 80 was used as a surfactant due to miscibility among all components. An excess amount of DTGE (200 mg) was added into two mL of each component until undissolved extract was observed, followed by stirring for two days. Thereafter, a total phenolic content was applied to quantify the DGTE solubility on each component using a Folin-Chiocalteu colorimetric assay. This method is based on the previously reported study with minor modifications.[24] Each component, along with saturated DGTE, was withdrawn accurately and dissolved in ethanol. A 300 µL sample and 1.5 mL 10% Folin reagent were mixed homogeneously for 5 min. A 1.2 mL of 7.5% sodium carbonate was added and incubated for 30 min. The sample was scanned using a Thermo Genesis S10 spectrophotometer at 730 nm. The gallic acid (10-150 µg/mL) was utilized as a standard for calculating the solubility of DGTE based on the gallic acid equivalent. Furthermore, the selected components also evaluated the miscibility among them using a matrix system to obtain miscible components.[15]

Self-nanoemulsion formulation development

The highest solubility and miscible components were further developed using simplex lattice design utilizing State Ease 360 software (StatEase; Minneapolis, MN) to determine the optimized formulation. Oil, surfactant, and co-surfactant were 30-60, 30-60, and 10-40%, respectively, and were used to design and build according to the minimum design point based on simplex lattice design. The optimized formulation was determined by statistical evaluation depending on transmittance value and emulsification time. DGTE loading was also evaluated as the response. An overlay plot between those responses determined the optimized formulation.[23]

Preparation and characterization of DGTE nanoemulsion

An excess amount of DGTE (100 mg/mL) was incorporated into the mixture of selected oil, surfactant, and co-surfactant at a predetermined ratio according to the simplex lattice design. Unloaded DGTE was eliminated by centrifugation, and the supernatant was used as a preconcentrated formulation.

Nanoemulsion characterization was carried out by transmittance and emulsification time for formulation development. The nanoemulsion was formed when 1 mL of preconcentrate nanoemulsion was diluted in 100 mL water at 100 rpm. The time required for complete dispersing was noted as emulsification time. Furthermore, the nanoemulsion transmittance at 650 nm was also characterized. They were applied to depict the spontaneous emulsification and nanodroplet formation behaviors, respectively.

Determination and characterization of the optimized formulation

Multiple linear regression analysis was applied to assess nanoemulsion components' effect on the quality critical attributes. The model was analyzed using an analysis of variance along with a 95% confidence level. The coefficient determination (R^2), adjusted R^2, and predicted R^2 were applied for model fitting parameters and validation.[25] The optimized formulation was determined by overlaying two response contour plots according to the quality target product profiles: emulsification time and transmittance of < 30 s and $>80\%$, respectively.

The optimized formulation was evaluated according to the optimization parameters (transmittance and emulsification time) for model verification. In addition, particle size, distribution, and zeta potential were characterized using a Nano Zeta Sizer SZ100 (Malvern, UK). The sample was placed into a glass cuvette and scanned at 633 nm along with scattering angle, gate time, and temperature of 173°, 2.56-10.28 ms, and 25 °C, respectively. The zeta potential was also assessed using a similar instrument but utilized a carbon cuvette with an electrophoretic mobility principle.[23]

Peel-off mask formulation

Optimized formulation containing DGTE was incorporated into the hydrogel system. The amount of loaded DGTE was based on the 100 × IC_{50} of DGTE. Therefore, the requirement for the preconcentrated formulation was adjusted to the DGTE in the hydrogel system. The nanoemulsion was obtained when preconcentrated was introduced to the medium.

On the other side, the peel-off mask base was formulated according to the different combinations of polymers, namely PVA (A)-HPMC (B), for instance, 10.5%A-0.5%B, 10.25%A-0.75%B, and 10%A-1%B for F1, F2, and F3, respectively. In addition, several excipients were also added to obtain better physical characteristics of the peel-off mask, for example, propylene glycol 5% as a humectant, phenoxyethanol 1% as a preservative, and water 100%. Furthermore, the peel-off bases were obtained by hydrating polymers in water at 80 °C using an IKA T25 ultraturax homogenizer (Staufen, Germany) and adding the preservative and humectant. Each formulation contained

DGTE preconcentrated nanoemulsion equivalent to 100 × IC_{50}; hence, the amount of water was divided for nanoemulsion and peel-off base preparations. The preparation of nanoemulsion was carried out according to the aforementioned methods. Nanoemulsion formulation was added into a peel-off mask hydrogel-based, then mixed homogeneously (nanoemulgel).

Peel-off mask characterization

Several characterizations of the peel-off mask were evaluated by pH, viscosity, spreadability, and drying time.

pH assay

pH was evaluated using a benchtop Hanna HI5222 pH meter (Smithfield, RI) equipped with a semisolid probe electrode. Calibration was conducted before the measurement process at pH 4.01 and 7.00, respectively. The calibration was completed when the slope and offset were 100±5% and +30 ~ 30, respectively. This evaluation was in triplicates, and the measurement was carried out without any dilution.[26]

Viscosity evaluation

The viscosity was evaluated using a DVNext Rheometer (AMETEK Brookfield; Middleborough, MA) along with 25 rpm (temperature of 25 °C). The sample was placed into a sample holder, and the selected rotor was fitted to the chamber. Formulation viscosity was recorded for 1 min measurement. This evaluation was in triplicates.[26]

Spreadability

Spreadability was measured using modified tools according to the previous method with minor modifications.[27] A 0.5 g formulation was placed on the desk glass and covered by another glass, and a 100 g weight was put on the desk glass for a minute, and the increase of length and width was measured using a calibrated caliper (accuracy 0.1 mm). The area was calculated according to the diameters and noted as spreadability.

Drying time

Drying time was also evaluated according to the alteration of weight loss during drying. A 0.5 g sample was spread in 1 cm^2 on the glass and incubated in an oven at 32°C. The weight was measured every 5 min until the constant weight was achieved. The percentage of weight loss was plotted with time. The intersection of two lines between dramatic loss and steady weight was noted as drying time. In addition, the amount of water retained in the formulation was also carried out.[28]

Ex-vivo dermatokinetics study

Ex-vivo transport study used a Franz diffusion cell and an abdominal rat skin as a membrane. This method was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Universitas Sebelas Maret, along with an ethical clearance No. 111/UN.27.06.11/KEP/EC/2022. This cell comprises two compartments separated by a nearly 750 µm thickness membrane along with 2.14 cm^2 of the contact area. The donor compartment was the nanoemulsion-based and hydrogel-based peel-off mask formulations, respectively. Meanwhile, the acceptor compartment was phosphate buffer saline pH 7.4 (25 mL), and the temperature was kept constant at 37± 1 °C under a stirring rate of 600 rpm. A 1.0 mL sample was withdrawn at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 18, and 24 and replaced by the same volume and temperature. The permeated antioxidant compound was calculated using a Folin-Ciocalteau assay. A 300 µL of the sample was added in 1.5 mL 25% Folin-Ciocalteau reagent. After reacting for 5 min, 1.2 mL of 5% sodium bicarbonate was added and vortexed homogeneously, then incubated for 20 min. A blue-colored sample was scanned at 769 nm.[24] Gallic acid was used as a standard and applied for constructing a validated calibration curve in the range of 5 – 60 µg/ml gallic acid equivalent (GAE); absorbance = 0.0132 × concentration GAE (µg/ml) + 0.0513 (R^2 0.995; and adjusted R^2 0.994). The amount of retained antioxidant compounds in the membrane was also calculated. The extraction was performed using methanol assisted by ultrasonication followed by an appropriate dilution. The assay was also conducted using a Folin-Ciocalteau assay.

Results and Discussion

Green tea leaf extract

The extraction process was carried out to obtain phenolic compounds as powerful antioxidants in tea leaves, followed by lyophilization. The yield was 12.28% (w/w) from dried tea leaves. In addition, the extract contained 0.59% (w/w) quercetin equivalent. In order to evaluate the dried extract, the in-vitro antioxidant profiles were evaluated. It is presented in Figure 1. Quercetin and DGTE had a similar pattern, a sigmoidal shape. The IC_{50} of quercetin and DGTE was 2.78 and 6.58 µg/mL, respectively. Although the DGTE contained less flavonoid than the previously reported study,[29] the antioxidant capacity was two times higher than that of it.[29] The DGTE had powerful antioxidant capacity because it contained phenolic and flavonoid compounds responsible for scavenging free radicals.[5] Although the flavonoid content was relatively low, it proved that the

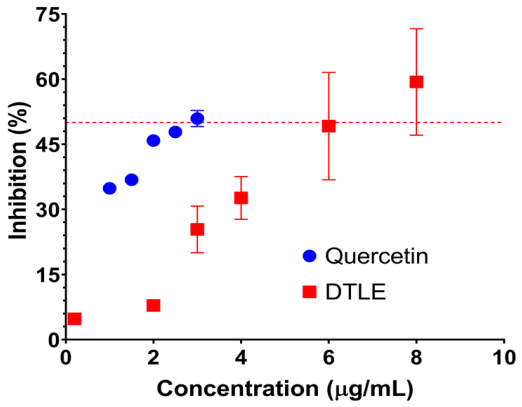

Figure 1. *In-vitro* antioxidant assay of dried tea leaf extract (DGTE) and quercetin using DPPH assay.

polyphenolic compounds contained in the DGTE had a more dominant contribution to the antioxidant activity. Epigallocatechin gallate has been proven to be a powerful antioxidant compound in tea leaves. Therefore, for further evaluation, a polyphenolic assay was applied.

Furthermore, the DGTE loading could be determined by $100 \times IC_{50}$ of the DGTE to obtain an adequate antioxidant level in the peel-off mask formulation; thus, the DGTE loading in the peel-off mask formulation was 0.658% (w/v). However, in this study, nanoformulation was applied to improve the permeation of antioxidant compounds. Hence, the loading should be a particular consideration.

Formulation and Optimisation of Nanoemulsion of DGTE

The selection of nanoemulsion components faces a significant challenge in determining this formulation's performance.[15] Therefore, this study applied a selection of oil, surfactant, and co-surfactant based on the critical requirements of nanoemulsion formulation, namely miscibility and DGTE solubility. The solubility of DGTE in each component was the main feature of obtaining the optimized formulation and a high drug loading.[23] Conversely, the miscibility was evaluated from the formation of the isotropic mixture. This critical parameter contributes to the formation of the self and spontaneous emulsification process during dilution.[16] Tween 80 (HLB 15.4) was only selected as a surfactant due to immiscibility with other surfactants, for instance, Kolliphor EL (HLB 12-14), Cremophor RH40 (HLB 14-16), Labrafil M1944CS (HLB 9), and Labrasol (HLB 12). Due to its miscibility, virgin coconut oil and oleic acid were used as the oil phase component, while Transcutol CG, propylene glycol, and PEG 400 were also selected as co-surfactants. The solubility of DGTE in VCO (29.22 mg/g) was more excellent than that of oleic acid (20.87 mg/g), and thus, VCO (HLB 8) was selected as the oil phase in this formulation. In addition, the solubility of DGTE in PEG 400 (93.14 mg/g) was higher than that of Transcutol CG and propylene glycol (91.22 and 74.96 mg/g, respectively). Moreover, the solubility and miscibility between components were also studied. As a result, VCO, Tween 80, and PEG 400 were chosen as components in the nanoemulsion formulation. A similar result with different drug models was reported that those materials provided nanodroplet size and good physical stabilization.[30,31]

When introduced to the medium, spontaneous and self-nanoemulsion required an isotropic mixture that produces nano-sized droplets.[32] Hence, formulation optimization was performed by simultaneous assessment using a statistical approach, a mixture design model for this study to provide optimized formulation based on the integrated evaluation.[23] The formulation development was determined on the critical quality attributes, for instance, spontaneous formation, nanodroplet formation, and extract loading. Spontaneous emulsification was the primary performance of self-nanoemulsion formulation, and it can be assessed by the time required to emulsify completely and emulsification time (ET).[14] The ET of all formulations ranged from 8.9 – 14.3 s. It proved that the emulsion process happened suddenly and spontaneously in less than 30 seconds. In order to assess the effect of each component, for example, VCO (A), Tween 80 (B), and PEG 400 (C) in the emulsification behavior, a multiple linear regression was applied. The statistical analysis showed that the model was built in a special cubic model. However, the model was insignificant ($p>0.05$; 0.1417), and thus, the model could not be utilized to assess each component's effect on the emulsification time. Conversely, all components had an insignificant effect on the emulsification time ($p>0.05$). The data showed a narrow gap between the lowest and the highest data, while the component changes were more than 20%. Therefore, the model could not be used for predicting the optimized formulation because alteration of the components was meaningless on emulsification time properties.[25]

The clarity of the nanoemulsion indirectly reflected the nanodroplet formation after dilution.[15] Therefore, the transmittance (%T) was applied to assess the nanodroplet formation. The higher the transmittance, the smaller the oil droplet size was.[15,33] The transmittance value was in the range of 7.11 to 95.32%. The multiple linear regression and quadratic models were applied (Eq. 1). The model was significant ($p<0.05$); thus, the factors affected the %T value. The R^2 and predicted R^2 were 0.985 and 0.927, respectively. The statistical parameters indicated the validation of the model; the data depicted the low tendency of misguiding for predicting the response.[25]

$$\%T = 6.72\times A + 31.21\times B + 25.56\times C + 33.76\times A\times B - 39.39\times A\times C - 6.27\times B\times C \quad \text{Eq. (1)}$$

According to the model's coefficient regression, the oil phase was the most outstanding contribution, affecting the reduction of %T (4.7%), followed by Transcutol CG (17.9%) and Tween 80 (21.8%). The effect of oil phase composition changes was 4-5 times greater than co-surfactant and surfactant in reducing the %T. The interaction between oil and surfactant increased the %T significantly ($p<0.05$); meanwhile, the interaction between oil and co-surfactant reduced the %T ($p<0.05$). In addition, there was no significant interaction between surfactant and co-surfactant. It proved that the surfactant played a fundamental role in reducing the size of the nanodroplet. However, the oil increased the droplet size by reducing the %T value.[23] The contour plot of %T (Figure 2a) showed that the Tween 80% at the highest proportion was responsible for increasing the %T value, and the lowest %T value was observed at around the high proportion of the oil phase. The change was observed linearly around the low to the mid proportion of co-surfactant. Previous work reported that the modulation of nanodroplet formation quantitatively was affected by the contribution of surfactant co-stabilized by co-surfactant. However, both components should be in a miscible and isotropic mixture.[15]

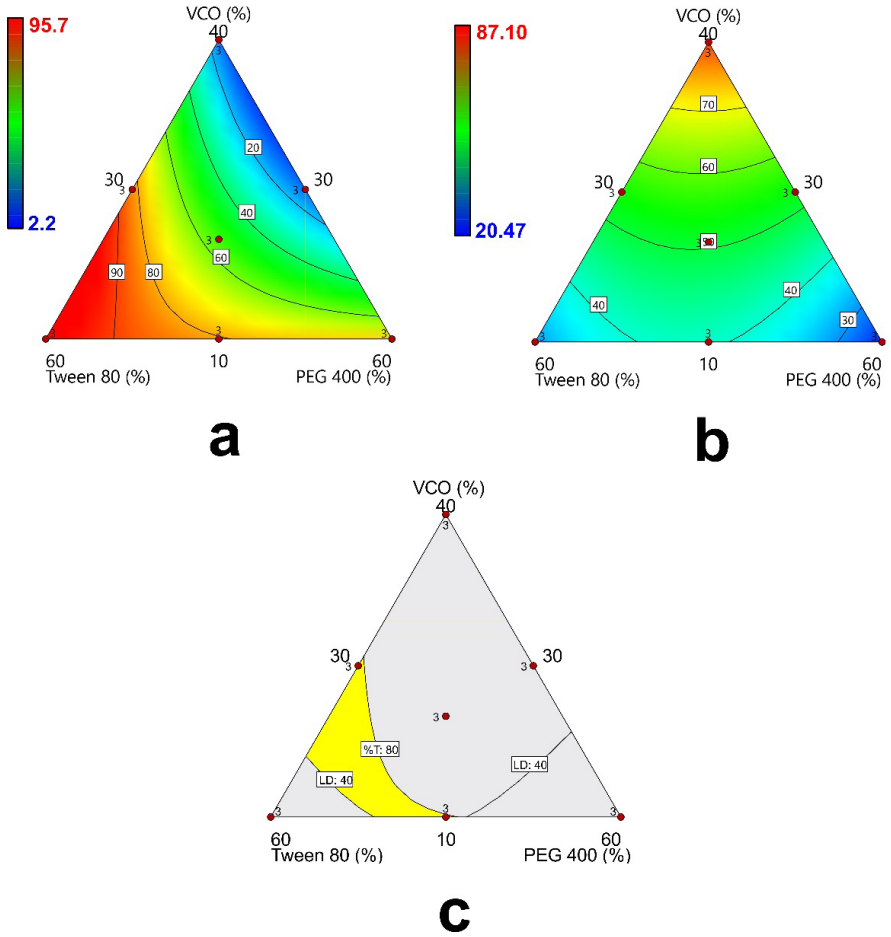

Figure 2. Contour plot of transmittance (a) and extract loading (b) of dried tea leaf extract nanoemulsion and superimposed contour plot for determination of optimized formulation (c).

The extract loading was the main feature of the preconcentrated nanoemulsion system.[34] The higher the drug loading, the better system was due to a low surfactant content. However, the use of surfactant is less toxic due to topical use. In addition, the surfactant promotes the permeation of active compounds due to disturbing the stratum corneum layer.[35] The DGTE loading was 22.94 – 82.82 mg/g. In order to assess the effect of the factors on the DGTE loading, a multiple linear regression model was constructed (Eq. 3). The model was significant ($p<0.05$); therefore, the factors influenced the DGTE loading. Statistical parameters were applied for model validation. The R^2, adjusted R^2, and predicted R^2 were 0.975, 0.928, and 0.872, respectively. The predicted R^2 was generated by the cross-validation technique. Therefore, the gap between adjusted R^2 and predicted R^2 below 0.2 was recommended for the model validation.[25] Oil was the dominant factor affecting the increase of DGTE loading compared to the surfactant and co-surfactant (around 3-4 times). The interaction between oil and surfactant had no significant effect on the DGTE loading and the interaction between oil and co-surfactant ($p>0.05$). On the other hand, the interaction between surfactant and co-surfactant significantly increased the DGTE loading ($p<0.05$). The co-surfactant and surfactant synergistically affected the rise of DGTE loading. The co-surfactant helps the lipophilic drug dissolve in the oil.[16] The previous report showed that the co-surfactant fundamentally enhanced the drug loading to the isotropic mixture. The concentration of co-surfactant raised the pitavastatin loading dramatically in a nanoemulsion system.[15]

$$\text{DGTE loading} = 82.84\times A + 30.97\times B + 22.96\times C - 8.71\times A\times B + 0.66\times A\times C + 54.84\times B\times C \quad \text{Eq. (2)}$$

The contour plot of the DGTE loading (Figure 2b) showed the effect of alteration of DGTE value. The highest DGTE loading was observed on the high proportion of VCO; meanwhile, the surfactant and co-surfactant at the highest proportion had the lowest DGTE loading. The change in DGTE value was affected by reducing the oil phase proportion; meanwhile, the interaction was observed in the middle proportion of surfactant and co-surfactant.

The optimized formulation was determined by overlaying the contour plots of %T and the DGTE. It is presented in Figure 2c. The optimized formulation was controlled by quality target product profiles, namely the %T value, and DGTE loading was not more or less than 80% and 40 mg/g, respectively. The optimized formulation consisted of 21.62% VCO, 48.38% Tween 80, and 30% PEG 400, which had a desirability value of 40%. It was predicted

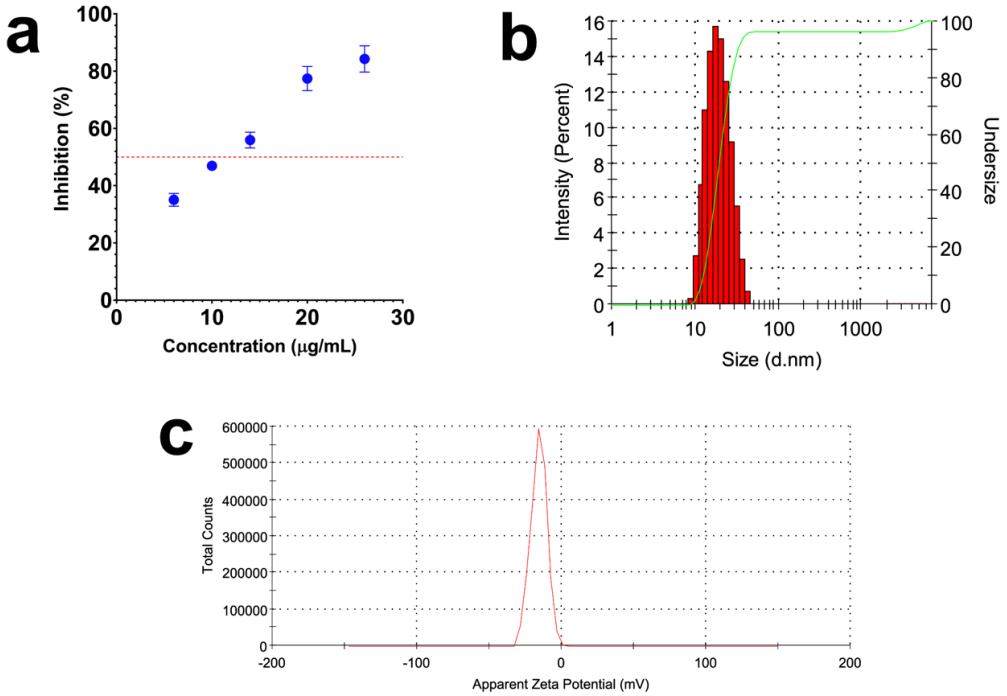

Figure 3. *In-vitro* DPPH antioxidant profiles (a), droplet size distribution (b), and zeta potential distribution (c) of dried tea leaf extract nanoemulsion.

to have the %T value and the DGTE loading of 92.57% and 48.99 mg/g. In order to verify the model, a statistical test was applied and showed no significant differences ($p>0.05$) between predicted and observed (T% 93.61±3.01%, $p = 0.225$ and DGTE loading of 55.07±4.84 mg/g, $p = 0.077$). The optimized formulation was also characterized by antioxidant activity, droplet size, and zeta potential. The antioxidant profile of nanoemulsion is presented in Figure 3a. A distinctive pattern was observed compared to the DGTE. The sigmoidal pattern disappeared, and the linear correlation was observed around 30% to 80% inhibition. The IC_{50} was 11.43 µg/mL; it showed a reduction in the antioxidant activity due to incorporation into oily droplets. The droplet size of the optimized formulation was 19.38± 0.19 nm along with a polydispersity index (PDI) of 0.190± 0.004 (Figure 3b). It indicated that the droplet size was below 100 nm and probably had better permeation properties due to the tiny droplet. The droplet size distribution was narrow due to a PDI of less than one, and a monodisperse system was achieved. The zeta potential of the nanoemulsion droplet (Figure 3c) was -15.69± 0.62 mV), indicating a tendency of high attraction force compared to the repulsion force.[32] However, based on the hydrogel formulation, this formulation was incorporated into the peel-off mask. Therefore, the nearly neutral zeta potential value had a negligible effect on the thermodynamic and kinetics droplet stabilization due to being entrapped into the three-dimensional network of the hydrogel system [36,37]

Formulation of DGTE nanoemulsion-based peel-off mask

The main focus of this peel-off mask formulation was the effect of polymers on their physical characteristics. In this study, physical stability was observed during storage for 28 days (Figure 4). The pH value of this formulation is presented in Figure 4a. A pH was the key feature of the percutaneous formulation. A fair and stable pH value is a benefit due to improving patient adherence. In addition, pH promotes different ionic or anionic forms of active compounds. Hence, it affects the permeation properties of active moiety.[26] The pH value between all formulations was insignificant ($p>0.05$), and all pH values were 5.5 to 7. It was the range of safe and comfortable pH values for the skin. However, the pH was increased during the stability test, and all formulations had a similar pattern.

The viscosity of all formulations was 600 to 1200 dPas, and it affected the physical stability of the peel-off mask formulation. The F1 was the lowest viscosity, while the F2 was the highest. The interaction between polymers influenced this effect. A slight rise was observed in all formulations during the storage, but those were insignificant differences ($p>0.05$). The spread-ability pattern was proportionally inverse to the viscosity model. The higher the viscosity, the lower the spread area was. However, all formulations indicated no significant difference between formulation and during storage ($p>0.05$). Drying time is the main feature of the peel-off mask formulation. PVA addition was intended to reduce the drying time, while HPMC played as an elasticity characteristic. The results revealed that the F1, the highest PVA proportion, had the shortest drying time. Conversely, the highest HPMC value (F3) was the longest drying time. It was observed that the drying time was affected by the contribution of the polymers and their interaction. During the drying process,

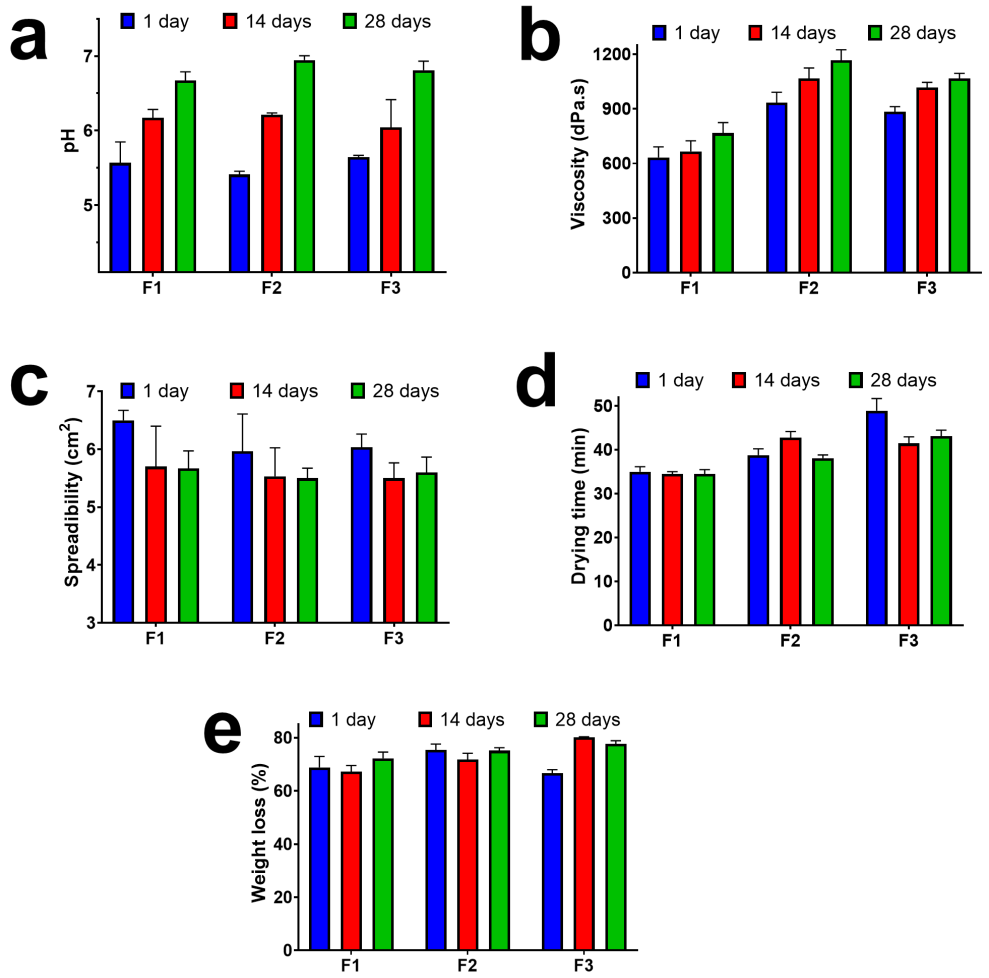

Figure 4. Characterization of peel-off mask formulation: pH (a), viscosity (b), spreadability (c), drying time (d), and weight loss during drying (e). F1 (10.5% polyvinyl alcohol, PVA-0.5% hydroxypropyl methylcellulose, HPMC), F2 (10.25% PVA-0.75% HPMC), and F3 (10% PVA-1% HPMC).

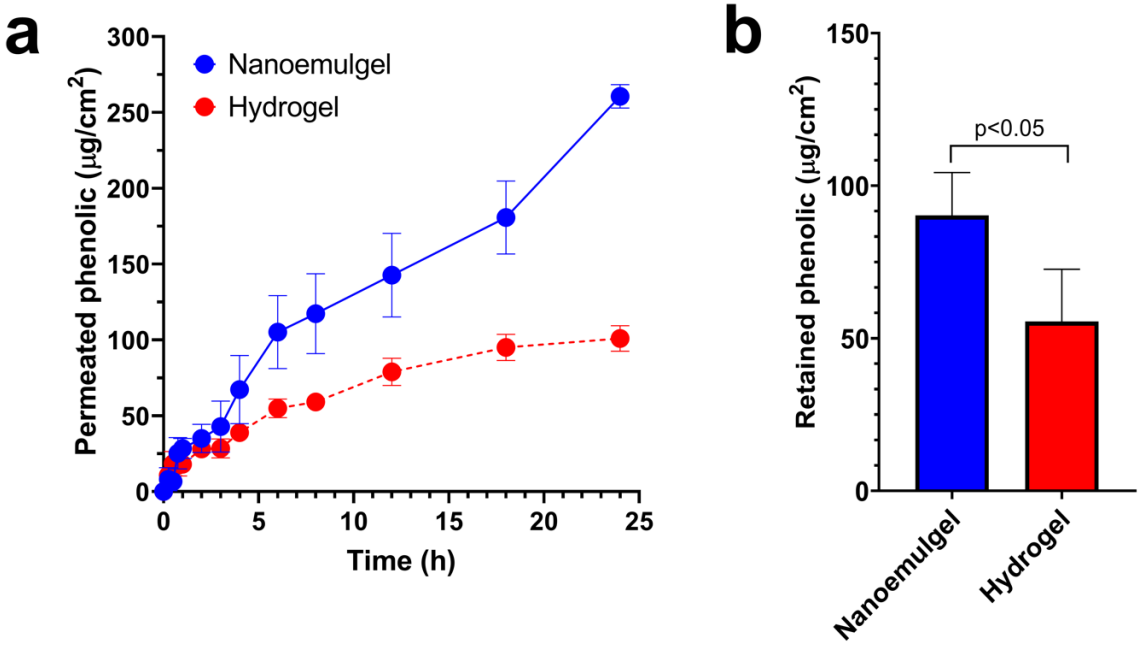

Figure 5. *Ex-vivo* transport study of dried tea leaf extract in the nanoemulgel-based and hydrogel-based formulation in permeated phenolic profiles (a) and retained phenolic in the skin membrane (b).

when the formulation was applied to the skin, the drying phenomenon involved the time and the water loss system. It has a direct correlation to the drying time. The longer the drying time, the lower the weight loss.[20] The data (Figure 4e) proved that both F1 and F2 have no significant weight loss difference; however, it was significant, particularly in the initial formulation of F3. The storage contributed to the stabilization of polymers' 3D network and involved their interaction; therefore, it was considered to play a fundamental role in similar characteristics of drying kinetics properties. However, F1 was the most acceptable formulation due to its high spreadability, lowest drying time, and adequate physical stability. Furthermore, it was used for *ex-vivo* evaluation.

Ex-vivo transport study

In order to evaluate the effect of nanoemulgel-based formulation on the permeation properties of DGTE, an *ex-vivo* transport study using rat skin was carried out. The permeation profile is presented in Figure 5a. The data proved that the nanoemulgel formulation was more remarkable in amount and flux than the hydrogel-based formulation. The hydrogel-based permeation profile only had a two-phase transition during permeation profiles, indicating that there were before and after membrane saturation. However, there were more than two phases observed in the nanoemulgel-based formulation. According to the total amount of permeated, the nanoemulgel formulation improved by 2.5 times higher than the conventional formulation, hydrogel. According to the calculated data, the area under the curve, the nanoemulgel-based, rose the permeation ability by 201%. In addition, the fluxes in the steady state of nanoemulgel-based and hydrogel-based were 8.31 and 3.35 μg/cm^2.h, respectively; hence, it confirmed that the nanoemulsion blatantly enhanced the permeation properties. Moreover, the saturated membrane was achieved at 0.98 and 1.11 h for nanoemulgel and hydrogel, respectively. Therefore, the nanoemulsion formulation influenced the permeation through its transport mechanism and size.[38] In addition, a surfactant-like system enhances permeation.[39] Previous work also proved that the permeation of active compounds was increased by nanoemulsion formulation incorporated into a gel formulation by nearly 250% in terms of cumulative permeated drug and retention.[35]

The amount of permeated phenolic compounds and retained DGTE in the membrane also proved that the nanoemulsion had better retention in the skin membrane due to skin disposition and distribution (Figure 5b). The amount of retained phenolic compounds in the nanoemulsion-based formulation was nearly 50% greater than that of the hydrogel-based formulation. The droplet size and lipophilic characteristics gained particular consideration in the permeation phenomenon through intracellular transport. The nanoemulsion of polyphenol compounds proved that it broke the limitation of the transport mechanism due to its hydrophilicity.[40]

Conclusion

The phyto-nanoemulsion containing DGTE incorporated into the peel-off mask formulation was successfully developed. DGTE has potent antioxidants, and it is maintained in the preconcentrated formulation. The optimized phyto-nanoemulsion comprised 21.62% virgin coconut oil, 48.38% Tween 80, and 30% PEG 400, producing less than 50 nm droplet sizes. The peel-off formulation was successfully formulated, and PVA was determined on the drying kinetics and peel-off features. The nanoemulgel formulation increased the permeation parameters, namely the amount of permeated phyto-antioxidant compounds and flux, by roughly 200%. Moreover, this formulation also increased the deposited antioxidant compounds in the skin and reduced the saturated membrane time.

Ethical Issues

This method was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Universitas Sebelas Maret, along with an ethical clearance No. 111/UN.27.06.11/KEP/EC/2022.

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Author Contributions

Dinar S.C. Wahyuni: Validation, Funding Acquisition, Project Administration, Writing - Original Draft. Ratih P. Febrinasari: Methodology, Validation. Sakinah: Investigation, Validation, Data Curation. Ana Mardiyah: Data Curation, Validation. Syaiful Choiri: Conceptualization, Software, Validation, Formal analysis, resources, Visualization, Supervision, Writing - Review & Editing.

Conflict of Interest

The authors report no conflicts of interest.

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