

PREPARATION, CHARACTERIZATION, AND *IN VITRO* SKIN PENETRATION OF *MORUS ALBA* ROOT EXTRACT NANOEMULSION

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ABSTRACT

Objective: White mulberry (*Morus alba*) root extract has terpenoid, flavonoid, and stilbene compounds. The stilbenes, oxyresveratrol and resveratrol, have antioxidant and antityrosinase activities. Nanocarriers can help active ingredients to be delivered in a more efficient manner. The advantages of nanoemulsion on products include increased penetration, biocompatibility, and low toxicity due to its non-ionic properties and have the ability to combine the properties of lipophilic and hydrophilic active ingredients. The objective of this study was to prepare, characterize, and evaluate the *in vitro* skin penetration of *M. alba* root extract nanoemulsion.

Methods: The *M. alba* root extract was prepared by ionic liquid-based microwave-assisted extraction method. Nanoemulsion was optimized and prepared using virgin coconut oil (VCO), Tween 80, and polyethylene glycol 400 (PEG 400) by aqueous phase-titration method to construct pseudoternary phase diagram. *M. alba* root extract nanoemulsion was characterized for droplet size, viscosity, zeta potential, and physical stability tests for 12 weeks. *In vitro* skin penetration of oxyresveratrol from nanoemulsion was determined by the Franz diffusion cell and was compared by macroemulsion preparation, then analyzed by high-performance liquid chromatography method.

Results: Based on pseudoternary phase diagram, nanoemulsion of white mulberry root extract contained of 2% VCO and 18% mixture of surfactant Tween 80 and PEG 400 (1:1) was chosen. Nanoemulsion has average globule size of 81.61 nm, with polydispersity index 0.22, and potential zeta -1.56 mV. The cumulative penetration of oxyresveratrol from nanoemulsion was 55.86 $\mu\text{g}/\text{cm}^2$ with flux of 6.53 $\mu\text{g}/\text{cm}^2/\text{h}$, while regular emulsion was 32.45 $\mu\text{g}/\text{cm}^2$ with flux of 3.5501 $\mu\text{g}/\text{cm}^2/\text{h}$.

Conclusion: Nanoemulsion of white mulberry root extract was penetrated deeper than regular emulsion.

Keywords: *Morus alba*, Nanoemulsion, Oxyresveratrol, Skin lightning.

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INTRODUCTION

The white mulberry (*Morus alba*) has been used in traditional Chinese medicine to treat inflammatory diseases. White mulberry root extract has been known to contain terpenoids, flavonoids, and stilbene compounds [1], including oxyresveratrol and resveratrol which have antioxidant and antityrosinase activity [2]. In Indonesia, the white mulberry plant is widely cultivated as the main source of silkworm feed. The utilization of this plant is still limited by fruits and leaves only, while the root part which has antityrosinase activity has not been widely developed. At present, in the market, there are many foreign cosmetic preparations use extracts from the white mulberry root as an active substance along with other natural ingredients. While local cosmetic products have not been utilize the potential of this root. However, oxyresveratrol development in cosmetics is still limited because it has low water solubility so the ability to diffuse through the stratum corneum is low. In addition, oxyresveratrol is unstable in aqueous systems, which are susceptible to discoloration and oxidative isomerization, especially when exposed to light [3]. Therefore, the development of appropriate delivery systems is a need to include the maximum amount of oxyresveratrol in the cosmetics and immediately penetrating into the skin.

Emulsions are a scattering of an immiscible fluid into another balanced out by the nearness of surfactant [4]. Emulsions which have bead sizes between 5 and 200 nm are named as nanoemulsions, ultrafine emulsions, submicron emulsions, translucent emulsions, and miniemulsions [5,6]. Nanoemulsions are dynamically steady yet thermodynamically flimsy frameworks [7]. The nanosize bead can

decrease the event of flocculation, sedimentation, and creaming amid capacity [8].

According to Moghassemi and Hadjizadeh (2014), a rational design of skin delivery systems based on nanocarriers can help active ingredients to be delivered in a more efficient manner. The advantages of nanoemulsion on products include increased penetration, biocompatibility, and low toxicity due to its non-ionic properties and have the ability to combine the properties of lipophilic and hydrophilic active ingredients [9]. This study aims to test a preparation of white mulberry root extract nanoemulsion on containing oxyresveratrol as skin lightening.

MATERIALS AND METHODS

Materials

M. alba root extract was produced by ionic liquid-based microwave-assisted extraction method as described before by Fadhila, Jufri, and Mun'im, 2018. Chemicals reagent used was ethanol 80%, oxyresveratrol standard (Shanghai Yuanye Bio-Technology Co., Ltd.), propylene glycol, Tween 80, polyethylene glycol 400 (PEG 400), virgin coconut oil (VCO), methylparaben, and propylparaben.

Instrument

The instruments were magnetic stirrer, pH-meter type 510 (Eutech), Ostwald viscometer, zetasizer nano ZS (Malvern Instruments Ltd., United Kingdom), Franz diffusion cell, and high-performance liquid chromatography (HPLC) system (LC-20AT, Shimadzu, Kyoto, Japan).

Methods

Construction of pseudoternary phase diagram

To make nanoemulsion, oil stage that we pick was VCO, Tween 80 as the surfactant, PEG 400 as cosurfactant, water as the watery stage, and propylene glycol as entrance enhancer. Surfactant and cosurfactant in proportion 1:1 (v/v) were blended as Smix. The pseudoternary stage graph was created by the water titration strategy at room temperature. The oil stage and Smix were blended at various volume proportions from 1:9 to 1:9 (v/v) and, afterward, were dropped by water carefully under delicate fomentation to every blend. The nanoemulsions were seen as a straightforward fluid. In view of the pseudoternary stage outline, the chose measure of oil stage, surfactant, and cosurfactant and the watery period of nanoemulsion were kept on getting ready white mulberry root separate nanoemulsion [10].

Formulation of nanoemulsion containing *M. alba* root extract

Incorporation of white mulberry root extract into nanoemulsion was performed by dissolving white mulberry root extract in oily phase and surfactant. White mulberry root extract containing surfactant and cosurfactant was mixed and added by amount of water. The oily phase containing dissolved extract was added by drops. Target content of extract in nanoemulsion was set to be 1%.

Characterization of nanoemulsion

To decide the normal bead size and drop measure circulation of the nanoemulsion by unique light dispersing technique, Zetasizer Nano (Malvern Instruments, Worcestershire, UK) was utilized. A similar instrument was utilized to decide the bead surface charge (zeta potential) and polydispersity file (polydispersity index [PDI]). The polydispersity record (PDI) is a list that portrays the variety in size [11]. The viscosity of nanoemulsion was determined using Ostwald viscometer. A calibrated digital pH meter was used to determine pH for evaluation and stability test in triplicate at room temperature.

Stability studies

Stability studies on nanoemulsion were performed by first, rotator the sample at 3500 rpm for 30 min, taking the sample for the heating and cooling for 6 cycles. Sample was storage in oven and refrigerator temperatures (40°C and 4°C) for not <24 h each temperature; third, keeping the sample at refrigerator temperature (4°C), room temperature (25°C), and oven temperature (40°C). These studies were performed for the period of 3 months. The organoleptic and pH were determined at every 2 weeks. The concentration of oxyresveratrol was determined at 0 and 3 months.

In vitro skin permeation study

As skin pervasion study, rodents (200 g) were gotten from the Laboratory Animal Center of Pharmacy Faculty, Universitas Indonesia and approved by Komisi Etik Penelitian Kesehatan Fakultas Kedokteran, Universitas Indonesia, number 379/UN2.FI/ETIK/2018. The rats were sacrificed after abdominal hair was removed and then, the full skin was excised from the abdominal region. At that point, the skin was expelled from subcutaneous tissue and fat, washed with 0.9% saline, and after that put away at 4°C to use inside 24 h.

Franz diffusion cells were utilized to decide *in vitro* skin penetration with an extracted rodent skins (1.76 cm²) and phosphate buffer pH 7.4 as receptor compartment (15 ml) at 37±0.5°C. The receptor compartment was filled and attractively mixed at 300 rpm all through the investigation. About 2 ml of tests (emulsion and nanoemulsion) were mounted on the epidermal surface of rodent skin. Tests were pulled back at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h. For each time interim, 1 ml receptor arrangement was gathered and separated with a 0.45 µm syringe and investigated by HPLC to the determinate amount of oxyresveratrol. To keep up sink conditions, fresh phosphate buffer was included in the receptor compartment in the meantime [10].

HPLC analysis

HPLC analysis was performed to examination oxyresveratrol in nanoemulsion and macroemulsion. The compound was utilized C18 fortified silica gel, 5 µm, 150×4.6 mm molecule measure, YMC section from Phenomenex (Torrance, USA). The indicator was ultraviolet (SPD-20A, Shimadzu, Japan) at 320 nm. The mobile stage was acetonitrile and water containing 1% acetic acid (25:75 v/v) and stream rate of 1 ml/min in isocratic mode. The infusion volume was 20 µl and runtime of 15 min. Oxyresveratrol stock arrangement 1 mg/ml in 80% ethanol was arranged and diluted into 0.0005, 0.001, 0.005, 0.01, and 0.05 mg/ml to make alignment bend. The two emulsions were diluted in 80% ethanol each [12].

Data analysis

The two emulsions were resolved the aggregate sum (Q, µg/cm²) of oxyresveratrol penetrated through rodent skin. The flux (J, µg/cm²/h) of oxyresveratrol was gotten by plotting the total measure of penetrated per unit territory of skin versus time.

RESULTS

Pseudoternary phase diagram

Fig. 1 showed the pseudoternary phase diagram of VCO, Smix (mixture of surfactant and cosurfactant 1:1), and water. The obtained nanoemulsion area was present in the area passed by the yellow line. From the experimental results, nanoemulsion was formed by composition of VCO and surfactant mixture with ratio of 9:1 in all tested compositions (10–50%). The amount of surfactant mixture that was less than that ratio is not sufficient to form nanoemulsion. Based on these preliminary results, the selected formula consisted of 2% VCO, 18% surfactant mixture, and 80% water phase and then was continued with evaluation and *in vitro* assay.

Characterization of nanoemulsion

The nanoemulsion was evaluated organoleptically and visual homogeneity and then was measured pH value. Fig. 2 and Table 1 show the organoleptic of nanoemulsion. Nanoemulsion of white mulberry root extract was a yellow-brown liquid, aromatic odor and

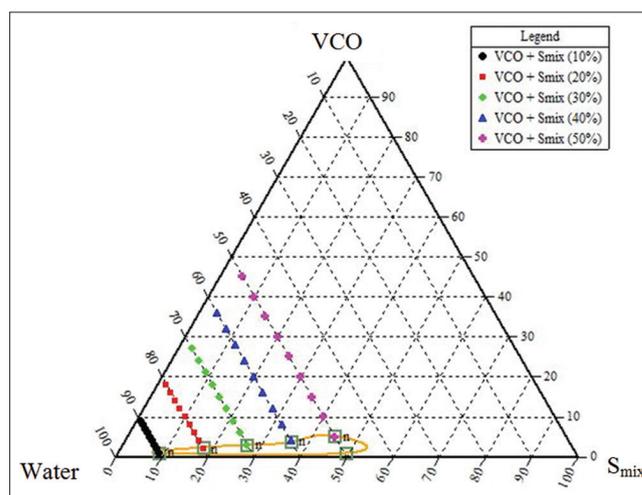


Fig. 1: Pseudoternary diagram of virgin coconut oil -water-Smix

Table 1: Organoleptic, pH, and homogeneity of nanoemulsion

Organoleptic	Properties
Color	Yellow-brown
Odor	Aromatic
Texture on skin	No sticky
Homogeneity	Homogeny
pH	6.5

had a non-sticky texture when applied to the skin. The yellow-brown color of nanoemulsion came from the added extract. Homogeneity was tested with object glass and showed the nanoemulsion distributed homogeneously. The pH value of the white mulberry root nanoemulsion was 6.5 and still within the range of skin pH (4.5–6.5).

The viscosity was determined by Ostwald viscometer and the result showed viscosity value of 4.03×10^{-3} Pa.s. Table 2 shows that nanoemulsion had average globule size of 81.61 nm and a PDI value of 0.220 with a normal distribution curve as shown in Fig. 3. The small globule size appeared as clear or opaque liquid that is distinct from the milky white color associated with regular emulsions [13]. The zeta potential of white mulberry root extract nanoemulsion was -1.56 mV.

Stability test

The result of the cycling test was showed in Table 3. There are no visible changes in nanoemulsions organoleptic, include color, odor, texture and homogeneity of nanoemulsion. After 6 cycles, there was a decrease in pH value of 0.49 (from 6.5 to 6.01). The pH changed that occurred was still acceptable within the pH range of the skin.

Physical stability tests were performed at three different temperatures; low temperature (4°C), room temperature (28°C), and oven temperature (40°C) for 3 months (12 weeks). Observations of changes in nanoemulsion's organoleptic and pH measurements were carried out every 2 weeks. The 12-week test results showed no change in odor organoleptic, texture when applied, and homogeneity of preparations at all three storage temperatures. No phase separation and creaming occurred. Changes that occurred were seen in the color of the nanoemulsion on which became darker at 40°C and became brighter at 4°C storage. The preparations which were stored at 28°C showed no color changed.

Changes in the pH of the preparations in the three storage temperatures are shown in Fig. 4. The pH measurement results for 12 weeks indicate a gradual decrease in each storage temperature, but still within acceptable limits the pH of the skin (4.5–6.5).

In vitro skin penetration test

The penetration test was performed to analyze the oxyresveratrol penetration ability from nanoemulsion and to compare with that regular emulsion. The nanoemulsion was prepared based on the previous formulation and method, wherein Tween 80, PEG 400, and VCO had a ratio of 9:9:2 while for regular emulsion was used Tween 80, PEG 400, and VCO in the ratio of 8:8:4 with both the same compositions of excipient. Both nanoemulsion and regular emulsions were made by homogenizing using a magnetic stirrer at a speed of 1250 rpm.

Fig. 5 shows the *in vitro* release profile of oxyresveratrol from nanoemulsion and from regular emulsion using phosphate buffer pH 7.4 as receptor media. The results of the *in vitro* penetration test showed that the cumulative amount of penetrated oxyresveratrol from



Fig. 2: Nanoemulsion of *Morus alba* root extract

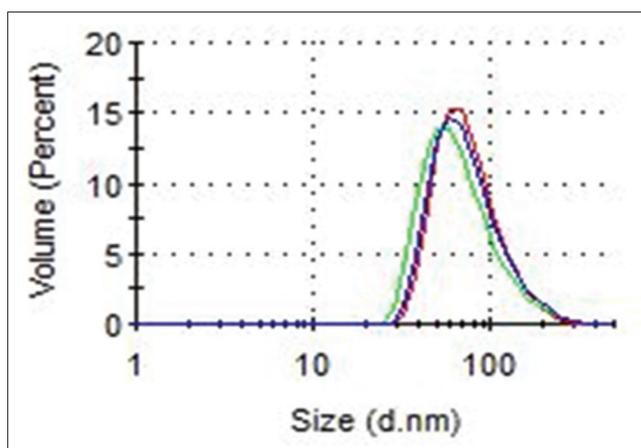


Fig. 3: Distribution of globule size

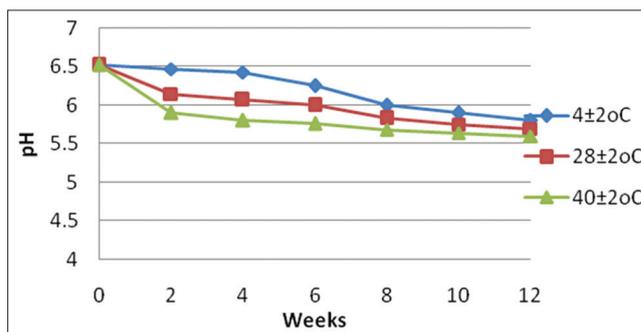


Fig. 4: PH of nanoemulsions for 12 weeks

Table 2: Globule size and polydispersity index

Z-average (nm)	Polydispersity index
81.61	0.220

Table 3: Cycling test

Observation	Fresh condition	After 6 cycles
Color	Brownish-yellow	Brownish-yellow
Texture on skin	No sticky	No sticky
Odor	Aromatic	Aromatic
Homogeneity	Homogeny	Homogeny
pH	6.52	6.01

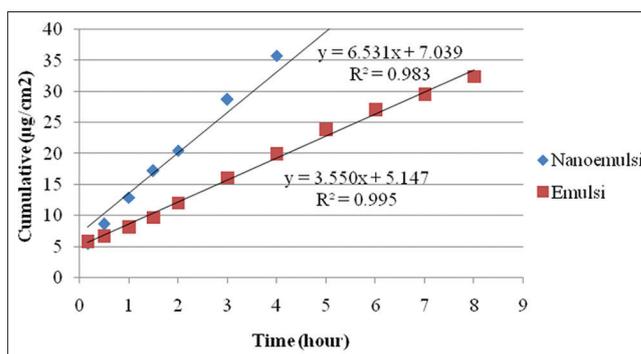


Fig. 5: Franz diffusion cell penetration test

nanoemulsion was 55.86 $\mu\text{g}/\text{cm}^2$ with a flux of 6.5316 $\mu\text{g}/\text{cm}^2/\text{h}$ and from a regular emulsion of 32.45 $\mu\text{g}/\text{cm}^2$ with a flux of 3.5501 $\mu\text{g}/\text{cm}^2/\text{h}$. The percentage of oxyresveratrol penetrated from nanoemulsion on for 8 h was 25.44%. These results indicate that nanoemulsion on increases the penetration ability of oxyresveratrol was 1.8 times better than regular emulsion.

DISCUSSION

Nanoemulsions are the most promising mode of drug delivery system that captured the attention of many pharmaceutical scientists due to its tiny nanosized droplets that are spherical and also exhibit good kinetic stability [14]. There are two methods to make nanoemulsion, low-energy and high-energy methods. Sari *et al.* (2018) have been successfully made nanoemulsion by spontaneous emulsification method (low energy) with 5% red palm olein with 40% surfactant Tween 80 and 20% cosurfactant sorbitol [15] while another study suggests that the use of Ultra-Turrax (high energy) is a good strategy to prepare nanoemulsion [16].

The pseudoternary phase diagram is an auxiliary method for determining initial screening of surfactant and cosurfactant compositions for nanoemulsion on formulations. From the trial results, the nanoemulsion utilizing the least conceivable surfactant fixation while keeping up the strength and little globule estimate was picked as the following formulation. Research by He *et al.* (2017) showed that oxyresveratrol has a solubility of 168.90 mg/mL in Tween 80 and 445.37 mg/mL in PEG 400, so it becomes an option for use as a surfactant and cosurfactant [17].

The size or diameter of the globule of the nanoscale conductor system is the most important parameter since the entrance of a compound into the skin can happen through the pores of pilosebaceous (10–70 μm), pores of sweat glands (60–80 μm), and most often through the intercellular lipid matrix of stratum corneum (about 75 nm) [18-20]. In addition, due to its small size, considerable increase in surface area causes increased contact with the skin and increases absorption [21].

According to McClements (2015), the general thickness of two stages, the scattered stage and the constant stage impact the consequences of the size decrease process. At the point, when the thickness is generally too high, globules end up impervious to break and rather start to pivot without anyone else hub when given erosion. The type of oil and oil volume also affect the size of globule. Nanoemulsion which made with high-viscosity oil has a larger globule size [22].

The consistency of the bead estimate dispersion is estimated as the PDI. The PDI is a file that portrays the size variety. The higher the PDI esteem, the more extensive the span of the disseminated bead [11]. The index value is in the range of values between 0 (uniform size distribution) and 0.5 (width size distribution). Nanoemulsion is called as “monodisperse” if the PDI is <0.2; while a much higher polydispersity (>0.4) is indicated by ordinary emulsions. This PDI provides information about the physical stability of a dispersion system. The low PDI value indicates that the dispersion system that is formed is more stable for the long term [23].

The dispersed phase globules are known to acquire an electric charge during the process of emulsification. The globules gather to charge layers around themselves called Helmholtz double layer; zeta potential in which the difference in charge potentials between points on the fixed charge layer on the globule to the point where both positive and negative charge exist. Zeta potential can be measured by zeta meter and helps in the prediction of flocculation behavior of the system. Dispersed phase systems having a zeta potential value higher than 0.25 mV are likely to be stable because the intensity of the charge on the particles repels them from one other [24].

As a standard guideline, the total estimation of zeta potential over 30 mV give great stability or more 60 mV give excellent stability [25,26].

Research by Nurdianti *et al.* (2017), nanoemulsion on astaxanthin developed with sunflower oil composition, Kolliphor RH40, and PEG 400 (1:8:1) has a potential zeta value of -5.38 mV [27]. Compared with white mulberry root extract nanoemulsion, the two nanoemulsions have a potential zeta value which is close to 0 because it used a surfactant and non-ionic surfactant.

Numerous segments of nanoemulsion can influence the capacity to enter through the skin. The nearness of sebum in hair follicles, it is conceivable that oil-based nanoemulsion, surfactants, and alcohols may encourage transport of both the hydrophilic and lipophilic compound. Numerous excipients from nanoemulsion go about as entrance enhancers in topical applications. Due to the hydrophobic attributes of the skin, the oil period of nanoemulsion which additionally has hydrophobic properties can expand the entrance of dynamic fixings [28]. Surfactants are otherwise called skin infiltration enhancers since they can break up lipids in the stratum corneum as a noteworthy hindrance to the take-up of topical medications [29]. The infiltration of surfactant atoms into the lipid lamellae of the stratum corneum depends by and large on the solvency and surfactant segment [30]. Cosurfactant can likewise influence the conduct of medication transport on the skin. For instance, ethanol, it can increment lipophilic transport of the stratum corneum through the arrangement of pores in the stratum corneum [31,32]. What's more, hydrated skin is commonly progressively penetrable, with the goal that the water contained in nanoemulsion is additionally a perfect hydration enhancer [33].

CONCLUSION

Nanoemulsion of white mulberry root extract was made by composition of 2% VCO, 18% mixture of surfactants Tween 80 and PEG 400 (1:1), and 80% water. *In vitro* penetration tests showed the cumulative amount of penetrated oxyresveratrol from nanoemulsion on preparations (55.86 $\mu\text{g}/\text{cm}^2$) was higher than that of regular emulsion (32.45 $\mu\text{g}/\text{cm}^2$).

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AUTHORS' CONTRIBUTIONS

All authors certify that they have participated sufficiently in work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

CONFLICTS OF INTEREST

There are no any conflicts of interest in this study.

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