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RESEARCH ARTICLE

Antioxidant Activity, Formulation, and Evaluation of Roselle Seed Oil Extract Nanoemulsions

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Abstract

Roselle Hibiscus is a medicinal plant used in folk medicine. The parts of the flower, calyx, and bracts, are regularly used but the seed of roselle has not been used and studied for antioxidant activities. The objective of the present work was to determine the antioxidant activity of roselle seed oil extract extracted using dichloromethane maceration. The extraction then was formulated as nanoemulsions and evaluated for its physicochemical properties and stability. The oil obtained from *Roselle Hibiscus* seed demonstrated good antioxidant activity, according to the DPPH assays. Roselle seed oil extract nanoemulsions were developed using non-ionic surfactants and water by ultrasonic emulsification method and evaluated to achieve optimum stability. Nanoemulsions formulated using Tween 60 and Tween 80 had a relatively small droplet size ranging from 20-30 nm. After a seven-day stability test at the room temperature and a 6-cycle heating-cooling study, phase separation or creaming did not occur to the nanoemulsions formulated by Tween 60 and Tween 80. The ratio of roselle seed oil extract and Tween 60 that yielded the best physical properties for the nanoemulsions was 1% and 5%, respectively. The results indicated that nanoemulsions had nanoscale droplet size and were stable under the normal and stress storage condition.

Keywords: *Nanoemulsions, Roselle seed oil, Stability.*

Introduction

The skin barrier has both hydrophilic and lipophilic regions. Hydrophilic substances have difficulty passing through lipid-rich regions, while lipophilic substances have a low probability of crossing the aqueous regions. Several studies have been reported nanoemulsions could improve the transdermal permeation of many drugs when compared to conventional topical formulations.

Nanoemulsion, an isotropic, transparent (translucent) system consisting of oil, water, and surfactant, has a droplet diameter generally in a range of 10 to 100 nm [1]. Nanoemulsion can improve the solubility of drugs, is relatively uncomplicated to fabricate, and has longer stability than the conventional topical formulations, e.g. emulsions and ointments. In addition, nanoemulsion requires a lower quantity of surfactant, in general, 5-20% w/w lower than microemulsions. Hence, they could reduce the opportunity of skin irritation and production costs [2, 3].

These characteristics make it an interesting technology for the pharmaceutical and cosmetic industries. Hibiscus sabdariffa L., a member of family Malvaceae, is known as roselle and is one of the most famous medicinal plants in several countries. The useful parts of the plant are the flower, calyx, and bracts. Those parts are used to make beverages in Egypt. The tender stem, leaves, and chalice are used as vegetables in Africa.

Fermented roselle seeds are used as a spice in northern Nigeria. The seed oil is richer in gamma-tocopherol [4], which is an antioxidant used widely in food and cosmetic industries [5], than the sepals, leaves, and stems extracts [4]. Topical antioxidants from natural sources are one of the approaches used to reverse signs of skin aging. The nanoemulsions containing roselle seed oil extract might enhance its skin penetration and have significant implications in skincare. Therefore, the purpose of this study was to measure the antioxidant capacity of roselle seed oil extract; to fabricate nanoemulsions containing roselle seed oil extract, and; to determine the effects of type and quantity of surfactants on the physicochemical properties of nanoemulsions.

Materials and Methods

Materials

Roselle seed oil was extracted and provided by the second author. The following reagents were obtained from P.C. Drug Center Co., Ltd., (Bangkok, Thailand): polyethoxylated sorbitan esters (Tween) 20, Tween 60, Tween 80 and polyethoxylated glycerides (Cremophor) RH 40. Sorbitan esters (Span) 80 was obtained from CT chemical (Bangkok, Thailand). Polyethoxylated fatty acid ester (Myrj) 52 and ascorbic acid were supplied by Sigma-Aldrich (Saint-Louis, MO, USA). 6 – hydroxy - 2, 5,7,8-tetramethylchroman – 2 - carboxylic acid (Trolox) and Hydroxyl (OH) and 2-2′-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Fluka (Switzerland). All other chemicals and reagents were of analytical or chromatographic grade.

Antioxidant Activities of Roselle Seed Oil

The strength of the antioxidants is quantified in vitro by measuring its efficacy in quenching a known concentration of typically DPPH. Antioxidant activities of the extract and standard solutions were determined based on their radical-scavenging ability in reacting with a stable DPPH free radical. Ascorbic acid and Trolox were used as a reference standard. A 100 µl sample of the roselle seed oil extract at the concentrations of 12.5 to 200 µg/ml or the standard was mixed with 100 ml of DPPH in absolute ethanol solution (0.1 mM). After the incubation at 37°C for 30 min, the absorbance of each solution was determined at 520 nm using a spectrophotometer (Hitachi®, Japan); corresponding blank readings were also taken, and % inhibition was then calculated. The scavenging activity percentage (AA%) was determined according to Habu et al [6].

$$AA\% = \left(\frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}}\right) \times 100$$

The antioxidant activity of the extract was expressed as IC50, defined as the concentration of the extract required to inhibit DPPH radicals by 50%, using an exponential curve. A solution of DPPH in ethanol was used as a control solution, whereas ethanol was used as a blank. The experiment was performed in triplicate.

Preparation of Roselle Seed Oil Nanoemulsions

Nanoemulsions of roselle seed oil extract were prepared by the ultrasonication method. Tween 20, Tween 60, Tween 80, Cremophor RH 40, Span 80 and Myrj 52 were used as surfactants. The oil concentration to surfactant concentration ratios were 1:1, 1:5 and 1:10. Briefly, roselle seed oil extract, the surfactant, and water were mixed in a beaker under continuous stirring for 30 minutes (Table 1). The mixture solution was sonicated using an ultrasonic probe sonicator (Vibra Cell VCX 750, Sonics Materials Inc., USA) for five minutes at a 40% amplitude resulting in the formation of nanoemulsions.

The prepared formulations with the appearance as cloudy or milky white solution were excluded from this study, whereas the nanoemulsion formulations which were transparent and clear were selected for further evaluation.

Table 1: Composition of roselle seed oil extract nanoemulsions formulation (100 mg)

Formulation code	Oil (mg)	Surfactant (mg)		Water (mg)
F1	1	Tween 20	1	98
F2			5	95
F3			10	90
F4		Tween 60	1	99
F5			5	95
F6			10	90
F7		Tween 80	1	99
F8			5	95
F9			10	90

F10		Cremophor RH 40	1	99
F11			5	95
F12			10	90
F13		Span 80	1	99
F14			5	95
F15			10	90
F16		Myrj 52	1	99
F17			5	95
F18			10	90

Determination of pH value

The pH of the nanoemulsions formulations was measured by a pH meter (Mettler Toledo MP 220, Greifensee, Switzerland) in triplicate at 25 °C [7].

Evaluation of Droplet Size and Zeta Potential

Twenty microliters of each nanoemulsion formulations were then diluted with distilled water to 2000 µl in a microtube (Eppendorf, NY, USA). Droplet size, polydispersity index (PDI), and zeta potential of the prepared samples were measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instrument Ltd., Worcestershire, UK). The measurements were conducted at 25 °C at a 90° angle and each measurement was performed with three readings per sample [7].

Stability Study of Nanoemulsion

Each formulation was stored in a glass bottle, tightly sealed with a cap and then stored at 25°C for seven days. A heating-cooling cycle was also performed to evaluate the stability of formulated nanoemulsions at the extreme temperature changes. This test consisted of storing the samples at 40°C in a hot air oven (Memmert® CTC256, Buchenbach, Germany) for 48 hours and then at 4°C in a refrigerator (Panasonic® NR-BU303 SSTH, Osaka, Japan) for another period of 48 hours. The cycle was repeated six times. Physicochemical characteristics including droplet size, PDI, stratification, and pH were evaluated before and after the heating-cooling cycle (n = 3).

The nanoemulsions were observed visually for any turbidity or phase separation. Furthermore, the nanoemulsions were evaluated for physical stability characterized by the percentage of creaming and cracking. Cracking of nanoemulsion refers to the separation of the dispersion phase that dose not re-disperse by gentle shaking. The creaming index (%CI) was calculated as the following: %CI = (CC/CT) × 100; where CC is the total height of the cream layer and CT is the total height of the emulsion layer [4, 8].

Statistical Analysis

Data were expressed as the mean ± standard deviation (SD) and analyzed by one-way ANOVA. Differences between formulations were set to be significant at $p \leq 0.05$.

Results and Discussion

Antioxidant Activities of Roselle Seed Oil

The antioxidant potential of roselle seed oil (*Hibiscus sabdariffa* L.) extract was determined by measuring the DPPH radical scavenging activity of the extract. The IC50 value was calculated to determine the concentration of the extract required to scavenge 50% of the DPPH free radicals under the employed investigational conditions. The smaller the IC50 value, the higher the antioxidant activity [9]. The calculated IC50 values of the roselle seed oil extract, ascorbic acid, and Trolox were 247.08 ± 14.73 µg/ml, 4.10 ± 2.94 µg/ml and 5.61 ± 0.41 µg/ml, respectively. These results showed that roselle seed oil extract had antioxidant capacity lower than the standard controls (ascorbic acid and Trolox).

Table 2: Physical characteristics of freshly prepared roselle seed oil extract nanoemulsions

Formulation code	Physical property	pH	Size (nm)	Zeta potential (mV)
F1	turbid			
F2	turbid			
F3	turbid			
F4	turbid			
F5	transparent	3.60±0.03	33.71±3.41	-22.48±13.10
F6	transparent	3.48±0.02	22.82±1.79	-10.71±4.73
F7	turbid			

F8	turbid			
F9	transparent	6.74±0.22	19.12±3.30	-11.37±3.95
F10	turbid			
F11	turbid			
F12	turbid			
F13	turbid			
F14	turbid			
F15	turbid			
F16	turbid			
F17	turbid			
F18	turbid			

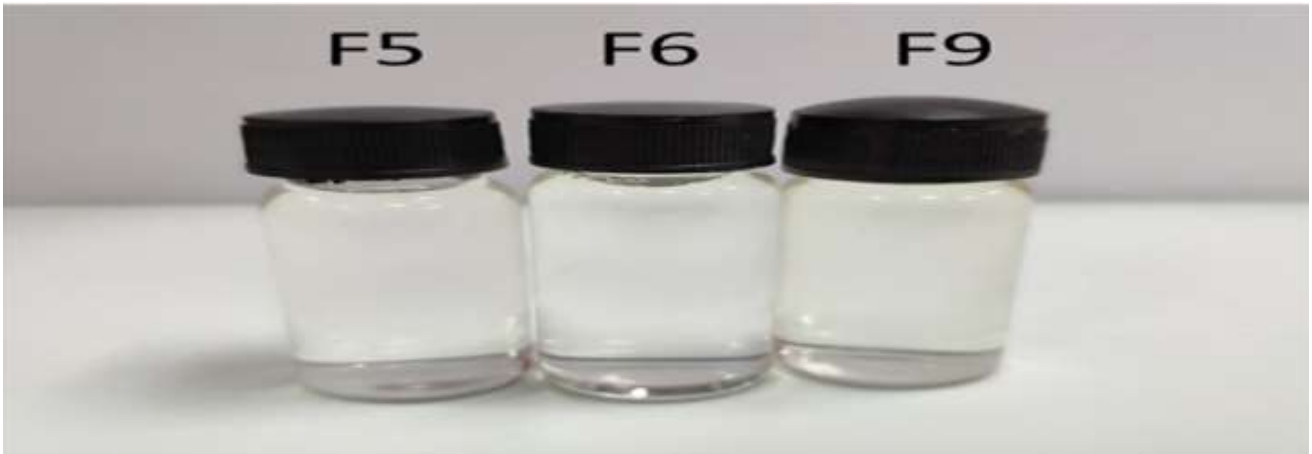

Fig. 1: The physical appearance of the roselle seed oil nanoemulsions (F5, F6, and F9)

Physicochemical Properties and Characterization

Roselle seed oil extract was used as an oil phase and Myrj, Tweens, Spans, and Cremophors were used as nonionic surfactants in this study. The oil/surfactant ratios were 1:1, 1:5 and 1:10. The ratio of oil to the surfactant that resulted in nanoemulsions were 1:5 (Tween 60 and Tween 80) and 1:10 (Tween 80) (Table 2). The selected formulations as shown in fig. 1 (F5, F6, and F9) were transparent and clear in solutions and they were selected for characterization in further studies.

Roselle seed oil extract could effectively form nanoemulsions with Tween 60 and Tween 80 while Tween 20, Cremophor RH 40, Span 80 and Myrj 52 were not effective surfactants for roselle seed oil nanoemulsions. The reason of these phenomenons might be from the structure of these surfactants which be unsuitable to form nanoemulsion containing roselle seed oil extract.

The structure of surfactants may have affected the nanoemulsion forming potential of the surfactants. The various polysorbate surfactant contain the fatty acid portion of the molecule including oleic acid (Tween 80), lauric acid (Tween 20), and stearic acid (Tween 60). Stearic acid and oleic acid are long-chain fatty acid with an 18-carbon atom. Stearic acid is a saturated fatty acid whereas oleic acid is a monounsaturated fatty acid. Lauric acid is a saturated fatty acid with a 12-carbon atom chain. For these reasons, at 5% of surfactant formulation, only Tween 60 (F5) could be used to successfully fabricated the nanoemulsions while Tween 20 and Tween 80 could not. The nanoemulsions with Tween 80 could be prepared at the highest amount of surfactant (10%; F9).

This might be a monounsaturated fatty acid of Tween 80. Type of nanoemulsions could define by the hydrophilic-lipophilic balance (HLB), whose scale ranges from 0 to 20, of surfactant. Lower HLB surfactants (<10) are lipophilic and form a water-in-oil emulsion while higher HLB surfactants (>10) are hydrophilic and form an oil-in-water emulsion. Since the formulations consisted of hydrophilic surfactants (Tweens 60) with the HLB of 14.9, nanoemulsions in this study were oil-in-water nanoemulsions in which oil was dispersed in the continuous aqueous phase.

The pH measurement is an important experiment to monitor the stability of the nanoemulsions. Changing pH values indicates the happening of chemical reactions that may decrease the quality of the final product. The apparent pH of all formulations was measured by pH meter in triplicate at 25 ± 1 °C and found to be in between 3.48-6.74.

The pH of the nanoemulsions can be adjusted to close to pH range of 5 to 6 which is optimum for transdermal formulation without the effect on particle size and zeta potential [10]. The mean droplet size of the prepared nanoemulsions with 10% of Tween 60 (F6) and Tween 80 (F9) was approximately 20 nm. These close approximation in the size of nanoemulsions F6 and F8 might be due to the close HLB value of Tween 60 and Tween 80.

The HLB of Tween 60 was 14.9 and Tween 80 was 15.0. We also found that the droplet size decreased with surfactant concentration. At 5% and 10% concentration of the surfactant, the droplet size was approximately 33 nm to 22 nm, respectively (Table 2). This was supported by the fact that the increase in surfactant concentration decreases the interfacial tension of the oil phase and water phase, so the droplet size of the nanoemulsions with high surfactant concentrations is smaller [11, 12].

The decrease in the size of nanoemulsions when the concentration of the surfactant is increased can be explained by thermodynamics. From the equation □= Gs = Hs - TSs, an increase in the concentration of surfactant, the surface tension decreases and leads to a decrease in Gibbs free energy (Gs). then

The decrease in the size of nanoemulsion when the concentration of the surfactant was increase could also be explained by the emulsification mechanism. The formation of an emulsion is spontaneous if the Gibbs free energy is less than zero. Then, when increasing the concentration of surfactant, the surface tension was decreased which can be described by this equation ($\gamma = G^s = H^s - TS^s$). Hence, the reduction of surface tension results in a decrease in Gibbs free energy [13].

In general, zeta potential values greater than +30 mV or less than -30 mV are considered as stable nanoemulsions. Table 2 shows the zeta potential values of nanoemulsions. The result illustrated that all the nanoemulsions were negatively charged with the zeta-potential of more than -30 mV. Since Tween 60 is a nonionic surfactant that is not affecting the zeta potential, the negative charge should have come from the anions in the roselle seed extract. This explanation is plausible because the extract contains carboxylic groups as in the fatty acid.

Stability Study of Nanoemulsion

In this study, the stability of nanoemulsions was used to determine whether the formulation should be further pursued. The most common results from emulsion instability are sedimentation and creaming. The upward (creaming) or downward (sedimentation) movement of the particles in a colloidal dispersion is caused by the gravitational acceleration force. In this study, the selected formulations (F5, F6, and F9) with the ultrasonication method were stored at 25°C for seven days and were tested by the six-cycle of heating-cooling cycles.

Phase separation or creaming did not occur in all of the selected formulations. The droplet aggregation such as flocculation or coalescence of nanoemulsions by gravity force sparsely occurred as a result of their nanoscale droplet size. The driving force from the Brownian motion movement which occurs in nanoemulsions with a diameter lower than 70 nm prevents the creaming [14, 15].

The measurement of pH value, droplet size, and zeta potential were analyzed after storing the nanoemulsions under the storage conditions. The pH values (Fig. 2) remained relatively close to the values of the freshly prepared counterparts (3.53-6.47). Therefore, it could be speculated that chemical reactions that decline the product quality did not occur. The mean droplet size and zeta potential of the samples after seven days and six cycles of heating- cooling assessment did not significantly different from the value of the freshly prepared counterparts (Fig. 3).

All selected formulations demonstrated good stability without phase separation. However, the formulation F5 (1% of roselle seed oil extract and 5% of Tween 60) was selected as an optimized formulation because it had the lowest amount of surfactant. For further research, the nanoemulsions should be stored under long-term storage conditions (25±2°C/ 60±5%RH or 30±2°C/ 60±5%RH) for 12 months to determine the long-term stability. Additionally, toxicity tests in cell culture or animal model is an interesting experiment to define how safe is it.

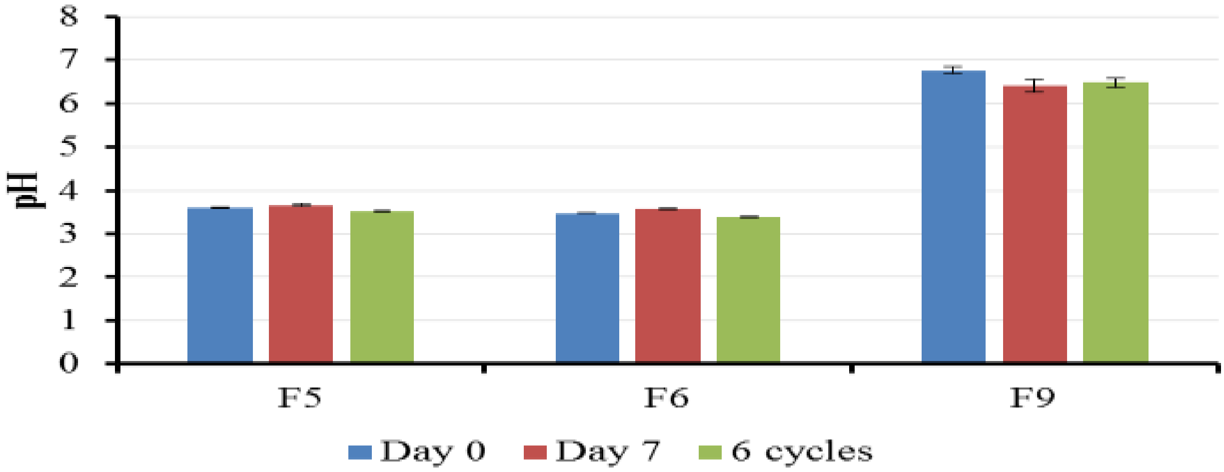

Fig. 2: pH values of each formulation at day 0, 25°C for 7 days and 6 cycles of heating-cooling cycles

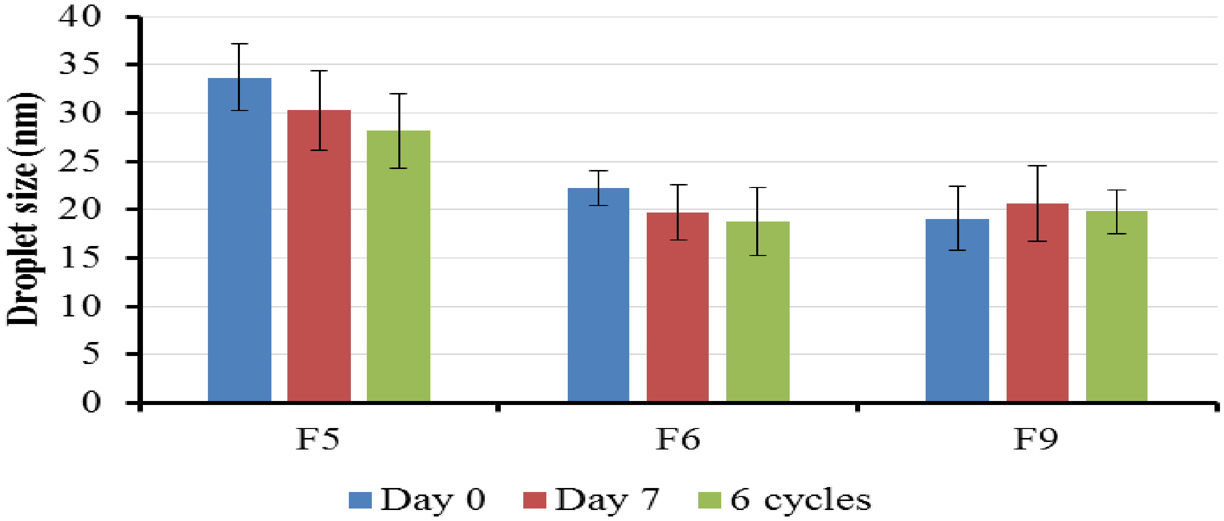

Fig. 3: Nanoemulsions Droplet Size at Day 0, 25°C for 7 days and 6 cycles of Heating-Cooling Cycles

Conclusion

Roselle seed oil extract showed good antioxidant capacity and it could successfully be fabricated to nanoemulsions by the ultrasonication method using 1% of roselle seed oil extract and 5% of tween 60.

The results suggested that nanoemulsions containing roselle seed oil extract were stable after being stored under the heating-cooling cycle. This research studied on physicochemical properties of roselle seed oil extract nanoemulsions. The measurement of toxicity and long-term stability are required to ensure the use of roselle seed extract nanoemulsion in pharmaceutical or cosmetic uses.

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References

1. Arora R, Aggarwal G, Harikumar SL, Kaur K (2014) Nanoemulsion based hydrogel for enhanced transdermal delivery of ketoprofen. Advances in Pharmaceutics, 1-12. doi:10.1155/2014/468456
2. Rinaldi F, Hanieh PN, Longhi C, Carradori S, Secci D, et al (2017) Neem oil nanoemulsions: characterisation and antioxidant activity. J. Enzyme Inhib. Med. Chem., 32(1): 1265-1273. doi:10.1080/14756366.2017.1378190
3. Maruno M, Rocha-Filho PA da (2010) O/W nanoemulsion after 15 years of preparation: a suitable vehicle for pharmaceutical and cosmetic applications. J. Disper Sci. Technol., 31(1): 17-22. doi:10.1080/01932690903123775

4. Mohamed R, Fernández J, Pineda M, Aguilar M (2007) Roselle (Hibiscus sabdariffa) seed oil is a rich source of γ-tocopherol. J. Food Sci., 72(3): S207-S211. doi:10.1111/j.1750-3841.2007.00285.x

5. Thiele JJ, Hsieh SN, Ekanayake-Mudiyanselage S (2005) Vitamin E: critical review of its current use in cosmetic and clinical dermatology. Dermatol Surg, 31: 805-813. discussion 813. doi:10.1111/j.1524-4725.2005.31724

6. Habu JB, Ibeh BO (2015) In vitro antioxidant capacity and free radical scavenging evaluation of active metabolite constituents of Newbouldia laevis ethanolic leaf extract. Biol. Res, 48(1): 16. doi:10.1186/s40659-015-0007-x

7. Pereira T, Guerreiro C, Maruno M, Ferrari M, Rocha-Filho P (2016) Exotic vegetable oils for cosmetic o/w nanoemulsions: in vivo evaluation. Molecules, 21(3):248. doi:10.3390/molecules21030248

8. Pengon S, Chinatangkul N, Limmatvapirat C, Limmatvapirat S (2018) The effect of surfactant on the physical properties of coconut oil nanoemulsions. Asian J. Pharm. Sci., 13(5): 409-414. doi:10.1016/j.ajps.2018.02.005

9. Wu H-Y, Yang K-M, Chiang P-Y (2018) Roselle anthocyanins: antioxidant properties and stability to heat and pH. Molecules 23(6): 13-57. doi:10.3390/molecules23061357

10. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, et al (2011) Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. J. Nanobiotechnology, 9: 44. doi:10.1186/1477-3155-9-44

11. Alliod O, Valour J-P, Urbaniak S, Fessi H, Dupin D, Charcosset C (2018) Preparation of oil-in-water nanoemulsions at large-scale using premix membrane emulsification and Shirasu Porous Glass (SPG) membranes. Colloids Surf, A Physicochem Eng. Asp, 557: 76-84. doi:10.1016/j.colsurfa.2018.04.045

12. Chang Y, McClements DJ (2014) Optimization of orange oil nanoemulsion formation by isothermal low-energy methods: influence of the oil phase, surfactant, and temperature. J. Agric. Food Chem., 62(10): 2306-2312. doi:10.1021/jf500160y

13. Goodarzi F, Zendehboudi S (2019) A comprehensive review on emulsions and emulsion stability in chemical and energy industries. Can J. Chem. Eng., 97(1): 281-309. doi:10.1002/cjce.23336

14. Walstra P (2003) Physical chemistry of foods. Food science and technology. New York: Marcel Dekker, CRC Press. doi:10.1201/9780203910436

15. McClements DJ, Rao J (2011) Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit. Rev. Food Sci. Nutr., 51(4): 285-330. doi:10.1080/10408398.2011.559558