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Research Article

Application of nanotechnology in *Eichhornia crassipes* extracts

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Abstract

Natural extracts have widespread application because some can be used as antioxidants in pharmacological cosmetics. In this study, the ethanol extract of the dried leaves of the *Eichhornia crassipes* was prepared. Nano-emulsion of crude extract was developed by multiple emulsion homogenization techniques. Span 80 and tween 80 were used as emulsifiers in which oil phase and surfactant were virgin coconut oils and nonyl phenol ethoxylate with ratios of water, emulsifier and oil of 80:10:10, 70:20:10 and 70:10:20 and different ratio of co-emulsifier between span 80 and tween 80 at 0:2, 0.5:1.5, 1:1, 1.5:0.5 and 2:0. Emulsion extract was homogenized for 10-60 minutes at 2000-4000 rpm. Particles size and electrokinetic potential of emulsion extracts were determined by dynamic light scattering method. The droplet size was decreased by increasing surfactant concentration and time of homogenization. The smallest droplet size of the nano-emulsion consisting of 80:10:10 of water, emulsifier and oil in emulsifier of tween 80: span 80 at the ratio of 7.5:2.5, homogenized for 60 minutes at 2000-4000 rpm. was in the range of 100 to 400 nm. The nano-encapsulation of E. crassipes extract would be potential for the delivery of bioactive compounds.

Keywords: emulsion, *Eichhornia crassipes* extract

Introduction

Plants extracts are nowadays highly demanded as food, medicine, health and care products. They contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which have properties like anti-inflammatory anti-cancer antioxidant and antibacterial (Kähkönen et al., 1999; Embuscado, 2015). Antioxidant is a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Shahidi & Zhong, 2015; Oroian & Escriche, 2015). *Eichhornia crassipes* is the aquatic weed that is very fast growing plant and causes serious problems (Ganguly et al., 2012). However, E. crassipes extracts have received considerable attention since they provide rich sources of natural bioactive compounds with antimicrobial, antitumor, antiviral and antioxidant activities (Ganguly et al., 2012; Silva et al, 2015; Shanab & Shalaby, 2012).

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The applications of nanotechnology and nanomaterials can be found in cosmetic products including moisturizers, hair care products, makeup and sunscreen. Emulsion is defined as a heterogeneous system, consisting at least two immiscible liquids or phases. One of which is dispersed as droplets in the other liquid, for example, oil dispersed in water (o/w emulsion) or water dispersed in oil (w/o emulsion) (Khan et al., 2011; Solans et al., 2005). The design and development of effective formulations are often referred to in the literature as nanoemulsion (Khan et al., 2011; Solans et al., 2005), miniemulsion (Landfester, 2003) and microemulsion (Solans & García-Celma, 1997). Nanoemulsion is referred as the droplets size range in 20-500 nm (Khan et al., 2011). However, the emulsion preparations still represent some drawbacks such as an instability that can be classified into four phenomenons: flocculation, creaming, coalescence and breaking. Important factors of emulsion properties are formulation and process parameter in the preparation (Gutiérrez et al., 2008; Gupta et al., 2016). The formulation of emulsion consists of oil water and surfactant. Emulsion can be stabilized by adding some emulsifiers such as surfactants, polymers and solid particles (Gupta et al.,2016; Bouchemal et al., 2004; Tadros et al., 2004). The hydrophilic – lipophilic balance (HLB) is one crucial parameter, which conventionally determines the type and stability of emulsion. The combinations of hydrophilic and hydrophobic surfactants can provide better performance than the pure surfactant (Lv et al., 2014; Zhang & Que, 2008; Orafidiya & Oladimeji, 2002; Anton et al., 2008). There are two methods of process parameters in the emulsion preparation, high-energy and low- energy. The high-energy emulsion method is able to make small droplet size and increase emulsion stability. The high-energy emulsification method includes high-shear stirrers, high pressure homogenizers and ultrasound generators. The low-energy emulsion method, making use of internal chemical energy stored in the components by changing the spontaneous curvature of the surfactant, includes emulsion inversion point and phase inversion temperature (Anton et al, 2008; Meesathien & Phromyothin, 2016; Fernandez et al., 2004).

In this work, the ethanol extract of the dried leaves of the *Eichhornia crassipes* was prepared. Nano-emulsion of the crude extract was developed by multiple emulsion homogenization technique. Span 80 and tween 80 were used as emulsifiers in the formulation of emulsion in which oil phase and surfactant were virgin coconut oils and nonyl phenol ethoxylate, respectively.

Materials and methods

Meterials

Eichhornia crassipes leaves were collected from Prawet burirom canal. Ethanol and hexane were obtained as reagent grade chemical from Carlo Erba and used without further purification. Disodium EDTA was obtained from Sigma Aldrich. Vergin coconut oil and propylene glycol were obtained in cosmetic quality. Sorbitan oleate (span 80) polysorbate 80 (tween 80) and nonylphenol ethoxylate (NP9) were obtained as reagent grade chemical from Acros Organics.

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Preparation of *Eichhornia crassipes* extract

Fresh leaves of *Eichhornia crassipes* were cleaned and dried with hot air oven at 40ºC for 24 hours. They were grinded and kept at 8ºC. *Eichhornia crassipes* powder (5 g) was leached with hexane (100 mL) and extracted with ethanol (100 mL) by Sohlxlet extraction at 70ºC for 24 hours. The extraction was concentrated by rotary evaporator at 40 rpm, 500 mbar, 30ºC and kept the crude extract in amber glass bottle at 8ºC.

Emulsion preparation

The emulsions were prepared in the ratio of water, emulsifier and oil as 80:10:10,70:20:10 and 70:10:20 with the different ratios of co-emulsifier between span 80 and tween 80 as 0:2, 0.5:1.5, 1:1, 1.5:0.5 and 2:0. Vergin coconut oil was stirred at speed of 300 rpm in a 100 mL beaker at room temperature. Span 80 and tween 80 were added into the oil phase and then stirred at 300 rpm for 5 min at 80oC, then water was added and stirred at 300 rpm for 30 min. The emulsion was homogenized by homogenizer at 2000-4000 rpm for 10-60 min. Finally, nano-emulsion was analyzed a particle size distribution and pH value.

Results and discussion

The emulsion formulations were prepared in different ratios of water, emulsifier, oil and co-emulsifier which are span 80 and tween 80.

The effect of ratio of water, emulsifier and oil

The pH value of the milky emulsion was measured at 25ºC by pH meter (starter 3100, ohaus). pH values of emulsion are in the range of 6.96 - 7.30 which is neutral. Therefore, it can be used on living organisms and does not irritate the skin. Considering the droplet size, the emulsion formulation of water, emulsifier and oil in the ratio of 80:10:10 presented the particle size smaller than those from the ratio of 70:20:10 and 70:10:20 whereas the co-emulsifier between tween 80 and span 80 in the ratio of 7.5:2.5 presented the smallest size at 1479.1 nm.

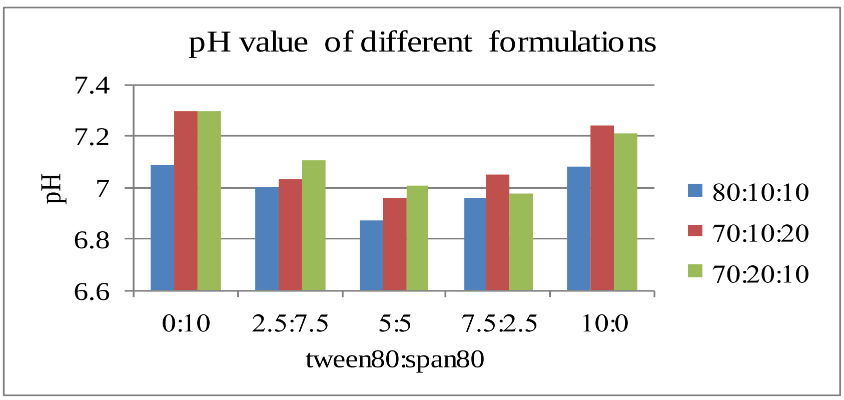

Fig. 1 pH values of different formulations (water: emulsifier: oil)

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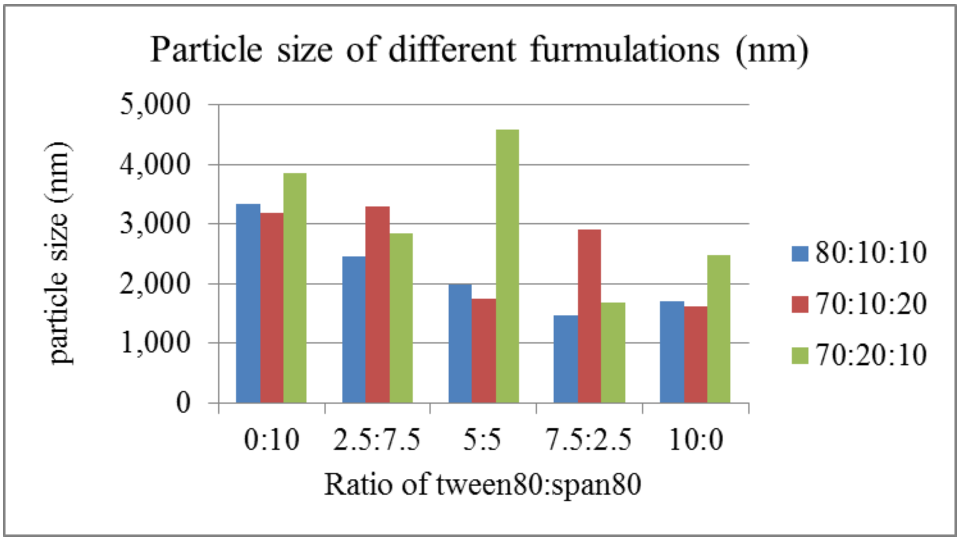

Fig. 2 Particale size (nm) of different formulations (water: emulsifier: oil)

Table 1 Characterization of pH value, Particle size (nm), Physical observation and phase after 24 hours of formulation.

Ratio of water: co-emulsifier: oil	Ratio of Tween80:span80	pH value	Particle size (nm)	Observation	Phase after 24 hour
80:10:10	0:10	7.09	3330.3	Milky emulsion	Separation
	2.5:7.5	7.00	2448.4	Milky emulsion	Separation
	5:5	6.87	1979.0	Milky emulsion	Separation
	7.5:2.5	6.96	1479.1	Milky emulsion	Stable
	10:0	7.08	1702.8	Milky emulsion	Separation
70:10:20	0:10	7.3.0	3177.5	Milky emulsion	Separation
	2.5:7.5	7.03	3297.7	Milky emulsion	Separation
	5:5	6.96	1756.9	Milky emulsion	Stable
	7.5:2.5	7.05	2898.7	Milky emulsion	Separation
	10:0	7.24	1613.3	Milky emulsion	Separation
70:20:10	0:10	7.30	3850.5	Milky emulsion	Stable
	2.5:7.5	7.11	2839.1	Milky emulsion	Stable
	5:5	7.01	4584.2	Milky emulsion	Separation
	7.5:2.5	6.98	1687.8	Milky emulsion	Separation
	10:0	7.21	2477.8	Milky emulsion	Separation

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The parameter of homogenization

The emulsion formulation selected for studying the homogenization parameter contained water:emulsifier:oil in the ratio of 80:10:10 and co-emulsifier with tween 80 and span 80 in the ratio of 7.5:2.5. The parameters of high homogenizer at speed test of 2000 and 4000 rpm for 10, 15, 30 and 60 min were studied. The results showed that the particle size at 10 and 60 min of homogenization at speed test of 2000 rpm was smaller than that of 4000 rpm whereas 15 and 30 min of homogenization at the speed test of 4000 rpm gave smaller particle size than that of 2000 rpm. The particle size obtained from the homogenization at speed test of 2000 and 4000 rpm for 10 min were 1764.5 and 1876 nm, respectively. Increasing the time of homogenization at both 2000 and 4000 rpm to 60 min resulted in the formation of smaller particle size at 302.1 and 312.7 nm., respectively. Therefore, speed and time test increasing of high homogenizer affect nano-emulsion particle size.

Table 2 Particles size of ratio water:co-emulsifier:oil (80:10:10) with the ratio of tween80:span80 (1.5:0.5) at different speed and time of homogenization

Speed (rpm)	Particle size (nm)			
	Time (min.)			
	10	15	30	60
2000	1764.5	1624.9	1044.4	302.1
4000	1876.0	1479.1	909.5	312.7

Conclusion

The best emulsion formulation which was suitable for preparation of nano-emulsion of *Eichhornia crassipes* extract consists of water, emulsifier and oil in a ratio of 80:10:10 and co-emulsifier having tween 80 and span 80 in the ratio of 7.5:2.5 at the homogenization time for 60 min at 2000 or 4000 rpm. The size of emulsion was in the range of 302.1-312.7 nm. High-homogenization parameter leads to smaller droplet size by increasing time or speed of homogenization. The nano-encapsulation of E. crassipes extract would be potential for the further delivery of bioactive compounds.

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References

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S. & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food chemistry*.

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Embuscado, M. E. (2015). Spices and herbs: natural sources of antioxidants–a mini review. *Journal of Functional Foods*, *18*, 811-819.

Shahidi, F. & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of functional foods*, *18*, 757-781.

Oroian, M. & Escriche, I. (2015). Antioxidants: characterization, natural sources, extraction and analysis. *Food Research International*, *74*, 10-36.

Ganguly, A., Chatterjee, P. K. & Dey, A. (2012). Studies on ethanol production from water hyacinth—A review. *Renewable and Sustainable Energy Reviews*, *16*(1), 966-972.

Silva, R. P., de Melo, M. M., Silvestre, A. J. & Silva, C. M. (2015). Polar and lipophilic extracts characterization of roots, stalks, leaves and flowers of water hyacinth (Eichhornia crassipes), and insights for its future valorization. *Industrial Crops and Products*, *76*, 1033-1038.

Shanab, S. M. & Shalaby, E. A. (2012). Biological activities and anticorrosion efficiency of water hyacinth (Eichhornia crassipes). *Journal of Medicinal Plants Research*, *6*(23), 3950-3962.

Khan, B. A., Akhtar, N., Khan, H. M. S., Waseem, K., Mahmood, T., Rasul, A. & Khan, H. (2011). Basics of pharmaceutical emulsions: A review. *African Journal of Pharmacy and Pharmacology*, *5*(25), 2715-2725.

Solans, C., Izquierdo, P., Nolla, J., Azemar, N. & Garcia-Celma, M. J. (2005). Nano-emulsions. *Current opinion in colloid & interface science*, *10*(3), 102-110.

Landfester, K. (2003). Miniemulsions for nanoparticle synthesis. In *Colloid chemistry II* (pp. 75-123). Springer Berlin Heidelberg.

Solans, C. & García-Celma, M. J. (1997). Surfactants for microemulsions. *Current opinion in colloid & interface science*, *2*(5), 464-471.

Gutiérrez, J. M., González, C., Maestro, A., Sole, I., Pey, C. M. & Nolla, J. (2008). Nano-emulsions: New applications and optimization of their preparation. *Current Opinion in Colloid & Interface Science*, *13*(4), 245-251.

Gupta, A., Eral, H. B., Hattona, T. A. & Doyle, P. S. (2016). Nanoemulsions: formation, properties and applications. *Soft Matter.*, *12*(11): 2826-41.

Bouchemal, K., Briançon, S., Perrier, E. & Fessi, H. (2004). Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. *International journal of pharmaceutics*, *280*(1), 241-251.

Tadros, T., Izquierdo, P., Esquena, J. & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in colloid and interface science*, *108*, 303-318.

Lv, G., Wang, F., Cai, W. & Zhang, X. (2014). Characterization of the addition of lipophilic Span 80 to the hydrophilic Tween 80-stabilized emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *447*, 8-13.

Zhang, L. & Que, G. (2008). Influence of the HLB parameter of surfactants on the dispersion properties of brine in residue. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *320*(1), 111-114.

Orafidiya, L. O. & Oladimeji, F. A. (2002). Determination of the required HLB values of some essential oils. *International Journal of Pharmaceutics*, *237*(1), 241-249.

Anton, N., Benoit, J. P. & Saulnier, P. (2008). Design and production of nanoparticles formulated from nano-emulsion templates—a review. *Journal of Controlled Release*, *128*(3), 185-199.

Meesathien, N. & Phromyothin, D. (2016). Preparation and Characterization of Nano-cream from Squalene in olive oil and Virgin Coconut Oil by Nanoemulsions method. *Thai Journal of Nanoscience and Nanotechnology*, *1*(1), 30-36.

Fernandez, P., André, V., Rieger, J. & Kühnle, A. (2004). Nano-emulsion formation by emulsion phase inversion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *251*(1), 53-58.