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Liposomes Encapsulating *Artocarpus lakoocha* Roxb. and *Glycyrrhiza glabra* L. Extracts: Characterization and Shelf Life of Freeze-Dried Vesicles

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Abstract

Liposome is the one way of encapsulation of extracts for reducing the extract degradation. This study was to prepare the liposome entrapped extracts of *Artocarpus lakoocha* Roxb. (L-Al), *Glycyrrhiza glabra* L. (L-Gg) alone and in combination of *A. lakoocha* and *G. glabra* extracts (L-AlGg). The liposomes were prepared by Mechanochemical method and freeze-drying. For stability of liposomes, storage at 4, 25 and 45°C for 8 weeks was performed. The trapping efficiency of liposomes and tyrosinase inhibitory activities of extracts entrapped in liposome were investigated. Results showed liposome morphology was the spherical vesicles evaluated by TEM. Before freeze-drying, liposomes had particle sizes of 156.966 ± 0.808, 140.8 ± 0.818 and 158.633 ± 4.193 nm for L-Al, L-Gg and L-AlGg, respectively. The entrapment efficiency of L-Al, L-Gg and L-AlGg was found to be 95.83 ± 13.48, 97.99 ± 5.23 and 93.90 ± 16.28 %, respectively. The tyrosinase inhibitory activities of released extracts from L-Al, L-Gg and L-AlGg were 81.57 ± 1.22, 68.92 ± 1.23 and 81.40 ± 0.64 %, respectively. After freeze-drying, the particle sizes of L-Al and L-AlGg were no significant changes, while L-Gg particle size was bigger ($p < 0.01$). The liposome entrapment and tyrosinase inhibitory activity of released extracts were not significantly changed after freeze-drying. This indicates good stability and no extract leakage of liposomes. In storage at 4°C for 8 weeks, the entrapment efficiency of L-Al, L-Gg, L-AlGg and tyrosinase inhibitory activity of released extracts were not significantly different, comparing with controls. When increasing temperature of storage effected on the significantly reduction of the entrapment of liposomes and the tyrosinase inhibitory activity of released extracts ($p < 0.01$). Therefore, the freeze-dried liposome and storage at low temperature is recommended for stabilizing liposome and extract quality.

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Introduction

Liposomes are small artificial vesicles of spherical shape that can be formed by the self-assembly of phospholipids in aqueous solution. They have been useful as a drug or food carrier because they are able to retain water-soluble substances in the inner aqueous phase and oil-substances in the bilayer wall (Bangham & Horne, 1964; Bangham et al., 1965; Takahashi et al., 2007). Liposomes also have proved to be applied carriers for gene or compound delivery to cells in culture and in preclinical trials. They are extensively used as carriers for numerous molecules in cosmetic industries and have been studied the use of liposome encapsulation to grow delivery systems that can entrap unstable compounds such as antioxidants, antimicrobials and other bioactive compounds (Inoh et al., 2004; Atrooz, 2011; Benech et al., 2002). Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combinations, and release the entrapped into deep skin (Shehata et al., 2008). This system has been reported to effectively enhance the skin permeation of plant extracts (Tasahashi et al., 2009; Pinsuwan et al., 2010). For freeze-dried liposomes, lyophilization still remains the main studied technique even if examples of marketed lyophilized drug or cosmetic products are very limited. This is due to the complexity of the process since the choice of excipients and process parameters to protect the membrane integrity from stresses due to freezing and dehydration is challenging (Jensen, 2017; Franzé et al., 2018). The cryoprotectants (lactose, trehalose and sucrose) are considered excipients of choice to include in outer aqueous phase of liposomal dispersions for stabilizing the liposome membrane during freeze-drying and reducing the detrimental effects (Viswanathan et al., 2019; Kannan et al., 2015; Wang et al., 2006).

A. lakoocha is a tropical tree widely distributed throughout the regions of Southeast such as Sri Lanka, India, Myanmar, Vietnam, Indonesia, Malaysia and Thailand. *A. lakoocha* has been used for parasite treatment and the ethanolic extract of *A. lakoocha* heartwood was reported to possess antioxidant and tyrosinase inhibitory activities (Sritularak et al., 1998; Singhatong et al., 2010; Panichakul et al., 2018). The main active compounds in *A. lakoocha* heartwood extract are oxyresveratrol and resveratrol (Likhitwitayawuid et al., 2006; Tengamnuany et al., 2006; Panichakul et al., 2018). Encapsulation of *A. lakoocha* extract into liposomes was able to provide prolonged release. A vivo skin whitening study demonstated that the lotion containing *A. lakoocha* extract-loaded liposomes exhibited better skin whitening effects on human volunteers than the lotion containing non-encapsulated *A. lakoocha* extract (Teeranachaideekul et al., 2013).

Licorice, the root of the *Glycyrrhiza glabra* as herbal medicine in Asia and southern Europe is known to have anti-oxidant, anti-melanogenic, anti-inflammatory, anti-viral, anti-allergic and anti-cancer activities (Mukhopadhyay & Panja, 2008; Ha et al., 2013; Han et al., 2013; Bae et al., 2014). Glabridin, the main component of licorice has tyrosinase inhibitory activity to skin-whitening in cosmetic products, and is used in dietary supplements, foods (Simmler et al., 2013). The ethanolic extract of *G. glabra* roots was incorporated in liposomes and hyalurosomes to promote the proliferation and migration of fibroblasts, favouring the closure of the scratched area, and reduced inflammation, favouring the re-epitelization process (Castangia et al., 2015). In addition, Liposomes of *G. glabra* extract had been developed for enhancing the anti-oxidative protection, the immune-modulating activity and anti-tubercular drugs of *G. glabra* (Castangia et al., 2015; Zhang et al., 2013; Viswanathan et al., 2019; Wu et al., 2017).

This study involves the development of liposome dry powder containing extracts of *A. lakoocha, G. glabra* or their mixtures for the cosmetic application. The physicochemical properties of liposomes, such as morphology, size and stability on storage were evaluated.

Materials and methods

1. Plant materials and extraction

A. lakoocha heartwoods (Al) and *G.glabra* roots (Gg), plant powders were purchased from Vejpongosot drugstore in Bangkok, Thailand. The plants powders were extracted in ethanol by maceration modified from previously described (Panichakul et al., 2018). Briefly, 200 g of plant powder was macerated in 1 liter of 95% ethanol at room temperature for 6 h. After three times of maceration, the alcoholic extracts were pooled, filtrated and evaporated under reduced pressure below 45°C. *A. lakoocha* and *G.glabra* extracts were kept at -20°C until used.

2. Determination of total phenolic content

The total phenolic compound in Al and Gg extracts was determined by Folin-Ciocalteu reagent, (Javanmardi et al., 2003). In 96-well plates, 4.5 µl of 1 mg/mL of

samples diluted with 126 µl of deionized water (DI) was mixed with 90 µl of 2% Na_2CO_3 for 3 min and then added with 4.5 µl of 50% Folin-Ciocalteu reagent. After the samples were incubated at room temperature for 30 min, resulting in a blue molybdenum-tungsten complex that was determined at 750 nm by microplate reader (Biochrom EZ Read 2000). The total phenolic content in each sample was calculated by comparing to standard gallic acid and shown as milligrams of gallic acid equivalents to one gram of extract (mg GAE/g extract).

3. Mushroom tyrosinase assay

The inhibition of mushroom tyrosinase activity was determined using L-3,4-dihydroxyphenylalanine (L-DOPA) as a substrate (Ko et al., 2013). Briefly, 20 µl of samples and 140 µl of 20 mM phosphate buffer (pH 6.8), and 20 µl of 461.68 unit/ml of mushroom tyrosinase were added to each well of a 96-well plate and mixed with gentle shaking at room temperature for 10 min. Then, 20 µl of 4 mM L-DOPA was added and incubated at 37 °C for 30 min. The relative amount of dopachrome formed in the mixture was determined at a wavelength of 475 nm by microplate reader (Biochrom EZ Read 2000). Tyrosinase and L-DOPA solution was used as the control and kojic acid at various concentrations of 0.0024 – 1.25 mg/ml was a positive control. The inhibition of tyrosinase activity was calculated and expressed as a percentage of control.

4. Liposome preparation

Liposome preparation was performed by the mechanochemical method using high speed homogenizer modified from a previous report (Takahashi et al., 2007). Soybean lecithin 20 g was added into deionized water 150 ml, and then mixed for 5 min into ultrasonic bath (Transsonic digitals, Elma, USA). This mixture was dispersed by high speed homogenizer (Ultra Turrax T25, IKA Labortechnik, USA) for 10 min at 11,000 rpm. After adding with 10 ml of propylene glycol, 40 ml of 1 g/ml trehalose in water and 1 ml Verstatill PC, the mixture was then continuously homogenized for 15 min at 22,000 rpm. Liposomes were determined particle size by Zetasizer Nanoseries model S4700 (Malvern Instrument, UK).

For preparation of liposomes encapsulating extracts of *A. lakoocha* (L-Al), *G. glabra* (L-Gg) or a combination of Al and Gg extracts (L-AlGg), 0.2 g of Al and Gg alone or combined extracts (Al : Gg at a ratio 9:1) were separately suspended into 10 ml propylene glycol and 70 ml deionized water. Eighty milliliters of extracts and 80 ml of soybean lecithin were mixed for 5 min into ultrasonic bath. The mixtures were dispersed by high speed homogenizer for 10 min at 11,000 rpm, then added with 40 ml of 1 g/ml trehalose and 1 ml Verstatill PC, and continuously homogenized for 15 min at 22,000 rpm, respectively.

5. Measurement of particle size and morphology of liposomes

The Z-average particle size (hydrodynamic diameter), size distribution and polydispersity index (PI) of liposomes were determined by Dynamic laser light scattering method using Zetasizer Nanoseries model S4700 (Malvern Instrument, UK) with a wavelength of 532 nm at 25°C. The scattering angle was fixed at 90°. Prior to analysis, 0.1 ml of fresh or rehydrated liposomes suspension sample was diluted with 10 ml of deionized water. Particle size of liposomes were also monitored during leakage and experiments carried out at 4, 25 and 45°C for 8 weeks to assess the stability of liposome formations in storage conditions. Each measurement was repeated three times.

The morphology of liposomes was detected by Transmission electron microscopy (TEM). Liposome suspension was diluted at 1:10 with deionized water. One drop of diluted sample was left alone on a copper grid for 5 min and stained with 1% phosphotungstic acid for 2 min. After the excess, liquid was absorbed by the filter papers, the stained sample was air-dried at room temperature and then observed under TEM (JEOL, JEM-1400, Japan).

6. Determination of the entrapment efficiency of liposomes

To assess the entrapment efficiency (EE) of L-Al, L-Gg and L-AlGg, a phenolic compound used as a marker was determined by Folin-Ciocalteu assay. Briefly, 1 ml of each liposome suspension was centrifuged at 13,000 rpm for 15 min. The floated liposomes were collected and added 1 ml of ethanol and 1 ml of hexane. After shaking for 15 min, samples were centrifuged at 5,000 rpm for 15 min. The ethanol parts were collected and then determined the total phenolic contents. In 96-well plates, 4.5 µl of samples diluted with 126 µl of deionized water (DI) was mixed with 90 µl of 2 % Na_2CO_3 for 3 min and then added with 4.5 µl of 50 % Folin-Ciocalteu reagent. After 30 min of incubation, a blue molybdenum-tungsten complex was determined at 750 nm by microplate reader (Biochrom EZ Read 2000, UK). The total phenolic content in each sample was calculated by comparing to standard gallic acid. The EE% was calculated from the total phenolic contents of

incorporated extract divided by the total phenolic contents of extracts used at the beginning of preparation multiplied by 100.

7. Lyoprotection of liposomes

Freshly prepared liposomes with or without the adding of extracts were mixed with trehalose. Liposomes suspension was quickly frozen with iced acetone, stored in freezer at -20°C 48 h. After that frozen liposomes were dried for 48 h using Supermodulyo-230 freeze dryer (Thermo, USA) with a condenser temperature of -55°C and pressure at $1\text{-}10^{-1}$ mbar. The dried samples were stored at 4°C.

8. Stability of liposome formation

After lyoprotection of liposomes, the stability of dried liposomes was studied. The samples of dried liposomes kept for 8 weeks at 4, 25 and 45°C were analyzed and compared to freshly prepared liposomes as controls.

Two grams of each dried L-Al, L-Gg and L-AlGg were in glass bottles sealed with foil and then kept at 4, 25 and 45°C for 8 weeks. To determination of particle size of liposome after 8 weeks of storage, 0.1 g of lyophilized liposomes were reconstituted in 400 µl of 2% xanthan gum solution and diluted at 1:100 with deionize water. The liposome suspension was determined particle size by Dynamic laser light scattering method.

After storage for 1, 2, 4, 6 and 8 weeks, one hundred milligrams of dried liposomes were reconstituted in 400 µl of deionize water, added 400 µl of ethanol and 400 µl of hexane. After shaking for 15 min, the mixtures were centrifuged at 5,000 rpm for 5 min. The ethanol part was collected and used to determine total phenolic contents by Folin-Ciocalteu assay and tyrosinase inhibitory activity by mushroom tyrosinase assay. The color of freeze-dried liposomes at 8 weeks was determined by Chroma meter (Konica Minolta, CR-400).

9. Statistical analysis

The data were analyzed by the SPSS version 17. These experiments were expressed as mean values ± standard deviation (SD). Data were subjected to statistical analysis using Paired – Samples T Test and One-way ANOVA, and p values < 0.01 were regarded as significant.

Results and discussion

The total phenolic contents and tyrosinase inhibitory activity of extracts from *A. lakoocha* heartwood (Al) and *G. glabra* root (Gg) were determined. Results showed that the total phenolic contents of Al and Gg alone and in combination with Al and Gg at a ratio of 9:1 were 103.42 ± 0.71, 75.03 ± 0.57 and 97.80 ± 5.16 mg GAE/g extract, respectively. The inhibitory effects of Al and Gg alone and in combined Al and Gg extracts on mushroom tyrosinase were analyzed and presented as the concentration that inhibited 50% of the mushroom tyrosinase activity (IC_{50}) (Table1). Al and Gg alone and in combined Al and Gg extracts inhibited mushroom tyrosinase activity with IC_{50} values of 0.074 ± 0.040, 0.137 ± 0.065 and 0.071 ± 0.028 mg/ml, respectively. These values indicate that the Al, Gg alone and in combined Al and Gg extracts were more potent than kojic acid (IC_{50} = 0.256 ± 0.005 mg/ml) as tyrosinase inhibitors. Al alone and combined Al and Gg extracts had higher tyrosinase inhibitory activities than those of Gg extracts in Table 1. The bioactivity of Al and Gg extracts as known is tyrosinase inhibitory activity (Povichit et al., 2010; Velvizhi & Annapurani, 2018; Panichakul et al., 2018). Our previous reports found that the tyrosinase inhibitory activity of extracts from Al alone and combined Al and Gg extracts was higher than those of Gg extracts (Panichakul et al., 2018).

Table 1 The total phenolic contents and tyrosinase inhibitory activity of extracts from *A. lakoocha* heartwood (Al) and *G. glabra* root (Gg) and in combination of Al and Gg

Extracts	Total phenolic contents mg GAE/g extract	Tyrosinase inhibitory activity (IC_{50}) (mg/ml)
Al	103.42 ± 0.71	0.074 ± 0.040*
Gg	75.03 ± 0.57	0.137 ± 0.065*
Al and Gg	97.80 ± 5.16	0.071 ± 0.028*
Kojic acid	-	0.256 ± 0.005

Remark: Data are mean ± S.D. from three independent experiments.
* indicates significant different at $p < 0.01$.

1. Production of liposomes of Al, Gg alone and in combined Al and Gg extracts

Al and Gg alone and in combination of Al and Gg extracts were encapsulated in liposomes by the mechanochemical method using high speed homogenizer. Liposomes containing extracts were evaluated for particle size and morphology, % entrapment and stability of liposome formation. TEM images showed liposomes were spherical in shape (Fig. 1) as similar as previously described (Rangsimawong & Ngawhirunpat, 2015). The particle sizes of empty liposomes and liposomes entrapped Gg extract (L-Gg) were 138.133 ± 0.35 and 140.8 ± 0.818 nm, respectively that was

slightly smaller than particle sizes of liposomes entrapped with Al alone (L-Al) and in combination of Al and Gg extracts (L-AlGg) (156.96 ± 0.80, and 158.633 ± 4.193 nm, respectively) (Table 2). The entrapping efficiency of the individual liposome, L-Al, L-Gg and L-AlGg was 95.83 ± 13.48, 97.99 ± 5.23 and 93.90 ± 16.28 %, respectively (Table 3). Results indicated that no significantly different efficiency of liposome entrapment of Al and Gg alone and in combination of Al and Gg extracts. According to liposomes composed of phospholipid bilayers and an aqueous cavity has been reported to effectively enhance the skin permeation of plant extracts (Takahashi et al., 2009). Encapsulation of Al and Gg extracts in liposomes was able to enhance the quality of extracts bioactivity as previously described (Teeranachaideekul et al., 2013; Wu et al., 2017).

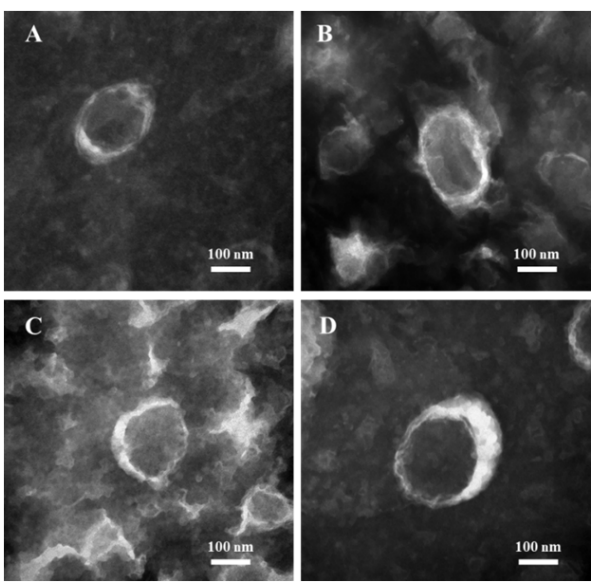

Fig. 1 TEM (Transmission electron microscopy) images of liposomes A) empty liposome; B), C) and D) liposomes entrapped Al, Gg alone and in combined Al and Gg extracts, respectively. Magnification x 300,000

Table 2 Mean particle size and polydispersity index of liposomes before and after freeze-drying

Liposomes	Before Freeze-drying		After Freeze-drying	
	Particle size (nm)	Polydispersity Index : PDI	Particle size (nm)	Polydispersity Index : PDI
L-Empty	138.133 ± 0.350	0.157 ± 0.004	ND	ND
L-Al	156.966 ± 0.808	0.201 ± 0.018	163.5 ± 2.95	0.301 ± 0.021
L-Gg	140.8 ± 0.818	0.154 ± 0.019	151.40 ± 0.96*	0.222 ± 0.009
L-AlGg	158.633 ± 4.193	0.204 ± 0.018	180.6 ± 2.26	0.343 ± 0.014

Remark: Data are mean ± S.D. from three independent experiments.
* indicates significant different at $p < 0.01$.
ND is not done.

Table 3 Percentages of liposome entrapment before and after freeze-drying

Liposomes	% of Encapsulation Efficiency	
	Before freeze-drying	After freeze-drying
L-Al	95.833 ± 13.480	83.333 ± 1.990
L-Gg	97.992 ± 5.230	94.779 ± 5.016
L-AlGg	93.898 ± 16.280	90.847 ± 2.560

Remark: Data are mean ± S.D. from three independent experiments.
* indicates significant different at $p < 0.01$.

2. Freeze-drying of liposomes

To overcome the instability of liposomes, the freeze-drying of liposomes with adding cryprotectants (lactose, trehalose and sucrose) was previously reported. Trehalose at a lipid : cryoprotectant ratio of 1:4 was found to be the most suitable as compared to lactose and sucrose (Viswanathan et al., 2019). In this study, trehalose was used as a cryprotectant to achieve reproducible results after freeze drying liposomes. All freeze dried liposomes, L-Al, L-Gg and L-AlGg (Fig. 2) were redispered on reconstitution and then determined particle size, % entrapment and tyrosinase inhibitory activity as shown in Table 2, 3 and 4. Results showed after freeze drying, there was no significant change in the particle size of L-Al and L-AlGg, but particle size of L-Gg was bigger ($P < 0.01$) (Table 2). In addition, % entrapment and tyrosinase inhibitory activity of released extracts were not significantly changed after freeze-drying reconstitution (Table 3 and 4). These results indicate that freeze-dried liposomes were stable and no significantly extract leakage from liposome particles was found. Thus, the cryprotectants on the nature of freeze-dried liposomes was able to achieve reproducible results after freeze drying of liposomes and reduce the aggregation of liposomes as previously reported (Viswanathan et al., 2019; Mohammed et al., 2006; Yang et al., 2013).

Fig. 2 Before and after freeze-drying of liposomes

Table 4 Tyrosinase inhibitory activity of extracts released from liposomes before and after freeze drying

Liposomes	%Tyrosinase inhibition/ 1 mg/ml extract	
	Before freeze-drying	After freeze-drying
L-Al	81.570 ± 1.220	77.223 ± 1.841
L-Gg	70.340 ± 1.229	68.070 ± 2.500
L-AlGg	81.400 ± 0.640	79.920 ± 1.700

Remark: Data are mean ± S.D. from three independent experiments.
* indicates significant different at $p < 0.01$.

3. Long-term stability of freeze-dried liposomes of L-Al, L-Gg and L-AlGg

Freeze-dried liposomes of L-Al, L-Gg and L-AlGg were stored for 8 weeks at different temperatures of 4, 25 and 45°C for evaluating the liposome stability. The particle size, % entrapment and tyrosinase inhibitory activity were determined in week 1, 2, 4, 6 and 8 as shown in Table 5 and Fig. 3 and 4. Results showed after storage for 8 weeks, at 4, 25 and 45°C, particle sizes of L-Al and L-Gg were significantly changed ($P < 0.01$), compared with those before storage as controls. For L-AlGg, its particle size had no change when was kept at 4°C, but storage at 25, 45°C, the particle size was significantly smaller ($P< 0.01$), compared with a control (Table 5). At 8 weeks of storage, the color of freeze-dried L-Al, L-Gg and L-AlGg kept at 4°C was not changed, while these liposomes kept at 25 and 45°C had more color intensity, compared with controls. The entrapment efficiency of L-Al, L-Gg and L-AlGg evaluated during 8 weeks (at 1, 2, 4, 6, and 8 week) was found that all liposomes kept at 4°C had no significantly difference ($P < 0.01$), compared with those before storage as controls. At 25 and 45°C, the % entrapment of all liposomes was reduced as shown in Fig. 3. In addition, tyrosinase inhibitory activity of released Gg and in combined Al and Gg extracts had no change when kept at 4°C compared with controls, while the released-Al extract had significantly reducing tyrosinase inhibitory activity ($P < 0.01$). When temperature of storage was up to 25 and 45°C, the tyrosinase inhibitory activity of all released extracts was significantly reduced, compared with those activities of extracts as controls ($P < 0.01$) (Fig. 4). This indicates that the increasing temperature for liposome storage was able to effect on instability of liposomes while the low temperature, at 4°C is still to keep the quality of liposomes. As a previous report, auto-oxidation of beta-carotene in liposomes kept at low temperature (4°C) was reduced beta-carotrene (Moraes et al., 2013). Interestingly, the combination of Al and Gg extracts could be encapsulated in the same vesicle of liposomes. The bioactivity of released L-AlGg extracts in tyrosinase inhibition was the same level before storage, while tyrosinase inhibitory activity of released- L-Al extracts after storage was reduced. Therefore, the encapsulation of combined *A. lakoocha* and *G. glabra* extracts in liposome is a considered technique for liposome development. In further study, liposomes of Al, Gg alone or in combination of Al and Gg extracts will be applied to develop cosmetic products and evaluation of the efficiency in human volunteer. Finally, this finding is an alternative way for improving quality and enhancing the shelf-life of liposomes.

Table 5 Mean particle size and polydispersity index of liposomes before and after storage at 4, 25 and 45°C for 8 weeks

Liposomes	Temperature (°C)	Before storage		After storage for 8 weeks	
		Particle size (nm)	PDI	Particle size (nm)	PDI
L-Al	4	163.5 ± 2.959	0.30 ± 0.021	184.7 ± 4.222*	0.33 ± 0.005
	25			201.3 ± 1.4*	0.39 ± 0.007
	45			341.633 ± 3.394*	0.370 ± 0.070
L-Gg	4	151.4 ± 0.960	0.222 ± 0.010	168.267 ± 1.193*	0.288 ± 0.026
	25			170.067 ± 0.709*	0.39 ± 0.018
	45			135.933 ± 2.138*	0.255 ± 0.019
L-AlGg	4	180.6 ± 2.260	0.34 ± 0.014	186.367 ± 3.360	0.33 ± 0.025
	25			163.267 ± 2.272*	0.32 ± 0.022
	45			149.367 ± 1.157*	0.304 ± 0.032

Remark: Data are mean ± S.D. from three independent experiments.
* indicates significant different at $p < 0.01$.

Conclusion

The encapsulation of Al and Gg alone or in combination of Al and Gg extracts in liposomes is an alternative way to enhancing the delivery systems of extracts. The long-term stability of liposomes can be improved by formulating the systems as freeze-dried products. To reduce liposome aggregation, carbohydrates such as trehalose is required in systems of freeze-drying. In addition, the low temperature of liposome storage is able to keep liposome quality.

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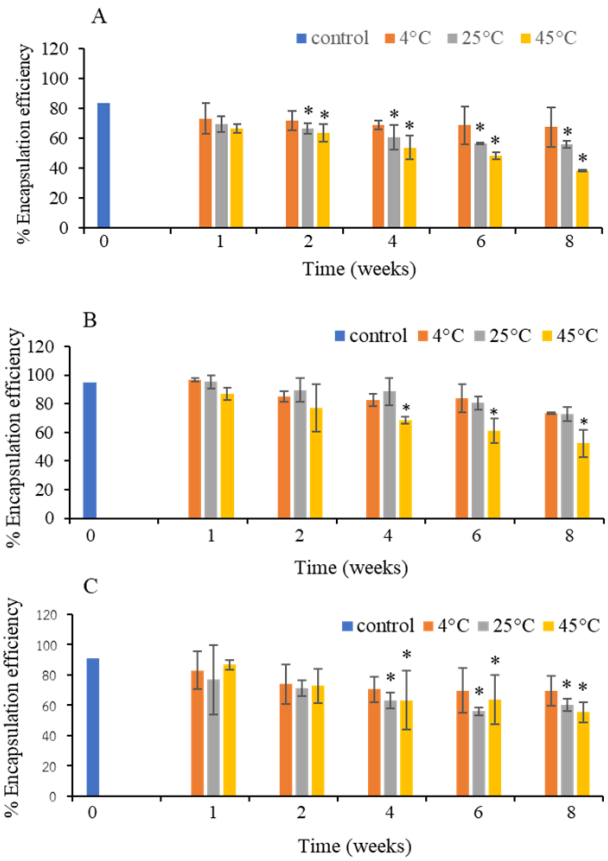

Fig. 3 Encapsulation efficiency of L-Al, L-Gg and L-AlGg after kept for 8 weeks at 4, 25 and 45°C. A) L-AL, B) L-Gg and C) L-AlGg, liposomes were determined the % entrapment in 1, 2, 4, 6 and 8 weeks. Data are mean ± S.D. from three independent experiments. * indicates significant different at $p < 0.01$

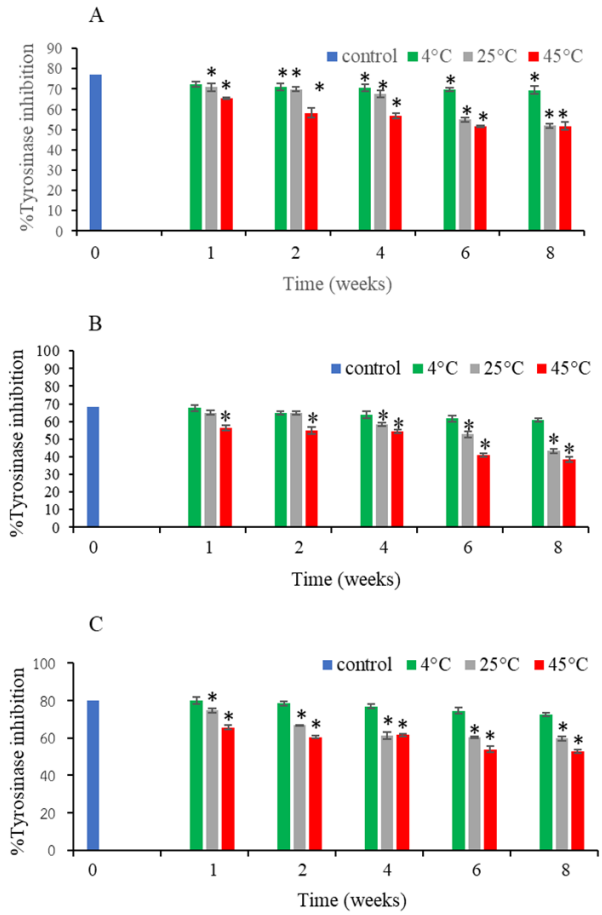

Fig. 4 Tyrosinase inhibitory activity of released extracts from L-Al, L-Gg and L-AlGg after kept for 8 weeks at 4, 25 and 45°C. A) L-AL, B) L-Gg and C) L-AlGg, liposomes were evaluated the % of tyrosinase inhibitory activity in 1, 2, 4, 6 and 8 weeks. Data are mean ± S.D. from three independent experiments. * indicates significant different at $p < 0.01$

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References

Atrooz, O.M. (2011). Efects of alkylresorcinolic lipids obtained from acetonic extract of Jordanian wheat grains on liposome properties. *International Journal of Biological Chemistry, 5*(5), 314-321.

Bae, J.Y., Jang, H.N., Ha, J.H., & Park, S.N. (2014). Antimicrobial activities of licorice extracts from various countries of origin according to extraction conditions. *Korean Journal of Microbiology and Biotechnology, 42*(4), 361-366.

Bangham, A.D, Standish, M.M., & Watkins, J.C. (1965). Diffusion of univalent ions across the lamellae of swollen phospholipids. *Journal of Molecular Biology, 13,* 238-252.

Bangham, A.D., & Horne, R.W. (1964). Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. *Journal of Molecular Biology, 8,* 660-668.

Benech, R.O., Kheadr, E.E., Laridi, R., Lacroix, C., & Fliss, I. (2002). Inhibition of *Listeria innocua* in cheddar cheese by addition of nisin Z in liposomes or by in situ production in mixed culture. *Applied and Environmental Microbiology, 68,* 3683-3690.

Castangia, I., Caddeo, C., Manca, M.L., Casu, L., Latorre, A.C., Díez-Sales, O., ... Manconi, M. (2015). Delivery of liquorice extract by liposomes and hyalurosomes to protect the skin against oxidative stress injuries. *Carbohydrate Polymers, 134*(10), 657-663.

Franzé, S., Selmin, F., Samaritani, E., Minghetti, P., & Cilurzo, F. (2018). Lyophilization of liposomal formulations: Still necessary, still challenging. *Pharmaceutics, 10*(3), 139.

Ha, J.H., Lee, H.M., Kwon, S.S., Kim, H.S., Kim, M.J., & Jeon, S.H. (2013). Screening of effective extraction conditions for increasing antioxidant activities of licorice extracts from various countries of origin. *Journal of Society of cosmetic scientists of Korea, 39*(4), 259-269.

Han, S.B., Gu, H.A., Kim, S.J., Kim, H.J., Kwon, S.S., & Kim, H.S. (2013). Comparative study on antioxidative activity of *Glycyrrhiza uralensis* and *Glycyrrhiza glabra* extracts by country of origin. *Journal of Society of cosmetic scientists of Korea, 39*(1), 1-8.

Inoh, Y., Kitamoto, D., Hirashima, N., & Nakanishi, M. (2004). Biosurfactant MEL-A dramatically increases gene transfection via membrane fusion. *Journal of Controlled Release, 94,*423-431.

Javanmardi, J., Stushnoff, C., Lockeb, E., & Vivanco, J.M. (2003). Antioxidant activity and total phenolic content of Iranian Ocimum accessions. *Food Chemistry, 83*(4), 547-550.

Jensen, G.M. (2017). The care and feeding of a commercial liposomal product: Liposomal amphotericin B (AmBisome). *Journal of Liposome Research, 27,* 173–179.

Kannan, V., Balabathula, P., Thoma, L.A. & Wood, G.C. (2015). Effect of sucrose as a lyoprotectant on the integrity of paclitaxel-loaded liposomes during lyophilization. *Journal of Liposome Research, 25,* 270–278.

Ko, H.H., Tsai, Y.T., Yen, M.H., Lin, C.C., Liang, C.J., Yang, T.H., . . . Yen, F.L. (2013). Norartocarpetin from a folk medicine *Artocarpus communis* plays a melanogenesis inhibitor without cytotoxicity in B16F10 cell and skin irritation in mice. *BMC Complementary and Alternative Medicine, 13,* 348, doi:10.1186/1472-6882-13-348.

Likhitwitayawuid, K., Sornsute, A., Sritularak, B., & Ploypradith, P. (2006). Chemical transformations of oxyresveratrol (trans-2,4,3′,5′-tetrahydroxystilbene) into a potent tyrosinase inhibitor and a strong cytotoxic agent. *Bioorganic & Medicinal Chemistry Letters, 16,* 5650-5653.

Mohammed, A.R., Bramwell, B., Coombes, A.G.A., & Perrie, Y. (2006). Lyophilisation and sterilization of liposomal vaccines to produce stable and sterile products. *Methods, 40,* 30-38.

Moraes, J.M., Carvalho, M.P., Silva, C.R., Cho, S., Sola, M.R., & Pinho, S.C. (2013). Liposomes encapsulating beta-carotene produced by the proliposomes method: characterisation and shelf life of powders and phospholipid vesicles. *International Journal of Food Science & Technology, 48,* 274–282.

Mukhopadhyay, M. & Panja, P. (2008). A novel process for extraction of natural sweetener from licorice (*Glycyrrhiza glabra*) roots. *Separation and Purification Technology, 63*(3), 539-545.

Panichakul, T., Rodboon, T., Suwannalert, P., Rungruang, R., Boohuad, N., Tripetch, C., & Youdee, P. (2018). *Study of activities of Glycyrrhiza glabra and Artocarpus lakoocha to inhibit melanogenesis for developing whitening cream.* Bangkok: Suan Dusit University.

Panichakul, T., Youdeeb, P., Boohuada, N., Itsarasooka, K., & Suwannalert, P. (2019). *Study of activities of Glycyrrhiza glabra and Artocarpus lakoocha to inhibit melanogenesis for developing whitening cream)*. Bangkok: Suan Dusit University.

Pinsuwan, S., Amnuaikit, T., Ungphaiboon, S., & Itharat, A. (2010). Liposome-containing *Hibiscus sabdariffa* Calyx extract formulations with increased antioxidant activity, improved dermal penetration and reduced dermal toxicity. *Journal of the Medical Association of Thailand, 93,* S216-226.

Povichit, N., phrutivorapongkul, A., Suttajit, M., & Leelapornpisid, P. (2010). Antiglycation and antioxidant activities of oxyresveratrol extracted from the heartwood of *Artocarpus lakoocha* Roxb. *Maejo International Journal of Science and Technology, 4*(03), 454-461.31.

Rangsimawong, W., & Ngawhirunpat, T. (2015). Characterization of liposomes for transdermal drug delivery system. *Thai Bulletin of Pharmaceutical Sciences, 10*(1), 61-74.

Shehata, T., Ogawara, K., Higaki, K., & Kimura, T. (2008). Prolongation of residence time of liposome by surface-modification with mixture of hydrophilic polymers. *International Journal of Pharmaceutics, 359*(1-2): 272-9.

Simmler, C., Pauli, G.F., & Chen, S. (2013). Phytochemistry and biological properties of glabridin. *Fitoterapia, 90,* 160-184.

Singhatong, S., Leelarungrayub, D., & Chaiyasut, C. (2010). Antioxidant and toxicity activities of *Artocarpus lakoocha* Roxb. heartwood extract. *Journal of medicinal plant research, 4*(10), 947-953.

Sritularak, B., De-Eknamkul, W., & Likhitwitayawuid, K. (1998). Tyrosinase inhibitors from *Artocarpus lakoocha. Thai Journal of Pharmaceutical Sciences, 22,* 149-155.

Takahashi, M., Inafuku, K.I., Miyagi, T., Oku, H., Wada, K., Imura, T., & Kitamoto, T. (2007). Efficient preparation of liposomes encapsuling food materials using lecithins by a mechanochemical method. *Journal of Oleo Science, 56*(1), 35-42.

Tasahashi, M., Kitamoto, D., Asikin, Y., Takara, K., & Wada, K. (2009). Liposome encapsulating Aloe vera leaf gel extract significantly enhance proliferation and collagen synthesis in human skin cell lines. *Journal of Oleo Science, 58,* 643-650.

Teeranachaideekul, V., Nithitanakool, S., Junhunkil, T., Ponpanich, L., Nopporn, N., Detamornrat, U., & Chulasiri, M. (2013). Liposomes: A novel carrier system for *Artocarpus lakoocha* extract to improve skin whitening. *Journal of Asian Association of Schools of Pharmacy, 2,* 243-253.

Tengamnuay, P., Pengrungruangwong, K., Pheansri, I., & Likhitwitayawuid, K. (2006). *Artocarpus lakoocha* heartwood extract as a novel cosmetic ingredient: evaluation of the in vitro anti-tyrosinase and in vivo skin whitening activities. *International Journal of Cosmetic Science, 28,* 269–276.

Velvizhi, S. & Annapurani, S. (2018). Estimation of total flavonoid, phenolic content, and free radical scavenging potential of *Glycyrrhiza glabra* root extract. *Asian Journal of Pharmaceutical and Clinical Research, 11*(4), 231.

Viswanathan, V., Pharande, R., Bannalikar, A., Gupta, P., Gupta, U., & Mukne, A. (2019). Inhalable liposomes of *Glycyrrhiza glabra* extract for use in tuberculosis: formulation, in vitro characterization, in vivo lung deposition, and in vivo pharmacodynamic studies. *Drug Development and Industrial Pharmacy, 45*(1), 11-20.

Wang, T., Deng, Y., Geng, Y., Gao, Z., Zou, J., & Wang, Z. (2006). Preparation of submicron unilamellar liposomes by freeze-drying double emulsion. *Biochimica et Biophysica Acta, 1758,* 222–231.

Wu, Y., Yi, L., Li, E., Li, Y., Lu, Y., Wang, P., … Wang, D. (2017). Optimization of Glycyrrhiza polysaccharide liposome by response surface methodology and its immune activities. *International Journal of Biological Macromolecules, 102,* 68-75.

Yang, S., Liu, C., Liu, W., Yu, H., Zheng, H., Zhou W., & Hu, Y. (2013). Preparation and characterization of nanoliposomes entrapping medium-chain fatty acids and vitamin c by lyophilization. *International Journal of Molecular Sciences, 14,* 19763-19773.

Zhang, X., Xiong, Y.L., Chen, J., & Zhou, L. (2013). Synergistic inhibition of lipid oxidation by pea protein hydrolysate coupled with licorice extract in a liposomal model system. *Journal of Agricultural and Food Chemistry, 61*(35), 8452-61.