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Salak Plum Peel Extract as a Safe and Efficient Antioxidant Appraisal for Cosmetics

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The antioxidant activities of Salak plum (*Salacca edulis*) peel extracts were assessed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′–azino-bis(3-ethylbenzothiaizoline)-6-sulfonic acid (ABTS), and ferric reducing ability of plasma (FRAP) assays. The ethyl acetate (EtOAc) fraction was the most potent ($DPPH_{IC50}$ = 2.932 ± 0.030 μg/mL, $ABTS_{IC50}$ = 7.933 ± 0.049 μg/mL, $FRAP_{EC}$ = 7,844.44 ± 40.734). Chlorogenic acid was detected as the marker (1.400 ± 0.102 g/kg). The EtOAc fraction was non-cytotoxic in vero and normal human fibroblast (NHF) cells. It exhibited cellular oxidative prevention and damage treatment at 5–40 μg/mL in NHF cells. Salak plum peel loaded liposome consisting of lecithin and hydrophobically modified hydroxyethylcellulose (HMHEC) was developed and found stable with adequate entrapment efficacy. Thus Salak plum peel was highlighted as a potential ecological antioxidant for health promotion aspects, and for cosmetics.

Key words: cellular antioxidant; chlorogenic acid; salak plum; *Salacca edulis*; liposome

Human gain antioxidants from dietary supplements to suppress the adverse effects of metabolized and polluted free radicals on health. Fruits, vegetables, and beverages are the main sources of antioxidants having biological, physiological, and therapeutic effects.[1,2] High consumption of fruits and vegetables provides more health benefits,[3] including anti-aging effects.[4] Antioxidants scavenge free radicals, limiting oxidative damage to cellular proteins, and lipids, and carbohydrates alleviate cell death.[5] Apoptosis and necrosis of human epidermis cells including fibroblasts[6,7] induced by free radicals propagate oxidants from lipid peroxidation generating, inflammatory cytokines, that accumulate in the skin.[8] Adverse reactions of oxidants also deactivate collagenase and elastase, degrading collagen and elastin as well.[9] Antioxidants are therefore regarded as an efficient in treating aging.[9] In addition to fruits, and vegetables, their residues and non-edible parts are regarded as potential sources of antioxidants, with respect to enrichment of polyphenols.[10,11] The application of antioxidants from biological sources is widely accepted as a safe and efficient treatment of aging.[9,12] Those of ecological products that are well known in terms of organic or green products, are therefore highly in demand, including cosmetics.[13]

Several tropical and subtropical fruits are well known for their health protective and promotion effects. New exotic fruits possess biological activities that are important for human health, are being highly investigated, including Salak plum, or snake fruit (*Salacca edulis*). Salak plum is an edible plant in the Plamae family with a scaly brown oval shape. This potential antioxidant fruit is widely cultivated in Southeast Asia.[14] Its pulp is consumed fresh in Thailand and prepared as a dessert popularly served during the summer. The fruit was found to reduce cholesterol in rodents,[15] which is relevant to its strong antioxidant activity, evaluated by means of ABTS[16] and DPPH assays to be comparable to kiwi fruit.[17] Its biological activities are contributed by polyphenols,[15–17] chlorogenic acid and catechins.[16] However, biological activity of the peel has never been presented, including characterization of the polyphenols.

Thus Salak plum peel was first extracted, and evaluated comparatively as to antioxidant activity by means of DPPH, ABTS, and FRAP assays. The polyphenols were characterized. The safety of the extract was examined against vero, and normal human fibroblast cells. Cellular antioxidant activities relating to skin wrinkles were conducted in human fibroblast cells. Salak plum peel extract was further encapsulated in liposome. The physical and chemical stabilities of the developed nanoparticles were evaluated at different temperatures for 1 and 3 months. This ecological antioxidant was appraised for health care and cosmetic applications both in the extract form and as nanoparticles.

Materials and Methods

Salak plum peel extract preparation. Ripped Salak plum fruits (Sumalee cultivar) were purchased from a local market in Chiang Rai, Thailand. This plant material was identified by Dr. Nijsiri Ruangrungsri, Faculty of Pharmaceutical Sciences, Department of Pharmacognosy, Chulalongkorn University, Bangkok, Thailand. The voucher specimen (MKSP 0511) was deposited in our laboratory

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Abbreviations: ABTS, 2,2′–azino-bis(3-ethylbenzothiaizoline)-6-sulfonic acid; Aq, aqueous; DPPH, 1, 1-diphenyl-2-picrylhydrazyl; EE, entrapment efficacy; EtOH, ethyl alcohol; EtOAc, ethyl acetate; FRAP, ferric reducing ability of plasma; HMHEC, hydrophobically modified hydroxyethylcellulose; IC_{50}, inhibitory concentration at 50%; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NHF, normal human fibroblast; TPTZ, 2,4,6-tri(2-pyridyl)-*S*-triazine

herbarium at Mae Fah Luang University, Chiang Rai. The sharply pointed needles of the fruit hulls were removed prior to removal of the peels from the pulp. The peels were air dried and ground into powder (143.38 g). The powder was extracted with 70% EtOH (1,000 mL) with shaking (150 rpm) for 24 h at ambient temperature. The filtrate was concentrated to dryness under reduced pressure and the residue was extracted for 2 more times. The obtained crude 70% EtOH extract was defatted with *n*-hexane and further partitioned with EtOAc giving *n*-hexane and EtOAc fractions respectively. The aqueous layer was lyophilized, yielding an aqueous fraction (Aq.).[18)]

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. A serial dilution of samples was prepared in absolute EtOH (Merck, Darmstadt, Germany). Ascorbic acid (Fluka, Missouri, USA) was used as standard to prepare a calibration curve ($r > 0.999$). The ability to scavenge the stable free DPPH radical (Fluka) was tested by mixing a portion of the sample solution with an equal volume of DPPH (6×10^{-5} M), and was allowed to react in a light protected vessel for 30 min at room temperature. Reduction of the DPPH radical was monitored at 517 nm by microplate reader (ASYS, UVM340, Cambridge, UK). The concentration of the samples causing 50% inhibition (IC_{50}) of DPPH radical was compared with the standard, ascorbic acid. All determinations were done in triplicate.[18)]

2,2′–Azino-bis(3-ethylbenzothaiazoline)-6-sulfonic acid (ABTS) radical scavenging activity. A stock solution containing 7 mM ABTS (Fluka) and 2.450 mM potassium persulfate (Fluka) stored in a light protected vessel for 16 h at ambient temperature was diluted with EtOH (Merck). The working solution, having an absorbance of 0.700 ± 0.200 at 750 nm, was applied in the assay. The absorbance at 750 nm of the mixture of samples (20 µL), and the working ABTS solution (180 µL) was determined following 5 min of mixing. Antioxidant activity was calculated. The IC_{50} of ascorbic acid was compared to the IC_{50} of the extracts. All measurements were done in triplicate.[18)]

Ferric reducing ability of plasma (FRAP). FRAP reagent was prepared in 2,4,6-tri(2-pyridyl)-*S*-triazine (TPTZ) solution (10 mM, 2.5 mL) with 40 mM HCl, $FeCl_3$ (20 mM, 2.5 mL), and acetate buffer pH 3.6 (0.3 M, 25 mL) (Fluka). The samples (3 µg/mL, 20 µL) in EtOH were reacted with FRAP reagent (180 µL). Absorbance was recorded by microplate reader at 595 nm. Reducing power was expressed as equivalent concentration (EC) to that of 1 mM $FeSO_4$ (Fluka), and ascorbic acid was used as positive control. The assay was determined in triplicate.[18)]

Standardization of polyphenols in the extracts. HPLC analysis was carried out on a Waters 2695 equipped with a Waters 2996 photodiode array detector (measured at 250 nm) using a reversed phase column (Alltech, Illinois, USA, Prevail C_{18} 5 µm, 250 × 4.6 mm) and a guard column (Alltech, Prevail all-guard cartridge C_{18} 5 µm, 7.5 × 4.6 mm). All solvents and standards were of HPLC grade. Ferulic acid, caffeic acid, gallic acid, kojic acid, chlorogenic acid, rosmarinic acid, and quercetin (Fluka) at various concentrations in AcCN (Labscan, Gliwice, Ireland) were adopted to prepare calibration curves ($r > 0.999$). The samples were successively separated by a gradient mobile phase consisting of AcCN (A) and 3% aq. AcOH (B). The eluent was programmed as follows: 0–3 min 100% B, 3–5 min 85% B, 5–10 min 80% B, 10–15 min 75% B, 15–20 min 70% B, and 20–30 min 50% B at a flow rate of 1 mL/min. Characterization of the polyphenols in the fractions (1 mg/mL) was done in 3 cycles. The analyzed content was converted to g/kg of crude extract.[18)]

Toxicity in vero cells. Safety of the extracts was evaluated by cytotoxicity against vero cells obtained from the National Center for Genetic Engineering and Biotechnology of Thailand. Toxicity tests were conducted by the green fluorescent protein detection method, and compared to elliptine.[19)]

Toxicity in normal human fibroblast (NHF) cell culture. NHF cell lines obtained from the National Nanotechnology Center of Thailand were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco, New York, USA) supplemented with 10% Fetal Bovine Serum (FBS; Gibco), 1% L-glutamine (Gibco), and 1% penicillin G/streptomycin (Gibco) in a 5% CO_2 chamber at 37 °C. Cytotoxicity assessments were performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT (USB, USA) assay. NHF cells (8×10^3 cells/well) were seeded and incubated in a 5% CO_2 incubator at 37 °C. The cells were treated with the Salak plum peel EtOAc fraction (0.1–50 µg/mL), incubated for 24 h, and replaced with fresh medium containing MTT solution (5 mg/mL), and further incubated for 4 h. The medium containing MTT was removed. The formazan crystals that formed in the viable cells were solubilized by DMSO (Sigma, Missouri, USA). Absorbance was recorded at 550 nm by microplate reader. The untreated cells were used as control. Cell viability (%) was calculated, and that of more than 75% compared with the control (100% cell viability) was interpreted as non-cytotoxic.[18)]

Cellular oxidative damage treatment. NHF cells were damaged by H_2O_2 (Merck) due to its pivotal role in generating reactive oxygen species from nearly all sources of oxidative stress, and oxygen radicals can diffuse freely in and out of cells and tissues.[6,20)] NHF cells (8×10^3 cells/well) were treated as above. Cytotoxicity was induced by H_2O_2 (1×10^{-3}–1×10^3 µg/mL) for 24 h. The untreated cells were used as control. The treatment of cells, and determination of cell viability was conducted as above. The concentration of H_2O_2 causing cell damaged was obtained. Thus that concentration was applied to treat NHF cells (8×10^3 cells/well) for 24 h, and the cells were treated with the samples for a further 24 h. Determination of cell viability was conducted as above.[18,21)]

Cellular oxidative prevention. The samples were mixed with H_2O_2 at the induced cell toxicity concentration. Incubation and treatment were performed similarly to the determination of cell viability, as done previously.[18,21)]

Preparation of Salak plum peel EtOAc fraction loaded liposome. Liposome composed of hydrophobically modified hydroxyethylcellulose (HMHEC; Natrosol, Kentucky, USA) and soybean lecithin (Epikuron 200, Hamburg, Germany) were prepared by a modified thin film-hydration technique from the literature.[22)] Briefly, lecithin was dissolved in diethyl ether, evaporated to dryness under reduced pressure (250 mmHg) at 45 °C for 15 min, and kept in a desiccator overnight to remove the remaining ether. HMHEC, and the Salak plum peel EtOAc fraction were dispersed in phosphate buffered saline (pH 7.4). The liposome was resuspended in the mixture with shaking at 300 rpm for 1 h. The particle size of the Salak plum liposome was reduced by means of sonication at 22–25 °C at 100 W (Vcx 130 pb, Sonics and Materials, Connecticut, USA) for 5 min, and pause for 1 min. Sonication was repeated for 3 cycles. The physicochemical properties of the liposome containing various proportions of HMHEC and lecithin, which are particle size, PDI (Polydispersity index), and zeta potential were measured by Nanosizer (Zetasizer Nano Z, Malvern, Worcestershire, UK).

Entrapment efficacy of the liposome. The efficacy of liposome to entrap Salak plum peel extract was evaluated by a modified ultracentrifugation method at 60,000 × *g* at 4 °C (Optimal-100k 220/240 vac, Beckman, Indiana, USA) for 2 h in an order to isolate the incorporated active form from the free form.[23,24)] The chlorogenic acid content of the supernatant was analyzed by HPLC. Entrapment efficacy (%) was calculated as follows: $[(T - t)/T] \times 100$, where T is total load active and t is active in free form of the supernatant.

Stability evaluation of the liposome. Particle size and PDI of each Salak plum liposome were determined following storage under different temperatures (5, 25, and 45 °C) in light protected vials for 1 and 3 months.[25)]

Statistical analysis. Results are reported as mean ± SD. Significance of differences was determined by SPSS for Windows. ANOVA and pair *t* test at $p < 0.05$ was regarded as statistically significant.

Table 1. Biological Activities of Salak Plum Peel Extracts

Samples	Antioxidant		
	DPPH (IC_{50}, μg/mL)	ABTS (IC_{50}, μg/mL)	FRAP (EC)
70% EtOH	8.870 ± 0.030	22.479 ± 0.152	1,848.889 ± 20.367
EtOAc	2.932 ± 0.030	7.933 ± 0.049	7,844.444 ± 40.734
Aq.	4.124 ± 0.030	21.363 ± 0.014	2,186.667 ± 35.277
Ascorbic acid	3.404 ± 0.025	6.347 ± 0.087	6,214.689 ± 28.249

Results and Discussion

Extraction, antioxidant activities, and standardization of Salak plum peel

Removal of the biologically active fractions of the Salak plum peels was conducted by an economically feasible method.[18] The peels were first extracted with 70% EtOH, which afforded the crude extract with the highest yield (13.13%), followed by Aq., EtOAc and *n*-hexane fractions (0.78, 0.62, and 0.16%). The scavenging of oxidants causing cellular damage causing aging has been further evaluated.[8,21] Antioxidant activities were comparatively assessed by DPPH, ABTS, and FRAP assays based on a single electron transferring mechanism to terminate the chain reaction of free radicals.[26] The *n*-hexane fraction was excluded from biological activity assessments, as this non-polar solvent was adopted for cleanup of fat. The entire fraction had antioxidant potential to scavenge the DPPH radical (Table 1), in particular the EtOAc fraction, which was significantly more potent than ascorbic acid ($p < 0.05$). The radical cation ($ABTS^{\bullet+}$) terminating efficacy of the Salak plum peel fractions were less than the positive control.

The EtOAc fraction was the strongest fraction, although its inhibitory effect against lipid peroxidation was exhibited by ABTS assay. FRAP reducing power was assessed to indicate the efficiency of the fractions to reduce the oxidized intermediates of the lipid peroxidation process. This assessment confirmed that the EtOAc fraction was the most active reducing agent, significantly better than ascorbic acid ($p < 0.05$). Thus Salak plum peel antioxidant can act as primary and secondary antioxidant.[18]

The antioxidant activities of Salak plum peel assessed by different assays were correlated. ABTS was positively correlated to FRAP ($r = 0.9996$) confirming a lipid peroxidation inhibitory effect as assessed by ABTS and FRAPS assays. In contrast, the correlation between DPPH, and other assays was found to be moderate ($r_{ABTS} = 0.4981$, $r_{FRAP} = 0.4793$). Polyphenols scavenge $DPPH^{\bullet}$ by a single electron transferring mechanism *via* the hydrogen atom. $ABTS^{\bullet+}$ scavenger functions through one anion radical or two neutral radicals, or a two-electron transferring mechanism. Therefore, polyphenols having greater molecular weights with less hydroxyl substitution more strongly terminate cation radical explicit reducing power.[27] In order to reveal the influence of antioxidant polyphenols in Salak plum peel extracts, characterization was conducted by means of HPLC. Identification of the polyphenols was delineated by absorption wavelength and retention time with a mobile phase system that affords an extracts' fingerprint. Chlorogenic acid was analyzed, as it has been reported in Salak plum pulps.[16] Other phenolics that are becoming of interest as biologically active agents in pharmaceutical and cosmetic products, gallic, caffeic, ferulic, and rosmarinic acids and quercetin,[1,2] (Fig. 1) were also characterized.

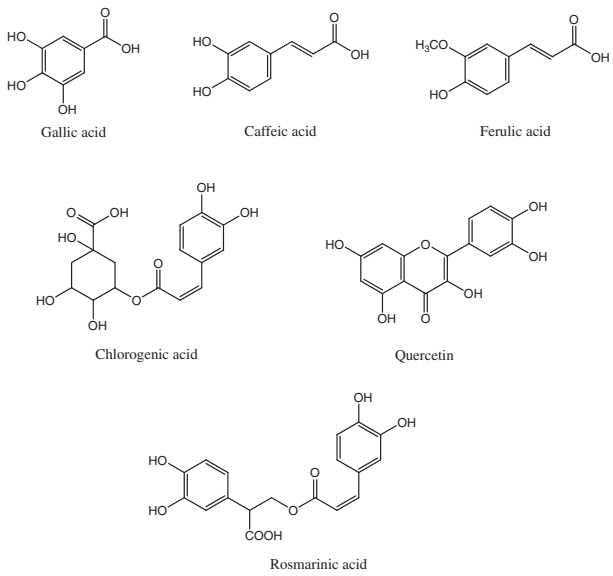

Fig. 1. Structures of Polyphenols Found in Salak Plum Peel.

Caffeic acid was found in greatest quantity in the 70% EtOH extract, whereas chlorogenic acid was detected as a marker in the EtOAc and Aq. fractions (Fig. 2 and Table 2).

Although the EtOAc fraction was found to be the main antioxidant, the analyzed polyphenol content was less than the crude 70% EtOH extract. The relation coefficient between the polyphenols and the activities was therefore examined (Table 2). Caffeic and gallic acids, small phenolic molecules, were found to be correlated to DPPH ($r_{caffeic\ acid} = 0.9583$ and $r_{gallic\ acid} = 0.9226$). On the other hand, greater molecular weight polyphenols (Fig. 1) were more closely correlated with ABTS ($r_{quercetin} = 0.9969$ and $r_{rosmarinic\ acid} = 0.9896$) and FRAP ($r_{quercetin} = 0.9987$ and $r_{rosmarinic\ acid} = 0.9931$). These correlations are in accordance with our suggestion that the polyphenols reactive in the ABTS and FRAP assays were similar but differed from the DPPH scavenger.

Cytotoxicity and cellular antioxidant activity of Salak plum peel

To apply this natural antioxidant to health care, toxicity in vero cells was conducted to ensure safety.[19] The entire fraction was non-cytotoxic in this mammalian cell at 50 μg/mL, which was the highest test concentration. Cytotoxicity against NHF cells was focused

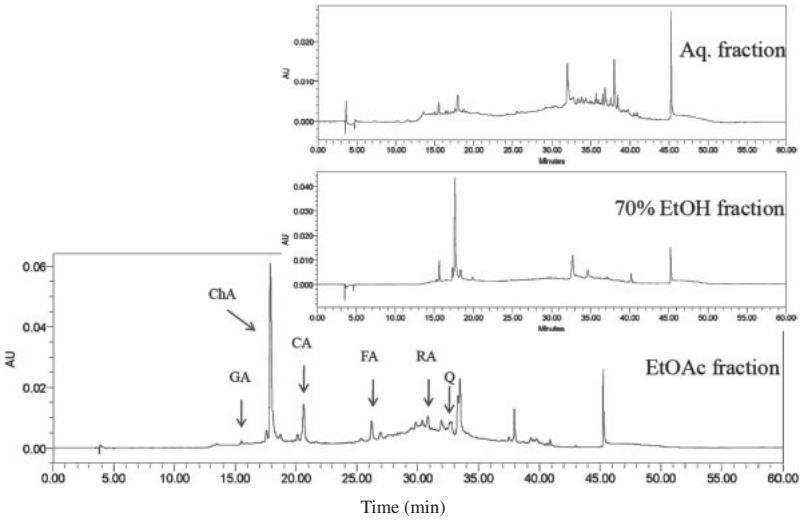

Fig. 2. HPLC Chromatograms Showing Gallic (GA), Chlorogenic (ChA), Caffeic (CA), Ferulic (FA), Rosmarinic (RA) Acids, and Quercetin (Q) in Salak Plum Peel EtOAc, 70% EtOH, and Aq. Fractions.

Table 2. Polyphenolic Contents and Correlations between Biological Activities

Polyphenols (g/kg)	Fractions			Relation coefficient (r)		
	70% EtOH	EtOAc	Aq.	DPPH	ABTS	FRAP
Gallic acid	2.859 ± 0.083	0.424 ± 0.020	0.153 ± 0.025	0.9226	0.2311	0.2155
Chlorogenic acid	5.984 ± 0.383	1.400 ± 0.102	0.294 ± 0.017	0.8657	0.1576	0.1441
Caffeic acid	8.448 ± 1.363	0.227 ± 0.010	0.086 ± 0.025	0.9583	0.2984	0.2813
Ferulic acid	0.304 ± 0.063	0.110 ± 0.003	0.049 ± 0.011	0.8322	0.1251	0.1129
Rosmarinic acid	0.028 ± 0.005	0.079 ± 0.027	0.026 ± 0.017	0.3965	0.9896	0.9931
Quercetin	0.018 ± 0.011	1.107 ± 0.027	0.035 ± 0.021	0.4428	0.9969	0.9987

Table 3. Antioxidant Activities towards NHF Cells

Sample	Concentration	Cellular oxidative prevention		Cellular oxidative damaged treatment	
		Cell viability (%)	*p*-Value	Cell viability (%)	*p*-Value
Salak plum peel EtOAc fraction	5 μg/mL	87.5 ± 6.4	0.006	71.7 ± 10.5	0.195
	10 μg/mL	95.0 ± 3.3	0.003	76.1 ± 6.9	0.045
	20 μg/mL	96.2 ± 7.3	0.003	77.4 ± 6.1	0.029
	30 μg/mL	96.6 ± 6.4	0.002	87.4 ± 4.6	0.004
	40 μg/mL	98.7 ± 1.2	0.001	88.2 ± 3.4	0.002
Control		100.0 ± 0.2	—	100.0 ± 5.1	—

according to the biosynthesis of collagen and elastin, biological fibers that function in skin elasticity and firming, in NHF cells. The production and degradation of these fibers are alleviated by collagenase and elastase, which are altered by free radicals.[5,6] The cellular antioxidant capacity of NHF cells prevents aging of the skin. The Salak plum peel EtOAc fraction (5–40 μg/mL) was found to be safe for NHF cells, as the viability of the cells was more than 75%.[18,21] Thereafter, the content of the oxidant, H_2O_2, mediating NHF cell death was observed at 3.8 pg/mL.[18] H_2O_2 is reactive oxygen species inducing apoptosis and necrosis of cells[7,8,21] that has been well studied and used as a model oxidant in cellular damage *via* intercellular, and intracellular oxidative stress[7] due to free diffusing of oxidants in, and out of cells, and tissues.[6,20] The healing efficacy of the Salak plum peel EtOAc fraction towards NHF cells lies in the fact that extracellular generated oxidants propagate their oxidative damaged internally for 24 h.[7,21] In addition, cellular antioxidant activity was also exhibited in the prevention mechanism that the oxidants were scavenged outside the cells (Table 3).

Thus oxidative stress inside the cells is limited. In addition, the prevention mechanism of this cellular antioxidant was more evident in a higher concentration range of the extract (10–40 μg/mL) significantly healed H_2O_2-induced cells damage. However, the precise mechanism of this cellular antioxidant should be determined to design a correspondent action, which finds more application in the development of pharmaceuticals that are beyond the scope of this study.

Preparation and stability of the Salak plum peel EtOAc fraction loaded liposomes

This natural antioxidant is therefore appreciable to health care as for its activity, but the stability of natural antioxidant polyphenols, limiting the safety and efficacy of the polyphenols is the main obstacle to application.[28] In addition, the polar nature of the polyphenols is drawback for a delivery through the skin barrier, and

Table 4. Components and Properties of the Salak Plum Peel EtOAc Fraction Loaded Liposomes 1-9

Liposome	Mole ratio of Lecithin:HMHEC:Cholesterol	Properties			
		pH	Entrapment efficacy (%)	Size (nm)	PDI
1	$7{:}1 \times 10^{-9}{:}0$	6.203 ± 0.012	87.869 ± 0.341	62.700 ± 2.616	0.257 ± 0.018
2	$7{:}2 \times 10^{-9}{:}0$	6.220 ± 0.000	89.235 ± 1.499	105.333 ± 1.747	0.263 ± 0.015
3	$7{:}3 \times 10^{-9}{:}0$	6.267 ± 0.040	87.778 ± 0.342	48.650 ± 0.563	0.217 ± 0.010
4	$7{:}4 \times 10^{-9}{:}0$	6.250 ± 0.000	86.816 ± 0.325	146.267 ± 0.850	0.329 ± 0.005
5	Pro-Lipo™S	3.573 ± 0.012	39.964 ± 0.738	94.227 ± 0.346	0.248 ± 0.009
6	$7{:}1 \times 10^{-9}{:}2$	6.250 ± 0.000	84.958 ± 5.342	62.947 ± 0.349	0.326 ± 0.030
7	$7{:}2 \times 10^{-9}{:}2$	6.843 ± 0.006	77.266 ± 1.933	104.433 ± 1.193	0.285 ± 0.003
8	$7{:}3 \times 10^{-9}{:}2$	6.833 ± 0.006	80.327 ± 1.212	99.133 ± 1.595	0.278 ± 0.002
9	$7{:}4 \times 10^{-9}{:}2$	6.923 ± 0.006	79.858 ± 1.189	132.167 ± 2.774	0.293 ± 0.005

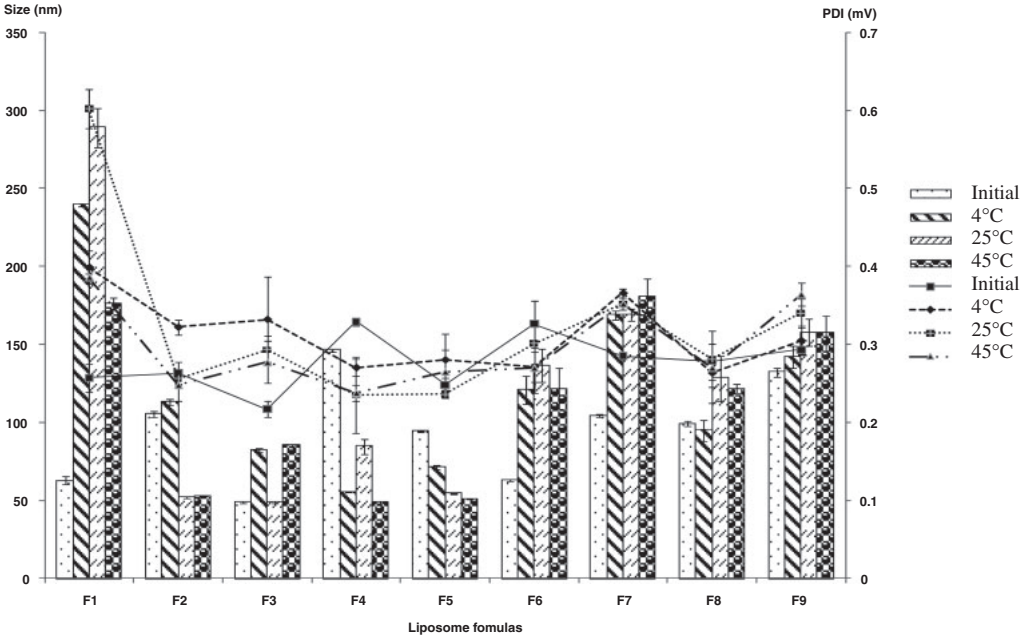

Fig. 3. Physical Stability Evaluation of the Salak Plum Peel EtOAc Fraction Loaded Liposomes 1-9 for 1 Month.

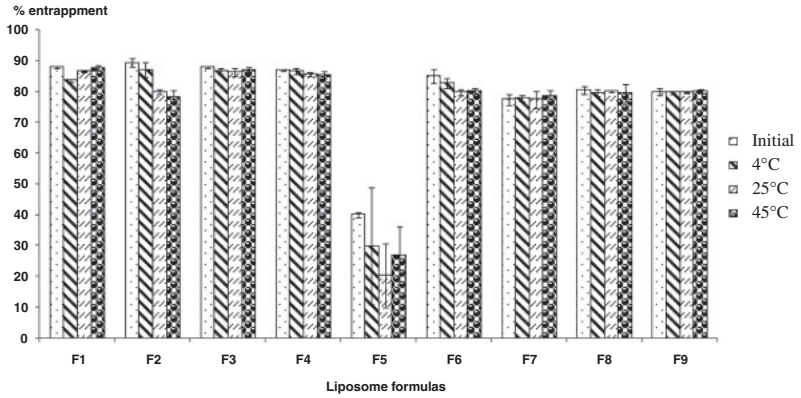

Fig. 4. Chemical Stability Evaluation of the Salak Plum Peel EtOAc Fraction Loaded Liposomes 1-9 for 1 Month.

diminishes the efficacy of their topical application. Liposome, important in cosmetics,[29] consisting of HMHEC and lecithin[30] of more than 95%,[31] was therefore prepared in this present study as a delivery system in a comparison with a commercial liposome (Table 4). The content of cellulose polymer was increased to improve steric stabilization, providing more surface coverage area and higher excluded volume limiting the nucleation process of the liposome,[32] and loaded with 50 μg/mL the Salak plum peel EtOAc fraction. The mild acid Salak plum peel liposome, which in the range of 5.5–7 indicates stable formulation acceptably used in cosmetics,[33] was HMHEC-dependent, but the pH of the commercial liposome was lowest and out of the safety range of topical formulation. Liposomes 1-4 were further developed by the addition of cholesterol at 12.5%.[34,35] The addition of cholesterol neutralized the liposome as the pH increased. There-

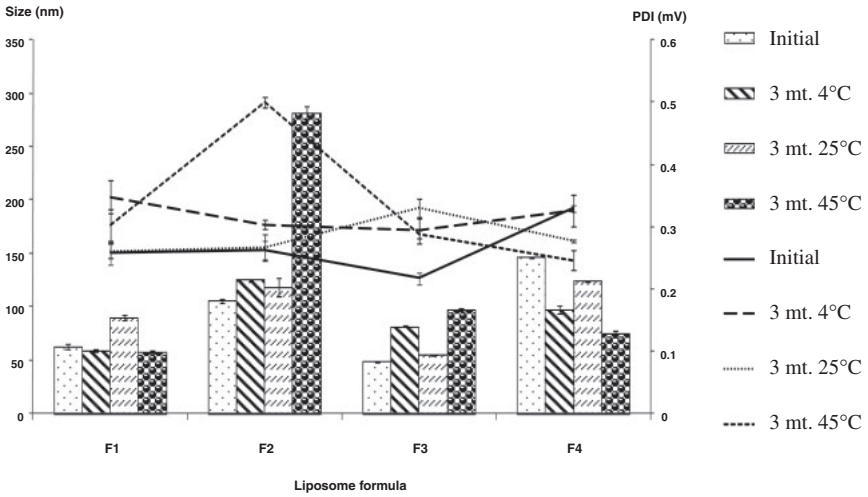

Fig. 5. Physical Stability Evaluation of the Salak Plum Peel EtOAc Fraction Loaded Liposomes 1-4 for 3 Months.

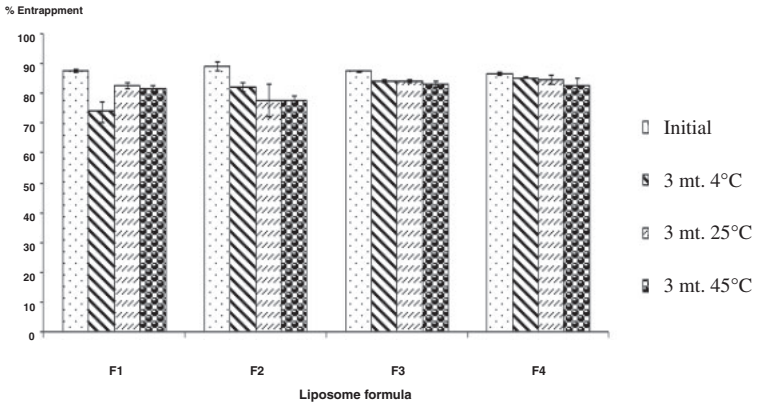

Fig. 6. Chemical Stability Evaluation of the Salak Plum Peel EtOAc Fraction Loaded Liposomes 1-4 for 3 Months.

after, the entrapment efficacy (%EE) of the liposome was determined by means of HPLC in terms of chlorogenic acid content as it was exhibited as the extract marker. These modified liposomes showed greater entrapment (86–89%EE) than that of the lecithin liposome prepared by the same method (68% EE),[34,35] but those with cholesterol with greater pH entrapped less of chlorogenic acid (77–85%EE) (Table 4). Liposomes 1-4 at a particle size of less than 150 nm, referring nanoparticles, is classified as Large, Unilamellar Vesicle (LUV; 50–150 nm).[36] A greater size liposome with cholesterol (liposome 6-9) corresponded to a reduced amount of lecithin.[37] Lecithin in the vesicle wall of greater than 95% controlled the size to less than 99 nm. The HMHEC content additionally governed size, particularly once the amount was close to 4 mg/mL, its critical aggregation concentration.[38] In addition, adequate PDI (Table 4), less than 0.35 indicating narrow size distribution with good homogeneity, ensured stability of the liposome. In addition, negative values of zeta potential (-8 to -19 mV) are recognized to indicate good stability of the suspension of natural extract liposome, as reported for caffeine.[24]

The long-term stability of the Salak plum peel EtOAc fraction loaded liposome was evaluated at 4, 25, and 45 °C. Although entrapment efficacy was reduced, the remaining chlorogenic acid was greater than 80% in the lecithin:HMHEC liposome, and 70% of lecithin:HMHEC:cholesterol, but the entrapment efficacy of liposome 5 was dramatically reduced. HMHEC content obviously controlled liposome size. At a lower ratio of HMHEC, the liposome tended to be flocculated, and coalescence occurred,[39] which corresponded with PDI. Twice as much of HMHEC aggregated the size under low temperature conditions, whereas Ostwald ripening was noticed at 25, and 45 °C. The coalescence phenomenon slowed and no size reduction was observed at 3 fold as much of HMHEC. Liposomes 6-9 showed rare stablity in terms of physicochemical properties, and the entrapment efficacy (Figs. 3 and 4).

Hence we included lecithin:HMHEC liposomes (1-4) for further 3 months stability evaluation (Figs. 5 and 6).

Entrapment efficacy was found to be decreased. However, those with 3 and 4 folds of HMHEC yielded better entrapment. Following coalescene, Ostwald ripening was observed in liposome 1, whereas a thickening effect of HMHEC at higher content conserved liposome stability[40] due to three-dimensional network of the elastic film covering the vesicle wall.[38] Liposome 3 was found to be the best vesicle for cosmetic products, followed by liposomes 4, 2, and 1.

Conclusions

This present study reveals the compatibility of an ecological antioxidant with an acceptable stable delivery

system. An additional anionic surfactant, SDS, having a synergistic effect in controlling particle growth and stabilization, is a future challenge in preventing agglomeration and Ostwald ripening,[32] but this is beyond the scope of our study, in addition to the releasing rate of chlorogenic acid from liposome. Therefore, Salak plum peel EtOAc fraction is an efficient antioxidant for health products, including anti-aging and rejuvenation cosmetics. The application of this natural antioxidant meets consumers' consideration for ecological conservation. In addition, the Aq. fraction can be regarded as a rich source of caffeic, chlorogenic, and gallic acids.

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