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Research Article

STABILITY ENHANCEMENT OF *CELASTRUS PANICULATUS* SEED OIL BY LOADING IN NIOSOMES

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ABSTRACT

Objective: To investigate the bioactive compounds, biological activities and also develop the stability enhancement system for bioactive compounds in *Celastrus paniculatus* seed oil (CPSO)

Method: This study has investigated the bioactive compounds including fatty acid profile and total phenolic content, antioxidative and tyrosinase inhibition activities of CPSO. Then, various blank niosomes formulations were prepared. The stability of CPSO was enhanced by loading CPSO in niosomes, CPSO loaded niosomes were determined their physical properties such as size, zeta potential and chemical stability of oleic acid.

Results: CPSO gave not only high content of fatty acids especially *cis*-9- oleic acid (43.99 % w/w) but also the total phenolic compounds, γ-tocopherol and α-tocopherol that played an important role for the tyrosinase inhibition activity. CPSO exhibited superior tyrosinase inhibition activity than the standard ascorbic acid, kojic acid and arbutin. The maximum loading of the CPSO in selected niosomes (1:1 molar ratio of Tween61:cholesterol) was 2.00 % w/v with the average particle size of loaded niosomes about 230 nm and zeta potential of -46 mV. In the stability study, the CPSO loaded niosomes showed higher oleic acid content than CPSO solution indicating the substance protection after loading in niosomes after 3 months storage.

Conclusion: Niosomes loaded with CPSO appeared to be a suitable method for the stability enhancement of the CPSO bioactive compounds because of not only the low toxicity, biodegradable, biocompatible, non-immunogenic but also skin penetration enhancer of niosomes as well.

Keywords: *Celastrus paniculatus* seed oil *(*CPSO), niosomes, stability enhancement, tyrosinase inhibition activity

INTRODUCTION

Celastrus paniculatus (or Ma taek in Thai) is a medicinal plant having a remarkable reputation such as the treatment of cognitive dysfunction, epilepsy, insomnia, rheumatism, gout and dyspepsia [1, 2], analgesic and anti-inflammatory [3] and antianxiety activity [4]. It also enhances learning and memory possibly by decreasing biogenic amines turnover or antioxidant effect. Moreover, Celastrus oil or Malkanguni oil has been reported to exert a number of additional pharmacological actions such as hypolipidemic and antiatherosclerotic activities as well as anti-spermatogenic action [5]. The *C. paniculatus* seeds extracted with petroleum ether yield dark brown oil. Early reports on the seed oil using paper chromatography and gas chromatography techniques stated that oil mainly contains palmitic, stearic, oleic, linoleic and linolenic acids [6]. Since *C. paniculatus* seeds are a rich source of oil. The amounts of neutral lipids in the oil were the highest, followed by glycolipids and phospholipids [7]. As known, the double bonds in the chemical structures of these unsaturated fatty acids or other lipids can be oxidized and give the rapid onset of oil rancidity.

Many approaches have been introduced to solve this problem including the application of vesicular systems such as liposomes and niosomes, which are widely used to deliver drugs, cosmetics, and plant extracts for pharmaceutical and cosmeceutical purposes [8], especially for the continuous release and targeted delivery [9]. Although liposomes can encapsulate a wide variety of drugs and deliver them to the target sites, liposomes have high cost with short shelf life because of the phospholipid composition which may be hydrolysed [10]. Nonionic surfactants e.g. Span, Tween 61 can be used as the substitution for phospholipid to form bilayer vesicles because of not only lower cost, but also higher chemical stability than the phospholipids [11]. Smaller vesicular size can also be obtained from niosomes than liposomes which will be beneficial for transdermal delivery [12, 13]. The bilayer spheroidal structures of these vesicles are usually composed of phospholipids or non-ionic surfactants mixed with cholesterol. These vesicles are advantageous for drug and cosmetic delivery because of their low toxicity, modification of pharmacokinetics and bioavailability and environment protection of the entrapped substances. They are widely used to deliver drugs, cosmetics, and plant extracts for pharmaceutical and cosmeceutical purpose. Niosomes are preferred in comparing to liposomes for topical delivery because they are chemical stable, low toxicity, biodegradable, biocompatible and non-immunogenic.

Thus, the present study has investigated the bioactive compounds including fatty acid profile and total phenolic content, antioxidative and tyrosinase inhibition activities of *C. paniculatus* seeds oil (CPSO) prepared by cold press technique. Then, the stability of CPSO was enhanced by loading CPSO in niosomes, CPSO loaded niosomes were determined their physical properties such as size, zeta potential and chemical stability of oleic acid.

MATERIALS AND METHODS

Materials

Tween61 (polyoxyethylene sorbitan monostearate), gallic acid, vitamin C (L-(+)-ascorbic acid), 2,2-diphenyl-1-picryhydrazyl radical (DPPH), EDTA, dimethyl sulfoxide (DMSO), kojic acid, ferrozine and ferric chloride ($FeCl_2$) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tyrosinase from mushroom (4187 U/mg) and L-tyrosine were purchased from Fluka (Switzerland). Cholesterol, linoleic acid, oleic acid and 1,6 diphenyl-1,3,5-hexatriene from Wako Pure Chemical Industrial Ltd. (Osaka, Japan) were used. Gamma linolenic acid was purchased from Tokyo Chemical Industrial Ltd.

(Tokyo, Japan). All other chemicals and reagents were analytical grade.

Methods

Preparation of the *C. paniculatus* seed oil (CPSO)

The seed of *C. paniculatus* was collected from Samoeng district, Chiang Mai, Thailand during January to June 2013. Voucher specimens were deposited at Highland Research and Development Institute, Thailand for future reference. The dried seeds of *C. paniculatus* were then cold pressed by a Thai traditional wood cold press machine to yield yellowish-orange oil of cold pressed *C. paniculatus* seed oil (CPSO). The CPSO was stored in a desiccator until use.

Determination of bioactive compounds of *C. paniculatus* seed oil

Fatty acid analysis of CPSO

One mg of CPSO was used to prepare the fatty acid methyl esters (FAME) for gas chromatograph (GC) analysis according to a procedure previously described [14]. The GC analysis of the FA composition was performed using a Shimadzu GC-14B equipped with FID and a capillary column RT®-2560 (biscyanopropyl polysilozane) 100 m x 0.25 mm I.D., df: 0.2 um (Serial # 47147-04) (Restek®, USA). The following temperature program was used: 120 °C for 5 min followed by a 4 °C/min increase to 230 °C, which was then held for 25 min.

Determination of total phenolic contents (TPC)

Total phenolic contents (TPC) in the form of gallic acid in the CPSO were determined using the Folin–Ciocalteu reagent [15]. Briefly, 100 mg/ml of the CPSO were mixed with Folin–Ciocalteu reagent and 20% w/v of sodium carbonate (Na_2CO_3) at ambient temperature (27±2ºC). After incubation for 30 minutes, the absorbance of blue color developed in each assay mixture was recorded at 760 nm by a well reader. The TPC of the extracts were expressed in mg of gallic acid equivalents (GAE) per gram of extract.

Antioxidative activities of the CPSO

DPPH radical scavenging assay

Free radical scavenging activities of the CPSO, standard antioxidants (vitamin C and E) and standard unsaturated fatty acids were determined by a modified DPPH assay [16]. Briefly, 50 µl of five serial concentrations of the CPSO or the standard unsaturated fatty acids including gamma linolenic acid, linoleic acid and oleic acid (at 0.01-100 mg/ml) dissolved in 95% v/v ethanol and 50 µl of ethanol solution of DPPH were put into each well of a 96-well microplate (Nalge Nunc International, NY, USA). The reaction mixtures were allowed to stand for 30 minutes at 27 ± 2ºC, and the absorbance was measured at 515 nm by a well reader (Bio-Rad, model 680 microplate reader, USA) against a blank (95% v/v ethanol). Vitamin C and E (0.001-10 mg/ml) were used as positive controls. The experiments were done in triplicate. The percentages of radical scavenging activity were calculated as follows: scavenging (%) = [(A-B)/A] x 100, where A was the absorbance of the control and B was the absorbance of the sample. The sample concentrations providing 50% of scavenging (SC_{50}) were calculated from the graph plotted between the percentages of scavenging and the sample concentrations.

Lipid peroxidation inhibition activity

The antioxidant activity of the CPSO was assayed by the modified Ferric-thiocyanate method [17]. An amount of 50 µl of five serial concentrations of the CPSO and the standard unsaturated fatty acids at 0.01-100 mg/ml dissolved in DMSO was added to 50 µl of linoleic acid in 50% v/v DMSO. The reaction was initiated by the addition of 50 µl of NH_4SCN (5 mM) and 50 µl of $FeCl_2$ (2 mM). The mixture was incubated at 37 ± 2ºC in a 96-well microplate for 1 hour. During the oxidation of linoleic acid, peroxides are formed leading to the oxidation of Fe^{+2} to Fe^{+3}. The latter ions forming a complex with thiocyanate can be detected at 490 nm. The solution without the sample was used as a negative control. Vitamin C and E at 0.001-10 mg/ml were used as positive controls. All determinations were performed in triplicate. The inhibition percentages of lipid peroxidation of linoleic acid were calculated by the following equation: Inhibition of lipid peroxidation (%) = [(A-B)/A] x 100, where A was the absorbance of the control and B was the absorbance of the sample. The sample concentrations providing 50% inhibition of lipid peroxidation (IPC_{50}) were calculated from the graph plotted between the percentages of lipid peroxidation inhibition and the sample concentrations.

Metal ion chelating assay

The metal ion chelating activity of the CPSO was assayed by the modified ferrous ion chelating method [18]. Briefly, 100 µl of five serial concentrations of the CPSO or the standard unsaturated fatty acids at 0.01-100 mg/ml dissolved in 95% v/v ethanol were added to the solution of 2 mM $FeCl_2$ (50 µl) in distilled water. The reaction was initiated by the addition of 5 mM ferrozine (50 µl) and the total volume was adjusted to 300 µl by distilled water. Then, the mixture was left at 27 ± 2ºC for 15 minutes. Absorbance of the resulting solution was then measured at 570 nm by a microplate reader. EDTA (0.001-10 mg/ml) was used as a positive control. The negative control contains $FeCl_2$ and ferrozine, which were complex formation molecules. All experiments were performed in triplicate. The inhibition percentages of ferrozine-Fe^{2+} complex formation were calculated by the following equation: Metal chelating activity (%) = [(A-B)/A] x 100, where A was the absorbance of the control and B was the absorbance of the sample. The sample concentrations providing 50% metal chelating activity (MC_{50}) were calculated from the graph plotted between the percentages of metal chelating activity and the sample concentrations.

Tyrosinase inhibition assay

The tyrosinase inhibition activity of the CPSO was assayed by the modified dopachrome method using tyrosine as a substrate [19]. Briefly, 50 µl of five serial concentrations of the CPSO or the standard unsaturated fatty acids at 0.01-100 mg/ml dissolved in DMSO, 50 µl of 200 units tyrosinase solution in 0.1M phosphate buffer, and 50 µl of 1 mg/ml tyrosine solution in 0.1M phosphate buffer, and 50 µl of 0.1M phosphate buffer were added into a 96-well plate. The mixture was incubated at 37 ± 2°C for 60 minutes and the absorbance at 450 nm was measured. Vitamin C and kojic acid (0.001-10 mg/ml) were used as positive controls. All experiments were performed in triplicate. The percentages of tyrosinase inhibition were calculated according to the following equation: Tyrosinase inhibition activity (%) = [(A-B)-(C-D)/(A-B)] x 100, where A was the absorbance of the blank after incubation, B was the absorbance of the blank before incubation, C was the absorbance of the sample after incubation, and D was the absorbance of the sample before incubation. The sample concentrations providing 50% inhibition (IC_{50}) was calculated from the graph plotted between the percentages of tyrosinase inhibition activity and the sample concentrations.

Niosomes preparation

Preparation of blank and loaded niosomes

For blank niosomes, the total amounts of 20 mM of Tween61 mixed with cholesterol (at 1:1, 3:7 and 7:3 molar ratio) were dissolved with chloroform in a round bottom flask. For CPSO loaded niosomes, the amounts of 20 mM of Tween61 mixed with cholesterol (at 1:1 molar ratio) together with the CPSO at various concentrations (1.0, 2.0 and 3.0 % w/v) were dissolved with chloroform in a round bottom flask. The solvent was removed by a rotary evaporator (Eyela, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) to get a thin film and the residual solvent was dried overnight in a vacuum desiccator. An amount of 20 ml of distilled water was added to the film and mixed at 50 ± 1°C for 15 minutes by a rotary evaporator. The dispersion put in the ice bath was sonicated for 2 minutes by an ultrasonic generator (US-300T, Nissei, Tokyo, Japan).

Physicochemical characteristics of the blank and loaded niosomes

The blank niosomes were stored in transparent vials covered with aluminum cap for 1 month at various temperatures (4±2, 25±2 and

45±2°C) and investigate at initial and after stored for 1 month. Moreover, the various blank niosomes was evaluated their stability by the accelerated heat cool cycle for 6 cycles. For the CPSO loaded niosomes, the loaded niosomes at various concentrations were stored at 4±2, 25±2 and 45±2°C and investigate at initial and 1, 2, 3 months, respectively. In the evaluating steps, the samples were withdrawn and assayed for physical characteristics (appearance, particle size and zeta potential) and the oleic acid contents by HPLC.

Physical characteristics of niosomes

Appearances

The blank and CPSO loaded niosomes were investigated for the sedimentation, separation layer and color optically at initial and 1, 2, 3 months.

The maximum loading of the CPSO

The concentrations of the CPSO entrapped in niosomes were increased from 1.0 to 3.0 % w/v. The maximum loading of the CPSO in niosomes was determined from the maximum concentration of the CPSO which gave no precipitation.

Particle sizes

The particle sizes of CPSO loaded niosomes and blank niosomes were measured by dynamic light scattering (DLS) by the Zetasizer 300HSA (Malvern Instruments, Malvern, UK) based on photon correlation spectroscopy. The niosomal dispersions were diluted to 15 times with distilled water. The diameters of loaded niosomes were carried out for 100 s at room temperature (25±2°C). The time-dependent correlation function on the scattered light intensity was measured at a scattering angle of 90° and wavelength at 535 nm.

Zeta potential

The charges of CPSO loaded niosomes and blank niosomes were determined using the Zetasizer 300HSA (Malvern Instruments, Malvern, UK). The analysis time was kept for 60 s. The average zeta potential and charges were determined. The time-dependent correlation function on the scattered light intensity was measured at a scattering angle of 90°. Samples were diluted 15 times with freshly filtrated Millipore water for the particle size and zeta potential measurement.

Chemical stability of the oleic acid in niosomes loaded with CPSO

The remaining oleic acid contents in the loaded niosomes were determined at 0, 1, 2 and 3 months in comparing to the oleic acid solution which dispersed in 70% (v/v) propylene glycol. The 500 µl of niosomes loaded with CPSO or oleic solution were mixed with 1000 µl of the extraction mixture [50% (v/v) triton X-100 and 50% (v/v) of 95% v/v of acetonitrile with 0.1% glacial acetic acid]. Then, the oleic acid contents were determined by HPLC using the same condition as previously described [20]. Briefly, Oleic acid content was determined by HPLC (Luna® C18 10 micron 250 x 4.60 mm Phenomenex USA Column, LC1200UV/VIS Detector and LC1100HPLC pump) using the mobile phase of 95% v/v acetonitrile mixed with 5% v/v of 0.1% v/v glacial acetic acid, injection volume at 20 µl, flow rate of 1 ml/min and the UV detector at 205 nm. The oleic acid contents in the extracts were determined from the HPLC chromatogram in comparing to the oleic acid.

Statistical analysis

The results were presented as the mean ± SD of three independent experiments. ANOVA was used for the analysis of the test results (LSD test) at the significance level of *p*-value <0.05.

RESULTS AND DISCUSSION

Bioactive compounds of *C. paniculatus* seed oil

Fatty acid profile of *C. paniculatus* seed oil

C. paniculatus seed oil (CPSO) was yellowish-orange oil which contained high amount of several fatty acids. **Table 1** showed the fatty acids profile of CPSO. Total monounsaturated fatty acid group showed the highest content of fatty acid in CPSO of 44.10 % w/w, followed by total monounsaturated fatty acid group and total polyunsaturated fatty acid group of 28.03 and 27.86 % w/w, respectively. The highest monounsaturated fatty acid content of CPSO was mainly from *cis*-9- oleic acid (43.99% w/w) and palmitoleic acid (0.11% w/w). For the total saturated fatty acid group, palmitic acid showed the highest content, followed by stearic acid and myristic acid of 24.35, 3.06, 0.48 % w/w, respectively. In the total polyunsaturated fatty acids group, *cis*-9,12- linoleic acid and α-linolenic acid were the main composition of this group with the content of 16.33 and 11.23 % w/w, respectively. Hence, CPSO in this study composed of top 3 fatty acids including *cis*-9- oleic acid (43.99% w/w), palmitic acid (24.35 %w/w) and *cis*-9,12- linoleic acid (16.33 %w/w), respectively. So, oleic acid was selected to be used as the main content of fatty acid in CPSO in this study. This agreed with previous study that the oil from *C. paniculatus* seed contained high amount of oleic acid, palmitic acid and linoleic acid, respectively [21].

Table 1: **Fatty acid composition in *C. paniculatus* seed oil (CPSO)**

Fatty acid	Molecular formula	Fatty acid composition of CPSO (% wt)
Butyric acid	C4:0	-
Caproic acid	C6:0	-
Caprylic acid	C8:O	-
Capric acid	C10:0	-
Undecanoic acid	C11:0	-
Lauric acid	C12:0	0.07
Tridecanoic acid	C13:0	-
Myristic acid	C14:0	0.48
Pentadecanoic acid	C15:0	-
Palmitic acid	C16:0	24.35
Heptadecanoic acid	C17:0	0.07
Stearic acid	C18:0	3.06
Arachidic acid	C20:0	-
Heneicosanoic acid	C21:0	-
Behenic acid	C22:0	-
Tricosanoic acid	C23:0	-
Lignoceric acid	C24:0	-
Total saturated fatty acid		28.03
Myristoleic acid	C14:1	-
cis-10-Pentadecenoic acid	C15:1n10	-
Palmitoleic acid	C16:1n16	0.11
cis-10-Heptadecenoic acid	C17:1n10	-
trans-9-Eiaidic acid	C18:1n9t	-
cis-9-0leic acid	C18:1n9c	43.99
cis-11-Eicosenoic acid	C20:1n11	-
Erucic acid	C22:1n9	-
Nervonic acid	C24:1n9	-
Total monounsaturated fatty acid		44.1
trans-Linolelaidic acid	C18:2n6t	-
cis-9,12-Linoleic acid	C18:2n6	16.33
gamma-Linolenic acid	C18:3n6	-
alpha-Linolenic acid	C18:3n3	11.23
cis-11, 14-Eicosadienoic acid	C20:2	-
cis-11, 14-Eicosatrienoic acid	C20:3n6	-
cis-11,14,17-Eicosatrienoic acid	C20:3n3	-
Arachidonic acid	C20:4n6	0.3
cis-13,16 Docosadienoic acid	C22:2	-
cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5n3	-
4,7,10,13,16,19-Docosahexanoic acid	C22:6n3	-
Total polyunsaturated fatty acid		27.86

Total phenolic contents in CPSO

Total phenolic content in the form of gallic acid in the CPSO was 1.25 ± 0.45 mg GAE/ gram of extract. These phenoilc compounds may interrupt with the free-radical chain of oxidation and donate hydrogen from phenolic hydroxy groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation

reaction [22]. Moreover, the phenolic compound which used as therapeutic agents or as food preservatives are chemically capable of acting as alternative substrates or competitive inhibitors of tyrosinase in *in vitro* and also *in vivo* on melanocytes *[23]*.

Antioxidative activities of CPSO

Table 2 showed the antioxidative activities of CPSO from several mechanisms. CPSO exhibited DPPH scavenging activity as SC_{50} of 28.11±1.97 mg/ml which was lower than standard ascorbic acid (SC_{50}= 0.11±0.02 mg/ml) of about 255 times. CPSO exhibited the metal chelating activity (MC_{50}) and inhibition of lipid peroxidation activity (IPC_{50}) at 314.61±12.45 and 56.24±8.85 mg/ml, respectively, which was lower than the standard EDTA (MC_{50}= 0.48±0.07 mg/ml) and standard ascorbic acid (IPC_{50}= 0.83±0.02 mg/ml) of about 655 and 68 times, respectively. But CPSO showed higher DPPH scavenging activity than standard oleic acid (SC_{50}= 70.45±15.14 mg/ml) of about 3 times. This might be due to the other substances containing in CPSO except the main constituent oleic acid played the DPPH scavenging role such as phytosterols or alkaloid compounds. This results agreed with the previous study was reported that *C. paniculatus* seed oil being characterized by a relatively high amount of phytosterols, wherein the sterol marker was β-sitosterol followed by campesterol and stigmasterol [21]. Moreover, sesquiterpene alkaloids such as celapanin, celapanigin and celapagin were found in *C. paniculatus* seed oil [3].

Note: "-" means not found

Tyrosinase inhibition activity of CPSO

Table 2 presented the tyrosinase inhibition activity of CPSO in comparing to the standard unsaturated fatty acids, ascorbic acid, kojic acid and arbutin. CPSO showed the highest tyrosinase inhibition activity with IC_{50} of 0.043±0.034 mg/ml which was higher than standard oleic acid (IC_{50}= 9.14±1.14 mg/ml), standard ascorbic acid (IC_{50}= 0.09±0.01 mg/ml), standard kojic acid (IC_{50}= 0.066±0.011 mg/ml) and standard arbutin (IC_{50}= 0.057±0.007 mg/ml) of about 212, 2, 1.5 and 1.3 times, respectively. This might be due to the tocopherol played this important role [24]. Previous study reported that γ-tocopherol was the major tocopherol isomer while the rest being α-tocopherol [3]. Moreover, CPSO from this study has shown the γ-tocopherol and α-tocopherol contents at 95.5, 165.3 mcg/ml, respectively, which was different from previous study. This might be due to the different origin of *C. paniculatus* seed oil.

Table 2: Antioxidative activities and tyrosinase activity of CPSO in comparing to various standards

Sample	Antioxidative activity			Tyrosinase inhibition activity (IC_{50})
	DPPH scavenging activity (SC_{50})	Metal ion chelating activity (MC_{50})	Inhibition of lipid peroxidation activity (IPC_{50})	
CPSO	28.11±1.97	314.61±12.45	56.24±8.85	0.043±0.034
linolenic acid	19.45±1.25	1.89±0.45	5.93±0.87	0.89±0.05
linoleic acid	48.92±8.47	6.74±2.43	5.25±0.45	3.56±0.87
oleic acid	70.45±15.14	26.28±5.45	11.25±1.13	9.14±1.14
ascorbic acid	0.11±0.02	NA	0.83±0.02	0.09±0.01
EDTA	NA	0.48±0.07	NA	NA
kojic acid	NA	NA	NA	0.066±0.011
arbutin	NA	NA	NA	0.057±0.007

Note: NA means not applicable

Characteristics of various blank niosomes

The mean sizes, zeta potential of blank niosomes at various ratios were shown in **Figure 1**. Niosomes with molar ratio of Tween61: cholesterol at 1:1 gave the white opalescent dispersion with the mean particle size at 195.20±15.61 nm with the negative value of zeta potential of -46.40±3.72 mV indicating the good physical stability. After stored at various temperature for 1 month, size of 1:1 molar ratio niosomes were in the acceptable range of 200-300 nm and zeta potential value were lower than -30 mV which was outside the range of ± 30 mV range demonstrating the physical stability of the dispersion [25]. While, the 7:3 (molar ratio of Tween61: cholesterol) niosomes exhibited the size of 431.50±21.57 nm at initial and fluctuated in the range of 240-290 nm when stored at 4±2, 25±2 and 45±2°C. But its size was rose to 370 nm after subjected to the accelerated heat cool cycles which use to evaluate the stability of nanoparticles resulting in the unstable formulation of 7:3 molar ratio. The 3:7 molar ratio niosomes showed the size of 431.50±21.57 nm at initial, but the sizes of niosomes were decreased to about 200 nm after stored at 45±2 C for 1 month. This might be due to the high temperature of 45°C was not suitable for this formulation and might cause the leakage of niosomes when loaded with substances especially oil substance. So, the 1:1 molar ratio of Tween61:cholesterol was selected for further study due to its stable physical property.

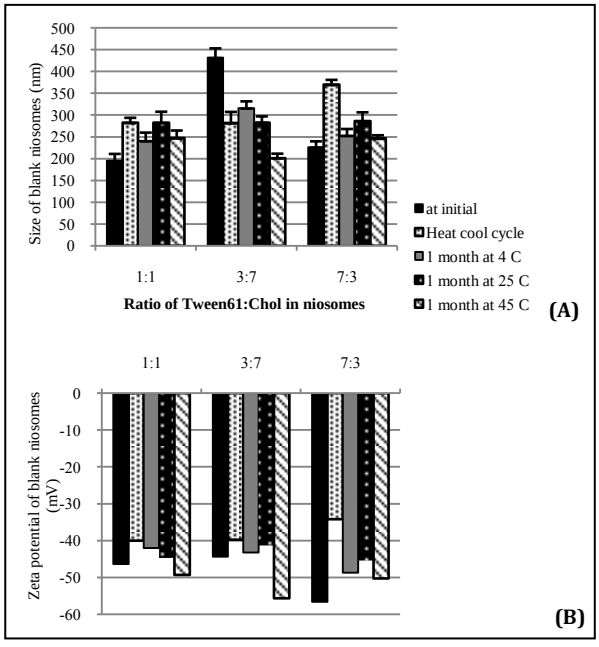

Fig.1: The mean sizes (A) and zeta potential (B) of blank niosomes at various molar ratios of Tween61:cholesterol (1:1, 3:7 and 7:3)

Physicochemical stability of niosomes loaded with CPSO

The maximum loading of CPSO in niosomes

When the loading concentration of CPSO was more than 2.00% (w/v) of CPSO, yellowish orange separate layers on the surface of niosomal dispersion were observed. The maximum loading of OSF3 in niosomes (1:1 molar ratio) was 2.00% (w/v). CPSO was intervened between the non-polar head of Tween61 and cholesterol structure at the niosomal surface membrane as in the previously study [26].

Size and zeta potential of niosomes loaded with OSF3

Size and zeta potential of the loaded niosomes were presented in **Figure 2**. Sizes of CPSO loaded niosomes were slightly larger than the blank niosomes of about 1.2 times. This might be due to the loaded CPSO intervened the niosomal membrane leading to the less rigidity of the membrane after loaded with CPSO [26]. After stored at 4 and 25°C for 3 months, the loaded niosomes showed the range of size of about 230-280 nm, while the niosomes at 45°C showed more fluctuate size of about 230-330 nm. This might be due to the heat from the storage temperature changed the gel structure of niosomal membrane from the closely packed gel to the loosely packed liquid crystalline structure resulting in the variation of niosomal size. However, the niosomal size after stored at three different temperatures for 3 months were in the acceptable range (230-330 nm). Moreover, the CPSO loaded niosomes which had negative charge from their zeta potential values in the blank niosomes (-46.40±3.72 mV) may repulse the negative charges of the unsaturated fatty acids resulting the larger vesicular sizes [27] and more negative value of zeta potential after stored for 3 months (-45 to -50 mV) except the niosomes stored at 45°C. This may be due to the leakage of loaded CPSO from the niosomal membrane resulting in the smaller size and more positive value of zeta potential of niosomes stored at 45°C. So, the storage condition of CPSO loaded niosomes should be not more than 30°C.

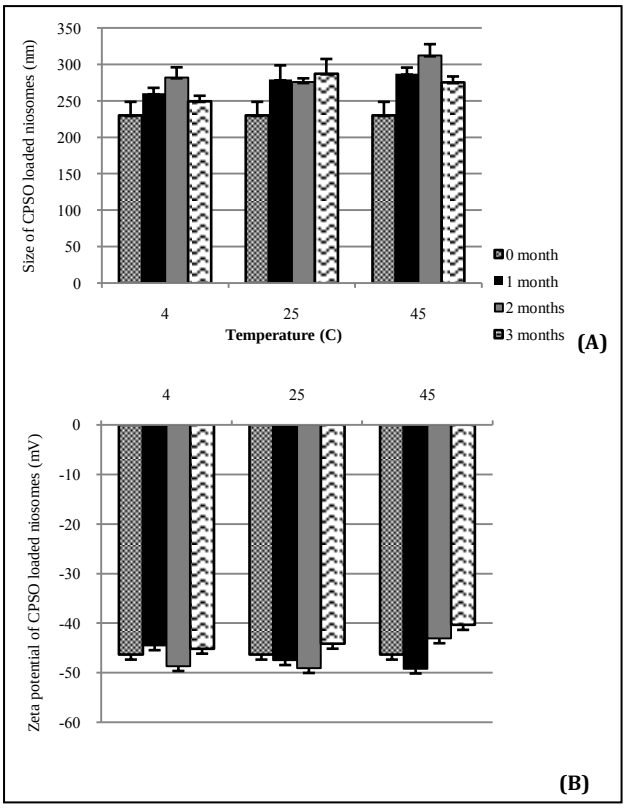

Fig.2: The mean sizes (A) and zeta potential (B) of CPSO loaded niosomes at various storage temperatures (4±2, 25±2 and 45±2°C)

Chemical stability of niosomes loaded with CPSO

The percentage (%) remaining of the oleic acid in the CPSO solution and CPSO niosomes were presented in **figure 3**. Temperatures appeared to affect the stability of the oleic acid in CPSO. The oleic acid content in CPSO niosomes after stored at 45°C for 3 months were lower than those at 4 and 25°C. At 4°C for 3 months, CPSO niosomes gave the highest remaining oleic acid than other storage condition. The storage times and temperatures of niosomes also affected the contents of oleic acid. Moreover, the CPSO loaded niosomes exhibited significantly higher oleic acid content than the CPSO solution at 25°C for 3 months (p<0.05). This may be due to the stability enhancement and the protection of the degradation of the unsaturated fatty acids from niosomes. Not only the protection from niosomes of oleic acid but the other contents were also protected such as total phenolic compounds, γ-tocopherol and α-tocopherol. The hydrophilic substance, total phenolic compounds were located in the core of niosomes, while the hydrophobic substance were at the niosomal membrane next to oleic acid [28]. Furthermore, niosomes are not only low toxicity, biodegradable, biocompatible, non-immunogenic, skin penetration enhancer but also showed environment protection of the loaded substances.

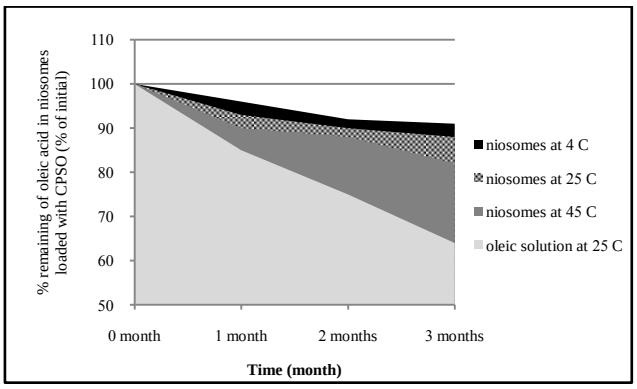

Fig.3: The percentage (%) remaining of the oleic acid in the CPSO solution and CPSO niosomes at various storage temperatures for 3 months

CONCLUSION

C. paniculatus seed oil (CPSO) gave not only high contents of fatty acids which consisted of *cis*-9- oleic acid (43.99 % w/w), palmitic acid (24.35 % w/w) and *cis*-9,12- linoleic acid (16.33 % w/w) but also the total phenolic compounds, γ-tocopherol and α-tocopherol that played an important role for the tyrosinase inhibition and antioxidative activities. CPSO exhibited superior tyrosinase inhibition activity than the standard ascorbic acid, kojic acid and arbutin. The CPSO was loaded in blank niosomes (1:1 molar ratio of Tween61:cholesterol) which performed the best stability according to enhanced the stability of bioactive compounds in CPSO. The maximum loading of the CPSO in niosomes was 2.00 % w/v with the average particle size of loaded niosomes about 230 nm and zeta potential of -46 mV. In the stability study, the CPSO loaded niosomes showed higher oleic acid content than CPSO solution indicating the substance protection after loading in niosomes after 3 months storage. Niosomes loaded with CPSO appeared to be a suitable method for the enhancement stability of the CPSO bioactive compounds because of not only the low toxicity, biodegradable, biocompatible, non-immunogenic but also skin penetration enhancer of niosomes as well.

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