Research Article
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Life Sciences, Medicine and Biomedicine

Microencapsulation of *Lysiphyllum strychnifolium* extract using pectin as a carrier matrix and its characterization

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Received: 1 December 2021; **Revised:** 25 March 2022; **Accepted:** 26 March 2022; **Published:** 3 April 2022
DOI https://doi.org/10.28916/lsmb.6.1.2022.102

ABSTRACT

Lysiphyllum strychnifolium (Craib) A. Schmitz (LS, Fabaceae) is one of the folklore medicines in Thailand. The previous studies have demonstrated several pharmacological activities and high polyphenolic substances possessed by this plant. However, the suitable encapsulation of LS extract has not been discovered. This study aimed to develop LS microcapsules using spray-drying technique with pectin as a carrier. Moreover, the powder analysis and characterization were also conducted. The effects of inlet temperatures (80, 100, and 120°C) and carrier concentrations (1, 5, and 10 %w/v) on the encapsulation yield (EY), encapsulation efficiency (EE), total phenolic content (TPC), and main markers (trilobatin and yanangdaengin) of LS microcapsules were studied. Finally, the characterization was investigated by Fourier transform infrared (FTIR), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). The obtained results indicated that S2 microcapsule formulation, pectin to extract ratio 10:1 (w/w) at inlet temperature of 100°C, was chosen as the optimal condition because of the positive tendency to acquire higher EE as pectin level was increased. On the contrary, the level of TPC and markers was reduced due to the more addition of pectin. The FTIR, XRD, and DSC results suggested that the well-encapsulated microcapsules were obtained for S2 formulation and SEM represented the semi-spherical structure of its microstructures. The development of LS microcapsules with the proximity to gain the advantageous powder analysis and characteristic has been achieved. Therefore, this approach could be used for the subsequent manufacturing of LS extract.

Keywords: *Lysiphyllum strychnifolium; pectin; microencapsulation; spray-drying; characterization*

INTRODUCTION

Lysiphyllum strychnifolium (Craib) A. Schmitz (LS) (synonym; *Bauhinia strychnifolium*), known as Ya-nang-daeng or Khayan in Thai, belongs to a large family Fabaceae distributed throughout north and northeast of Thailand (Hao et al., 2003; Larsen & Larsen, 1984). The decoction of leaves, stems, and roots from this plant has been traditionally used for the treatment of cancer, food poisoning, fever, allergy, diarrhea, and intoxication (Chamratpan & Homchuen, 2005; Luengthong et al., 2016).

The previous study found that LS leaf extract obviously contained high amount of total phenolic compound (Maitree et al., 2018). Notably, some chemical compounds identified from LS are quercetin, 3,5,7,3′,5′-pentahydroxy-flavanonol-3-O-α-L-rhamnopyranoside, 3,5,7-trihydroxy-chromone-3-O-α-L-rhamnopyranoside, β-sitosterol, stigmasterol, and astilbin from stems (Sampaopan et al., 2021; Yuenyongsawad et al., 2013), gallic acid, trilobatin, and yanangdaengin from leaves (Kongkiatpaiboon et al., 2020). The previous studies have revealed the diverse pharmacological effects investigated from LS such as anticancer (Yuenyongsawad et al., 2013), antiallergic (Bunluepuech et al., 2013), antimicrobial (Bunluepuech et al., 2013; Sukprasert et al., 2020b), anti-inflammatory and anti-hyperuricemic (Sato et al., 2019), antidote (Sukprasert et al., 2020a), antioxidant (Itharat & Sayompark, 2016; Maitree et al., 2018), and antidiabetic (Bunluepuech et al., 2019). As such, LS extract can be utilized in several functional applications for the treatment and prevention of diseases.

Up to date, there has no evidence revealing the suitable encapsulation of LS extract available even though it possesses beneficial activities. Mostly, LS comprises some phenolic and phytosterol compounds which can render unsuitability when formulated as a plain extract powder due to the instability of those substances to the adverse condition in environment during processing and storage (Gharsallaoui et al., 2012; Xu et al., 2009). In addition, as a plain extract, it has to be stored in low temperature condition because its deliquescence characteristic can turn the dried powder into sticky-black materials during short-term exposure in room temperature (Sato et al., 2019). Microencapsulation is the solution of these challenges to protect the active compounds by the incorporation with the protective carrier matrix leading to shielding the active compounds, preserving the pharmacological activities, improving the bioavailability, and maintaining the quality of the product consequently (Ribeiro et al., 2019). In this study, spray-drying technique was employed to encapsulate the plant extract into microcapsules because of its economical, good efficiency, and potentially used in large scale production (Silva et al., 2014).

The general carriers used for spray-drying process are polysaccharide, protein-based, semi synthetic, and synthetic polymers (Akbarbaglu et al., 2021; Luo et al., 2015; Mishra et al., 2014). Each carrier has the advantageous and disadvantageous regarding to desirable properties, cost, and the ability to generate the proper matrix. Pectin is a negatively charged polysaccharide carrier sourced from vegetables and fruits. In industry, pectin is usually applied as stabilizing, thickening, emulsifying, and coating agent (Rahmani et al., 2020). As biopolymer coats, pectin has the multifaceted properties such as biodegradable, high biocompatibility, low toxicity, and film forming capacity which presumably able to act as carrier for active compounds (Ribeiro et al., 2021; Luo et al., 2015).

Therefore, the present study aimed to develop LS microcapsules using spray-drying technique with pectin as a carrier. The effect of the various inlet temperatures and pectin concentrations on encapsulation yield (EY), encapsulation efficiency (EE), total phenol content (TPC), and markers content were investigated. The characterization of microcapsules obtained using Fourier transform infrared (FTIR), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM) were studied.

MATERIALS AND METHODOLOGY

Chemicals and plant materials

Pectin extra pure (degree of esterification 63-66%) was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Gallic acid and sodium carbonate were obtained from Sigma-Aldrich Chemical Co. (MO, USA) and Ajax Chemicals Pty Ltd. (Sydney, Australia), respectively. Folin-Ciocalteu's phenol reagent was purchased from Merck KGaA (Darmstadt, Germany). Reference standards for trilobatin and yanangdaengin were donated by Dr. Sumet Kongkiatpaiboon and their isolation and characterization have been reported previously (Kongkiatpaiboon et al., 2020). All other reagents and solvents used were purchased from local suppliers and were of analytical grade.

The leaves of LS were collected at Bangprakok, Ratchaburana, Bangkok, Thailand in November 2020 and authenticated by Asst. Prof. Dr. Bhanubong Bongcheewin at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand in which the voucher specimen was deposited (voucher no. PBM-005667). The leaves were separated from the whole plant before being soaked in running tap water. They were oven-dried at 50°C for 48 h and further grinded into powder.

Preparation of LS extract

The powder was extracted in a boiling water with water to leaf ratio at 10:1 (mL/g) for 15 min in triplicate. The aqueous extract resulted was filtered by using filter paper Whatman no.1. The residue was pooled and concentrated using a controlled-vacuum rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland) at 60°C with the estimated reduction portion of water at about 80% and further lyophilized by a freeze dryer (Labconco, MO, USA) (Sato et al., 2019). The coarse extract powder obtained was kept at -20°C until used. The percentage yield based on the dried weight of plant material was calculated using the following formula:

$$\text{The percentage yield (\%)} = \left(\frac{\text{weight of the extract}}{\text{weight of the powdered leaves}}\right) \times 100$$

Preparation of the microcapsules

LS microcapsules were prepared by varying the level of pectin concentration and inlet temperature as described in Table 1. The carriers were dispersed in water and homogenized overnight using magnetic stirring prior to combining with the solution of freeze-dried extract powder. Then, the solution resulted was spray-dried by a B-290 mini spray dryer (BÜCHI Labortechnik AG, Flawil, Switzerland). The pump power was kept at 10% to maintain the feed flow rate at 3.41 ± 0.19 mL/min and the airflow rate was 40 m^3/h with aspirator level at 100%. To maintain the homogeneity of liquid feed during drying process, the solution was gently stirred using magnetic stirring. As a reference, the extract-free carrier and carrier-free extract were prepared by spray-drying under the same experimental condition.

Table 1: Experimental design of LS microcapsules produced by spray-drying.

Formulations	Pectin:LS extract ratio (w/w)	Inlet temperature (°C)
S1	10:1	80
S2	10:1	100
S3	10:1	120
S4	5:1	80
S5	5:1	100
S6	5:1	120
S7	1:1	80
S8	1:1	100
S9	1:1	120

Powder analysis

Encapsulation yield (EY)

The microcapsules derived was weight and related with the theoretical weight using the amount of total powder before microencapsulation process. The percentage yield was expressed as % and calculated using the following formula:

$$\text{EY (\%)} = \left(\frac{\text{weight of the obtained powder}}{\text{theoretical weight}}\right) \times 100$$

Total and surface phenolic contents (TPC and SPC)

TPC and SPC were determined as the main parameters for the effective microencapsulation process. Briefly, the TPC was determined by dissolving the desirable amount of microcapsules (10-40 mg) in 1 mL methanol. The solution resulted was vortex (10 s) and further sonicated for 20 min. Following this, it was centrifuged at 4,000 rpm for 5 min and the supernatant was collected to measure the TPC. The absorbance was read at 765 nm using Infinite 200 Pro-microplate reader (Tecan, Männedorf, Switzerland) (Díaz-Bandera et al., 2015). For SPC, the quantification was tested under the same condition with an exception by removing the sonication step. The TPC and SPC were determined in triplicate and calculated as gram gallic acid equivalents (GAE) per 100 g of microcapsules (% w/w) using $y = 0.0019x - 0.0022$ ($r^2 = 0.9999$), as calibration formula.

Encapsulation efficiency (EE)

EE was carried out using the indirect method measured by Folin-Ciocalteu spectrophotometry as previously described (Saenz et al., 2009) with few modifications. EE was calculated using the following formula:

$$\text{EE (\%)} = \frac{(\text{TPC} - \text{SPC})}{\text{TPC}} \times 100$$

Where TPC and SPC are total phenolic content and surface phenolic content of microcapsules, respectively.

High-performance liquid chromatography (HPLC) analysis

The Thermo HPLC system (Thermo Separation Products, MA, USA) equipped with a vacuum solvent degasser, quaternary pump, autosampler, and UV-visible diode-array detector was applied to determine the markers such as trilobatin and yanangdaengin in LS extract or microcapsules based on the previously described by Kongkiatpaiboon et al. (2020). The standard concentrations of 31-1,000 µg/mL for trilobatin and 15-500 µg/mL for yanangdaengin dissolved in methanol were used for calibrations. The desirable amount of extract (5 mg) or microcapsules (10-40 mg) were dissolved in 1 mL methanol and further sonicated for 20 min. The solution obtained was filtered by means of 0.45 µm nylon membrane and 10 µL of solution filtered was injected into the HPLC with a BDS Hypersil C18 column (150 mm × 4.6 mm, i.d. 5 µm) (Thermo Fisher Scientific, MA, USA) for separation. The chromatographic separation was carried out using a mobile phase with 0.5% acetic acid as solvent A and methanol as solvent B with the flow rate of 1 mL/min at 25°C. The elution gradient program was as follows: 0% to 100% B (40 min), 100% B (10 min), and subsequent column equilibration with 100% A for 10 min. The peaks were detected at 254 nm and further identified by comparing the retention time with standards. The calibration equation of $y = 2519.7x - 20599$ ($r^2 = 0.9995$) and $y = 7058.1x - 43413$ ($r^2 = 0.9978$) were used to quantify the content of trilobatin and yanangdaengin, respectively, in sample.

Powder characterization

Fourier-transform infrared (FTIR)

The FTIR spectra of samples were investigated using Nicolet iS5 FTIR Spectrometer (Thermo Fisher Scientific Inc., MA, USA) equipped with iD7 attenuated total reflectance (ATR). The spectra were obtained at a resolution of 4 cm^{-1} and recorded in the range of 400 to 4000 cm^{-1}.

X-ray diffraction (XRD)

The analysis of XRD of samples was performed using MiniFlex 600 XRD diffractometer (Rigaku Corporation, Tokyo, Japan). The measurement condition was kept in the diffraction angle (2θ) between 2 and 60° with the scanning step of 0.02° and speed at 4°/min.

Differential scanning calorimetry (DSC)

Thermal analysis of microcapsules was conducted using DSC8000 (PerkinElmer Inc., MA, USA). The amount of 5 mg of each sample was placed in 40 µL aluminum pan which was sealed and pressed afterwards. The thermograms were obtained in the range of 25 to 300°C using heating rate at 10°C/min under dynamic atmosphere of nitrogen.

Scanning electron microscopy (SEM)

The morphology of microcapsules was examined using field emission scanning electron microscopy (FE-SEM, JSM-761F, JEOL, MA, USA). The sample was scattered on the aluminum stub and further metalized with gold. The analysis was performed at 10 kV with required magnification.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and statistically analyzed using one-way analysis of variance (ANOVA) in SPSS program version 26. The difference was further estimated by Tukey's multiple comparison and the significance level was taken at $p < 0.05$.

RESULTS

Yield of freeze-dried extract

The yield of freeze-dried extract from the amount of 200 g crude LS leaves was 24.51% and the physical appearance of LS extract was coarse powder with the color of brownish orange. This result is in accordance with 23.81% of yield reported by Sato et al. (2019).

Powder analysis

Yield of microcapsules

The EY in this study ranged from 36.81 to 66.18% (Table 2). The drying process of S1 and S3 formulations had to be terminated due to the formation of sticky materials over the nozzle cap which further obstructed the flow of liquid feed leading to squirting the solution out of the nozzle body (Fig. 1).

Table 2: Effects of spray-drying variables on drying outcomes and properties of LS microcapsules.

Formulations	EY (%)	EE (%)	TPC (%w/w)	Markers (%w/w)	
				Trilobatin	Yanangdaengin
S1	NA	NA	NA	1.48 ± 0.29	0.34 ± 0.10
S2	45.06	18.03 ± 0.15	1.13 ± 0.02	1.46 ± 0.04	0.32 ± 0.01
S3	NA	NA	NA	1.47 ± 0.05	0.33 ± 0.01
S4	36.81	4.08 ± 1.00*	1.39 ± 0.06	2.49 ± 0.02	0.54 ± 0.01
S5	55.70	6.62 ± 0.91*	1.38 ± 0.04	2.51 ± 0.08	0.56 ± 0.03
S6	57.12	8.60 ± 1.53*	1.48 ± 0.06	2.41 ± 0.03	0.55 ± 0.03
S7	60.78	3.54 ± 0.02*	1.90 ± 0.15*	6.94 ± 0.46*	1.47 ± 0.07*
S8	64.08	3.82 ± 0.48*	4.38 ± 0.31*	7.11 ± 0.19*	1.58 ± 0.04*
S9	66.18	5.33 ± 1.93*	4.43 ± 0.61*	6.10 ± 1.36*	1.40 ± 0.33*

EY, encapsulation yield; EE, encapsulation efficiency; TPC, total phenolic content; NA, not applicable; EE, TPC, and markers data are expressed as mean ± SD (n = 3);
* indicates significant difference from S2 ($p < 0.05$).

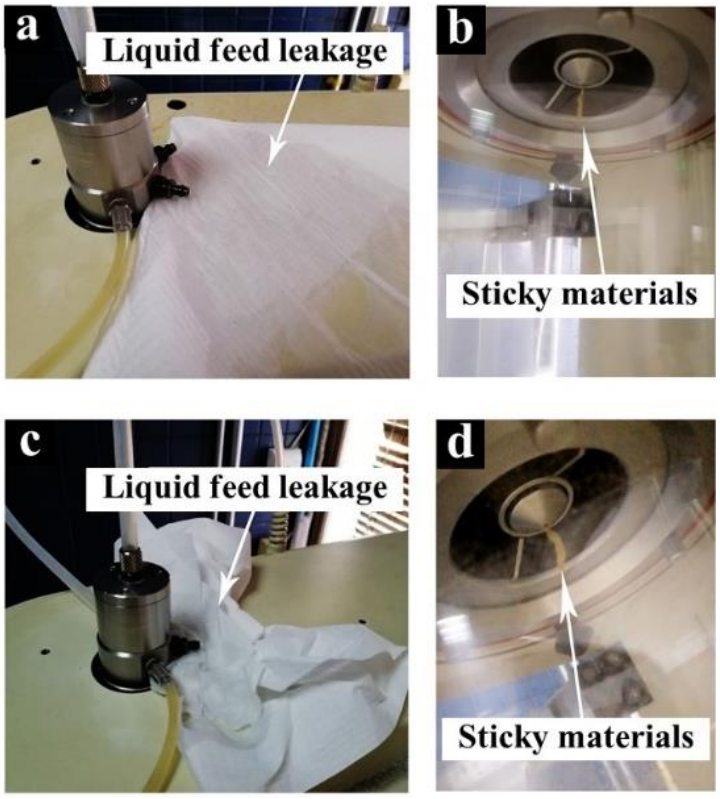

Fig. 1: The presence of liquid feed leakage and sticky materials in S1 (a & b) and S3 (c & d) formulations.

Fig. 2 represents the physical appearance of microcapsules obtained. The forms were in fine powder with characteristic odor and bitter taste. The color was turned from brownish orange to light brown when the level of pectin was added.

TPC analysis

Pectin concentration and drying inlet temperature affected TPC value significantly ($p < 0.05$). As seen in Table 2, The TPCs of pectin: extract formulation ratio (w/w) at 5:1 (1.38-1.48% w/w) and 10:1 (1.13% w/w) were significantly lower than that of ratio 1:1 (1.90-4.43% w/w) ($p < 0.05$). Moreover, the TPC of S7 was lower than that of S8 and S9 formulations ($p < 0.05$).

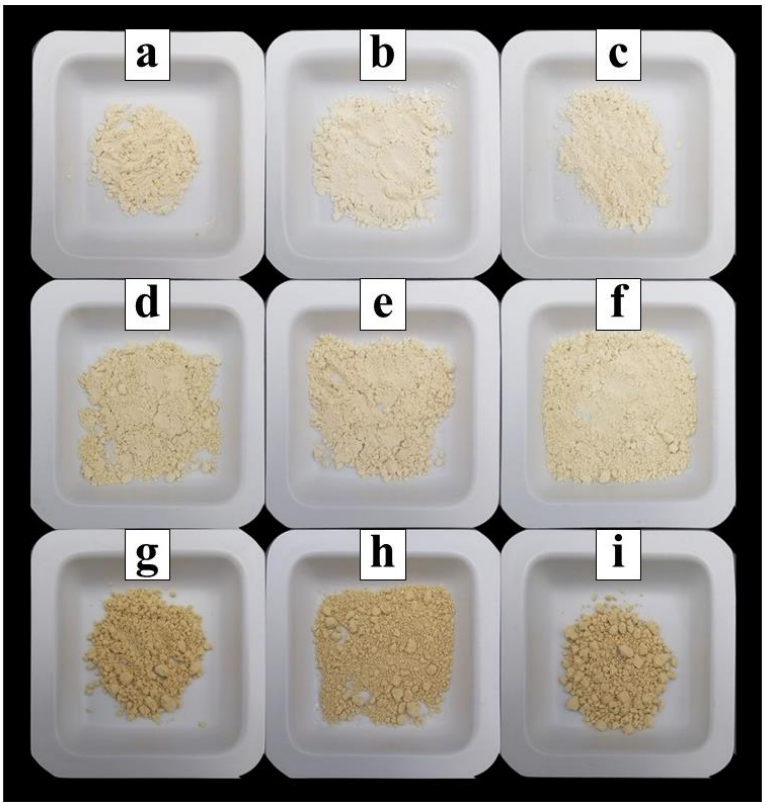

Fig. 2: Physical appearance of S1 (a), S2 (b), S3 (c), S4 (d), S5 (e), S6 (f), S7 (g), S8 (h), S9 (i) formulations.

EE analysis

As shown in Table 2, EE ranged from between 3.54 and 18.03%. The EE value of microcapsules was significantly affected by inlet temperature and pectin concentration levels. S2 microcapsule formulation had the significant highest EE at 18.03 ± 0.15% among of all formulations ($p < 0.05$). In addition, EE value of S6 formulation was significantly higher than that of S4, S7, S8 ($p < 0.05$).

HPLC analysis

The HPLC chromatogram of LS extract showed 2 prominent peaks at the retention times of 25.41 ± 0.06 min for trilobatin and 26.23 ± 0.07 min for yanangdaengin (Fig. 3) which corresponded to the previous work by Kongkiatpaiboon et al. (2020). Additionally, trilobatin and yanangdaengin contents in LS extract were 15.16 ± 0.22 and 3.31 ± 0.15% w/w, respectively.

For the developed microcapsules, the formulations using 5% and 10% pectin concentration showed the statistically significant lower amount of both markers than that of the formulation with 1% pectin concentration ($p < 0.05$) (Table 2).

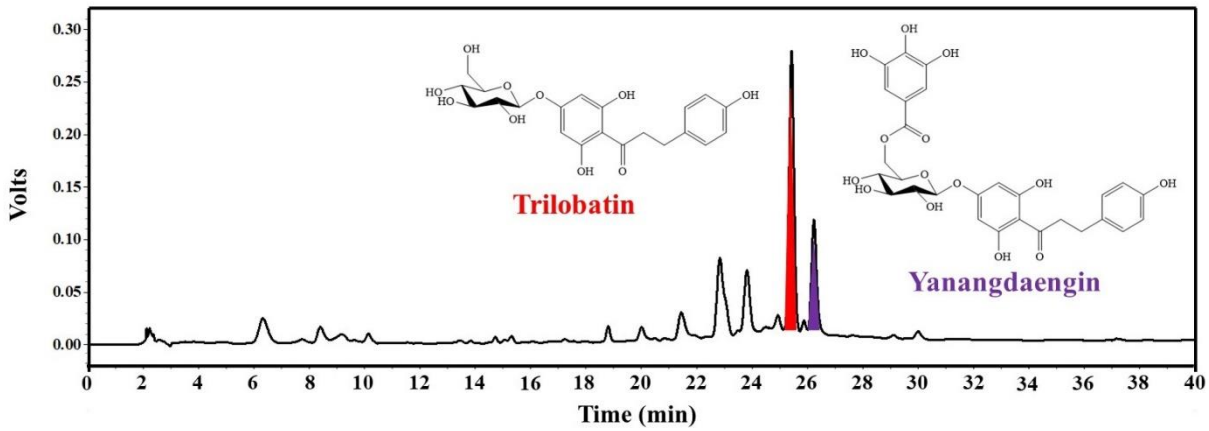

Fig. 3: The HPLC chromatogram of LS extract.

Powder characterization

FTIR characterization

Corresponding to the result reported by Vityazev et al. (2017) and Ribeiro et al. (2021), the spectrum pattern of pectin obtained indicated the characteristic peaks at 3362 cm^{-1} (O-H stretching), 2930 cm^{-1} (C-H stretching), 1737 cm^{-1} (C=H stretching), 1638 cm^{-1} (COO^- stretching), 1440 cm^{-1} (-COOH stretching), 1228 cm^{-1} (methyl ester COC groups stretching). The bands at 1143, 1072, 1011 cm^{-1} are pointed to pyranoside ring and 832 cm^{-1} is out-of-plane bending of OH groups (Fig. 4).

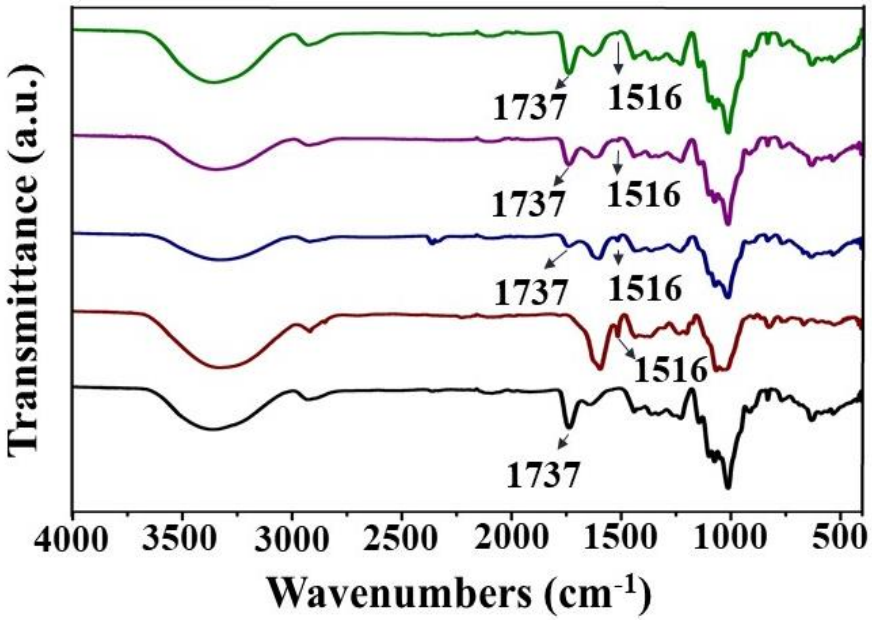

Fig. 4: FTIR spectra of pectin (▲), LS extract (▲), S8 (▲), S5 (▲), and S2 (▲) formulations.

In the spectrum of LS extract, the peak at 3327 cm^{-1} is attributed to O-H stretching and the bands at 2919 and 2850 cm^{-1} are referred to methylene chains. According to the literature, the peaks at 1594, 1516, 1433, 1199, 1066, 1019, and 822 cm^{-1} are the fingerprint region of trilobatin as the main marker of LS extract (Kurahayashi et al., 2018).

The FTIR spectra of LS microcapsules illustrated that the peaks at about 3350, 2926, and 1628 cm^{-1} were shifting close to those of pectin and the peaks at 1737 and 1011 cm^{-1} was also widened when the amount of pectin used was increased to encapsulate LS extract. The intensive absorption peak was obtained in S8 formulation at 2362 cm^{-1}.

XRD characterization

Fig. 5 has displayed the diffractogram of pectin confirming its amorphous structure. As reported by Rangelova et al. (2017), there was a wide peak at 21°, on the 2 theta scale, as the main characteristic of pectin. The existence of prominent peaks at 14.4°, 20-21.6° and 32.3° indicating the crystalline structures of compounds consisted in the LS extract. For the LS microcapsules, the pattern of crystalline was absence because the intensity of predominant peaks was reduced.

DSC characterization

Thermal profiles by DSC are reflected in Fig. 6. The DSC curve of pectin exhibited the endothermic peak at 125°C and combination of exothermic and endothermic peaks in the range of 225-270°C suggesting the signs of water evaporation and polymer degradation, respectively (Emadzadeh et al., 2021). The LS extract showed a series of endothermic and exothermic peaks ranging from 150°C to 200°C contributing to the breakdown of LS extract-comprised polyphenols. This finding is in accordance with the evidence presenting the melting point of its main markers (Kongkiatpaiboon et al., 2020). The DSC curve of S2 microcapsule formulation was more resemblant with that of pectin as compared to other formulations.

SEM characterization

Fig. 7 demonstrates the SEM image of pectin showing the semi-spherical particles with cavities on the surface due to water evaporation during spray-drying process (Kang et al., 2015). In LS extract micrograph, glassy structure with flake debris on the surface was observed as its indigenous form. Interestingly, S2 microcapsule formulation demonstrated the complete formation of microcapsules with semi-spherical structure.

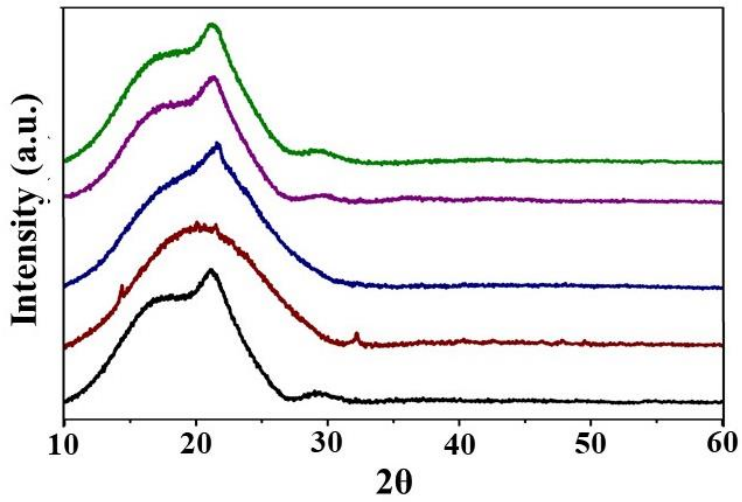

Fig. 5: X-ray diffractograms of pectin (▲), LS extract (▲), S8 (▲), S5 (▲), and S2 (▲) formulations.

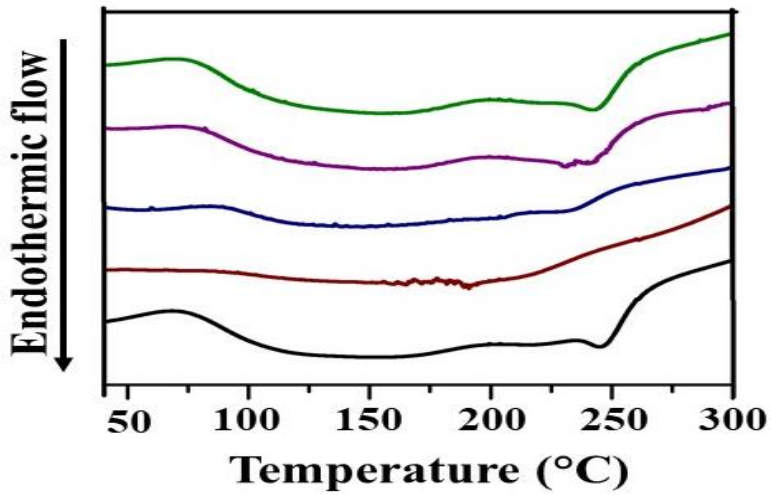

Fig. 6: DSC thermograms of pectin (▲), LS extract (▲), S8 (▲), S5 (▲), and S2 (▲) formulations.

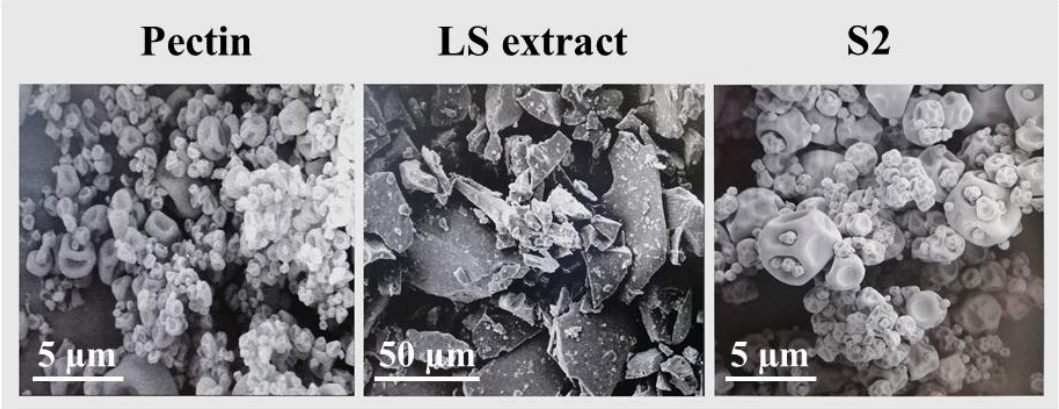

Fig. 7: SEM micrographs of pectin (3000 ×), LS extract (500 ×), and S2 (3000 ×) formulations.

DISCUSSION

Despite the various pharmacological effects and high bioactive substances of LS extract, the development of its nutraceutical product is essential to be widely consumed by the society. The present study developed LS microcapsules using a pectin as carrier by spray-drying technique. Our findings have shown favorable results for S2 microcapsule formulation exhibiting the highest EE value with the promising well-encapsulated form of powder obtained as indicated by FTIR, XRD, DSC, and SEM results. Moreover, we observed the reduction of TPC and markers levels due to higher amount of carrier used.

When the extraction process of LS leaves was conducted, our preliminary study showed the reduction of yield up to 40% when LS extract was produced using spray-drying technique. Regardless of the deliquescent character of LS extract, the reduction of yield was also observed during spray-drying process. It is attributable with the condition where only some particles could reach the collecting vessel because others adhered to the wall of chamber and cyclone or were blown away to the filter reservoir. This concept is supported by the previously reported work (Guo et al., 2020). Thus, the use of freeze-drying technique in extraction process of LS leaves was the proper choice to ascertain higher yield than that in spray-drying technique.

Microencapsulation using spray-drying technique is one of the conspicuous solutions to protect the moisture-sensitive powder from the adverse environmental impact. We found that this solution was fitted with LS extract powder due to its deliquescence characteristic. This process is a well-known process with high beneficial output to produce the consumable product in a large scale (Silva et al., 2014). In the microencapsulation part, S5 to S9 formulations were noticed to earn the successful yield because the production yield was higher than 50% as regarded to be the acceptable criterion for effective drying process (Moghbeli et al., 2019). Nonetheless, the reduction of yield in S2 and S4 formulations revealed the effects of temperature and level of pectin concentration used towards drying process. The use of larger amount of pectin and low temperature led to increasing the viscosity with unchanged glass transition temperature and prolonging the drying rates, respectively, resulting in incomplete drying process and reduction of yield as favor (Fernandes et al., 2012). The sticky materials formed in S1 and S3 formulations are also attributed to the optimum condition to produce microcapsule because regardless of the disadvantage using low temperature during atomization, higher temperature also enhance the adhesiveness and stickiness (Moghbeli et al., 2019).

Encapsulation efficiency is the crucial parameter reflecting the ability of carrier to encapsulate the active compounds (Indrawati et al., 2015). In this study, we observed that S2 formulation acquired the highest EE as the level of pectin was increased. A similar trend was found by Nunes et al. (2015), who worked with *Ilex paraguariensis* extract encapsulation using maltodextrin, asserting higher amount of carrier used was efficient to increase the entrapment of core material. Besides, the higher EE obtained by S6 than S4 formulation also explain the impact of optimum temperature at 120°C towards the effective microencapsulation process in regards of constructing the rapid formation of wall materials that can promote the retention of core (Balakrishnan et al., 2021). However, all of EE obtained were lower than 20% which was similar with the result reported by Correa-Filho et al. (2019), using gum arabic in the encapsulation of β-carotene, who also obtained low EE (< 20%). Due to the high viscosity of carrier, it promotes the vulnerability of core to be degraded by the drying temperature since the proximity of carrier stick over the wall of drying chamber. This also can be explained by the bigger particle size of core materials causing inefficient amount of carrier to coat the core (de Moura et al., 2018). Another factor that can be attributed is the possibility of LS extract ascribed to possess the negatively charged substances which once reacted with pectin as negatively charged polymer resulting in the repulsive interaction between both items and reducing the capacity of entrapment consequently (da Cruz et al., 2019).

To confirm the efficiency of microcapsules, the quantification of TPC and active markers is worthwhile to elucidate the effects of pectin concentration and inlet temperature on LS extract. Recently, there are two main phenolic markers, trilobatin and yanangdaengin, found in LS leaf extract as reported by Kongkiatpaiboon et al. (2020). The decrease levels of TPC and markers in this present study are considerably due to the higher quantity of pectin used. The similar result was found by Navidad-Murrieta et al. (2020) upon microencapsulation of *Hibiscus sabdariffa* extract in maltodextrin and gum arabic as carrier agents that acquired low total soluble polyphenols level in higher amount of carrier used. In addition, the higher level of TPC in S8 and S9 formulations than that in S7 formulation might be caused by the effect of high inlet temperature accelerating the polymerization and synthesis of polyphenols resulting in the increment of TPC level (Tolun et al., 2016). However, the level of markers was not affected by inlet temperature suggesting that trilobatin and yanangdaengin were not degraded because the range of temperature used was not exceeding the melting point of both markers at 165–166°C (Kongkiatpaiboon et al., 2020). Tolun et al. (2016), encapsulating grape polyphenols with maltodextrin and gum arabic, also observed that the use of inlet temperature at 140°C or lower did not reduce the main polyphenols amount analyzed by HPLC.

In FTIR spectra, the changes of wavenumbers and peaks in LS microcapsules can be explained by the reduction of intermolecular interaction between core and carrier when higher amount of carrier is applied. The same result was also observed by Emadzadeh et al. (2021), who encapsulated garlic oil in sugar beet pectin and β-cyclodextrin. Due to the intensive absorption peak in S8 formulation at 2362 cm^{-1}, this indicates that the new chemical bonds are created between LS extract and pectin. Meanwhile, the appearance of band at 1516 cm^{-1} is pointed to LS extract confirming the existence of physical interaction which further reflects the partially to almost entirely encapsulated

LS extract inside the pectin matrix. Due to the higher percentage of pectin in S2 formulation, mostly, the characteristic bands of LS extract were covered by pectin culminating in almost no new chemical bonds formed in this formulation. This idea is supported by Ribeiro et al. (2021) upon the microencapsulation of mangiferin using 10% pectin as a carrier. Furthermore, XRD diffractograms of LS microcapsules also revealed the transformation of crystalline to amorphous state which subsequent confirmed its successful encapsulation. Pertaining to the DSC results, the absence of prominent peaks of LS extract in S2 was also observed. This could be due to the elevation of thermal stability reflected by the expansion of the stability of S2 under harsh temperature. These alterations also demonstrated the presence of well-encapsulated LS extract inside pectin and, thus, it could improve the stability of S2. These results agreed with the study of Sansone et al. (2011) who encapsulated Fadogia extract with maltodextrin and pectin. In addition, the morphology of S2 powder by SEM demonstrating the physical protection of pectin to LS extract, as also indicated by FTIR, XRD, and DSC results.

CONCLUSION

Our study thereby demonstrated that pectin at high concentration (10% w/v) with an inlet temperature of 100°C was the optimum condition of spray-drying to produce the well-formed and encapsulated LS microcapsules. Our results suggest that this approach could be used as the novel formulation of LS extract to be applied as a consumable product for the treatment and prevention of disease in a society. The profound evaluations of microcapsules, the production of LS microcapsules-containing capsule, and the stability testing are suggested for the future study to provide the concrete evidence on the production and quality control parts of this product.

AUTHOR CONTRIBUTIONS

Vilasinee Hirunpanich Sato and Jiraporn Leanpolchareanchai suggested the conception of the work and led the project. Arman Syah Goli performed the experiment, analyzed and interpreted the data, and drafted the work. All authors edited, revised, and finally approved the manuscript.

ETHICS APPROVAL

Not applicable.

FUNDING

This project was supported by a grant from Thai Traditional Medical Knowledge Fund (42/2563) and partially supported by Mahidol University, Thailand.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this work.

ACKNOWLEDGEMENTS

We wish to thank Dr. Pilanthana Lertsatitthanakorn for providing the source of plant and Dr. Sumet Kongkiatpaiboon for generous supplies of the standards, trilobatin and yanangdaengin.

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Citation:

Goli, A. S., Leanpolchareanchai, J., Chewchinda, S., Yahuafai, J., Nontakham, J., Sato, H., & Sato, V. H. (2022). Microencapsulation of *Lysiphyllum strychnifolium* extract using pectin as a carrier matrix and its characterization. *Life Sciences, Medicine and Biomedicine*, *6*(1).
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Life Sciences, Medicine and Biomedicine
ISSN: 2600-7207