Indonesian Journal of Pharmacy

VOL 35 (2) 2024: 259–271 | RESEARCH ARTICLE

Optimizing the Spray Drying and Encapsulation of Mangiferin Extract from Mango Leaves (*Mangifera indica* L.) for Diabetes Management

Ngoc Nha Thao Nguyen*, Duc Linh Vo, Thi Thanh Yen Le and Thi Trang Dai Nguyen[1]

Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam

Article Info

Submitted: 24-05-2023
Revised: 02-10-2023
Accepted: 04-10-2023

*Corresponding author
Ngoc Nha Thao Nguyen

Email:
nnnthao@ctump.edu.vn

ABSTRACT

Mangiferin, a prominent phytochemical found abundantly in mango leaves, possesses a wide range of pharmacological properties, including antioxidant, antiviral, anticancer, and antidiabetic effects (Lee, Kim, & Kim, 2019). However, its minted solubility hinders its pharmaceutical applications. This study aimed to optimize the parameters of the spray-drying process to improve the dissolution of mangiferin in *Mangiferin indica* L. leaf extract and formulate capsules for diabetes treatment. Fourteen experiments were designed using Design Expert software and optimized with BC Pharsoft OPT software. Three independent variables were investigated: the ratio of mangiferin to excipients (X_1), the ratio of maltodextrin in spray fluid composition (X_2), and inlet air temperature (X_3). The dissolution of mangiferin was measured as the percentage released within 5 minutes (Y_1), 15 minutes (Y_2), and 30 minutes (Y_3), and the percentage of hygroscopicity at 25°C (Y_4). The results showed that the optimized spray drying process and formula, which included the ratios of mangiferin and maltodextrin in the spray drying solution of 8.30% and 9.63%, respectively, the inlet air temperature of 156.26°C, blowing speed of 50 m^3/s, and pump flow of 3 mL/min, possessed a dissolution of mangiferin in the spray drying powder of 91.89 ± 2.43% at 30 minutes, and a percentage hygroscopicity of 0.13 ± 0.13% at 25°C. This study, for the first time, explored the cause-effect relationships and optimization of the formulation of *Mangifera indica* L. leaf extract powder. The spray-dried powder capsule released more than 90% mangiferin within 30 minutes and showed significant hypoglycemic effects in inhibiting the α-glucosidase enzyme (IC 50 of 36.94 µg/mL equivalent to mangiferin in comparison with the commercial drug Glucobay (acarbose 176.09 µg/mL). In summary, the capsules containing the optimized formula of mangiferin spray-dried powder from *Mangifera indica* L. leaf extract have been successfully prepared and have potential antidiabetic effects in *in-vitro* settings.

Keywords: *Mangifera indica* L., mangiferin, spray-drying, optimization.

INTRODUCTION

Mangifera indica L. leaves, commonly known as mango leaves, have many pharmacological benefits such as anti-cancer (Palanuvej *et al.*, 2017), anti-diabetic (Samanta *et al.*, 2019), anti-oxidant (Ybañez-Julca *et al.*, 2020), anti-microbial (Jhaumeer Laulloo *et al.*, 2018), anti-obesity, lipid-lowering (Kumar *et al.*, 2021), hepato-protection, hair growth (Jung *et al.*, 2022) and anti-diarrheal properties (Yakubu & Salimon, 2015). The anti-diabetic potential of *Mangifera indica* L. leaf extract was assessed in albino mice. The results of the study indicated that the administration of *Mangifera indica* L. leaf extract had a discernible impact on diabetes-related parameters in the tested mice, and prevented the surge of glucose in the blood (Saleem *et al.*, 2019).

The extract induced a hypoglycemic effect in diabetic rats after four weeks of treatment. The extract was useful in maintaining the long-term effect, characterizing an anti-diabetic therapy (Villas Boas *et al.*, 2020). It is also confirmed that the extract of mango leaf at a dose of 400 mg/kg body weight reduced ($P < 0.05$) the blood glucose level in diabetic rats (Luka & Mohammed, 2012) significantly. However, the mechanism of action of plant extract was unknown (Senthilkumar *et al.*, 2020). Mangiferin is the predominant compound

found in mango leaves, with a substantial content ranging from 2.97% to 7.45% in dried leaves (Tayana *et al.,* 2019). This compound has a significant role in reducing cholesterol and fatty acids, while still serving as an anti-diabetic agent. It also helps the body to regulate metabolism and enhance and strengthen heart tissue (Kulkarni *et al*., 2018; Imran *et al*., 2017).

Numerous studies have been conducted globally to investigate the anti-diabetic potential of mangiferin and to compare the bioactivity of *Mangifera indica L*. leaf extract with other compounds. *Mangifera indica L*. leaf extract at a concentration of 500 mg/mL caused up to 95.7% inhibition of α-glucosidase while mangiferin at a concentration of 50 mg/mL resulted in 99.11% inhibition of α-glucosidase. Mangiferin is considered an active ingredient in the inhibition of α-glucosidase enzyme activity and in managing diabetic conditions (Kulkarni & Rathod, 2018). In a separate study, mangiferin demonstrated substantial inhibition of rat α-glucosidase with an IC50 value of 433.3 µg/mL, and *Mangifera indica* L. leaf extract showed potent inhibition of yeast α-glucosidase with an IC50 value of 50.3 µg/mL (Palanuvej *et al*., 2017). Manindicin A and B, mangiferin, and norathyriol have been isolated from the *Mangifera indica* L. leaf extract and demonstrated their anti-diabetic potential (Gu C. *et al.*, 2019). This inhibitory activity was notably four times more effective than that of the commercial inhibitor acarbose, which had an IC50 value of 16.28 ± 1.22 µg/mL. In contrast, mangiferin exhibited relatively weaker α-glucosidase inhibition, with an IC50 value of 32.11 ± 2.01 µg/mL. Moreover, manindicin A and manindicin B displayed even lower inhibitory activity against α-glucosidase, with IC50 values exceeding 300 µg/mL (indicating weak or no inhibition) for both compounds. The less inhibitory potential of mangiferin may be due to its molecular size and polarity (Gu C *et al*., 2019). The anti-diabetic potential of mangiferin was also demonstrated, as it increases insulin sensitivity (Roberto Villas Boas *et al.*, 2020) and inhibits α-glucosidase (Gupta, 2018). However, the main disadvantage of mangiferin is its poor solubility in water. That affects its bioavailability and reduces therapeutic efficacy (Acosta *et al*., 2016). Therefore, there is a compelling need to develop a formulation that increases the solubility and bioavailability of mangiferin. Due to its poor solubility, not only in water but in organic solvents as well, the technology applied to formulate this compound was dry amorphization using mesoporous silica. The solubility of these samples increased from 0.32 to 0.50 mg/ml, and the particle size decreased from 35.5 µm to around 7 µm (Adrienn Baán *et al.,* 2019). In a study by Liu *et al.* (2020), supercritical antisolvent technology was used to prepare mangiferin microparticles to improve their water solubility, antioxidant capacity, and oral bioavailability. The mangiferin microparticles had a higher solubility and were about 4.26, 2.1, and 2.5 times more soluble than free mangiferin in water, artificial gastric juice, and artificial intestinal juice, respectively. The dissolution rate of the mangiferin microparticles was also higher than that of free mangiferin.

Although various techniques were reported to improve the drug's solubility (Sudibyo *et al*., 2020; Windriyati *et al*., 2020), the use of the spray-drying method employing water-soluble carriers, considered a novel approach for addressing solubility issues, has not yet been reported for mangiferin extract derived from mango leaves. The primary objective of this study was to explore the optimization of a spray-drying powder formulation derived from mango leaves. This optimization process encompassed the determination of ideal spray drying conditions and the composition of excipients. Furthermore, the study aimed to ensure the stability of the properties of the spray drying powder under varying temperatures and storage conditions. An additional goal was to enhance the dissolution characteristics of mangiferin within the formulation. Subsequently, the optimized formulation was intended for encapsulation, followed by an assessment of its anti-diabetic potential *in vitro* models, using the α-glucosidase inhibitory assay as the method of evaluation.

MATERIALS AND METHODS

The mango leaf powder was obtained from mango leaves collected in O Mon, Can Tho City, in April 2022, and it had a recorded moisture content of 8.62%. These leaves were subsequently identified at the Department of Pharmacognosy-Botany-Traditional Medicine, Can Tho University of Medicine and Pharmacy, Vietnam.

The mangiferin standard used in the study was sourced from the National Institute of Drug Quality Control in Vietnam, with a control number of WS.0111280.01. Additionally, maltodextrin and lactose monohydrate were obtained from Merck in Germany. It is important to note that all materials and chemicals used in the study met the standards

required for pharmaceutical use and testing. The equipment used in the research included a spray dryer from Lab-plant in the UK, a UV-visible spectrophotometer (V-730, Jasco-Japan), a centrifuge (PLC-012E, Gemmy-Taiwan), a vortex (IKA Vortex 3, IKA-Germany), and a moisture analyzer (A&D MX-50, AND-Japan).

Preparation of dried mango leaf extracts

A total of twenty kilograms of dried mango leaf powder underwent extraction using the ultrasonic-assisted extraction method. The extraction process involved the use of a 44% ethanol solvent, with a ratio of medicinal herbs to solvent of 1:20 (w:v), and ultrasonic energy at 40 W. Following the collection of mango leaf powder, the extraction process involved several steps. Initially, the powder was subjected to filtration, and the resulting filtrate was evaporated to achieve a volume ratio of 1:10 in relation to the total filtrate volume. Subsequently, this concentrated extract was combined with distilled water in a 1:1 ratio and allowed to settle at a temperature of 5°C for 24 h. Following this settling period, the extract's precipitate was filtered and underwent multiple washes with distilled water until the filtrate no longer exhibited a reddish-brown coloration. Each batch processed according to this protocol had an initial weight of 500g. The precipitate from each batch was subsequently amalgamated and subjected to drying at 60°C until the moisture content decreased to below 5%. The quantification of mangiferin content within the dried extract was performed using a UV-visible spectrophotometer (V-730, Jasco-Japan)

Process of preparing spray-dried powder from mango leaves

The composition of the spray drying solution included dried mango leaf extract, lactose, maltodextrin, and distilled water. The spray drying powder formulation process involved several sequential steps. Initially, lactose and maltodextrin were dissolved in distilled water to create an excipient solution. Subsequently, the dried mango leaf extracts were dispersed into this excipient solution with the assistance of an IKA stirrer operating at a speed of 1000 rpm. The spray drying process was conducted with specific parameters, including a blowing speed of 50 m^3/s, a spray-dried fluid volume of 3 ml/min, an internal nozzle diameter of 0.5 mm, and the inlet air temperature as the variable of interest.

Throughout the spray-drying process, the continuous stirring of the spray-dried fluid was maintained using a magnetic stirrer. After completion of the process, the resulting dried powder was collected and placed in a desiccator for 24 h to ensure proper drying and stabilization. Subsequently, the powder was stored in a tightly sealed container at a constant temperature of 25°C. These steps were crucial to the successful production and preservation of the spray-dried mango leaf extract powder.

Experimental design and data analysis

A total of 14 experimental runs according to the D-optimal design were generated by Design Expert software (v7.0,, Stat-Ease-USA) to study the effects of independent variables on dependent variables. The content of mangiferin in total spray-dried fluid composition (X_1), the content of maltodextrin in total spray-dried fluid composition (X_2), and the inlet air temperature (X_3) were selected as three independent variables, whereas the percentage of mangiferin released within 5 min (Y_1), 15 min (Y_2), 30 min (Y_3), and the percentage of hygroscopicity at 25°C (Y_4) were chosen as four dependent variables. The ratio of mangiferin to excipients (X_1) and inlet air temperature (X_3) was studied at three levels, and spray-dried fluid composition (X_2) was studied at two levels (Table I).

The results of the experimental formulations were analyzed using BCPharSoft OPT software (University of Medicine and Pharmacy HCMC-Vietnam). The best-fitting model was chosen. To evaluate the cause-and-effect relations between the independent and dependent variables, the 3D diagrams of the fitted models were depicted (Table I). The optimized formulation was conducted in triplicate for further validation. The experimental data of the optimized formulation were compared with the predicted data created by BCPharSoft OPT software using Excel 2016 (Microsoft, USA).

Analytical method of mangiferin

The measurements were performed using a UV-visible spectrophotometer (V-730, Jasco-Japan). Standard solution*:* accurately weigh 10 mg of mangiferin standard, and dissolve in ethanol-water (1:1) to obtain 100 µg/ml as a stock standard solution. After that, the stock standard was diluted with ethanol-water (1:1) to give solutions with different concentrations (10, 15, 20, 25, and 30 µg/ml). The samples were measured at 367 nm.

Blank sample: ethanol-water (1:1).

Test solution*:* Weigh 0.1 g of dried mango leaf extract or spray-dried mangiferin powder, in 100 ml of ethanol-water (1:1). The test sample was extracted in an ultrasonic bath for 20 minutes. Then, the sample was centrifuged, and the supernatant was taken. The solution was diluted with ethanol-water (1:1) to the appropriate absorbance (from 0.2 to 0.8) and photometrically measured at 367 nm.

$$\text{Mangiferin content (\%)} = \frac{C_{mangiferin} \times V \times k}{m\,(100 - H) \times 1000} \times 10$$

$C_{mangiferin}$: the concentration extrapolated from the standard curve in the test solution (µg/ml). V: the volume of test solution (ml); k: the dilution factor; m: the mass of leaf extract/spray-dried mangiferin powder (g); H: the moisture content of leaf extract/spray-dried mangiferin powder (%).

Evaluation of the hygroscopicity of spray-dried powder

Two grams of spray-dried powder were stored tightly in a weighing cup and kept in a room followed by a temperature of 25 ± 1°C and a humidity of 75 ± 5% RH. After 7 days, the powder was weighed and the mass gain was determined due to the hygroscopicity of the spray-dried powder.

$$\text{Powder hygroscopicity (\%)} = \frac{\alpha' - \alpha}{\alpha} \times 100$$

Where a is the mass of the spray-dried powder at the beginning of the experiment; α' is the mass of the spray-dried powder after 7 days.

Evaluation of the dissolution of mangiferin in spray-dried powder products

The sample was stirred with a speed of 75 rpm in a pH 1.2 buffer solution (volume of 900 mL) maintained at 37 ± 0.5°C. At sampling times of 5, 15, and 30 min, the sample volume of 10 mL was compensated for the corresponding volume after sampling. The sample was filtered through a 0.45 µm membrane and quantified by the UV Spectrophotometer (V-730, Jasco-Japan) at wavelengths of 367 nm.

The pH 1.2 buffer solution used in this experiment was prepared as follows: 2.0 g of sodium chloride were dissolved in water, followed by the addition of 7.0 mL of hydrochloric acid. The solution was then further diluted with water to a final volume of 1 liter. This pH 1.2 buffer solution served as the medium for conducting the dissolution experiments.

Characterization of mangiferin before and after spray drying

Solubility

The equilibrium solubility of mangiferin, both before and after the spray drying process, was determined using the following procedure: A surplus amount of the sample was added to the dissolution medium at a concentration of 50 mg per 10 mL. The mixture was then subjected to continuous shaking for 72 h. This agitation was performed in a water bath shaker, maintaining a constant temperature of 37 ± 1°C throughout the duration of the experiment. This procedure aimed to assess the solubility characteristics of mangiferin under the specified conditions and to compare its solubility before and after the spray drying process. To remove undissolved mangiferin, the equilibrated samples were centrifuged at 5000 rpm for 10 min. The supernatant was then examined using the UV Spectrophotometer (V-730, Jasco-Japan) at a λ max of 367 nm.

UV spectrum determination

The UV-vis absorption spectra were recorded in the range of 200–600 nm with a UV Spectrophotometer (V-730, Jasco-Japan).

TGA/DSC evaluation

A DSC (Labsys Evo, Setaram-France) was used to define the physical state of mangiferin. The sample for TGA/DSC measurement was prepared by standard procedures using a sealed aluminum pan. About 3-6 mg of sample was used in the analysis. The analyses were performed by heating the samples from 25°C to 625°C at a heating rate of 5°C/min with N_2 as the purge gas.

FTIR analysis

An FTIR-4600 spectrophotometer (4600, Jasco-Japan) was used to investigate the differences in functional group spectra among the mangiferin extract, spray spray-drying mangiferin. The spectral range of samples was 4,000 to 400 cm^{-1}.

Encapsulation of raw powder, spray-dried mixture, and assessment of *in-vitro* drug release

Following the guidelines outlined in the Vietnamese Pharmacopoeia of 2018, a series of quality criteria were evaluated for both the spray-dried powder and the raw powder. These criteria

encompass sensory attributes, weight loss due to drying, and quantitative measures. To conduct these assessments, spray-dried powder labeled as S1 and raw powder labeled as R1 were encapsulated. The encapsulation process involved the use of hard gelatine capsules, specifically those of size number 0. The composition of the capsule fillings included ingredients and excipients. Talc was added at a concentration of 1%, while Ludipress was used to account for the remaining 100% of the total weight, which amounted to 500 mg. The capsule filler powder was prepared by simply mixing either the spray-dried powder or the raw powder with the specified excipients. The encapsulation of these materials was carried out using a manual encapsulation machine.

Uniformity of weight test of capsules

The capsules R1 and S1 were evaluated for uniformity of weight. Twenty randomly selected capsules were individually weighed using an analytical balance (Kern, Germany). Each capsule was carefully opened, and the contents were completely removed. The difference between the weight of the intact capsule and the empty shell was calculated for each capsule. The mean weight and percentage deviations of the twenty capsules were calculated.

Assessment of in-vitro drug release

This study was carried out using USP Dissolution Apparatus 1 (Pharmatest-Germany) in 900 mL of pH 1.2 buffer solution at a speed of 100 rpm and a temperature of 37±0.5°C. The test proceeded with R1 and S1 capsule formulations. Six capsules from each formulation were introduced into the baskets, and the procedure was conducted. At 5, 15, and 30 min, 10 mL of each sample were withdrawn and replaced with fresh dissolution medium maintained at 37±0.5 °C. The samples withdrawn were then filtered through 0.45 µm filter papers and assayed using the UV Spectrophotometer (Jasco V-730, Japan) at wavelengths of 367 nm for R1 and S1 capsules, respectively, using the data obtained from the calibration plots. The cumulative drug release was calculated and plotted against time.

***In-vitro* α-glucosidase inhibitory assay**

The capsules containing 100 mg mangiferin in spray-dried powder, and the reference product/positive control Glucobay (acarbose) were evaluated *in-vitro* for their anti-diabetic properties using the standard α-glucosidase inhibitory assay at 37°C, pH 7.0, with slight modifications (Kazeem *et al.,* 2013; Nguyen *et al.,* 2023). For this, the test samples (0.80 ml) were mixed with 0.8 ml of 0.1M phosphate buffer pH 7.0 and 0.80 ml of the α-glucosidase (1.0 U/ml; one unit is the required amount of α-glucosidase to catalyze the substrate 4-nitrophenyl β-D-glucopyranoside (pNPG) to form 1 µmol of p-nitrophenol (product) per minute) for 15 minutes at 37°C. Then, 0.80 ml of pNPG, at a concentration of 2 mM, was added to the mixture to start the reaction, followed by another 15-minute incubation. Finally, the reaction was halted with the addition of 3.2 ml of Na_2CO_3 (0.2M), and the solution was UV-Vis spectroscopically (Jasco V-730, Japan) measured at 400 nm. The blank was processed with the same protocol, with the sample being DMSO, the solvent for dissolving mangiferin. The percentages of α-glucosidase inhibition of the samples were calculated according to % α-glucosidase inhibition = $A_{Blank} - A_{Sample} \times 100\ A_{Blank}$ (1) where A_{Blank} and A_{Sample} are the absorbance values of the blank and the test samples, respectively.

Statistical analysis

All measurements were conducted in triplicate to ensure robust and reliable data. The results obtained were presented as the mean value along with the corresponding standard deviation (SD), reflecting the variability within the data set. Significant differences between the data were analyzed using Student's *t*-test and one-way analysis of variance. A P-value less than 0.05 was considered significant. Statistical analysis of the results was performed using Microsoft Excel (Version 2016).

RESULTS AND DISCUSSION

Preparation of dried mango leaf extract

In the initial phase of the study, the objective was to create a mango leaf extract with a heightened mangiferin content suitable for the subsequent spray-drying process. The results of this phase revealed that from 20 kilograms of dried mango leaf powder, a total of 429 grams of dried mango leaf extract was obtained. The moisture content of this extracted material was determined to be 4.25% with a standard deviation of 0.07%. Remarkably, the mangiferin content within this extract was found to be 83.17%, with a standard deviation of 1.01%. In a prior study, mango leaf extracts were typically derived from *M. indica* leaves sourced from Chinese trees using either water or ethanol extraction processes, resulting in

mangiferin contents ranging from 60% to 65%. The immersion method, employing ethanol as the extracting solvent, was identified as the optimal condition for mango leaf extraction (Reddeman *et al*., 2019). In contrast, our study used the ultrasonic-assisted extraction method with a 44% ethanol solvent, followed by precipitation of the extract with distilled water. The precipitate was subsequently filtered and washed multiple times with distilled water to obtain an extract with a significantly elevated mangiferin content exceeding 80%. It is noteworthy that compared to certain other studies, our extraction efficiency, while relatively moderate, was still notably higher. For instance, studies in Thailand reported mangiferin ethanolic extraction with efficiencies ranging from 1.3% to 2.8% (Aranya *et al*., 2010). This underscores the substantial influence of the chosen extraction conditions on key factors such as extract moisture content, extraction efficiency, and the *in-vitro* α-glucosidase inhibitory activity of the extract.

Optimization data analysis

The formula and process for spray-drying mango leaf extract were investigated under experimental conditions, designed by Design Expert software v7.0 (Table I).

Table I. The independent variables of formulations (F1-F14) and their responses

F	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	10	0	140	15.44	20.11	25.97	3.20
2	8	10	140	38.45	49.15	53.19	0.02
3	8	10	120	26.88	35.11	35.11	0.01
4	10	10	160	48.40	60.01	63.90	0.01
5	12	0	120	13.64	17.97	23.94	2.60
6	10	0	160	14.49	18.88	30.30	3.50
7	12	10	160	21.75	27.85	30.51	0.02
8	12	0	140	14.71	19.25	22.30	3.12
9	8	10	160	69.26	84.89	96.61	0.03
10	8	0	140	22.97	27.46	31.14	3.12
11	12	10	120	24.50	31.86	36.35	0.02
12	10	10	140	23.83	25.77	60.50	0.02
13	10	0	120	15.18	19.92	22.37	2.30
14	8	0	120	23.90	28.65	31.94	2.10

X_1: the content of mangiferin in total spray-dried fluid composition (%); X_2: the content of maltodextrin in total spray-dried fluid composition (%); X_3: inlet air temperature (°C); Y_1: % mangiferin released within 5 min; Y_2: % mangiferin released within 15 min; Y_3: % mangiferin released within 30 min; Y_4: % hygroscopicity.

The data (Table I) used as inputs for BCPharSoft OPT software to study cause-effect relations and optimize the formulation of spray drying powder from mango leaves. Training parameters were set as follows: Test groups were Y_1 [4, 6], Y_2 [4, 6], Y_3 [4, 13], and Y_4 [1, 11]; Transfer function: Back Propagation Learning; The three-dimensional response surface plots were used to study the interaction effects of two independent variables on the dependent variables at one time when the third variable was kept at a constant level. All R^2 values that were greater than 0.9 indicated very good reliability of the models. Therefore, these models could be used for multivariate optimization.

Validation of the optimization model

The optimization results by the intelligent software BCPharsoft OPT, including the optimal parameters of the formulation and the predicted values of the product properties.

Optimal parameters

X_1 (the content of mangiferin in total spray-dried fluid composition) = 8.30%; X_2 (the content of maltodextrin in total spray-dried fluid composition) = 9.63%; X_3 (inlet air temperature) = 156.26°C

Predicted Responses

Y_1 (the percentage of mangiferin released within 5 min) = 60.08%; Y_2 (the percentage of mangiferin released within 15 min) = 81.57%; Y_3 (the percentage of mangiferin released within 30 min) = 89.26%; Y_4 (the percentage of hygroscopicity) = 0.27%.

Perform the test on three lots using the independent variable values that have been optimized by BCPharSoft OPT software. Observed Responses: Y_1 (the percentage of mangiferin released within 5 min) = 60.20 ± 3.83%; Y_2 (the percentage of mangiferin released within 15 min) = 81.11 ± 2.21%; Y_3 (the percentage of mangiferin released within 30 min) = 91.89 ± 2.43%; Y_4 (the percentage of hygroscopicity) = 0.13 ± 0.13%. The results demonstrate that the observed values were in good agreement with the predicted values. The P-values of Y_1, Y_2, Y_3, and Y_4 are 0.22, 0.98, 0.07, and 0.56, respectively. All of the P-values are greater than 0.05.

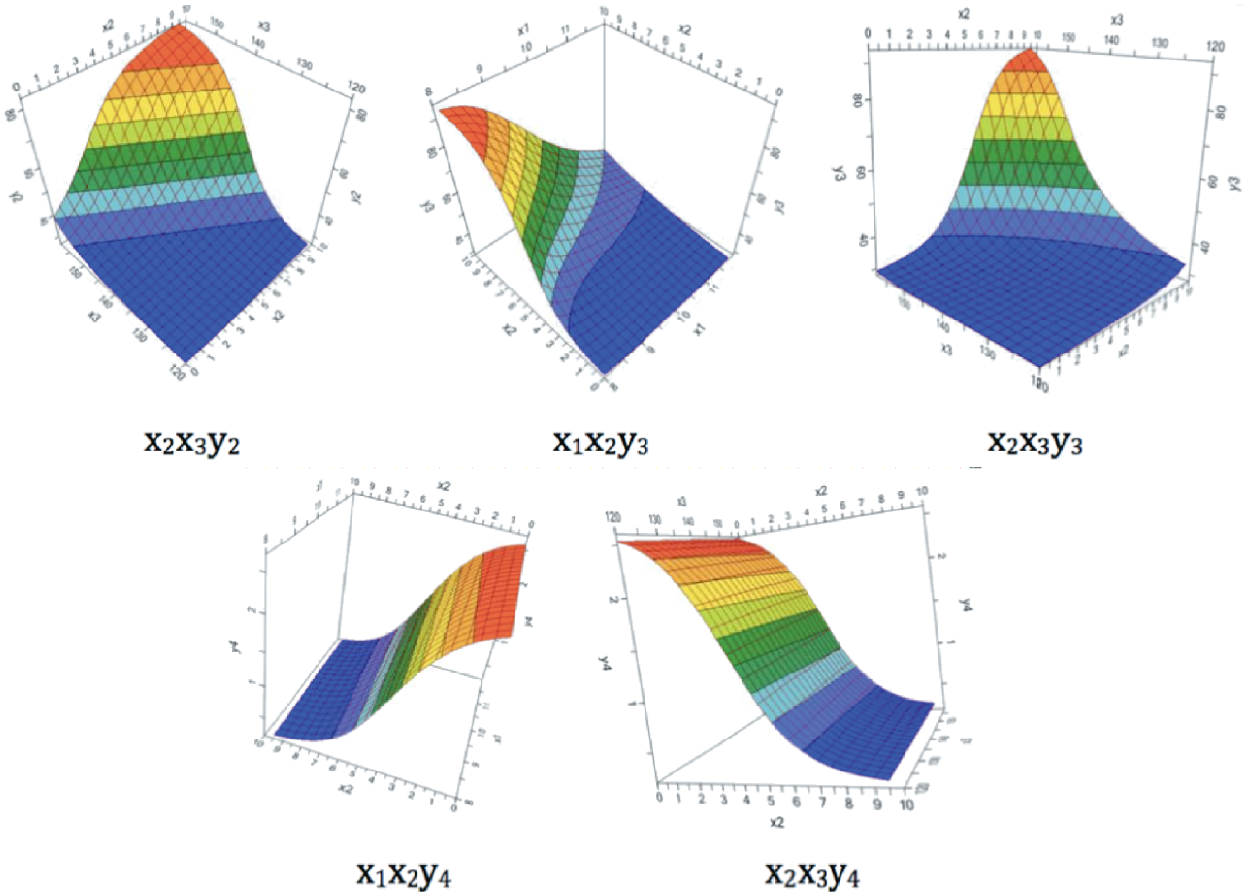

Figure 1. Response surface plots showing the effect of the content of mangiferin, the content of maltodextrin, and inlet air temperature on the percentage of mangiferin released and hygroscopicity (X_1: the content of mangiferin in total spray-dried fluid composition (%); X_2: the content of maltodextrin in total spray-dried fluid composition (%); X_3: inlet air temperature (°C); Y_1: % mangiferin released within 5 min; Y_2: % mangiferin released within 15 min; Y_3: % mangiferin released within 30 min; Y_4: % hygroscopicity.)

In the process of applying the spray-drying method for preparation, selecting an appropriate carrier with the right ratio is crucial. In this particular study, maltodextrin was chosen as the carrier, and its ratio was varied to explore its capacity to release active ingredients and its propensity to regain hygroscopicity within the formulations. Maltodextrin is an ideal choice for this method due to its favorable attributes, including excellent water solubility and a low tendency to reabsorb moisture after drying. Moreover, maltodextrin, when used in the correct ratio within the formulation, addresses a common issue associated with spray-dried powders, which is their susceptibility to re-hygroscopicity. This issue arises because the spray-drying process involves high temperatures and hot air, resulting in very low moisture levels in the freshly prepared product. The substantial temperature differential between the drying chamber and the external environment makes the powder highly susceptible to moisture reabsorption. Hence, selecting the right ratio of maltodextrin is essential to mitigate this re-hygroscopicity concern. So, the desiccant excipients in the formula should be enough, and if any, let the temperature reduce to 60°C for 15 minutes before collecting the powder. Then, collect and store in a desiccator right after finishing the process.

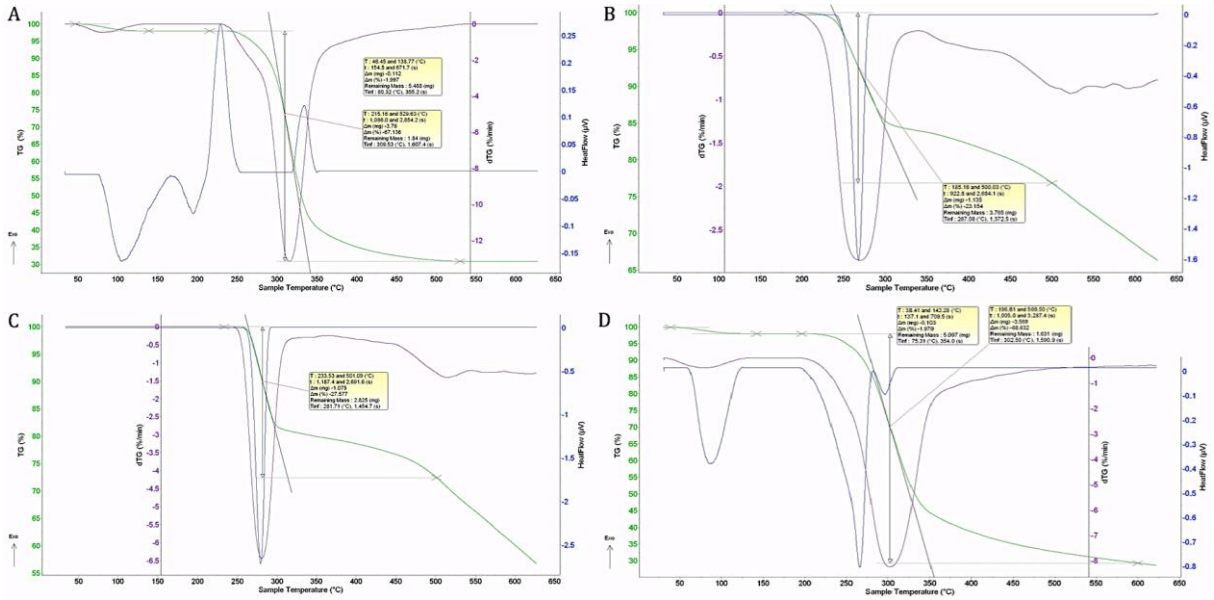

Figure 2. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) curves from 25 to 625°C. Green represents TGA and Blue represents DSC. (a) maltodextrin; (b) mangiferin extract; (c) mangiferin standard; (d) spray-dried mangiferin.

An examination of the causal relationship between the independent variable and the dependent variable (Figure 1) revealed a noteworthy trend. Specifically, it was observed that as the ratio of mangiferin increased, there was a corresponding decrease in the capability to release the active substance. This phenomenon can be attributed to the necessity of increasing the quantity of maltodextrin in tandem with the rise in mangiferin concentration.

This augmented presence of maltodextrin is crucial as it functions as a carrier, facilitating the dissolution of the active ingredient. Furthermore, as the percentage of maltodextrin increased, there was a concomitant improvement in the ability to release the active ingredient, and concurrently, a reduction in the moisture absorption capacity of the spray-dried powder was observed. This shift in characteristics can be attributed to the intrinsic properties of maltodextrin, which is known for its favorable solubility attributes and low moisture absorption capacity. This may be because maltodextrin has good solubility and low moisture absorption capacity, similar to studies by Karaaslan *et al.* (2014) and Yunita *et al.* (2020). When the drying temperature was increased, the ability to releasee the active ingredient and the moisture absorption capacity of the spray-dried powder rose. The main reason is the high temperature and the low moisture content of the recovered powder after spray drying, so the possibility of being re-hygroscopic is higher, similar to the study of Goula *et al.* (2005).

Characterization of mangiferin before and after spray drying

Solubility

The results of the saturation solubility study of mangiferin before and after spray drying were 0.23mg/mL and 0.42mg/ml, respectively. These data confirm that the drug release study was carried out in a sink condition (100 mg/900 ml).

UV spectrum determination

The spectra of mangiferin extract, spray-dried mangiferin, and the reference standard are shown using UV-visible spectrophotometry; mangiferin presented three significant peaks that were, respectively, 266, 319, 369; 265, 318, 368; and 264, 317, 367 (Figure 3). Notably, spray-dried mangiferin had the same UV spectrum as the mangiferin reference standard.

TGA/DSC evaluation

The thermal degradation profile helps to determine the temperature profile during the spray drying process to avoid any compound thermal decomposition. In the present study, the thermal degradation profiles of mangiferin and maltodextrin were evaluated using TGA. The mass loss of all samples during heating. According to the results analysis (Figure 2), spray-dried mangiferin experiences an initial small mass loss (1.98% of the initial sample weight) up to 142.28 °C due to residual moisture, then a second mass loss stage beginning at 196.81 °C and continuing until 599.50 °C (Figure 2d). Hence, based on the above thermal decomposition analysis, mangiferin and maltodextrin show good thermal stability, ensuring safe processing at high temperatures.

Mangiferin extract had only one sharp peak at 267.08°C. Maltodextrin had four different kinds of peaks. DSC of spray-dried mangiferin showed that the endothermal peak of mangiferin remained at 265.23°C, nearly the same as the onset temperature of mangiferin. This result showed that mangiferin is still in the amorphous state, and the high dissolution rate could result from reducing the particle size and wrapping it with maltodextrin.

FTIR analysis

Mangiferin compatibility with excipients was studied by FT-IR spectroscopy. From the spectra of the spray-dried mangiferin, shown in Figure 3, it was seen that peaks at 3368 cm^{-1} showed the presence of a secondary OH-bond, a peak at 2929 cm^{-1} showed the presence of C-H anti-symmetric stretching, and peaks at 1645 cm^{-1}, 1411 cm^{-1}, and 1152 cm^{-1} showed the presence of C-O stretching, CH-CH bending, and a CO bond. The peak at 1024 cm^{-1} showed the presence of C-C stretching in the mangiferin structure. The mangiferin is spray-dried with maltodextrin, as all the characteristic peaks of the drug were retained. Hence, there is no incompatibility between the mangiferin and this excipient.

Encapsulation and assessment of *in-vitro* mangiferin release

In the case of the type 2 diabetic model, the body weight of diabetic rats was significantly restored by treating them with mangiferin (at a dose of 10 and 20 mg/kg, administered intraperitoneally (i.p.) daily for 30 days when compared with standard glibenclamide (10 mg/kg) (B Dineshkumar *et al.*, 2010). Thus, based on the mangiferin content of the spray-dried powder (35.7% ± 0.3%), we chose to use 0.280 g of spray-dried powder per hard gelatine capsule (equal to 100 mg mangiferin). The same active ingredient content of raw materials was also used. The encapsulated mixture contained a combination of Ludipress as a filler and talc as a glidant. The appearance of mangiferin extract (raw material), spray-drying mangiferin, and the encapsulated mixture (Figure 4). The data on the predicted theoretical mass and the observed mass of the encapsulated mixture showed that the capsules were similar in terms of mass uniformity (percentage deviation < 10%). One of the most important tests for assessing the quality of capsules is the determination of the dissolution of the active compounds. A dissolution test was applied using a basket-type dissolution apparatus to determine the dissolution of the formulated capsules (Figure 5).

Figure 4. Images of mangiferin. (a) mangiferin extract; (b) spray dried mangiferin; and (c) spray dried mangiferin mixture before encapsulation.

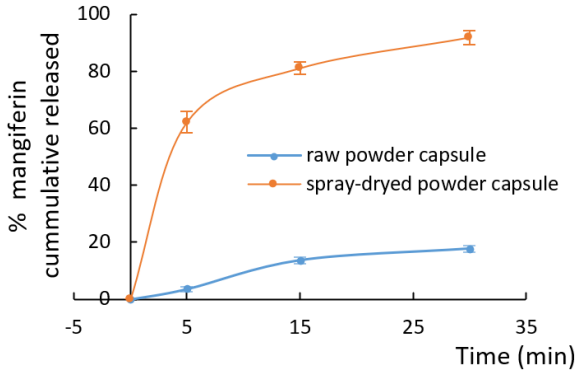

Figure 5. Dissolution profiles of the optimized spray-dried powder capsule compared with the raw powder capsule (n = 6, with data shown as mean ±SD).

In terms of the ratio of excipients and optimal spray drying conditions, two main problems have been solved simultaneously: improving the solubility of mangiferin 4.5 times compared to dry high-quality raw materials with the same active ingredient content (releasing more than 90% active ingredients in 30 min), and

reducing the ability of spray drying powder to absorb moisture back to less than 0.3%. This result was somehow moderate, or rather, better than another study.

Mixtures of mangiferin and polymers at the ratio of 1:1:2 were also prepared and analyzed in a previous study. The solubility of these samples increased nearly two-fold from 0.32 to 0.50 mg/ml and the particle size decreased from 35.5 μm to around 7 μm (Adrienn Baán *et al.,* 2019). In the study of Liu *et al.* (2020), supercritical antisolvent technology was used to prepare mangiferin microparticles with N, N-dimethylformamide as a solvent, and carbon dioxide as an antisolvent to improve their water solubility, antioxidant capacity, and oral bioavailability. The mangiferin microparticles had a higher solubility and were about 4.26, 2.1, and 2.5 times more soluble than free mangiferin in water, artificial gastric juice, and artificial intestinal juice, respectively. The dissolution rate of the mangiferin microparticles was also obviously higher than that of free mangiferin. In a study by Telange *et al*. (2021), self-assembled nanoparticles encapsulated with phospholipid complex using a combination of nanoprecipitation and solvent evaporation methods were developed to enhance the biopharmaceutical and antioxidant potential of mangiferin and the dissolution rate of mangiferin gained around 98% via a biphasic release pattern. To this end, by using the spray-drying technique, the dissolution of the mangiferin was significantly improved.

***In-vitro* α-glucosidase inhibitory assay**

In the *in-vitro* study, the mixture in the capsule of spray-dried mango leaf extract containing 100 mg mangiferin content, at a concentration of 36.94 ± 0,35 μg/ml, demonstrated α-glucosidase inhibitory activity of 50%, which is 4.5 times lower in comparison with the commercial drug Glucobay (acarbose) (176.09 ± 0,26 μg/ml).

The mangiferin action in our result was equal to that in the previous study in India, which showed that the IC50 of mangiferin was 36.84 μg/ml (Sekar *et al.,* 2019). But when compared to the standard acarbose (IC 50 = 21.33 μg/ml in the study of Sekar), mangiferin in our study showed higher activity. Another result showed that mangiferin inhibited α-glucosidase with an IC50 value of 358.54 μM, which is more potent than the positive drug acarbose (IC50 = 479.2 μM) in the zymological experiment (Shi *et al.*, 2017). Mango leaf extract and mangiferin exhibited IC50 values of 0.0503 and 0.5813 mg/ml, respectively, against α-glucosidase, compared to acarbose, which had an IC50 of 0.4493 mg/ml (Palanuvej *et al.,* 2017).

Interestingly, our study revealed a higher inhibition efficiency in the mango leaf extract, despite mangiferin being the primary component. Consequently, it is imperative to conduct a thorough investigation into the interconnections between the extracted mass, mangiferin content, and the biological activities exhibited by the extract. This inquiry is essential as these factors may not exhibit a straightforward correlation with the proportion of mangiferin alone.

In terms of capsule efficacy, the capsules containing equivalent amounts of mangiferin demonstrated a similar IC50 value compared to the respective extract. This indicates that the spray-drying processes and the capsule excipients did not significantly affect the mangiferin activity. In addition, the IC50 value of a capsule containing spray-dried mango leaf extract is significantly lower compared to that of an acarbose tablet.

CONCLUSION

For the first time, efforts were made to optimize the parameters of the spray-drying process to enhance the dissolution properties of mangiferin in *Mangifera indica* L. leaf extract. This optimized powder, enriched with a high content of mangiferin, was subsequently encapsulated for potential use in diabetes treatment. The key parameters for the preparation of the spray-dried mango leaf powder, where mangiferin is the predominant compound, were as follows: the ratio of mangiferin to maltodextrin in the spray-dried solution was 8.30% and 9.63%, respectively; the air inlet temperature was set at 156.26°C, the blowing speed at 50 m^3/s, and the pump flow rate at 3 ml/min. The process of preparing spray-dried mangiferin powder not only demonstrated its effectiveness in improving dissolution characteristics but also ensured the stability of the spray-dried powder, preventing hygroscopicity during storage. Moreover, capsules containing the spray-dried mango leaf powder exhibited noteworthy anti-diabetic activities in *in-vitro* α-glucosidase inhibitory assays. The capsules containing the spray-dried mango leaf powder hold promise and warrant further investigation, particularly in terms of in-vitro, subclinical, and clinical trials, with the potential to evolve into a valuable pharmaceutical product for managing diabetes.

ACKNOWLEDGMENTS

The authors thank Can Tho University of Medicine and Pharmacy for supporting this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Acosta, J., Sevilla, I., Salomón, S., Nuevas, L., Romero, A., & Amaro, D. (2016). Determination of mangiferin solubility in solvents used in the biopharmaceutical industry. *Journal of Pharmacy & Pharmacognosy Research*, 4(2), 49-53.

Dineshkumar, B., Analava Mitra, Manjunatha, M. (2010). Studies on the anti-diabetic and hypolipidemic potentials of mangiferin (Xanthone Glucoside) in streptozotocin-induced Type 1 and Type 2 diabetic model rats. *International Journal of Advances in Pharmaceutical Sciences*, 75-85. https://doi.org/10.5138/ijaps.2010.0976.1055.01009

Goula, A.M., & Adamopulos, K.G. (2005). Spray drying of tomato pulp in dehumidified air: The effect on powder properties. *Journal of Food Engineering*, 66, 35-42. https://doi.org/10.1016/j.jfoodeng.2004.02.029

Gu, C., Yang, M., Zhou, Z., Khan A., Cao, J., & Cheng, G. (2019). Purification and characterization of four benzophenone derivatives from *Mangifera indica* L. leaves and their antioxidant, immunosuppressive and α-glucosidase inhibitory activities. *Journal of Functional Foods*, 52, 709–714. https://doi.org/10.1016/j.jff.2018.11.045

Gupta, R. (2018). Active phytoconstituents for diabetes management: A review. *Journal of Complementary and Integrative Medicine*, 15, 15. https://doi.org/10.1515/jcim-2017-0123

Imran, M., Arshad, M.S., Butt, M.S., Kwon, J.H., Arshad, M.U., & Sultan, M.T. (2017). Mangiferin: a natural miracle bioactive compound against lifestyle related disorders. *Lipids in Health and Disease*, 16(1), 84. https://doi.org/10.1186/s12944-017-0449-y

Jhaumeer, L. S., Bhowon, M. G., Soyfoo, S., & Chua, L. S. (2018). Nutritional and biological evaluation of leaves of *Mangifera indica* from Mauritius. *Journal of Chemistry*, 1-8. https://doi.org/10.1155/2018/6869294

Jung, H., Jung, D.M., Lee, S.S., Kim, E.M., Yoon, K., & Kim, K.K. (2022). *Mangifera Indica* leaf extracts promote hair growth via activation of Wnt signaling pathway in human dermal papilla cells. *Anim Cells Syst (Seoul),* 26(3), 129-136. https://doi.org/10.1080/19768354.2022.2085790

Karaaslan, I., & Dalgic, A. (2014). Spray drying of liquorice (*Glycyrhiza glabra*) extract. *Journal of Food Science and Technology*, 51(11), 3014–3025. https://doi.org/10.1007/s13197-012-0847-0

Kazeem, M.I., Dansu, T.V., & Adeola, S.A. (2013). Inhibitory effect of *Azadirachta Indica* A. juss leaf extract on the activities of α-amylase and α-glucosidase. *Pakistan Journal of Biological Sciences*, 16(21), 1358-62. https://doi.org/10.3923/pjbs.2013.1358.1362

Kulkarni, V. M., & Rathod, V. K. (2018). Exploring the potential of *Mangifera indica* leaves extract versus mangiferin for therapeutic application. *Agriculture and Natural Resources*, 52(2), 155-161. https://doi.org/10.1016/j.anres.2018.07.001

Kumar, M., Saurabh, V., Tomar, M., Hasan, M., Changan, S., Sasi, M., Maheshwari, C., Prajapati, U., Singh, S., Prajapat, R.K. et al (2021). Mango (*Mangifera indica* L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. *Antioxidants*, 10, 299. https://doi.org/10.3390/antiox10020299

Liu, M., Liu, Y., Ge, Y., Zhong, Z., Wang, Z., Wu, T., Zhao, X., & Zu, Y. (2020). Solubility, Antioxidation, and Oral Bioavailability Improvement of Mangiferin Microparticles Prepared Using the Supercritical Antisolvent Method. *Pharmaceutics*, 12(2), 90. https://doi.org/10.3390/pharmaceutics12020090

Luka, C.D., & Mohammed, A. (2012). Evaluation of the anti diabetic property of aqueous extract of *Mangifera indica* leaf on normal and alloxan-induced diabetic rats. *Journal of Natural Product and Plant Resource*. 2(2), 239–243.

Nguyen, N.N.T., Duong, X.C., Nguyen, K.N., Van, Nguyen T.N., Dai, Nguyen T.T., Le, T.T.Y., Le, T.C.T., Nguyen, T.T.T., Pham, D.T. (2023). Development and *in-vitro/in-vivo* evaluation of film-coated tablets containing *Azadirachta indica* A. Juss leaf extracts for diabetes treatment. *Journal of Applied Pharmaceutical*

Science, 13(01), 193–200. https://doi.org/10.7324/JAPS.2023.130119

Palanuvej, C., Ganogpichayagrai, A., & Ruangrungsi, N. (2017). Antidiabetic and anticancer activities of *Mangifera indica* cv. *Okrong* leaves. *Journal of Advanced Pharmaceutical Technology & Research*, 8(1), 19–24. https://doi.org/10.4103/2231-4040.197371

Reddeman, R.A., Glávits, R., Endres, J.R., Clewell, A.E., Hirka, G., Vértesi, A., Béres, E., & Szakonyiné, I.P. (2019). A Toxicological Evaluation of Mango Leaf Extract (*Mangifera indica*) Containing 60% Mangiferin. *Journal of Toxicology*, 4763015. https://doi.org/ 10.1155/2019/4763015

Saleem, M., Tanvir, M., Akhtar, M.F., Iqbal, M., & Saleem, A. (2019). Antidiabetic Potential of *Mangifera indica* L. cv. *Anwar Ratol* Leaves: Medicinal Application of Food Wastes. *Medicina*, 55:353. https://doi.org/ 10.3390/medicina55070353

Samanta, S., Chanda, R., Ganguli, S., Gopi, Reddy, A., Banerjee, J. (2019). Anti-diabetic activity of mango (*Mangifera indica*): a review. *MOJ Bioequivalence & Bioavailability*. *6*(2), 23–26. https://doi.org/10.15406/mojbb.2019.06.00131

Sekar, V., Chakraborty, S., Mani, S., Sali, V.K., & Vasanthi, H.R. (2019). Mangiferin from *Mangifera indica* fruits reduces post-prandial glucose level by inhibiting α-glucosidase and α-amylase activity. *South African Journal of Botany*, 120, 129-134. https://doi.org/10.1016/j.sajb.2018.02.001

Senthilkumar, R., Muragod, P.P., & Muruli, N.V. (2020). Anti-diabetic Activity of Mango (*Mangifera indica*), *Indian Journal of Pure & Applied Biosciences*. 8(1), 213-216. http://dx.doi.org/10.18782/2582-2845.7950

Shi, Z.L., Liu, Y.D., Yuan, Y.Y., Song, D., Qi M.F., Yang, X.J., Wang, P., Li, X.Y., Shang, J.H., Yang, Z.X. (2017). *In Vitro* and *In Vivo* Effects of Norathyriol and Mangiferin on α-Glucosidase. *Biochemistry Research International,* 1206015. https://doi.org/ 10.1155/2017/1206015

Sudibyo, R. S., Mahdi, L., & Martien, R. (2020). Self Nano-Emulsifying Drug Delivery System (SNEDDS) of *Curcuma Mangga* Val. Essential Oil and The Stability Study. *Indonesian Journal of Pharmacy*, 31(4), 238-243. https://doi.org/10.22146/ijp.584

Tayana N., Inthakusol W., Duangdee N., Chewchinda S., Pandith H., and Kongkiatpaiboon S. (2019). Mangiferin content in different parts of mango tree (*Mangifera indica* L.) in Thailand. *Songklanakarin Journal of Science and Technology*, 41(3), 522-528.

Telange, D.R., Sohail, N.K., Hemke, A.T., Kharkar, P.S., Pethe, A.M. (2021). Phospholipid complex-loaded self-assembled phytosomal soft nanoparticles: evidence of enhanced solubility, dissolution rate, ex vivo permeability, oral bioavailability, and antioxidant potential of mangiferin. *Drug Delivery and Translational Research*, 11(3), 1056-1083. https://doi.org/10.1007/s13346-020-00822-4

Vietnam Pharmacopoeia Association (2018). *Vietnamese Pharmacopoeia*. 5th ed. Medical Publishing House, Vietnam, p. PL9, PL13.

Villas, Boas, G.R., Rodrigues, Lemos, J.M., de Oliveira, M.W., Dos, Santos, R.C., Stefanello da Silveira, A.P., Barbieri Bacha, F., Ito C.N.A., Bortolotte Cornelius, E., Brioli Lima, F., Sachilarid Rodrigues, A.M., Belmal Costa, N., Francisco Bittencourt, F., Freitas de Lima, F., Meirelles Paes, M., Gubert P., & Oesterreich, S.A. (2020). Aqueous extract from *Mangifera indica* Linn. (Anacardiaceae) leaves exerts long-term hypoglycemic effect, increases insulin sensitivity and plasma insulin levels on diabetic Wistar rats. *Plos One*, 15(1), e0227105. https://doi.org/10.1371/journal.pone.0227105

Windriyati, Y. N., Badriyah, M., Kusumaningtyas, D. A., & Riesmalia, R. L. (2020). Liquisolid Tablets Formulation of Atorvastatin Calcium Using Polyethylene Glycol 400 as Solvent and Some Carrier Materials. *Indonesian Journal of Pharmacy*, 31(4), 305–311. https://doi.org/10.22146/ijp.1138

Yakubu, M. T., & Salimon, S. S. (2015). Antidiarrhoeal activity of aqueous extract of *Mangifera indica* L. leaves in female albino rats. *Journal of Ethnopharmacology*, 163, 135-41. https://doi.org/10.1016/j.jep.2014.12.060

Ybañez-Julca, R.O., Asunción-Alvarez, D., Quispe-Díaz, I.M., Palacios, J., Bórquez, J., Simirgiotis, M.J., Perveen S., Nwokocha C.R., Cifuentes F., & Paredes, A. (2020). Metabolomic Profiling of Mango (*Mangifera indica* Linn) Leaf Extract

and Its Intestinal Protective Effect and Antioxidant Activity in Different Biological Models. *Molecules*, 25(21), 5149. https://doi.org/10.3390/molecules25215149

Yunita, O., Nuniek Winantari, A., Permana Sugiarto, R., Sutanto Prayitna, G., & Hwa, L. (2022). Characterization of Spray Dried Extract Katuk (*Sauropus androgynus*). *Indonesian Journal of Pharmacy*, 33(3), 455-464. https://doi.org/10.22146/ijp.4443