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Formulation and evaluation of physical stability and antidiabetic activity from nanoliposomes containing an ethyl acetate extract of *Solanum xanthocarpum* Schrader & Wendland fruit in rats

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ABSTRACT

Introduction: The extract of *Solanum xanthocarpum* fruit has antidiabetic activity. This research aims to formulate nanoliposomes containing ethyl acetate extracts of *S. xanthocarpum* selected from multilevel extraction, evaluate their physical stability and antidiabetic activity, and compare them with the extract.

Methods: The extracts were prepared using n-hexane, ethyl acetate, ethanol, and water as solvents, and their antidiabetic activities were evaluated to select the extract most effective in lowering blood sugar levels in rats. That extract was formulated into three nanoliposomes using varying amounts of phospholipid, cholesterol, and Span 60, i.e., F1 (40 mmol), F2 (50 mmol), and F3 (60 mmol). Various sonication times ranging from 10 to 30 minutes were evaluated for particle size, entrapment efficiency, and physical stability. The selected formulations were evaluated for antidiabetic activity.

Results: The ethyl acetate extract showed the highest decrease in glucose in rats and was selected for nanoliposome formulation. The nanoliposomes obtained were physically stable at low temperatures for 12 weeks. F2 had the smallest particle size (143.97 nm) and the greatest entrapment efficiency (92.981% ± 0.35%) with a sonication time of 30 minutes and was significantly different from F1 and F3 ($P<0.05$). The highest percentage reduction in blood sugar levels was with F2 at 74.57% and significantly differed ($P<0.05$) from the ethyl acetate extract of *S. xanthocarpum* at 73.98% and the positive control rat group.

Conclusion: The results show the potential uses of the prepared nanoparticles, especially the F2 formulation, as an antidiabetic formula.

Implication for health policy/practice/research/medical education:
The article reports the formulation and pharmacological effects from nanoliposomes containing an ethyl acetat extract of *Solanum xanthocarpum* fruits. The findings of this study show the potential of these nanoparticles as an alternative treatment for diabetes disease. However, clinical and toxicological studies are needed to confirm this.

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Introduction

Solanum xanthocarpum is a Solanaceae family plant that contains secondary metabolite components, including alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, glycosides, and phenolic compounds (1). The fruits of *S. xanthocarpum* contain β-sitosterol

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and stigmasterol (2), solasodine (3), solanocarpidine, carpesterol, glycoalkaloid, and α-solamargine (4). *S. xanthocarpum* fruit has antibacterial (5), antihyperlipidemic (6), analgesic, anti-inflammatory, antioxidant (7), antiurolithic (8), antidiabetic (9), and estrogenic activities (10).

The total flavonoid contents in ethyl acetate extracts of *S. xanthocarpum* were 162.49±0.15 µg quercetin equivalents/mg extract (11). Numerous pharmacological activities have been reported as evidence of the health benefits of quercetin, which include antioxidant, anti-inflammatory, anti-cancer, antidiabetic, antihypertensive, antihyperlipidemic, antibacterial, antiviral, antiallergy, anti-inflammatory, and anti-hyperuricemic activities (12-14). Quercetin has limited water solubility, low level of oral bioavailability and therapeutic action as a medication (15).

Liposomes are effective carrier systems for delivering herbal extracts with improved therapeutic effects by enhancing their solubility, stability, and bioavailability (16). Liposomes are considered excellent drug carriers due to their advantages in improving solubility and penetrating compounds with poor solubility (17). They are elastic, allowing them to be carriers for hydrophilic, lipophilic, or amphiphilic pharmaceuticals (18). In this study, a combination method of thin-film hydration and ultrasonication was used for 10, 20, and 30 minutes to prepare nanoliposome. It is an easy and quick method to perform with inexpensive instruments (19). This approach generates hydrated films with bilayers, such as multi-lamellar vesicles (MLV) and giant unilamellar vesicles (GULV); however, the particle sizes (PSs) are large. Size reduction is required to increase uniformity (20). Liposome particles of MLVs are >0.5 µm, and GULVs are >1 µm (21).

Ultrasonication is a method for reducing PS (22). Ultrasonication is the application of high-frequency sound waves that can decrease the size of the particles. The duration and frequency of the ultrasonic process influence the resulting nanoliposome diameter. The longer the ultrasound duration, the smaller and more homogeneous the PS (23). However, longer ultrasonication will cause the particles to become too small; the nanoliposome particles will agglomerate with each other, increasing the PS that can be measured using a particle size analyzer (PSA) (24).

In 2011, Poongothai et al studied a methanol extract of *S. xanthocarpum*, which could reduce blood glucose levels by 47.130% at 100 mg/kg BW and 61.40% at 200 mg/kg BW rats (25). Therefore, nanoliposome preparations were used in the current study. Nanoliposomes, or bilayer phospholipid vesicles measuring in nanometers, are a promising encapsulation technology. Some of the advantages offered by nanoliposome technology are the protection of sensitive compound molecules, storage stability, high loading capacity, increased bioavailability, increased solubility of otherwise insoluble drugs, such as secondary metabolites contained in extracts from plants, and a sustained release mechanism that improves antidiabetic effectiveness. This research aimed to formulate and evaluate the antidiabetic activity of nanoliposomes containing *S. xanthocarpum* fruit extract in rats compared with the pure extract. The results obtained in this study are expected to provide a better diabetes treatment for patients after bioavailability and clinical trials.

Materials and Methods

Plant materials

The plants were collected from North Sumatra province, Medan, Indonesia. A scientist from the Herbarium Medanense (MEDA), Jalan Biotechnology Number 1, University of Sumatera Utara, Medan, recognized the plant sample and assigned identity number 4618/MEDA/2019 for the deposited specimen.

Chemical reagents

The chemical reagents utilized in this study included lipoid S75 (Lipoid GMBH, Germany), lipoid DMPG-Na (Lipoid GMBH, Germany), cholesterol (Sigma-Aldrich, Germany), phosphate-buffered saline (Oxoid, United Kingdom), chloroform Pro Analysis (PA) (Merck, USA), methanol PA (Merck, USA), distilled water (Ikapharmindo, Indonesia), nicotinamide (Sigma, Germany), and streptozotocin (Sigma, Germany).

Instruments

The instruments used in this study included a digital pH meter (Hanna Instrument, Indonesia), transmission electron microscope (TEM) (JEOL JEM 1400, Japan), PSA (Fritsch Analysette 22 nanoTech, Germany), Nano Particle Analyzer SZ-100 and zeta sizer (Horiba Scientific, Japan), ultrasonic homogenizer probes (Model 150VT Biologics, USA), UV-Vis spectrophotometer (Shimadzu 1800, Japan), vortexer (Heidolph, Germany), and rotary evaporator (Heidolph, Germany).

Preparation of *Solanum xanthocarpum* fruit powder

Fresh *S. xanthocarpum* fruits were washed and dried in a 30 to 40 °C drying cabinet. The dried sample was then milled into powder and the loss on drying was determined.

Preparation of *Solanum xanthocarpum* fruit extract using various solvents

In this study, the extract was prepared using a multilevel extraction method of n-hexane, ethyl acetate, ethanol, and water (26). First, 2000 g of *S. xanthocarpum* fruit powder was extracted using n-hexane as the solvent. The extraction process involved adding 5 L of n-hexane to a vessel, placing the covered vessel in sunshine for 5 days, and stirring occasionally. The dregs were rinsed with the balance of the filtered solvent so that the filtrate was obtained and left for 2 days. The residues were dried at 50 °C in an oven before being extracted with ethyl acetate,

96% ethanol, and water using the same method. A thick extract resulted after the filtrate was concentrated using a rotary evaporator at 50 °C.

Antidiabetic activity of Solanum xanthocarpum extracts

Male Wistar rats weighing 150–200 g were maintained in laboratory conditions for 14 days after permission was obtained from the Animal Research Ethics Committee (AREC), University of Sumatera Utara (0715/KEPH-FMIPA/2022), before starting the experiment. Each extract was given orally to diabetic rats induced with a dose of 230 mg/kg BW of nicotinamide and streptozotocin at 65 mg/kg BW intraperitoneally (27). The antidiabetic activity test of the *S. xanthocarpum* extract was carried out by administering a test solution to each subject in a dose of 200 mg/kg BW orally once daily. The percentage decrease in blood glucose levels was calculated on the day 15 (10).

Thirty rats were divided into 6 groups, each group containing 5 rats. Before treatment, the rats fasted for 18 hours (ad libitum). Blood samples were drawn from the tail of each rat to measure the first blood glucose levels (baseline). After 48 hours of treatment, blood samples were drawn from each rat to measure their blood glucose levels. If the blood glucose levels were greater than 200 mg/dL, then the rats were considered to have hyperglycemia. The antidiabetic activity test of the *S. xanthocarpum* extract was performed by administering a test solution in a dose of 200 mg/kg BW orally once daily. The group divisions were as follows:

- Group 1: Negative control, given a suspension solution of Na-CMC 0.5% kg BW
- Group 2: Positive control, given a glibenclamide dose of 0.45 mg/kg BW
- Group 3: Treatment, given n-hexane extract
- Group 4: Treatment, given ethyl acetate extract
- Group 5: Treatment, given ethanol extract
- Group 6: Treatment, given water extract
- The blood glucose levels were measured by glucometer on days 0, 6, 12, and 15 (10).

The preparation of nanoliposomes by thin-layer hydration

Nanoliposome preparations were made using the ethyl acetate extract, which was selected based on the percentage of glucose reduction in diabetic rats. The nanoliposome formula consisted of 100 mg of extract and varying quantities of 95% lipoid S75, 5% DMPG-Na as a phospholipid material, 5% cholesterol, and 5% Span 60, resulting in three different formulations with various nanoliposomes components: F1 (40 mmol), F2 (50 mmol), and F3 (60 mmol). The 95% lipoid S75, DMPG-Na, cholesterol, and Span 60 were combined in the nanoliposome preparation and referred to as mixture A. Another mixture (B) was created by dissolving 100 mg of the extract in a 5:5 mixture of methanol and chloroform. Subsequently, mixture A was added to mixture B and homogenized using a vortexer. The resulting mixture was transferred to a round-bottom flask and subjected to a rotary evaporator at 50 °C and 420 mBar for 1 hour until a thin film formed on the walls of the round-bottom flask. Phosphate buffer saline was then added to reach a volume of 10 mL (28). The resulting liposomes were further reduced in size using an ultrasonic homogenizer with sonication times of 10, 20, and 30 minutes. The resulting nanoliposomes were characterized, and the formulations are detailed in Table 1.

Characterization of nanoliposomes

Organoleptic observations

Organoleptic observations included the shape, smell, and color of the nanoliposomes. Visual observations were applied.

pH

The pH was measured at 25 ± 2 °C using a digital pH meter (29).

Measurement of particle size (PS) and polydispersity index (PDI)

The PS and PDI were determined using a PSA with dynamic light scattering (DLS) (30).

Determination of zeta potential

The zeta potential (ZP) of nanoliposomes was evaluated using a zeta sizer with DLS (31).

Morphology

A morphological analysis of nanoliposomes was conducted using a JEOL JEM 1400 TEM. TEM analysis was carried out at the Chemistry Laboratory, Gajah Mada University, Yogya, Indonesia. TEM analysis examined the inner morphology and PS of nanoliposome particles (32).

Entrapment efficiency

The entrapment efficiency was determined using 10 mL of nanoliposomes centrifuged at 12 000 rpm for 120 minutes

Table 1. Nanoliposome formulations of *Solanum xanthocarpum* ethyl acetate extract (10 mL)

Formula code	Quantity of nanoliposom components (mmol)	Extract (mg)	Lipoid S75 (95%) (mg)	DMPG-Na (5%) (mg)	Cholesterol (5%) (mg)	Span 60 (5%) (mg)
F1	40	100	345.3	9.4	7.7	8.6
F2	50	100	431.7	11.4	9.7	10.8
F3	60	100	518.0	13.6	11.6	12.9

Lipoid S75: lipoid soybean 75; DMPG-Na: Dimyristoylphosphatidylglycerol-Natrium.

at 4 °C until the supernatant and sediment separated. The sediment was added to 1 mL of methanol 0.1 ml of 10% $AlCl_3$, and 0.1 mL of 10% CH_3COONa and measured in a UV-Vis spectrophotometer at 432 nm. This sediment was a total flavonoid compound (quercetin), and its absorption was measured using UV-Vis spectrophotometry at 432 nm. The amount of quercetin in the supernatant and sediment was used to determine the total flavonoid content of the nanoliposomes (33). The entrapment efficiency (% EE) was calculated using the formula below:

$$\%Entrapment\ efficiency = \frac{Total\ flavonoid\ content\ of\ sediment}{Total\ flavonoid\ content\ of\ sediment\ nanoliposomes} \times 100\%$$

Antidiabetic activity of *Solanum xanthocarpum* extracts nanoliposomes

Twenty diabetic rats were divided into 4 groups, containing 5 animals in each group. Before treatment, the rats fasted for 18 hours (*ad libitum*). The antidiabetic activity test of the *S. xanthocarpum* extract nanoliposomes was performed by administering a test solution in a dose of 100 mg/kg BW orally once daily. Groups 1 and 2 were the control groups, and 3 and 4 were the treatment groups. The group division was as follows:

- Group 1: Negative control, given a suspension solution of Na-CMC 0.5% kg BW
- Group 2: Positive control, given Glibenclamide dose of 0.45 mg/kg BW
- Group 3: Treatment, given the ethyl acetate extract
- Group 4: Treatment, given nanoliposomes of the ethyl acetate extract

Blood glucose levels were measured by glucometer on days 0, 6, 12, and 15 (10).

Statistical analysis

The data are presented as the mean ± standard deviation (SD). The data resulting from the percentage reduction in blood glucose levels in rats were analyzed using one-way analysis of variance (ANOVA) and the data from the nanoliposome evaluation were analyzed using two-way ANOVA. $P < 0.05$ indicated a significant difference.

Results

The *S. xanthocarpum* fruit powder was brownish-yellow in color. The loss on drying of the *S. xanthocarpum* powder was 7.73%.

The extract yield and water content of the extract from *Solanum xanthocarpum* fruit

The results were obtained from 2000 g of powdered *S. xanthocarpum*, which was extracted by multilevel extraction based on the polarity level of the solvent starting from non-polar, semi-polar, and polar, namely, n-hexane, ethyl acetate, ethanol, and water. The extract yields and water contents of the extracts of *S. xanthocarpum* fruit are shown in Table 2. Photographs of the fresh fruit, powder, and *S. xanthocarpum* extract are presented in Figure 1.

Antidiabetic activity test of *Solanum xanthocarpum* extracts

The antidiabetic activity test determined a significant decrease in blood glucose levels on days 0 (7 hours), 6, 12, and 15 after treatment. The extract with the highest reduction in glucose levels was formulated into nanoliposome preparations. The largest blood glucose decrease on day 15 (72.47%) occurred in rats treated with the ethyl acetate extract, and the lowest was the water extract at 52.75%. The reductions in blood glucose levels are presented in Table 3.

Figure 1. *Solanum xanthocarpum* fruits: (a) fresh fruit, (b) powder, and (c) ethyl acetate extract.

Table 2. Extraction results of *Solanum xanthocarpum* fruit

Fruit powder weight (g)	Extraction results (g)			Water content (%)
	Extract	Weight (g)	Yield (%)	
2000	n-Hexane	46.910	2.3460	5.91
	Ethyl acetate	34.950	1.7480	7.99
	Ethanol	614.28	30.714	5.95
	Water	43.870	2.1940	4.62

Table 3. The antidiabetic activity test results for *Solanum xanthocarpum* extract

Treatment groups	Percentage of blood glucose decrease ± SD			
	Day 0	Day 6	Day 12	Day 15
Na-CMC 0.5%	0.14 ± 1.87	3.42 ± 0.27	7.72 ± 0.82	11.19 ± 1.27
Glibenclamide 0.45 mg/kg BW	0.74 ± 2.33	32.02 ± 3.87	60.18 ± 7.47	72.38 ± 4.63
n-Hexane 200 mg/kg BW	0.85 ± 3.51	18.63[a,b] ± 3.68	41.84[a,b] ± 8.20	54.85[a,b] ± 9.01
Ethyl acetate 200 mg/kg BW	0.90 ± 3.06	37.70[a,b] ± 7.39	63.70[a,b] ± 6.80	72.47[a,b] ± 3.40
Ethanol 200 mg/kg BW	0.39 ± 3.43	27.67[a,b] ± 5.12	54.34[a,b] ± 5.78	65.23[a,b] ± 5.57
Water 200 mg/kg BW	1.46[a] ± 2.56	18.86[a,b] ± 4.42	41.14[a,b] ± 10.04	52.75[a,b] ± 7.77

Results are presented as the mean ± standard deviation (SD) (n = 5).
[a] Significantly different from Na-CMC ($P < 0.05$)
[b] Significantly different from Glibenclamide ($P < 0.05$)

Characterization of nanoliposomes

Organoleptic results

The nanoliposomes obtained from *S. xanthocarpum* fruit extract formed a brownish-green viscous liquid, had no odor and taste, and did not experience phase separation. No organoleptic differences existed between the F1, F2, and F3 nanoliposomes. The difference in ultrasonication time during nanoliposome preparation did not affect the organoleptic results.

Viscosity, PS, PDI, and ZP

The results of the viscosity, PS, PDI, and ZP measurements can be seen in Table 4. The viscosity of nanoliposomes increased with an increase in the concentration of the nanoliposome material (mmol). The higher the concentration, the greater the viscosity produced. As the sonication time increased, the viscosity decreased. All nanoliposome formulations had nanoscale PSs of 100–200 nm, and the smallest particles were found in F2, with a sonication time of 30 minutes.

The significance of the viscosity, PS, PDI, and ZP based on differences in all the nanoliposome parameters containing the ethyl acetate extract of *S. xanthocarpum* fruit was set to $P < 0.05$ using two-way ANOVA. This showed significant differences ($P < 0.05$) in all formulations and sonication times.

Figure 2 shows that the particles had a regular shape, no agglomeration, and an even distribution. The particles appeared spherical or round and exhibited MLV. The TEM measurements indicated that F2 had the smallest average diameters.

Entrapment efficiency

The entrapment efficiency of nanoliposomes was measured to determine the amount of drug successfully trapped in the nanoliposomes. Table 5 shows the results of the average nanoliposome entrapment efficiency measurements.

The highest percentage of entrapment efficiency of *S. xanthocarpum* extract nanoliposomes was 92.981% in F2 (50 mMol) with an ultrasonication time of 30 minutes (Table 5).

The nanoliposomes made with an ultrasonication time of 30 minutes were selected for the stability test based on the viscosity, PS, PDI, and ZP results.

Results of the stability test

Organoleptic results

After 12 weeks of storage at room temperature (25 ± 2 °C), there was no change in the color, smell, or taste of the nanoliposomes; however, two-phase separation occurred in nanoliposomes F1, F2, and F3 after 4, 12, and 8 weeks, respectively.

Table 4. Viscosity, particle size (PS), polydispersity index (PDI), and zeta potential (ZP) of nanoliposomes containing ethyl acetate extract of *Solanum xanthocarpum* fruits

Formula	Sonication time											
	10 minutes				20 minutes				30 minutes			
	Viscosity (cPs)	PS (nm)	PDI	ZP (mV)	Viscosity (cPs)	PS (nm)	PDI	ZP (mV)	Viscosity (cPs)	PS (nm)	PDI	ZP (mV)
F1	3110 ± 5.57	167.80 ± 6.17	0.3 ± 0.02	−27.433 ± 0.84	2673 ± 2.65	162.57 ± 3.78	0.2 ± 0.05	−22.767 ± 0.12	2498 ± 5.57[a]	162.57 ± 6.37[a]	0.2 ± 0.02[a]	−42.133 ± 0.59[a]
F2	3659 ± 2.65	166.73 ± 4.67	0.2 ± 0.09	−23.333 ± 0.12	3433 ± 3.00	154.20 ± 4.39	0.2 ± 0.05	−27.900 ± 0.00	3261 ± 1.73[b]	143.97 ± 0.64[b]	0.2 ± 0.01[a]	−25.367 ± 0.40[b]
F3	3717 ± 4.58	180.37 ± 1.46	0.4 ± 0.01	−37.067 ± 1.45	3327 ± 3.61	149.90 ± 2.44	0.3 ± 0.02	−32.100 ± 0.10	3056 ± 3.61[c]	154.47 ± 0.51[c]	0.3 ± 0.01[a]	−53.367 ± 0.12[c]

Results are presented as the mean ± standard deviation (SD) (n=3). a, b, and c mean that values with different superscripts in the same column are significantly different at $P < 0.05$.

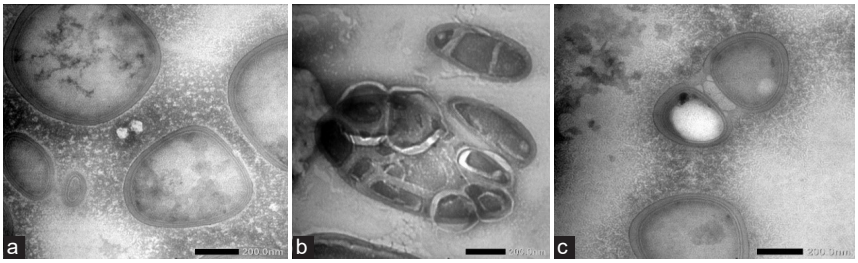

Figure 2. Transmission electron micrograph (40 000×) of nanoliposomes ethyl acetate extracts of Solanum xanthocarpum. (a) F1 (40 mmol): particle size diameter 500 nm, (b) F2 (50 mmol): particle size diameter 200 nm, and (c) F3 (60 mmol): particle size diameters 260 nm.

Organoleptic observation of nanoliposome after 12 weeks of storage at low temperatures (4 ± 2°C) showed that the color, smell, and taste did not change, and no phase separation was observed.

pH

The pH values obtained for formulas F1, F2, and F3 were 5.9 ± 0.06, 6.1 ± 0.06, and 6.0 ± 0.15 respectively; there was a slight insignificant decrease after 12 weeks of storage at room temperature (25 ± 2 °C) for each formula, namely F1 (5.5 ± 0.06), F2 (5.8 ± 0.06), and F3 (5.5 ± 0.15) and for low temperature (4 ± 2 °C), F1 (5.5 ± 0.06), F2 (5.9 ± 0.06), and F3 (5.7 ± 0.15).

Viscosity

The viscosity of nanoliposomes containing ethyl acetate extract of *S. xanthocarpum* fruit increased during storage. The viscosity values of nanoliposomes containing ethyl acetate extract of *S. xanthocarpum* fruit for formulas F1, F2, and F3 were 2498 ± 6.56, 3261 ± 6.24, and 3056 ± 3.61 respectively; there was a slight insignificant increase after 12 weeks of storage at room temperature (25 ± 2 °C) for each formula, namely F1 (3258 ± 6.93), F2 (3421 ± 6.08), and F3 (3522 ± 11.14) and for low temperature (4 ± 2 °C), F1 (2612 ± 8.72), F2 (3407 ± 4.36), and F3 (3255 ± 6.08).

Particle size

The nanoliposome PS increased with each observation after 12 weeks at room and low temperatures, as shown in Table 6.

Antidiabetic activity of nanoliposomes containing *Solanum xanthocarpum* ethyl acetate extract

The antidiabetic activity test was performed by determining the decrease in blood glucose levels on days 0 (7 hours), 6, 12, and 15 after treatment. The highest percentage of blood glucose decrease (74.57%) on day 15 occurred in rats treated with the nanoliposomes of ethyl acetate extract. The percentage decrease in blood glucose levels is presented in Table 7.

Discussion

Solanum xanthocarpum fruit samples were dried into powder; the loss on drying met the requirements for hindering microbial growth of herbal powder requirements. The loss on drying reflects the water content of the fruit during the drying process; evaporation of the water occurs during the extraction process. The extract yield and water

Table 5. Entrapment efficiency of nanoliposomes containing ethyl acetate extract of *Solanum xanthocarpum* fruit

Formulation	Entrapment efficiency (%) at variation of sonication time		
	10 minutes	20 minutes	30 minutes
F1 (40 mmol)	73.329 ± 1.18[a]	81.998 ± 2.06[a]	89.711 ± 0.87[a]
F2 (50 mmol)	86.114 ± 1.09[b]	89.555 ± 0.29[b]	92.981 ± 0.35[b]
F3 (60 mmol)	78.733 ± 2.44[c]	86.441± 0.16[c]	90.670 ± 0.10[c]

Results are given as mean ± standard deviation (SD) (n= 3).
Values with different superscript letters (a, b, and c) in the same column are significantly different at $P<0.05$.

Table 6. The particle sizes (PSs) of nanoliposomes containing ethyl acetate extract of *Solanum xanthocarpum* fruit storage at low temperature (4 ± 2 °C) and room temperature (25 ± 2 °C)

Formula	PS (nm)						
	0 week	4 weeks		8 weeks		12 weeks	
	LT and RT	LT	RT	LT	RT	LT	RT
F1 (40 mmol)	162.57	244.29	355.98	261.73	1041.9	264.67	1432.7
F2 (50 mmol)	143.97	144.54	154.21	147.78	322.81	153.67	388.98
F3(60 mmol)	154.47	164.21	233.84	211.45	492.45	221.54	611.95

LT: Low temperature; RT: Room temperature.

Table 7. The percentage decrease in blood glucose levels of each group of rats

Group of rats	Decreased blood sugar levels (%)			
	Day 0	Day 6	Day 12	Day 15
Na-CMC 0.5%	0.50± 0.20	1.28± 0.16	2.34± 0.32	2.78± 0.28
Glibenclamide (0.45 mg/kg)	1.58± 0.02	27.65± 1.52	66.67±1.39	70.89± 1.21
Ethyl acetate extract (100 mg/kg)	1.42± 0.24[a,b]	24.91± 0.22[a,b]	58.76±1.59[a,b]	73.08± 0.92[a,b]
Nanoliposome of ethyl acetate extract (100 mg/kg BW)	6.18±0.43[a,b,c]	72.11±0.55 [a,b,c]	74.31±0.59[a,b,c]	74.57±0.17[a,b,c]

Results are presented as the mean ± standard deviation (SD) (n = 5).
[a] Significantly different from Na-CMC ($P < 0.05$)
[b] Significantly different from Glibenclamide ($P < 0.05$).
[c] Significantly different from extract ($P < 0.05$).

content met that the qualified water content was ≤10%. The extract yield and water content were measured for each solvent used and related to the purity and presence of contaminants in the powder. Hence, reducing the water content to a certain amount helps extend the durability of the material during storage. In addition, excessive water content in the extract will accelerate microbial growth. It can also facilitate hydrolysis of the chemical content, decreasing the quality of traditional medicines (34).

From the four extracts obtained in this study, the highest yield was obtained from the ethanol extract, but the highest decrease in the blood glucose levels of the rats was obtained from the ethyl acetate extract. The high reduction in blood glucose levels in diabetic rats tested on ethyl acetate extract of *S. xanthocarpum* fruit was due to the flavonoid content, which was higher than that of the n-hexane, ethanol, and water extracts (11). Flavonoids have antioxidant activity, so they can regenerate the pancreas cells damaged by the formation of reactive oxygen, which can cause diabetes mellitus (10). Some tannins and saponins act as inhibitors of α-glucosidase in ethyl acetate, which is beneficial because it can delay glucose absorption after a meal, preventing postprandial hyperglycemia conditions (35).

The preparation of nanoliposomes containing the ethyl acetate extract of *S. xanthocarpum* fruit required Lipoid S75 and Lipoid DMPG-Na that act as phospholipids, components of nanoliposomes, cholesterol that interacts with the phospholipids to reduce porosity or leakage in the nanoliposome membrane, and Span 60, which increases the flexibility of the nanoliposome preparation (36). The thin-layer hydration method of the dry lipid phase can also increase the encapsulation efficiency of the liposomes in the form of MLVs (19). However, MLVs can have poor size homogeneity, so PS reduction is needed to homogenize them. Hence, PS needs to be considered when making liposomes.

One method of reducing PS is ultrasonication (22). The longer the ultrasonication duration, the more energy the particles receive, the more evenly distributed they are within the solution, and the resulting PSs will be more homogeneous (20). Ultrasonic vibrations during the sonication process lead to collisions between nanoliposome particles and shatter large liposomes into smaller ones. PS is a physical property parameter that must be considered when making nanoliposomes (37). Longer sonication times can reduce PS by destroying the membrane and causing the PS to become smaller, as occurred in formulations F1 and F2. However, F3, sonicated for 30 minutes, had a larger PS. This was caused by the longer ultrasonication duration, which caused the PS to become too small. In response, the nanoliposome particles agglomerated and increased the PS (24).

In this research, cholesterol was added to the nanoliposomes. The stability of a given liposome can be significantly influenced by physicochemical characteristics, including bilayer membrane composition, size, stiffness, and charge (38). Another critical factor influencing liposome stability is lipid composition (39). Cholesterol is an organic sterol molecule with amphiphilic properties. Structurally, this molecule has a hydroxyl group that can form hydrogen bonds with phospholipids. Various essential roles have been attributed to cholesterol, including regulating membrane permeability, elasticity, stiffness, and strength. Cholesterol is the most widely sterol used in liposome formulations, which can prevent liposome aggregation and increase liposome membrane stability (40-41).

All of the nanoliposomes in this study were more stable during storage at low temperatures than at room temperature. The increase in PS was greater at room temperature (25 ± 2 °C) than at low temperature (4 ± 2 °C) because physical processes, such as aggregation or flocculation, affect PS. Aggregation can accelerate coalescence or fusion, where new colloidal structures will be formed. Coalescence or fusion can be caused by Brownian motion, which causes intense collisions between particles; the new colloid that is formed will be challenging to redistribute (42).

All nanoliposome formulations in this study met the ZP value requirements and had good stability (43). The ZP can indicate the stability of colloidal dispersion systems during storage, specifically the possibility of aggregate formation in charged particles. The ZP values are generally used to determine the particle charge and nanoparticle stability (43); all formulations had good ZP values (−22 to −53 mV). Particles with ZPs greater than +25 mV or more negative than −25 mV are considered stable (44).

All formulations met the PDI value requirement for PS distribution. A PDI value less than 0.3 is considered acceptable and indicates the uniform size of the phospholipid vesicle population (45). A PDI value near zero suggests a uniform distribution of particles, whereas a PDI value of more than 0.5 indicates that the particles are very heterogeneous (46). A PDI value of 0.3–0.5 suggests a homogeneous population of phospholipid vesicles (34). The tendency of the particles to agglomerate to create larger particle aggregates might cause non-uniform PS. The PDI is affected by sonication duration, as ultrasonic waves can shatter large liposomes into smaller particles, rendering the size distribution homogeneous. Increasing sonication time still showed an acceptable PDI value (47). The pH indicates the stability of the nanoliposome. A decrease in pH during storage is caused by chemical reactions such as fat oxidation (48). All the nanoliposome formulations in this study experienced changes in pH when they were stored for 12 weeks at both room temperature (25 ± 2 °C) and low temperature (4 ± 2 °C) but still met the pH requirements for oral dosage, 4.5–5.5. When stored at low temperatures, the pH decreased slightly compared to room temperature (49).

Nanoliposome formula F3 showed the highest viscosity, this is due to the higher lipid component content. The viscosity value increased slightly after 12 weeks of storage at room temperature and low temperature.

Formulation F2 (50 mmol) had the highest entrapment efficiency. Entrapment efficiency was influenced by adding cholesterol to the nanoliposome preparation. Cholesterol in the nanoliposome formulation can reduce porosity or leakage through the bilayer membrane. Too much cholesterol will cause the liposome vesicles to be too stiff; too little can cause leakage in the nanoliposome preparation because the liposomes will be too elastic and trap too little quercetin in the extract (18). The amount of cholesterol in F2 (50 mmol) is the optimum amount to produce a high entrapment efficiency. The sonication time affected entrapment efficiency. The longer the sonication time, the smaller the PS, the larger the surface area, and the more dispersed the drug, increasing the drug entrapment (50).

The rat group given nanoliposomes containing ethyl acetate extract of *S. xanthocarpum* showed a higher reduction in blood sugar levels compared to other groups of rats; this was because the nanoliposome preparation had a smaller PS, which increased the solubility of the active ingredient, quercetin. Quercetin has low solubility, resulting in limited effects on bioavailability and absorption processes. Thus, nanoliposome preparations increase the solubility of quercetin, thereby increasing bioavailability and enhancing absorption and antidiabetic effectiveness (51).

Conclusion

Formula F2 of nanoliposomes containing an ethyl acetate extract of *S. xanthocarpum* fruit were stable at low temperatures (4 ± 2 °C) and unstable at room temperature (25 ± 2 °C) for 12 weeks of storage the antidiabetic activity was greater than the ethyl acetate extract of *S. xanthocarpum* fruit. However clinical and toxicological studies are needed to confirm this.

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Authors' contributions

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Conflict of interests

All authors of this article certify that they have no conflict of interest.

Ethical considerations

The animal experiments were carried out according to the Animal Research Ethics Committee's (AREC) policies. University of Sumatera Utara (Ethic Number: 00680/KEPH-FMIPA/2020).

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References

1. Saxena HO, Deshmukh A, Ganesh, Kakkar A, Singh N.

Phytochemical screening and assessment of secondary metabolites in different plant parts of *Solanum xanthocarpum*: a Dashmool species. Indian J Trop Biodiv. 2014;22(2):164-9.
2. Khanam S, Sultana R. Isolation of β-sitosterol & stigmasterol as active immunomodulatory constituents from fruits of *Solanum xanthocarpum* (Solanaceae). Int J Pharm Sci Res. 2012;3(4):1057-60.
3. Jayakumar K, Murugan K. *Solanum* alkaloids and their pharmaceutical roles: a review. J Anal Pharm Res. 2016;3(6):1-12. doi: 10.15406/japlr.2016.03.00075.
4. Singh OM, Singh TP. Phytochemistry of *Solanum xanthocarpum*: an amazing traditional healer. J Sci Ind Res. 2012;69:732-40.
5. Rana S, Prakash V, Sagar A. Antibacterial activity of *Solanum xanthocarpum* leaf extract. Int J Curr Microbiol Appl Sci. 2016;5(4):323-8. doi: 10.20546/ijcmas.2016.504.038.
6. Sridevi M, Senthil S, Pugalendi KV. Antihyperglycemic effect of *Solanum surattense* leaf-extract in streptozotocin induced diabetic rats. J Pharmacol Toxicol. 2011;2(7):621-9. doi: 10.3923/jpt.2007.621.629.
7. Pardhi P, Jain AP, Ganeshpurkar A, Rai G. Anti-microbial, anti-oxidant and anthelmintic activity of crude extract of *Solanum xanthocarpum*. Pharmacognosy Journal. 2010;2(11):400-4. doi: 10.1016/s0975-3575(10)80022-7.
8. Patel PK, Patel MA, Saralai MG, Gandhi TR. Antiurolithiatic effects of *Solanum xanthocarpum* fruit extract on ethylene-glycol-induced nephrolithiasis in rats. J Young Pharm. 2012;4(3):164-70. doi: 10.4103/0975-1483.100022.
9. Gupta AK, Ganguly P, Majumder UK, Ghosal S. Antidiabetic and antihyperlipidaemic effects of *Solanum xanthocarpum* total extract in alloxan induced diabetic rats. Pharmacologyonline. 2009;1:487-97.
10. Alam Q, Vijayanarayana K, Satyanarayana D. Evaluation of estrogenic activity of alcoholic extracts of fruits of *Solanum xanthocarpum* using percentage vaginal cornification and vaginal opening as parameters of assessment. Pharmacologyonline. 2010;3:495-502.
11. Kumar S, Pandey AK. Medicinal attributes of *Solanum xanthocarpum* fruit consumed by several tribal communities as food: an in vitro antioxidant, anticancer and anti-HIV perspective. BMC Complement Altern Med. 2014;14:112. doi: 10.1186/1472-6882-14-112.
12. Shabir I, Kumar Pandey V, Shams R, Dar AH, Dash KK, Khan SA, et al. Promising bioactive properties of quercetin for potential food applications and health benefits: a review. Front Nutr. 2022;9:999752. doi: 10.3389/fnut.2022.999752.
13. Aghababaei F, Hadidi M. Recent advances in potential health benefits of quercetin. Pharmaceuticals (Basel). 2023;16(7):1020. doi: 10.3390/ph16071020.
14. Kumar R, Vijayalakshmi S, Nadanasabapathi S. Health benefits of quercetin. Def Life Sci J. 2017;2(2):142-51. doi: 10.14429/dlsj.2.11359.
15. Khan YY, Suvarna V. Liposomes containing phytochemicals for cancer treatment-an update. Int J Curr Pharm Res. 2016;9(1):20-4. doi: 10.22159/ijcpr.2017v9i1.16629.
16. Sogut O, Aydemir Sezer U, Sezer S. Liposomal delivery systems for herbal extracts. J Drug Deliv Sci Technol. 2021;61:102147. doi: 10.1016/j.jddst.2020.102147.
17. Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H. Preparation, characterization and applications of liposomes: state of the art. J Colloid Sci Biotechnol. 2012;1(2):147-68. doi: 10.1166/jcsb.2012.1020.
18. Shashi K, Satinder K, Bharat P. A complete review on: liposomes. Int Res J Pharm. 2012;3(7):10-6.
19. Kalepu S, Sunilkumar KT, Betha S, Mohanvarma M. Liposomal drug delivery system—a comprehensive review. Int J Drug Dev Res. 2013;5(4):62-75.
20. Nkanga CI, Bapolisi AM, Okafor NI, Krause RW. General perception of liposomes: formation, manufacturing and applications. In: Angel C, ed. Liposomes-Advances and Perspectives. IntechOpen; 2019. doi: 10.5772/intechopen.84255.
21. Liu P, Chen G, Zhang J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives. Molecules. 2022;27:1372
22. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi S, Farhangi A, Verdi AA, et al. Induction of diabetes by streptozotocin in rats. Indian J Clin Biochem. 2007;22(2):60-4. doi: 10.1007/bf02913315.
23. Dua JS, Rana AC, Bhandari AK. Liposome: methods of preparation and applications. Int J Pharm Stud Res. 2012;3(2):14-20.
24. Aviana T, Hutajulu TF, Isyanti M. Pembuatan nano-karotenoid asal konsentrat minyak sawit dengan cara sonikasi. Jurnal Dinamika Penelitian Industri. 2015;26(1):11-8. doi: 10.28959/jdpi.v26i1.697.
25. Poongothai K, Ponmurugan P, Ahmed KS, Kumar BS, Sheriff SA. Antihyperglycemic and antioxidant effects of *Solanum xanthocarpum* leaves (field grown & in vitro raised) extracts on alloxan induced diabetic rats. Asian Pac J Trop Med. 2011;4(10):778-85. doi: 10.1016/s1995-7645(11)60193-4.
26. Sulmartiwi L, Pujiastuti DY, Tjahjaningsih W, Jariyah. Potential of mangrove *Avicennia rumphiana* extract as an antioxidant agent using multilevel extraction. IOP Conf Ser Earth Environ Sci. 2018;137(1):012075. doi: 10.1088/1755-1315/137/1/012075.
27. Szkudelski T. Streptozotocin–nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med. 2012;237(5):481-90. doi: 10.1258/ebm.2012.011372.
28. Zhang H. Thin-film hydration followed by extrusion method for liposome preparation. Methods Mol Biol. 2017;1522:17-22. doi: 10.1007/978-1-4939-6591-5_2.
29. Thermo Scientific. pH Measurement Handbook. 2014. p. 1-27.
30. Loquercio A, Castell-Perez E, Gomes C, Moreira RG. Preparation of chitosan-alginate nanoparticles for trans-cinnamaldehyde entrapment. J Food Sci. 2015;80(10):N2305-15. doi: 10.1111/1750-3841.12997.
31. Varenne F, Botton J, Merlet C, Vachon JJ, Geiger S, Infante IC, et al. Standardization and validation of a protocol of zeta potential measurements by electrophoretic light scattering for nanomaterial characterization. Colloids Surf A Physicochem Eng Asp. 2015;486:218-31. doi: 10.1016/j.colsurfa.2015.08.044.
32. Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomedicine. 2015;10:975-99. doi: 10.2147/ijn.s68861.
33. Munot N, Kandekar U, Giram PS, Khot K, Patil A, Cavalu

S. A comparative study of quercetin-loaded nanocochleates and liposomes: formulation, characterization, assessment of degradation and in vitro anticancer potential. Pharmaceutics. 2022;14(8):1601. doi: 10.3390/pharmaceutics14081601.
34. Indonesian Herbal Pharmacopoeia. 2nd ed. Jakarta: Health Department of Republic of Indonesia; 2017. p. 528.
35. Sales PM, Souza PM, Simeoni LA, Silveira D. α-Amylase inhibitors: a review of raw material and isolated compounds from plant source. J Pharm Pharm Sci. 2012;15(1):141-83. doi: 10.18433/j35s3k.
36. van Hoogevest P, Wendel A. The use of natural and synthetic phospholipids as pharmaceutical excipients. Eur J Lipid Sci Technol. 2014;116(9):1088-107. doi: 10.1002/ejlt.201400219.
37. Charumathy A, Ubaidulla U, Sinha P, Rathnam G. Recent update on liposome-based drug delivery system. Int J Curr Pharm Res. 2022;14(3):22-7. doi: 10.22159/ijcpr.2022v14i3.1991.
38. Gülseren I, Corredig M. Storage stability and physical characteristics of tea-polyphenol-bearing nanoliposomes prepared with milk fat globule membrane phospholipids. J Agric Food Chem. 2013;61(13):3242-51. doi: 10.1021/jf3045439.
39. Giulimondi F, Digiacomo L, Pozzi D, Palchetti S, Vulpis E, Capriotti AL, et al. Interplay of protein corona and immune cells controls blood residency of liposomes. Nat Commun. 2019;10(1):3686. doi: 10.1038/s41467-019-11642-7.
40. Ricci M, Oliva R, Del Vecchio P, Paolantoni M, Morresi A, Sassi P. DMSO-induced perturbation of thermotropic properties of cholesterol-containing DPPC liposomes. Biochim Biophys Acta. 2016;1858(12):3024-31. doi: 10.1016/j.bbamem.2016.09.012.
41. Trucillo P, Campardelli R, Reverchon E. Supercritical CO2 assisted liposomes formation: optimization of the lipidic layer for an efficient hydrophilic drug loading. J CO2 Util. 2017;18:181-8. doi: 10.1016/j.jcou.2017.02.001.
42. Stanich CA, Honerkamp-Smith AR, Putzel GG, Warth CS, Lamprecht AK, Mandal P, et al. Coarsening dynamics of domains in lipid membranes. Biophys J. 2013;105(2):444-54. doi: 10.1016/j.bpj.2013.06.013.
43. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (part 1). Trop J Pharm Res. 2013;12(2):255-64. doi: 10.4314/tjpr.v12i2.19.
44. Khorasani S, Danaei M, Mozafari MR. Nanoliposome technology for the food and nutraceutical industries. Trends Food Sci Technol. 2018;79:106-15. doi: 10.1016/j.tifs.2018.07.009.
45. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):57. doi: 10.3390/pharmaceutics10020057.
46. Avadi MR, Sadeghi AM, Mohammadpour N, Abedin S, Atyabi F, Dinarvand R, et al. Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method. Nanomedicine. 2010;6(1):58-63. doi: 10.1016/j.nano.2009.04.007.
47. Hariyanti H, Damayanti S, Tarini S. Optimization process and characterization cinchonine niosomes. Pharm J Indones. 2019;16(2):178-87.
48. SI Analytics. pH handbook helpful guide for practical application and use. USA: Xylem; 2011
49. Ansel HC, Allen LV Jr. Pharmaceutical Dosage Forms and Drug Delivery Systems. Philadelphia: Wolters Kluwer; 2014. p. 271-2.
50. Mavaddati MA, Moztarzadeh F, Baghbani F. Effect of formulation and processing variables on dexamethasone entrapment and release of niosomes. J Clust Sci. 2015;26(6):2065-78. doi: 10.1007/s10876-015-0908-4.
51. Kakran M, Shegokar R, Sahoo NG, Shaal LA, Li L, Müller RH. Fabrication of quercetin nanocrystals: comparison of different methods. Eur J Pharm Biopharm. 2012;80(1):113-21. doi: 10.1016/j.ejpb.2011.08.006.