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Evaluation of Sidr Leaf Ethanol (*Ziziphus mauritiana* Lam.) Extract-Based Tablets as Antihypercholesterolemia

Salmah Orbayinah*, Yunita P. Dewi, Fariez Kurniawan, Annisa Krisridwany
School of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta

Abstract
Sidr leaf extract (*Ziziphus mauritiana* Lam.) is effective against hypercholesterolemia by blocking the HMG-CoA reductase. This study seeks to identify the characteristics of tablets formulated from the ethanol extract of Sidr leaf and their efficacy in lowering total blood cholesterol levels. The tablets were produced using the wet granulation method and processed into three formulations, F1, F2, and F3, with differing dosages of 100, 150, and 250 mg, along with placebo. The granules produced were evaluated for their physical characteristics. The tablets were evaluated for their physical characteristics and tested for effectiveness in lowering cholesterol levels. The efficacy of tablet-form sidr leaf ethanol extract was assessed by analyzing cholesterol levels in mouse serum samples compared to simvastatin. The data were statistically analyzed using one-way ANOVA and post-hoc Tukey HSD test. In the disintegration time test, F1 and F2 tablets satisfied the criteria. However, F3 exceeded the limit of 60 minutes. Among the three tablet doses, the F2 tablet (150 mg) demonstrated superior efficacy in lowering total cholesterol levels relative to the F1 and F3 tablets. Consequently, tablets containing Sidr leaf extract demonstrate potential for hypercholesterolemia management at an effective dosage of 150 mg, warranting further investigation through clinical trials.

Keywords: Cholesterol total, Sidr leaf (*Ziziphus mauritiana* Lam.), Tablet

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*Corresponding author:
orbayinah_salmah@umy.ac.id

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Evaluasi Tablet Berbasis Ekstrak Etanol Daun Sidr (*Ziziphus mauritiana* Lam.) sebagai Antihiperkolesterolemia

Abstrak
Ekstrak daun sidr (*Ziziphus mauritiana* Lam.) mempunyai efektivitas sebagai antihiperkolesterolemia dengan menghambat HMG-CoA reduktase. Penelitian ini bertujuan untuk mengetahui karakteristik tablet ekstrak daun sidr dan efektivitasnya dalam menurunkan kolesterol total. Tablet dibuat dengan metode granulasi basah yang masing-masing diolah dalam tiga formula, F1, F2, dan F3, dengan dosis 100, 150, dan 250 mg serta satu formula plasebo. Granul yang dihasilkan diuji sifat fisiknya meliputi organoleptik, laju alir, kadar air, dan kompresibilitas. Tablet yang dihasilkan kemudian diuji sifat fisiknya, meliputi uji organoleptik, keseragaman ukuran dan berat, kekerasan, waktu hancur, kerapuhan, dan disolusi. Khasiat ekstrak etanol daun sidr berbentuk tablet ditentukan dengan mengukur kadar kolesterol pada serum mencit, lalu dibandingkan dengan simvastatin. Data hasil uji kemudian dianalisis menggunakan one way ANOVA dengan uji post-hoc Tukey HSD. Karakteristik ekstrak tablet pada setiap dosis memenuhi persyaratan, kecuali uji kerapuhan yang lebih tinggi dari standar. Uji waktu hancur menunjukkan tablet F1 dan F2 memenuhi syarat, namun F3 tidak memenuhi syarat (lebih dari 60 menit). Dari ketiga dosis tablet, tablet F2 (150 mg) paling efektif menurunkan kadar kolesterol total dibandingkan tablet lainnya. Oleh karena itu, dapat disimpulkan tablet ekstrak daun sidr berpotensi untuk pengobatan hiperkolesterolemia dengan dosis efektif 150 mg.

Kata Kunci: Kolesterol total, Daun sidr (*Ziziphus mauritiana* Lam.), Tablet

1. Introduction

Hypercholesterolemia is a condition where the cholesterol level in blood rises above its normal levels. Hypercholesterolemia is typically defined in humans as a cholesterol level exceeding 200 mg/dL. Both genetic predisposition and dietary factors influence the variation in serum cholesterol levels. Nutrition and genetics have been widely considered to contribute to the variation of serum cholesterol levels. Also, the oxidative stress due to the imbalance between radical production and the scavenging process is notably regarded as the cause of hypercholesterolemia and affects several serious complications. Overproduction of the very low-density lipoprotein (VLDL) has been widely known to cause hypercholesterolemia by elevating the low-density lipoprotein (LDL). Elevated serum cholesterol levels are strongly associated with an increased risk of cardiovascular diseases (CVD), including coronary heart disease (CHD) and atherosclerosis, which are significant global health concerns. High levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides further exacerbate these risks by contributing to plaque formation and vascular inflammation.[1,2,3]

Anti-hypercholesterolemia drugs like statins reduce total cholesterol by inhibiting the β-hydroxy-β-methylglutaryl coenzyme A reductase (HMGCR), promoting LDL-receptor production and cholesterol uptake. However, long-term consumption can lead to toxic effects like diabetes, neurological issues, and muscle disease. Studies are needed to explore alternative cholesterol-lowering drugs, such as phytomedicine, which offers potential as an effective and natural treatment option.[4] This is also supported by the World Health Organization (WHO), concerned with using herbal medicines to care for people's health.[5]

Sidr leaf is a part of a sidr plant, which has many benefits such as anti-diarrhea, antipyretic, and anti-diabetes.[6] Sidr leaf has historically been used to cure various illnesses in various regions worldwide. Sidr plant is typically grown in tropical or subtropical countries, such as Indonesia, with hot and dry temperatures and low altitudes.[7,8] Finally, the Sidr plant is considered to become a widespread and highly available plant.

Sidr leaf contains several bioactive compounds such as flavonoids and betulinic acids, which are antioxidants that prevent cardiovascular disease.[9] Antioxidant is crucial in treating complicated diseases such as atherosclerosis by inhibiting LDL oxidation and reactive oxidative species (ROS) production.[3] According to study by Jain et al.,10 the sidr leaf has a high value of ethanolic antioxidant of 182.93 ± 6.94 mg equivalent gallic acid (GAE) g−1 extract. The crude hydroethanolic extract of sidr leaf has the flavonoid content of 4 mg quercetin equivalents (QE) while the total phenolic content is between 58 and 84 mg GAE per 100 mg.[11]

Ethanolic extracts of various plants have demonstrated significant cholesterol-lowering effects, likely due to their rich content of bioactive compounds such as flavonoids, polyphenols, and antioxidants. For example, the ethanolic extract of *Buchholzia coriacea* significantly reduced total cholesterol in serum and liver by 45.9% and 44.2%, considerably decreasing LDL-C levels and lipid peroxidation in hypercholesterolemia-induced rats.[12] Similarly, the ethanol extract of *Punica granatum* Linn. lowered total cholesterol and LDL-C levels, demonstrating its potential as a natural lipid-lowering agent. These findings are particularly relevant as sidr leaf (*Ziziphus mauritiana* Lam.) is rich in bioactive compounds, including flavonoids (46.94 ± 1.55 µg QE/mg) and phenolics (84.69 ± 0.92 µg GAE/mg).[13] Quercetin 3-O-rhamnoglucoside 7-O-rhamnoside, a major flavonoid, contributes to its potent antioxidant potential.[14] The hydroethanolic extract and its fractions contain high polyphenol levels (58–84 mg GAE/100 mg)[15], with ethanol extracts showing the highest phenolic content (182.93 ± 6.94 mg GAE/g).[16] Methanol and chloroform extracts exhibit potent antioxidant activity, comparable to standard antioxidants,[17,18] with leaves demonstrating the highest efficiency among plant parts.[19] compounds such as catechin, rutin, syringic acid, and caffeic acid contribute to its antioxidant and antibacterial properties.[20] These findings support the traditional medicinal use of *Z. mauritiana* and highlight its potential for therapeutic applications.[21] These bioactive constituents are known to mitigate oxidative stress, inhibit lipid peroxidation, and reduce LDL cholesterol levels, which are key mechanisms in managing hypercholesterolemia. Therefore, studies on *Buchholzia coriacea* and *Punica granatum* Linn provide a foundational basis for investigating the potential cholesterol-lowering properties of ethanol extracts from Sidr leaves. However, further research and experimental validation are required to substantiate these effects.

The primary necessity and demand of today is the formulation of medications in a convenient form. Tablets are a type of dosage form widely used in medicine preparations due to the convenience of consumption, lower price in production, and better appearance. Tablets are generally easier than injections or liquid forms, which may require special equipment or measured doses. The tablet production needs several active drugs to acquire a simple and effective medication delivery system based on the route of drug administration for the treatment of the disease spectrum.[14,15] To the best of our knowledge, publications of the sidr leaf benefits are still scarce, especially as cholesterol-lowering substances in tablet form. Therefore, this study aims to characterize the ethanol extract of sidr

leaf (EESL) by the phytochemical screening, formulation, and testing of the tablet and measure the effectiveness of the EESL tablet in reducing the cholesterol level of hypercholesterolemia mice.

2. Materials and Methods

2.1. Materials

Sidr leaf powder (*Ziziphus mauritiana* Lam.), lactose monohydrate, PVP-K30, amprotab, sodium starch glycolate (SSG), magnesium stearate, talc, distilled water, pig oil, CHOD-PAP reagent, Wistar strain white rat. The test animals were carefully selected and assigned specific characteristics, including age, weight, and health conditions, to ensure consistency and reliability in the experimental results. The rats in this study were aged 8–10 weeks and weighed 150–200 grams. All animals were acclimatized to laboratory conditions for seven days before the experiment and were maintained on a standard diet and *ad libitum* access to water.

2.2. Tools

The tools used include evaluating granules and tablets, and measuring total cholesterol levels in rats. The tools consist of analytical balance (Mettler Toledo®), glassware (Iwaki Pyrex®), metal stirrer and wood stirrer, rotary evaporator (IKA RV 10 Digital V), rat cage (size 50×30 cm) complete with feed and drink, water bath (WNB 14 Ring 14L), PCR Tube 1.5 mL (Eppendorf), test tubes (Iwaki®) and racks, micropipette 1-10 µL and 100-1000 µL, Hettich EBA 20 centrifuge, spectrophotometer (Jasco V-730), stomach probe needle for the rat, mortar-stamper (16 cm), sieve mesh number 12 and 16, oven (Memmert UNB 500), Delta tablet printer, semi-automatic hardness tester (TH 3B, Copley scientific), friability tester (FR 2000 Copley scientific), disintegration tester (Erweka ZT 222), tablet dissolution tester (Dis 6000 Copley scientific), tapped density (JV 2000 Copley scientific), moisture analyzer (Bell 163L Mo), granule flow tester, Camac vessel, and 5cc syringe (OneMed).

2.3. Methods

2.3.1. Sidr Plant Determination

Determination of sidr plants was conducted in the Biology Laboratory of the Faculty of Applied Science and Technology, University Ahmad Dahlan, using intact sidr plants to ascertain the types of plants used.

2.3.2. Extraction

A total of 1016.9 grams of Sidr leaf powder was macerated in 70% ethanol at a 1:10 ratio for five days. During the preparation process, non-specific parameters such as moisture content, solubility in alcohol, and ash content should be monitored to ensure the quality and reproducibility of the extract.

2.3.3. Qualitative Phytochemical Screening

Flavonoid Test

The thin layer chromatography (TLC) method used a mobile phase combination of butanol: acetic acid: aquadest (4:1:5) and stationary phase silica gel GF254. The reference standards during the elution were rutin and quercetin.[6]

Alkaloid Test

100 mg extract was heated in a large test tube with 10 mL of HCl 1% for 30 minutes in a boiling water bath. Then, the suspension was filtered and added with three drops of Dragendroff reagent. The positive result was shown by precipitate forming, and the color changed to yellow or orange.[6]

Saponin Test

100 mg of the extract was put into a test tube and added with 10 mL of distilled water. It was then shaken vigorously for 30 minutes. The positive result was indicated by the foam arising up to ±3 cm from the surface of the tube.[6]

Tannin Test

100 mg of the extract was heated with 10 mL of water for 30 minutes in a water bath. Then, 5 mL of filtrate was taken, 1 mL of 2% NaCl and 5 mL of 1% gelatin solution were added. The positive result was indicated by the sedimentation forming.[6]

Polyphenol Test

100 mg of the extract was heated with 10 mL of water for 20 minutes in a boiling water bath. After it cooled down, FeCl3 reagent was added within three drops. If a blue-green color appeared, it indicated that the powder contained polyphenols.[6]

Anthraquinone Test

100 mg of the extract was boiled with 10 mL of KOH 0,5 N and 1 mL of hydrogen peroxide solution for two minutes. Then, 5 mL of the filtrate was taken, 10 drops

of acetic acid were added until it reached pH 5, and 10 mL of toluene was added and shaken. 0.5 N KOH was added to the top layer of phytate. The appearance of red in the water layer (base) indicated the presence of anthraquinone compounds.[6]

2.3.4. The Tablet Formulation

The formula for tablet extract of sidr leaf was made with varying doses: 100 mg (F1), 150 mg (F2), 250 mg (F3), and placebo (F4), with a total of 600 mg each tablet (Table 1). Tablets were made using the disintegrant's wet granulation and intra-extra granular methods. All ingredients were mixed (EESL, lactose, SSG, and amprotab extract) and added with PVP K-30, previously dissolved using 80°C distilled water. Next, the mixture was sieved using mesh number 12 to produce granules. The granules were then put into an oven at 50°C to be dried. After that, the granules were sifted again using mesh number 16, followed by external phase mixing.

2.3.5. Evaluation of Granules

Physical examination

The shape and size of the resulting granules were examined. The accepted granule is round, non-ovoid, and relatively the same size.[16]

Moisture Test

Briefly, 3.00 ± 0.5 grams of granules were placed on an aluminum plate of a moisture analyzer that was heated at 105°C for five minutes.[17]

Granule's Flow Rate Test

Approximately 100 grams of granules were put into the flow timer, and the flow time was calculated. Suitable granules have a flow time of no more than ten seconds.[18]

Granule Compressibility Index Test

Around 50 grams of granules were put into a 100 mL measuring cup, and the volume (V1) was recorded. Then, the extract was tapped 500 times, and the volume (V2) was calculated again. A good compressibility index value is less than 10%.

2.3.6. Evaluation of Tablet

Physical examination

Physical examination of the tablet was done by observing the tablet directly in terms of color, surface shape, and other physical disabilities.[19]

Weight Uniformity Test

Twenty tablets from each formula were weighed, with the average weight being. Then, each tablet in all formulas was weighed again.[19]

Size Uniformity Test

This test was done by measuring the width and height of each tablet using calipers, and its average size was calculated.

Hardness Test

A total of 20 tablets were tested one by one for hardness using a hardness tester.

Friability Test

Twenty tablets were weighed, put into a friability tester, and rotated for 4 minutes at 25 rpm. The tablets were weighed again after cleaning them from the remaining dust. Tablets would meet the requirements if their friability percentage were less than 1%.[20]

Disintegration Time Test

Six tablets were tested for disintegration using a disintegration tester. One tablet was placed in each tube in the basket, and the basket was then immersed in distilled water at 37°C for 30 minutes.

Dissolution Test

A total of six tablets were put into a container containing 900 mL of water with a rotation speed of 50 rpm. Each tablet was taken as much as 5 mL of sample with a five-minute break for 45 minutes. The samples were then measured for absorbance using ultraviolet-visible (UV-Vis) double beam spectrophotometry (V-730 Jasco) at the maximum wavelength of 254 nm. The obtained absorbance was put into a standard curve to see the drug contents.[21]

The absorbance of its drug contents was then calculated using the quercetin standard curve equation. The next step was to estimate the AUC from the relationship graph between time and the increase of drug contents dissolved in the dissolution media. The AUC was then compared with the total area of the graph (in percent) to determine the dissolution efficiency (DE) value.

2.3.7. In vivo Study of EESL Tablet

This study was conducted under ethical clearance issued by The Health Research Ethics Committee of The Faculty of Medicine and Health Science, University of Muhammadiyah Yogyakarta (no. 201/EP-FKIK-UMY/X/2019). In the in vivo test, forty male Wistar strain rats were used and divided into eight groups, namely normal group (B), base group (NA), negative group (KN), positive group (KP), group of 100-mg EESL tablet (F1), group of 150-mg EESL tablet (F2), group of 250-mg EESL tablet (F3), and the placebo tablet group (F4). The tablets, formulated with strengths of 100 mg, 150 mg, or 200 mg, were designed for dose calculations based on the rats' body weight (e.g., mg/kg or mg/200 g). The tablets were crushed for administration, and the appropriate amount of powder was calculated for each rat according to weight. The powder was then suspended in distilled water and administered orally to ensure precise dosing. This approach avoids the direct use of whole tablets, which may not align with individual dosing requirements. Each group, except the normal and the base group, was induced with 10 mL of pig oil daily for 14 days to increase cholesterol levels and mimic hypercholesterolemia conditions. The normal cholesterol level in Wistar rats typically ranges between 40–130 mg/dL. The induction with pig oil was designed to elevate cholesterol levels above this range, targeting a hypercholesterolemic state of approximately 150–200 mg/dL. This induction protocol allowed for the evaluation of the anti-hypercholesterolemic effects of the tested EESL formulations.

Then, it was followed by drug induction for 14 days; the positive control group was treated with 10 mg of simvastatin, followed by the treatment groups (100, 150, and 200 mg tablets of extract), and with a placebo. Whereas KN was still given pig oil induction, NA (Na-CMC 0.5%) and group B were given only standard feed. Cholesterol levels were measured 3 times, on day 0, day 14, and day 28. The cholesterol level was calculated using the mice's blood serum taken through the retro-orbital plexus in their eye. Mice blood serum was added with CHOD-PAP reagent, and the absorbance was read using UV-Vis double beam spectrophotometry at a wavelength of 500 nm (Cholesterol Oxidase Methode/CHOD-PAP).

2.4. Data Analysis

Both granule and tablet evaluation tests were tabulated in the table with RSD for specific parameters. The *in vivo* data of each tablet formulation were analyzed by one-way analysis of variance (ANOVA) using RStudio and the post-hoc Tukey HSD test was applied for the rejected null hypothesis ($p<0.05$). The histogram of the formulation versus cholesterol level was constructed using Microsoft Excel 2019.

3. Result

3.1. Determination of Sidr Plants

Determination aimed to determine the correctness of the plants, related to the microscopic morphological characteristics of the sidr plant, (*Ziziphus mauritiana* Lam.) in the literature, and to avoid mistakes in gathering the main ingredients and errors in the research result. The determination results showed that the plants were by the main ingredients used in this study, which were the sidr of the species *Ziziphus mauritiana* Lam.[22]

3.2. Plant Extraction

The crude EESL obtained from sifting 1.016,9 g of powder was 168.6106 g. This showed that the extraction process provides sufficient efficiency with a yield of 16.58%. The extract form was dark green with a distinctive aroma of leaves.

3.3. Qualitative Phytochemical Screening

The qualitative phytochemical screening of the crude EESL was performed to identify key chemical constituents, including flavonoids, alkaloids, anthraquinones, tannins, polyphenols, and saponins, using thin layer chromatography (TLC) methods and appropriate reagents. The presence of flavonoids was confirmed by yellow spots visible under UV light during the TLC analysis. The sidr leaf extract contained rutin and quercetin, as evidenced by the matching retardation factor (Rf) values and spot colors with The standards, as shown in Figure 1. The alkaloid test revealed an orange precipitate when Dragendorff's reagent was applied, indicating the presence of alkaloids. In contrast, the anthraquinone test showed no color change when potassium hydroxide (KOH) was added to the extract, confirming the absence of anthraquinones.[25]

Further tests identified the presence of other bioactive compounds. Tannins were confirmed by forming a water-insoluble precipitate when the heated and filtered extract was treated with gelatin. Similarly, the presence of polyphenols was established when ferric chloride ($FeCl_3$) caused a greenish-black discoloration in the sample. Lastly, saponins were detected through the characteristic foam formation observed when the extract was shaken with water. These results collectively highlight the phytochemical richness of the crude EESL, which contains flavonoids, alkaloids, tannins, polyphenols, and saponins, while anthraquinones are notably absent.[26]

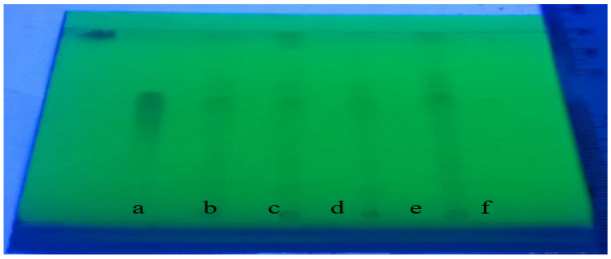

Figure 1. Thin Layer Chromatography (TLC) chromatogram showing flavonoid compounds in sidr leaf extract at 254 nm wavelength (λ). The analysis used silica gel GF254 as the stationary phase and a mobile phase comprising butanol: acetic acid: distilled water (4:1:5 v/v). The chromatogram illustrates the separation of standards quercetin (a) and rutin (b), alongside the sidr leaf extract (c), and formulations of tablets containing 100 mg (d), 150 mg (e), and 250 mg (f) of extract. The retardation factor (Rf) values and spot intensities confirm the presence of quercetin and rutin in the sidr leaf extract and its tablet formulations. Quercetin and rutin serve as reference standards to verify the accuracy of the separation and identification of flavonoids.

3.4. Evaluation of Tablet's Granules

3.4.1. Physical Examination Test

The physical appearance of granules from F1, F2, and F3 was irregularly round, with varying sizes and a little powder. For F4, round granules were obtained, some oval and of varying sizes.

3.4.2. Humidity Test

According to Farmakope Indonesia, the water content of natural material tablets must be less than or equal to 10 percent.25 Based on the results obtained in Table 2, the humidity in the four formulas ranged from 2.93 to 3.50%, so it can be concluded that all four formulas met the requirements. Ideally, the best and safest level is below 2%.

3.4.3. Compressibility test

The compressibility index of the four formulas showed promising results, and followed the standard (Table 1). The requirement for granules to have a good compressibility index is that after being compressed 500 times, the compressibility index is no more than 20%.[27]

3.5. Tablet Evaluation

3.5.1. Physical Examination Test

The first formula resulted in a tablet's color being evenly green, and F4 produced a white tablet. Meanwhile, in F2 and F3, brown and green brown tablets were obtained with mottling spread colors due to the less homogeneous mixing.

3.5.2. Size Uniformity Test

Tablets are considered uniform if their diameter is at least four-thirds and no more than three times their thickness.[19,28] The four tablet formulas, with a diameter of 110 mm and a thickness of 45 mm, met the requirements (Table 2).

3.5.3. Weight Uniformity Test

The four tablet formulas met the requirements of natural ingredients tablets,[29] with the two tablets having no more relative standard deviation (RSD) value than 5%, and there was no tablet more than 10%. The RSD values were 0.5; 0.6; and 1.0% for F1 and F2, F3, and F4, respectively (Table 2). According to the British Pharmacopoeia 1953,[30] the requirement for a tablet's weight uniformity is that when a sample of observed tablets was measured individually, the average weight percentage between two tablets is less than that specified. A tablet cannot have a deviation by more than double that percentage.

3.5.4. Hardness Test

The four formulas' hardness met conventional tablets' requirements, that is, between 4 to 8 kilogram force (kP) (Table 2).[31]

3.5.5. Friability Test

Table 1. Granule Evaluation Test Results

Granule Evaluation	1 (100 mg of EESL)	2 (150 mg of EESL)	3 (250 mg of EESL)	4 (Placebo)
Humidity Test (%)	3.44	3.50	2.93	3.40
Flow Rate Test (second)	6.50 ± 0.06	5.90 ± 0.06	4.71 ± 0.54	5.24 ± 0.19
Compressibility test (%)	12.00 ± 0.00	9.00 ± 1.41	15.00 ± 1.41	10.00 ± 2.83

Table 2. Tablet Evaluation Results

Evaluation Tests of Tablets	1 (100 mg of EESL)	2 (150 mg of EESL)	3 (250 mg of EESL)	4 (Placebo)
Weight uniformity (mg)	595.6 ± 3.0	602.2 ± 3.1	600.6 ± 3.8	684.7 ± 6.8
	RSD: 0.5%	RSD: 0.5%	RSD: 0.6%	RSD: 1%
Size uniformity (mm)	Diameter: 110	Diameter: 110	Diameter: 110	Diameter: 110
	Thickness: 45	Thickness: 45	Thickness: 45	Thickness: 45
Hardness (kg)	5.49 ± 0.66	6.14 ± 1.06	6.23 ± 0.57	6.15 ± 1.11
Friability (%)	2.15	1.83	1.52	1.21
Disintegration time (minutes)	16.39	24.42	>60	12.33
Dissolution (%)	90.83 ± 3.87	91.35 ± 0.01	95.17 ± 0.01	-

The tablets' friability evaluation results in all four formulas showed that none met the requirements (Table 2). This was due to using a punch whose edges were either worn or damaged, and the uneven mixing of lubricants.

3.5.6. Disintegration Time Test

Good natural-ingredient tablets have a disintegration time of no more than thirty minutes. 29 The evaluation results showed that all four formulas met the requirement for good disintegration time, except for F3 (Table 2). The disintegration time can be reduced by adding granule moisture, fine fraction (surface-area-to-volume ratio), reduction of lubricant concentration, and the compression force. The humidity level of F3 was the lowest, which might cause longer disintegration time.[32,33]

3.5.7. Dissolution Test

The dissolution test of the three formulas resulted in a value of dissolution efficiency (DE45) by more than 75% (Table 2). The typical standard is when a tablet can dissolve over 80% of the amount after 45 minutes of the test.34 Due to the typically single-point dissolution test of DE45, more accurate standard for the dissolution value was DE% stated by Putra et al.,35 which is defined as "the area under the dissolution curve up to a certain time, t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time".

3.6. In Vivo Test of Tablet Extract of Sidr Leaf

Pig oil was used as a cholesterol induction test animal in the *in vivo* test. Pig oil has been proven to induce hypercholesterolemia in mice, with a targeted total cholesterol level of >200 mg/dL. The induction was successful, as evidenced by the significant increase in total cholesterol levels measured on day 14 compared to baseline levels (e.g., from an average of 65.63 ± 23.15 mg/dL to 175.22 ± 99.76 mg/dL in the F2 group). After the total cholesterol level in mice increased, the tablet extract of sidr leaf was administered for fourteen days to determine its effectiveness in reducing the total cholesterol level. The administration of tablet extract of sidr leaf proved to be effective in lowering the total cholesterol level, as seen from the measurement of the total cholesterol level of the mice on day 28.

The total cholesterol results after drug administration were then statistically analyzed using RStudio with the preliminary test of the one-way ANOVA method to determine the effect of EESL tablet in reducing cholesterol level. According to the one-way ANOVA analysis, the eight treatment groups significantly differed in lowering total cholesterol levels ($p<0.05$). After that, the Post Hoc Test was conducted using Tukey HSD to determine which groups had the same cholesterol reduction.

The cholesterol-lowering effects of the tablet formulations were evaluated through a one-way ANOVA to determine whether significant differences existed among the treatment groups. The analysis revealed statistically significant differences ($p<0.05$) in total cholesterol levels among the groups. This suggests that the treatments had varying levels of effectiveness in reducing cholesterol. A post-hoc Tukey HSD test was conducted to identify which groups differed significantly. The results demonstrated that the 150-mg tablet group (F2) was as effective in reducing cholesterol levels as the positive control group (KP, treated with simvastatin) and the 250-mg tablet group (F3), with no significant differences observed among these groups ($p>0.05$). However, the 100-mg tablet group (F1) showed significantly lower efficacy compared to F2, F3, and KP ($p<0.05$). As expected, the placebo group (F4) exhibited no significant cholesterol-lowering effects compared to the untreated control groups (B and NA). Interestingly, the results also showed that the cholesterol-lowering effect of the 250-mg tablet (F3) did not significantly surpass that of the 150-mg tablet (F2), suggesting that 150 mg is the optimal dose under these experimental conditions. Additionally, the negative control group (KN), which continued receiv-

Table 3. Measurement of the total cholesterol level

Groups	Total cholesterol level average (mg/dL)			Statistical Analysis (Tukey HSD) Results
	Day 0	Day 14	Day 28	
B	61.91 ± 6. 73	87.39 ± 0.69	53.94 ± 1.21	$p < 0.05$ with all groups
NA	48.77 ± 5.10	100.63 ± 22.68	48.90 ± 5.46	$p < 0.05$ with all groups
F1	72.47 ± 26.56	123.67 ± 162.20	93.53 ± 10.14	$p < 0.05$ with F2, F3, KP, KN
F2	65.63 ± 23.15	175.22 ± 99.76	55.40 ± 10.77	$p > 0.05$ with KP, F3; $p < 0.05$ with F1, F4, KN
F3	47.61 ± 21.12	92.95 ± 10.90	58.64 ± 6.91	$p > 0.05$ with KP, F2; $p < 0.05$ with F1, F4
F4	55.65 ± 8.34	63.67 ± 7.32	52.01 ± 9.73	$p < 0.05$ with all groups except B, NA
KP	64.23 ± 17.43	122.01 ± 81.88	55.91 ± 2.53	$p > 0.05$ with F2, F3; $p < 0.05$ with F1, F4
KN	56.98 ± 19.34	97.02 ± 10.72	77.40 ± 4.72	$p < 0.05$ with KP, F2, F3

ing pig oil without any treatment, experienced slightly reduced cholesterol levels, potentially due to physiological adaptation. Despite this, KN showed significantly less reduction than KP, F2, and F3 ($p<0.05$).

On the fourteenth day after induction, groups B and NA demonstrated elevated cholesterol levels despite not being administered a pig oil diet. This increase was likely due to stress-induced corticosterone elevation, a known contributor to temporary changes in lipid metabolism. Additionally, natural interindividual variability among the rats could explain these minor fluctuations. Conversely, the F4 group did not exhibit a significant cholesterol increase despite pig oil induction. This outcome may be related to individual resistance to hypercholesterolemia, potentially driven by genetic or enzymatic factors. Furthermore, components of the placebo tablet formulation, such as sodium starch glycolate or lactose monohydrate, could have inadvertently influenced lipid absorption or metabolism, mitigating the cholesterol elevation typically induced by a high-fat diet.

4. Discussion

Tablet manufacturing is a complicated process involving many variables and technical concepts. Finding a tablet formulation with the desired physical and dissolving qualities while integrating herbal extracts is challenging. Granulation is the enlargement process of particles by agglomeration technique and has become the key process in producing pharmaceutical dosage forms, primarily tablets and capsules. Granulation transforms the fine powders of drugs into dust-free and free-flowing powders to compress them more efficiently. Granulation commences after the dry mixing process between the intended material powder and the active pharmaceutical ingredient (API) to get the uniformity of particle distribution of each ingredient. Wet granulation is a granulation process defined as the production of granules by wetting all the excipients and APIs using granulation liquid with or without a binder. The advantages of wet granulation are that it allows the particle size to be enlarged, flow ability and compressibility to be enlarged, wide application, continuous processing, shorter time of process, and the process not needing the drying process.[36]

Several studies have been carried out on the production of tablet-formed herbal extracts and their physical properties,[37] and demonstrated that the tablet contained dry hydroethanolic extract from *Lippia alba* leaves had the best physical properties including a weight variation of 617.1±4.1 mg, hardness for more than 30 N, friability of 0.02%, and disintegration time of 4.0 minutes,[38] also reported that the formulated tablet of *Pueraria tuberosa* water extract had the hardness level of 1-7.2 kg/cm2, less than 1% of friability, the weight variation was in accordance to the United States Pharmacopoeia (USP) limit by ±5%, the dissolution time was between five to six minutes, and the compact tablets possessed 3.9 to 5.5 mm of thickness parameter. According to Soeratri et al.,[39] the tablet of *Satureja kitaibelii* Wierzb. ex Heuff. (Lamiaceae) had several physical properties, including tensile strength at 0.55-1.89 Mpa, with the acceptable value being more significant than 1.7 MPa, and disintegration time at 646, 630, and 543 s for each of the three formulations, with sufficient time being less than 15 minutes for uncoated tablets according to the European Pharmacopoeia. Finally, this study can be inferred that the obtained tablet was similar to the previous studies, even though the friability and disintegration time of the EESL tablet were insufficient.

Sidr leaves (*Ziziphus mauritiana*) have been widely used due to the pharmacological properties. The aqueous extract of *Ziziphus mauritiana* leaf possesses the antioxidant effect on chronic alcohol-induced hepato-

toxicity by lowering the level of total bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase.[40] Other expanded properties of the sidr leaves extract, as the anti-microbial and antioxidant compounds, are analgesic, anti-inflammatory, anti-allergic, treatment for tuberculosis and blood related diseases, cancer, fever, and liver related diseases.[41] Due to the sidr leaves' properties as an antioxidant, it should be considered that the plant can treat the hypercholesterolemia more safely. Furthermore, the compacted extract into a tablet drug form can make oral administration more convenient.

Several materials are needed for the tablet manufacturing. This study used two phases, including a 94% phase and a 6% phase. The tablet ingredients include lactose monohydrate, polyvinylpyrrolidone K-30 polymer (PVP K-30), SSG 3%, amylum pro-tablet (amprotab), Mg stearate, and talc. Lactose monohydrate is a binder or diluent for sufficient hardness and disintegration properties.[42] PVP K-30 has a broad affinity for hydrophilic and hydrophobic medicines and is nontoxic, nonionic, inert, temperature-resistant, pH-stable, and biocompatible. This polymer is often used as an excellent binder in wet granulation to formulate a good hardness of the tablet and does not influence a drug's disintegration and dissolution rates.[43]

SSG is the superdisintegrant with the porous structures that enable the tablet to absorb water, which is necessary for disintegration.[44] Amprotab is a disintegrating agent that absorbs water into the tablet and elevates the dissolution rate.[45] Magnesium stearate plays as the lubricant with the typical level of 0.25-5% w/w for the immediate-release formulations.[46] Talc (hydrous Mg stearate) is a glidant which has better performance compared to starch, but it shows limitation on the retardation of the disintegration-dissolution profile. Talc is usually added into the formula in the 1-10 weight percent (wt)% range.[46,47]

In this study, rats were induced with pig oil for 14 days to elevate cholesterol levels. The average cholesterol level recorded was 175.22 ± 99.76 mg/dL. While this level does not exceed the hypercholesterolemic threshold for humans, the induction was considered partially effective as significant variability was observed among individual rats. Factors such as species-specific lipid metabolism and individual responses to dietary fat may have contributed to this outcome. Future studies will explore alternative induction methods or more extended exposure periods to achieve consistent results.

The total cholesterol results after drug administration were then statistically analyzed using RStudio with the preliminary test of the one-way ANOVA method to determine the effect of EESL tablet in reducing cholesterol level. According to the one-way ANOVA analysis, the eight treatment groups significantly differed in lowering total cholesterol levels ($p<0.05$).

After that, the Post Hoc Test was conducted using Tukey HSD to determine which groups had the same cholesterol reduction. Post-hoc test results of both KP and KN showed a significant difference ($p<0.05$). This means the KP, simvastatin, is more effective in lowering cholesterol levels than the KN. The F2 and F3 tablet extracts showed no significant difference ($p>0.05$) compared with KP, whereas the F1 showed a significant difference ($p<0.05$). The results mean that the tablet extracts with doses of 150 and 250 mg had the same effectiveness as the simvastatin lipid-lowering effect, and it was contrary to the 100 mg of extract, which was less effective in lowering the total cholesterol. The F1 significantly differed between F2 and F3 ($p<0.05$).

These results indicate that the higher the dose, the more effective the tablet in reducing cholesterol. However, the group of 150-mg and 250-mg EESL tablets did not provide a significant difference. It is caused by the bioactive compound within the EESL tablet having already reached a target saturation, i.e., nearly 100% of target occupancy, which then increased the affinity of drug-target and commenced the drug effect,[48] which means there was no difference in the effectiveness between the 150-mg dose and the 250-mg dose in reducing total cholesterol level. Nevertheless, this research has suggested doing a pharmacogenetic study to evaluate the genetic effect on the EESL tablet for further research to achieve a more precise modern herbal medication.

The comparison of each group with KN that showed a significant difference only existed in the KP, F2, and F4 tablet ($p<0.05$). This is due to physiological factors of mice in receiving pig oil, so the negative group also experienced a decrease in cholesterol. The 100-mg tablet extract and the 250-mg tablet extract showed significant results ($p<0.05$). This is due to the 100-mg tablet extract (F1) being less effective in reducing cholesterol levels in blood as compared to the positive group with F1. On a 250-mg tablet, this is due to the lack of homogeneity of the extract in the tablets, which resulted in effectiveness that was not so different from the negative control. The study focuses on the efficacy of sidr tablets in reducing cholesterol levels but does not evaluate their safety profile. Further research, including toxicological studies and clinical trials, is necessary to assess the safety, dosage tolerability, and potential adverse effects of Sidr tablets in both animals and humans.

5. Conclusion

EESL (*Ziziphus mauritiana* Lam.) positively contains flavonoids, quercetin, alkaloids, tannins, and polyphenols metabolites. The characteristics of each tablet formula showed they met all the requirements, except in the friability test, where none of the formula met the requirement (friability value >1%), and the 250-mg EESL tablet has the longest disintegration time (more than 60 minutes). Tablet extract of sidr leaf (*Ziziphus mauritiana* Lam.) reduces the total cholesterol level in pig oil-induced hypercholesterolemia in mice. The 150-mg tablet extract demonstrated the highest effectiveness in lowering total cholesterol levels, reducing 58.64 ± 6.91%, as determined through one-way ANOVA and post-hoc Tukey HSD analysis ($p<0.05$). Statistical comparisons revealed that the reduction in total cholesterol for the 150-mg tablet was significantly more significant than the 100-mg tablet (F1 group, $p<0.05$) and comparable to the 250-mg tablet (F3 group, $p>0.05$). These results suggest that 150 mg is the optimal dosage for cholesterol reduction under the study conditions. While the study highlights the potential of Sidr tablets for managing hypercholesterolemia, the safety profile of these tablets remains unexplored. Future research should prioritize comprehensive safety assessments alongside efficacy evaluations.

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Conflict of Interest

The authors declare no conflicts of interest related to this work.

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