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Journal of Multidisciplinary Applied Natural Science Vol. 6 No. 1 (2026) https://doi.org/10.47352/jmans.2774-3047.327
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Research Article

Antidiabetic and Antihyperlipidemic Activities of Nanoemulsion of *Epipremnum pinnatum* Leaf Extract in Alloxan-Induced Rats

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Received : January 31, 2025	Revised : July 15, 2025	Accepted : September 19, 2025	Online : December 20, 2025

Abstract
Diabetes mellitus is a serious threat to human health worldwide. Alternative therapy sources derived natural ingredients promise therapeutic benefits, safety, and cost savings. Nanoemulsion technology has demonstrated that natural extracts have improved bioavailability and therapeutic effects. This study aimed to demonstrate the efficacy of a nanoemulsion of *Epipremnum pinnatum* leaf extract, which has antidiabetic and antihyperlipidemic effects in alloxan-induced rats. The ideal nanoemulsion was formulated by testing four permutations of chitosan (0.1% or 0.2%; 18 mL), sodium tripolyphosphate (0.1% or 0.2%; 9 mL), combined with *Epipremnum pinnatum* extract (0.250 g) and Tween 80 (0.5%; 3 mL). The antidiabetic and antihyperlipidemic tests were divided into five treatment groups: normal group, negative control group (2 mL NaCMC/200g BW), positive control group (10 mg/kgBW of glibenclamide), treatment I (250 mg/kgBW of extract), and treatment II (250 mg/kgBW of nanoemulsion extract). The treatment lasted for 14 days, and the data taken were blood sugar, malondialdehyde (MDA), total cholesterol, triglyceride, LDL, and HDL levels. According to the results of *Epipremnum pinnatum* leaf extract nanoemulsion, the best nanoemulsion utilized in antidiabetic and antihyperlipidemic tests was one with a particle size of 165.70 nm and a zeta potential of 22.0 mV. The nanoemulsion administered at a dose of 250 mg/kg BW was more effective than the extract, and the effects were identical to those of glibenclamide. The data indicate that *E. pinnatum* leaf extract nanoemulsion could be used as an antidiabetic and antihyperlipidemic alternative treatment.

Keywords: alloxan, antidiabetic, antihyperlipidemic, *Epipremnum pinnatum*, nanoemulsion

1. INTRODUCTION

The major risk factors for cardiovascular disease are diabetes mellitus and hyperlipidemia. Diabetes often shows a pattern of increased triglyceride and low-density lipoprotein (LDL) levels [1]. This is one of the main causes of high morbidity and mortality in patients with diabetes mellitus. Diabetes is expected to affect 642 million people by 2040, with emerging countries accounting for most cases. This condition is expected to worsen owing to lifestyle changes [2]. Many antidiabetic and antihyperlipidemic medicines are available, from both natural and synthetic sources [3]. Existing modern medications have always been in terms of efficacy, cost, and various side effects. As these issues become more prevalent, conventional plant-based therapies are being used as alternative methods for diabetes management [4].

Epipremnum pinnatum, especially its leaves, has been used in traditional medicine. According to several studies, this plant has several qualities that make it beneficial as a medication. It inhibits cancer cell proliferation and can be used to treat burns [5]. It lowers the amount of triglycerides and has additional benefits. It also has antihyperuricemia [6], antihyperglycemic [7], anti-inflammatory [8], anti-hypercholesterolemia [9], and antioxidant effects [10]. It lowers the amount of triglycerides and has additional benefits. *E. pinnatum* leaf extract has significant antioxidant potential and can be used as an antidiabetic and antihyperlipidemic agent. *E. pinnatum* leaf contains the active chemical quercetin (0.1728 mg/g) in acetone extract [11]. Quercetin acts as an antidiabetic agent by inhibiting the enzymes alpha-glucosidase, alpha-amylase, aldose reductase, dipeptidyl peptidase-4, and protein tyrosine phosphatase 1B (PTP 1B), increasing insulin sensitivity and modulating oxidative stress [12][13]. Makhdalena (2006) stated that at a dose of 300 mg/kg BW, the ethanol extract of *E. pinnatum* leaf effectively reduced cholesterol and triglyceride levels, both of which are risk factors for cardiovascular disease [9]. In addition, further research indicates that *E. aureum*, a member

Table 1. Formula for the nanoemulsion of *E. pinnatum* leaf extract.

	Extract (g)	Chitosan (18mL)	S.TPP (9mL)	Tween 80 (3 mL)
Formula 1	0.250	0.1%	0.1%	0.5%
Formula 2	0.250	0.2%	0.2%	0.5%
Formula 3	0.250	0.1%	0.2%	0.5%
Formula 4	0.250	0.2%	0.1%	0.5%

of the Araceae family that includes *E. pinnatum*, has the potential to be an antidiabetic medicine. The results suggested that the ethanol extract of *E. aureum* reduced blood glucose levels by 61.07% with a 250mg/kgBW [14]. Until now, no scientific data has specifically investigated the antidiabetic effects of the *E. pinnatum* plant formulated in the form of nanoemulsion delivery. The results of initial phytochemical screening tests indicate the presence of bioactive compounds with potential effects as antidiabetic agents that have not been explored in nanoformula form [8].

Nanotechnology and nanoscience have evolved in various fields, including research. Nanoparticle production using polymer combination has attracted significant interest in the pharmaceutical and medical industries [15][16]. Nanoparticles are colloidal particles with diameter of 1–1000 nm. In the clinical phase, the utilization of natural products is focused on optimizing the bioavailability of preparations. The active ingredient's nanosize, which ranges between 50–500 nm, aids in absorption in the small intestinal walls, boosting bioavailability. Therefore, the application of nanotechnology in pharmaceutical preparations could increase the bioavailability of medication, manage the release of active substances, and improve the sensory qualities at the site of action. Nanoemulsion is one of the subtypes of nanotechnology-based systems that are liquid-based [17]. This study aimed to provide an overview of how changing the particle size of the extract into nanoemulsion affects the antidiabetic and antihyperlipidemic therapeutic effects of an ethanol extract of *E. pinnatum* leaf in male white rats.

2. MATERIALS AND METHODS

2.1. Materials

Fresh *E. pinnatum* leaves (synonym *Rhaphidophora pinnata*) were harvested in Mendalo, Jambi, Indonesia. Plant determination was conducted at the Biology Department, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University with a voucher number of 250/K-ID/ANDA/VI. The 70% ethanol (Brataco), acetonitrile, formic acid, sodium carboxymethyl cellulose (Brataco), aquadest, sulfuric acid, Dragendorff's reagent, Mayer's reagent, alloxan (Merck), TBA (Merck), TCA (Merck), glibenclamide, anhydrous acetic acid (Merck), concentrated sulfuric acid, concentrated HCl, 1% $FeCl_3$ (Merck), chitosan (Merck), sodium tripolyphosphate (Brataco), Tween 80 (Brataco), Mg powder (Merck), Folin-Ciocalteu reagent, gallic acid, $AlCl_3$, NaOH, quercetine, ketamine, xylazine, and glucose sticks (Easy Touch®). Male white Wistar rats were used in this study.

2.2. Extraction of E. pinnatum Leaf

E. pinnatum leaf powder was extracted using 70% ethanol via the maceration method. The bottle was filled with 500 g of simplicia, and the solvent was poured until the powder was completely submerged. The simplicia to the solvent ratio is 1:10. The mixture was left for 2 days and stirred 5-6 times a day at room temperature (25°C±2) and protected from light. The macerate was then strained, remacerated, and stored for 24 h. Remaceration was performed twice. The collected macerate was concentrated using a rotary evaporator at 50-60°C until a thick extract was obtained. The concentrated extract was subjected to phytochemical screening analysis and determination of total phenol and flavonoid contents according to the research procedures of Safitri et al. [18] and Alara et al. [19].

2.3. LC-MS Fingerprint Analysis

LC-MS was used to identify the chemicals in *E.*

pinnatum leaf extract with minimal adjustments to the work protocols used by Ismed et al. [20]. Ten milligrams of *E. pinnatum* leaf extract was weighed and dissolved in 10 mL of methanol using a measuring flask. This research used an ultra-performance liquid chromatography (UPLC, LC: ACQUITY UPLC H-Class System, Waters, USA) and a mass spectrometer (Xevo G2-S QTof, Waters, USA). This experiment used a C-18 column (1.8µm, 2.1 × 100 mm, ACQUITY UPLC HSS, Waters, USA) with a column temperature of 500°C and a room temperature of 250°C. The mobile phase used in this analysis consisted of mixture A (water + 5 mM ammonium formate) and mixture B (acetonitrile + 0.05% formic acid), flow rate using a gradual gradient of 0.2 mL/min for 23 min and injection volume of 5 µL, which had been previously filtered using a 0.2 µm filter syringe. MS analysis used electrospray ionization (ESI) positive charge mode with a mass range of 50–1200 m/z and source and desolvation temperatures of 100 and 350°C, respectively. Then cone gas flow rate and desolvation of 50 L/h and 800L/h were also used sequentially with collision energy varying between 4–60 eV. Polar compounds produced chromatograms first, followed by compounds with lower polarities. The chromatogram peaks were interpreted using the MassLynx application.

2.4. Nanoemulsion

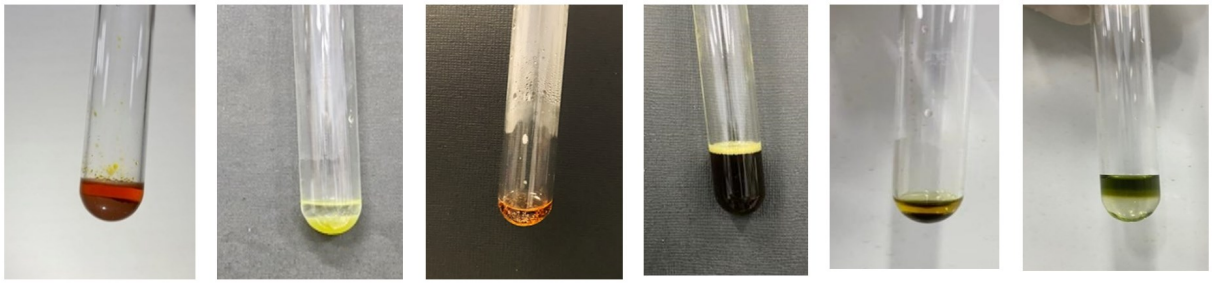
2.4.1. Preparation of Nanoemulsion of E. pinnatum Leaf Extract

Using a magnetic stirrer at 2,500 rpm for 30 min, 0.250 g of *E. pinnatum* leaf extract was combined with 18 mL of chitosan (at a concentration of 0.1% and 0.2%). Then, 9 mL of sodium tripolyphosphate (concentrations of 0.1% and 0.2%) was added dropwise with a magnetic stirrer at 2,500 rpm for 30 min. Finally, 3 mL of Tween 80 at a concentration of 0.5% was added dropwise and stirred with a magnetic stirrer at 2,500 rpm for 30 min to generate a nanoparticle emulsion solution [21]. Table 1 shows the process of preparing a nanoemulsion of the chitosan extract of *E. pinnatum*. The formula that produced the best results regarding particle size, zeta potential, and polydispersity index was used to test the antidiabetic and antihyperlipidemic effects in test animals.

2.4.2. Stability Test and Characterization of Nanoemulsions

The physical stability test of nanoemulsion was carried out using the freeze-thaw cycle test method. The freeze-thaw cycle test was carried out by storing the nanoemulsion preparation at a temperature of 4°C±2, then transferring to a temperature of 25°C±2, and each temperature for 24 h was recorded as one cycle. The test was carried out for six cycles, then observing changes in physical stability, including pH and organoleptic [22].

A HORIBA SZ-100 nanoparticle analyzer was used to characterize the samples. The average diameter of the nano-dispersed particles was measured using dynamic light scattering particle size analysis with a measuring instrument in the range of 0.3 nm – 8 µm. The samples were diluted at a ratio of 1:5 to prevent multiple scatterings. The temperature used for the measurements was 250°C. The zeta potential was measured by the electrophoretic mobility distribution of particles



(a) (b) (c) (d) (e) (f)

Figure 1. Phytochemical screening results of *E. pinnatum* leaf extract; (a) alkaloids with dragendorff reagent, (b) alkaloids with mayer reagent, (c) flavonoids, (d) saponins, (e) tannins, (f) steroids.

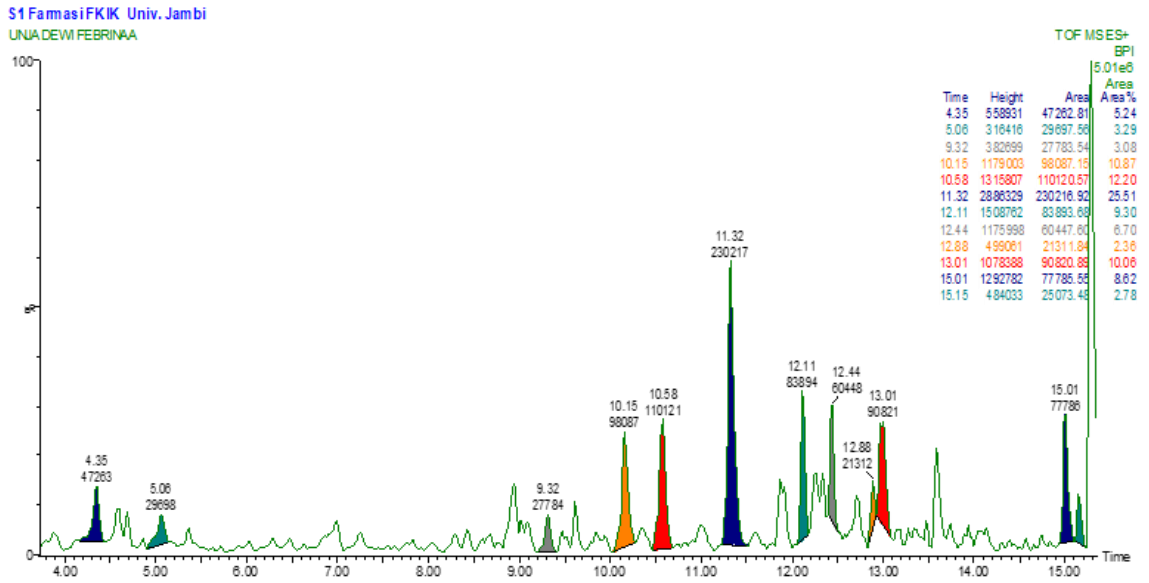


Figure 2. LC-MS chromatograms of *Epipprenum pinnatum* extracts.

using the laser Doppler velocimetry technique with a range of 2 and 200 mV. A 100 µL sample was obtained and placed in the instrument. The temperature used during the measurement was 25°C [23][24].

2.5. Antidiabetic and Antihyperlipidemic Effects of E. pinnatum Leaf Extract

The test animals for this study were healthy male white Wistar strain weighing 200–250 g. Six test animals were used in each treatment group. The experimental animals were divided into five groups. The normal control group consisted of non-diabetic rats that did not receive any treatment. The diabetic control group included diabetic rats that were not given any therapeutic intervention. The positive control group comprised diabetic rats treated with glibenclamide at a dose of 0.45 mg/kgBW. The first treatment group consisted of diabetic rats administreted with *E. pinnatum* leaf extract at a dose of 250 mg/kgBW, while the second treatment group consisted of diabetic rats treated with a nanoemulsion formulation of E. pinnatum leaf extract at the same dose of 250 mg/kgBW.

Before being induced by alloxan, the rat's blood sugar levels were measured using a glucometer by taking blood samples and wounding the tip of the tail. The rats were then fasted for 12 h. After fasting, the rats were induced with alloxan monohydrate at a concentration of 175 mg/kgBW, which had previously been dissolved in physiological NaCl 0.9%, and administered intraperitoneally at a rate of 2 mL/200g BW. Induction was given immediately after 5–10 min of alloxan preparation. After three days, if the rat's blood sugar level measurement results exceeded 200 mg/dL, the rat was classified as diabetic [25]. The potential antidiabetic effect of *E. pinnatum* leaf extract was assessed by measuring blood sugar levels on days 0, 7, 10, and 14 using a glucose stick with a glucometer (Easy Touch GCU®).

Malondialdehyde levels and antihyperlipidemic effects were measured on the last day of observation (day 14th). This blood collection has met ethical requirements according to the guidelines of National Center for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs), stating that the large blood collection process can be carried out for test animals in terminal conditions, namely, the condition of the test animals not being resuscitated [26]. In addition, the research stages have also received approval from the Ethics Committee of the Faculty of Medicine, Tadulako University (project number 3049/UN/28.1.30/KL/2021). Blood sampling began with anesthesia of the rats using high-dose anesthetics, which were ketamine (75 mg/kgBW) and xylazine (15 mg/kgBW), administered intraperitoneally. Blood was then collected from the heart of each rat. Three to five milliliters of blood were collected.

Malondialdehyde levels were measured using a

Table 2. Tentative metabolites identified in the *E. pinnatum* leaf extracts through LC-MS fragmentation using positive ionization.

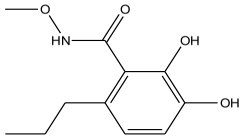
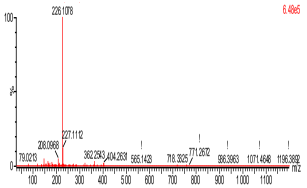
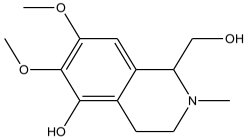
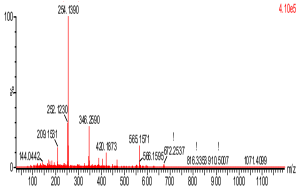
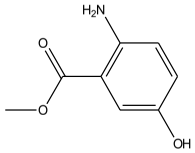
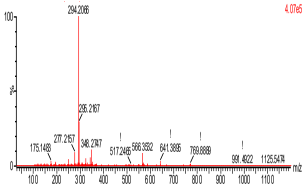
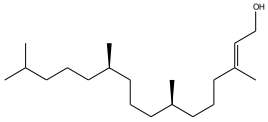
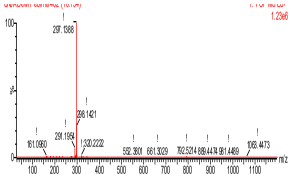
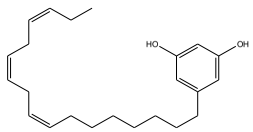
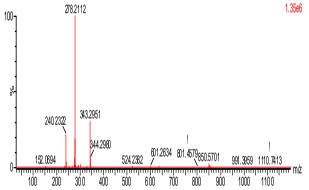
No.	Prediction Compound	Molecular Formula	Calculated Mass (m/z)	Molecular Weight	RT	Group	Chemical Structure	Mass Spectrum
1.	2,3-dihydroxy-*N*-methoxy-6-propylbenzenecarboximidic acid	$C_{11}H1_5NO_4$	226.1078	213.23	4.35	alkaloid		
2.	1-(hydroxymethyl)-6,7-dimethoxy-2-methyl-3,4-dihydro-1*H*-isoquinolin-5-ol	$C_{13}H_{19}NO_4$	254.1388	253.29	5.06	alkaloid		
3.	8-methylnonyl 2-amino-5-hydroxybenzoate	$C_{17}H_{27}NO_3$	294.2062	293.40	9.32	alkaloid		
4.	Phytol	$C_{20}H_{40}O$	297.1404	296.53	10.15	Hidrocarbon		
5.	5-(heptadeca-8,11,14-trien-1-yl) benzene-1,3-diol	$C_{23}H_{34}O_2$	343.2945	342.52	10.58	phenolic		

Table 2. *Cont.*

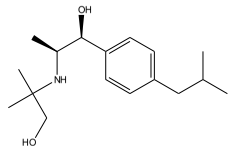
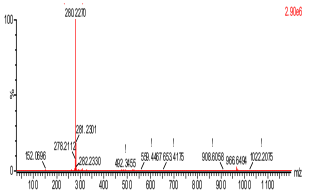
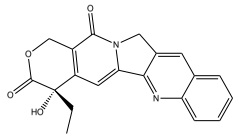
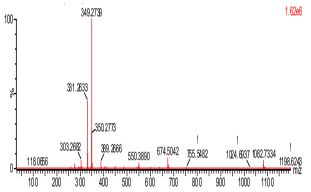
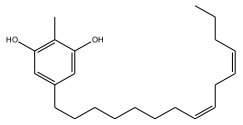
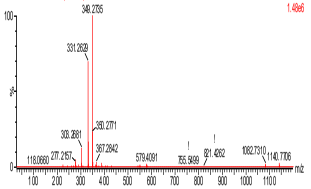
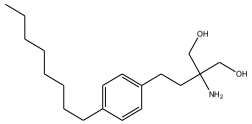
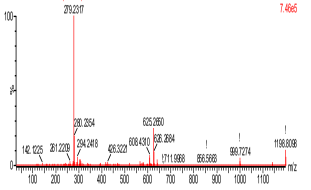
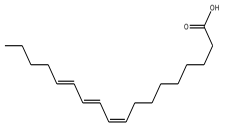
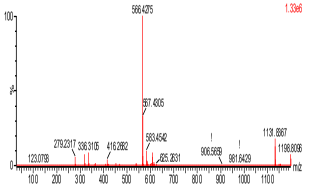
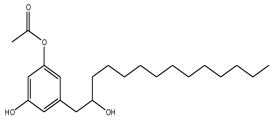
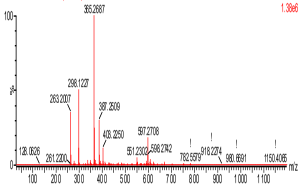
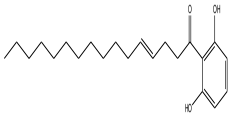
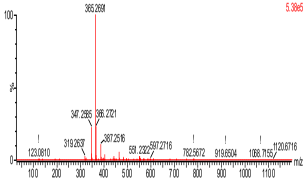
No.	Prediction Compound	Molecular Formula	Calculated Mass (m/z)	Molecular Weight	RT	Group	Chemical Structure	Mass Spectrum
6.	6-(deca-1,3,5-trien-1-yl)-2-(hydroxymethyl)-1-methylpiperidin-3-ol	$C_{17}H_{29}NO_2$	280.2306	279.42	11.32	alkaloid		
7.	Camptothecin	$C_{20}H_{16}N_2O_4$	349.2757	348.35	12.11	alkaloid		
8.	2-methyl-5-(pentadeca-8,11-dien-1-yl)benzene-1,3-diol	$C_{22}H_{34}O_2$	331.2633	330.50	12.44	phenolic		
9.	6-(6-hydroxy-2,5-dimethyloct-4-en-1-ylidene)-8-methyl-hexahydroindolizin-8-ol	$C_{19}H_{33}NO_2$	308.2607	307.47	12.88	alkaloid		
10.	Eleostearic acid	$C_{18}H_{30}O_2$	279.2315	278.43	13.01	fatty acid		

Table 2. *Cont.*

No.	Prediction Compound	Molecular Formula	Calculated Mass (m/z)	Molecular Weight	RT	Group	Chemical Structure	Mass Spectrum
11.	3-hydroxy-5-[(2*s*)-2-hydroxytetradecyl]phenyl acetate	$C_{22}H_{36}O_4$	365.2700	364.5197	15.01	phenolic		
12	1-(2,6-dihydroxyphenyl)hexadec-4-en-1-one	$C_{22}H_{34}O_3$	365.2687	346.50	15.15	phenolic		

Spectronic 21D spectrophotometer. Blood samples were centrifuged at 3,000 rpm for 15–20 min. Serum samples were taken as much as 100 µL, then put into a test tube, and 50 µL of 10% TCA solution and 300 µL of distilled water were added, then vortexed for 1 minute. The solution was centrifuged at 3000 rpm for 10 min. The supernatant formed was then taken and put into another test tube, and 1000 µL of 0.67% TBA was added. Furthermore, the tube was put into a water bath at 95–100ºC for 10 min. After that, the test tube was removed from the water bath and cooled. The reaction results were taken, and then their absorbance was measured using a spectrophotometer at 532 nm [12].

The parameters of the antihyperlipidemic effect were observed using a spectrophotometer with a kit from the Kairos company. The blood was centrifuged at 4,000 rpm for 5–10 min. Serum was collected and used for blood biochemical analysis of total cholesterol, triglycerides, HDL, and LDL using an automated chemistry analyzer.

2.6. Data Analysis

The results of this study were analyzed in two ways: descriptively (extract and nanoemulsion characteristics) and using a one-way ANOVA test (blood sugar, MDA, blood cholesterol, LDL, and HDL levels) with significant changes calculated at $p<0.01$. This was followed by Duncan's further test.

3. RESULTS AND DISCUSSIONS

E. pinnatum leaves were extracted using the maceration method, which involved soaking the plant material (simplicia powder) in a specific solvent. The advantage of this method is that the tools used are simple and do not require a heating process; thus, chemical compounds that cannot survive high temperatures are not harmed. Maceration of 500 g of powdered *E. pinnatum* leaf resulted in a 91 g thick extract, with an 18.2% yield. Phytochemical screening of *E. pinnatum* leaf extract revealed the presence of secondary metabolite compounds, such as alkaloids, flavonoids, tannins, steroids, and saponins (Figure 1).

Previous research has shown that phytochemical screening of *E. pinnatum* leaf extract revealed the presence of compounds such as alkaloids, flavonoids, anthraquinones, tannins, glycosides,

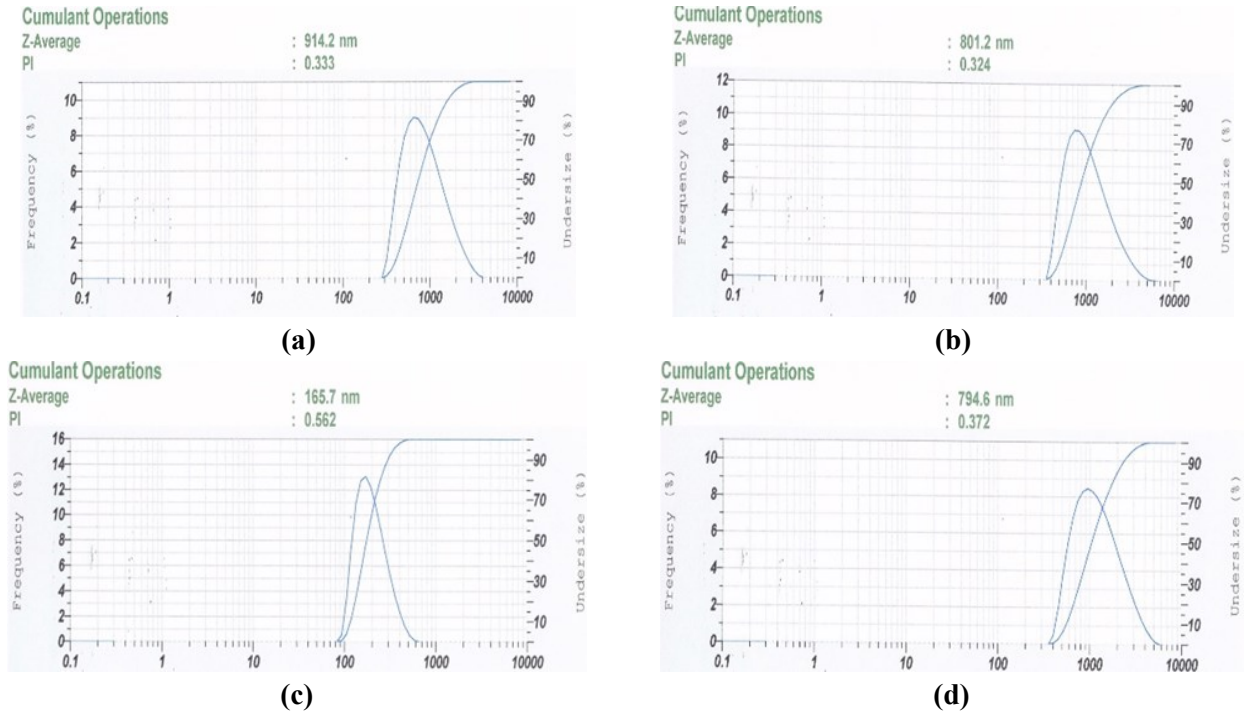


Figure 3. Particle size and polydispersity index results of *E. pinnatum* leaf extract nanoemulsions: (a) formula a 914.20 nm and polydispersity index 0.333, (b) formula b 801.50 nm and polydispersity index 0.324, (c) formula c 165.70 nm and polydispersity index 0.562, (d) formula d 794.60 nm and polydispersity index 0.372.

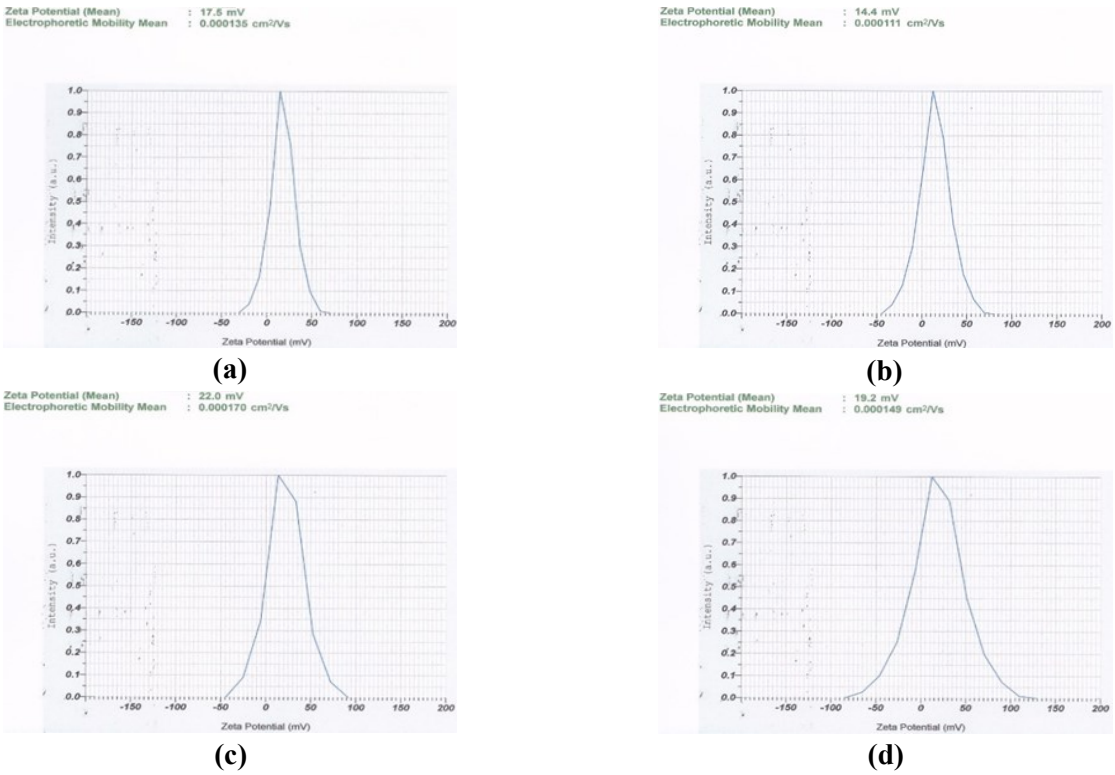


Figure 4. Zeta potential nanoemulsions of *E. pinnatum* leaf extract at formula (a) formula a 15.5 mV, (b) formula b 14.4 mV, (c) formula c 22.0 mV, and (d) formula d 19.2 mV.

phenols, phytosterols, carbohydrates, and steroids [27]. The overall phenolic content was 188.03 ± 2.35 GAE/g, and the total flavonoid content was 72.02 ± 4.78 QE/g. Phenolics and flavonoids are known to have high antioxidant activity. Its antidiabetic properties have been clinically demonstrated. Phenolic and flavonoid compounds exert an antidiabetic effect by activating the AMPK system, inhibiting α-glucosidase/α-amylase, inhibiting glucose absorption, enhancing insulin sensitivity, and activating PPAR [18]. LC-MS analysis yielded five bioactive compounds, which were identified as possible antidiabetic agents. Bioactive activity of compounds obtained from search results via the NCBI website and previous studies. The results are presented in Figure 2 and Table 2.

The first peak active compound suspected to be an antidiabetic agent was 1-(hydroxymethyl)-6,7-dimethoxy-2-methyl-3,4-dihydro-1*H*-isoquinolin-5-ol. This isoquinoline alkaloid compound has numerous pharmacologically established effects, including anticancer, antidiabetic, anti-inflammatory, and antimicrobial effects [28][29]. The second active compound, considered an antidiabetic, was a phytol compound. This hydrocarbon compound has been proven to have potential as an antidiabetic, anti-inflammatory, anticancer, anticonvulsant, antimicrobial, antihyperlipidemic, and immune adjuvant agent. As a result, the phytol compound has been identified as a potential novel medication candidate for various types of pharmacological therapies [30]. The third compound suspected to be an antidiabetic agent was 6-(deca-1,3,5-trien-1-yl)-2-(hydroxymethyl)-1-methylpiperidin-3-ol. This compound is an alkaloid based on the results of antidiabetic testing with DPP -4 inhibition testing results of IC_{50}<10nM. Additionally, this compound boosted plasma insulin levels with minimum toxicity. In addition, this plant may act as an antidiabetic agent due to its ability to produce α-glucosidase and α-amylase [31]. The fourth compound was camptothecin. Camptothecin has the potential to be an anticancer and antidiabetic agent [32]. Eleostearic acid was the last peak chemical with antidiabetic potential. Eleostearic acid is a fatty acid compound that exhibits antidiabetic and antitumor properties. The test results showed that glucose levels in the test animals decreased by 39.32% [33]. The antidiabetic

potential was also supported by the presence of seven tentative peaks of phenolic compounds in *E. pinnatum* leaf extract.

Nanoemulsion stability test using freeze thaw test method conducted in 6 cycles for 12 days with different temperatures of 4°C±2 then transferred to a temperature of 25°C±2 did not show any changes before and after treatment for formulas 2,3, and 4 with pH in the range of 6.56–6.45 statistically did not show any significant difference ($p<0.01$). However, in formula one, particle precipitation or phase separation indicates instability of the resulting nanoemulsion.

The nanoemulsion formulation used in this study utilized the ionic gelation method, which involves a cross-linking process between polyelectrolytes in multivalent ion pairs [34][35]. Several materials were used in this study. The first was chitosan (poly (b-1/4)-2-amino-2-deoxy-D-glucopyranose), a chitin-based biopolymer extracted from shell exoskeletons. Chitosan is commonly used as a carrier in the creation of nanotechnology products, utilizing the iconic gelation process because it improves drug stability [36][37]. The second was sodium tripolyphosphate, a negatively charged crosslinking agent. It was easier to interact with than other polyanion substances, such as sulfate and citrate. In the ionic gelation process for nanoemulsions, chitosan is a polycation that transports multivalent positive ions and interacts with sodium tripolyphosphate, which carries negative ions. Changes in ionic concentration affect the physical properties of the resulting nanoparticles [37]. The third was Tween 80, which served as a surfactant to maintain the diameter of the produced particles, hence limiting particle aggregation [21].

The goal of optimizing the nanoemulsion formula was to discover the formula with the most stable characteristics in terms of particle size, zeta potential, and polydispersity index. This study found that all nanoemulsion formulations were successfully synthesized with particle sizes ranging from 1 to 1000 nm [38][39]. However, formula III had the smallest particle size, measuring 165.7 nm. Previous research has discovered that nanoparticles ranging in size from 50–300 nm have good drug delivery capacity [40]. In addition, other factors that aided the optimization of formula III included a zeta potential value of 22 mV, which was closest to the standard, and the polydispersity index of 0.562. The literature states that a nanoemulsion preparation is homogeneous if the polydispersity index is 0–0.7 and the zeta potential is closest to ± 30 mV. The zeta potential is a parameter of the electric charge between colloidal particles [41]-[43]. This value was obtained from the difference between the electrical charge on the granules and the layer of dispersed colloidal particles. The zeta potential is crucial because it affects the stability of the nanoparticles. The stability of the nanoemulsion system is influenced by van der Waals forces of attraction and electrostatic repulsion. The higher the repulsive force between the particles, the greater the stability of the nanosystem [44]. The polydispersity index describes the homogeneity of a sample. A good polydispersity value indicates that the nanoemulsion is stable over time [45]. Thus, formula III was the nanoemulsion that was further used the *in vivo* testing as an antidiabetic and antihyperlipidemic agent (Figure 3 and Figure 4).

Table 3. Average blood glucose levels during 14-days treatment.

Treatments	Blood Glucose (mg/dL) ± SD				Percentage of Decrease
	Day 0	Day 7th	Day 10th	Day 14th	FBG Levels
Normal Control	96.6 ± 1.11	98.7 ± 1.85$^{\#}$	89.80 ± 0.21$^{\#}$	94.5 ± 1.03$^{\#}$	-
Diabetic Control	267.5 ± 3.71*	272.0 ± 2.83*	226.3 ± 3.42*	207.33 ± 3.28*	22.49%
Positive Control	240.0 ± 1.41*	149.7 ± 3.50$^{*\#}$	60.70 ± 3.77$^{*\#}$	54.33 ± 4.14$^{\#\&}$	77.36%
Treatment I	261.2 ± 2.78*	230.8 ± 3.73$^{*\#}$	221.0 ± 5.41$^{*\#}$	76.00 ± 4.66$^{\#}$	70.90%
Treatment II	271.0 ± 2.41*	179.5 ± 3.71$^{*\#}$	135.0 ± 4.24$^{*\#}$	50.00 ± 4.24$^{\#\&}$	81.55%

Note : Positive Control: Glibenclamide 0.45mg/kgBW; Treatment I: 250mg/kgBW Extract; Treatment II: 250 mg/kgBW Nanoemulsion Extract; FGB: Fasting Bloods Glucose; *$p<0.01$ significant difference compared to the normal control ; #$p<0.01$ significant difference compared to the diabetic control; $^{\&}p>0.01$ no significant difference compared to the positive control (Glibenclamide 0.45mg/KgBW); All the values are Mean ± SEM (n=6).

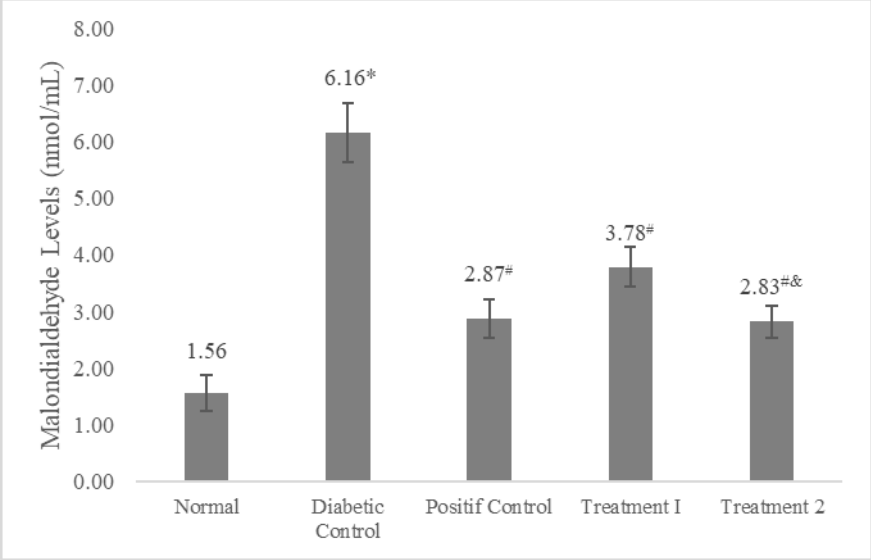


Figure 5. Average malondialdehyde levels after 14 days of treatment. Statistical tests using one-way ANOVA with Duncan's post hoc.

Note: Positive Control: Glibenclamide 0.45mg/kgBW; Treatment I: 250mg/kgBW Extract; Treatment II: 250 mg/kgBW Nanoemulsion Extract *$p<0.01$ significant difference compared to the normal control ; #$p<0.01$ significant difference compared to the diabetic control; &$p>0.01$ no significant difference compared to the positive control (Glibenclamide 0.45mg/kgBW).

The effectiveness of *E. pinnatum* leaf extracts nanoemulsions as an antidiabetic agent could be determined by blood sugar levels, while total cholesterol, triglyceride, LDL, and HDL levels could determine the antihyperlipidemic effect in diabetic conditions. All observation data were analyzed using one-way ANOVA, which had previously met the requirements for normality and homogeneity tests with a significance value greater than 0.05 ($p<0.05$). Blood glucose levels decreased significantly across the treatment groups ($p<0.01$), except for the negative control, which remained high on the 14th day. Table 3 shows the examination outcomes.

The antidiabetic activity of *E. pinnatum* leaf extract nanoemulsion in this study revealed that changes in particle size in the extract affected blood sugar levels. The one-way ANOVA statistical analysis revealed a significant difference when the extract was provided at the same dose (250 mg/kg BW), with a reduction percentage of 81.55% for the nanoemulsion extract.

Diabetes mellitus is one of the triggers for cell damage that can produce lipid peroxidation byproducts. MDA is one of the well-known biomarkers of oxidative stress and lipid peroxidation [12]. The results showed an improvement in MDA levels when compared to the diabetic control group. The best MDA level was in the nanoemulsion extract (250 mg/kgBW) with a 2.83 nmol/mL level (Figure 5). Cell damage caused by oxidative stress is repaired through the action mechanism of the phenolic antioxidant compounds found in *E. pinnatum* leaf extract.

This was also supported by evidence of increased HDL levels, a type of good cholesterol that protects blood vessels from fat accumulation and reduces plaque formation in nanoemulsion treatments (Table 4). Insulin is important in maintaining lipid homeostasis by inhibiting lipolysis in adipose tissue, stimulating lipogenesis in the liver, and increasing lipoprotein lipase activity. The condition of diabetes mellitus, as a result of the induction of the cytotoxic compound alloxan, is a trigger for the emergence of insulin deficiency conditions. This condition will disrupt normal lipid metabolism through increased lipolysis, excess production of triglyceride-rich lipoproteins in the liver, and decreased peripheral lipid clearance, which is the main trigger for the emergence of hyperlipidemia. Total cholesterol, triglyceride, and LDL levels have shown antihyperlipidemic effects. Changes in particle size in nanoemulsions will increase drug absorption through the absorption mechanism of enterocyte cells and microfold cells. This single epithelial layer

is differentiated into enterocyte cells along the intestinal lining. These cells can produce digestive enzymes and have microvilli, which play a role in expanding the surface area for drug absorption. This allows the chemical compounds contained in Epipremnum pinnatum leaf extract to work optimally [46].

The effectiveness of particle size-based therapies in in vivo testing has a critical size threshold for enhancing cellular interactions. Smaller particles can enhance cellular uptake. This study also aligns with the research conducted by Hoshyar et al., who discovered that drug-laden particles that enter cells with a size of less than 1000 nm have 2.5–250 times more absorption than larger particles [47]. Another study by Assi et al. showed that nano-sized drugs with a particle size range of 50–300 nm have good drug delivery capacity. These indicate that a nanoemulsion formula in this study, with a particle size of 165.7 nm, can increase the potential of *E. pinnatum* leaf extract as an antidiabetic and antihyperlipidemic agent [40]. According to Samudra et al. *Sargassum sp.* extract nanoemulsion has a better antihyperglycemic effect than *Sargassum sp.* extract [21]. In other research, *Gymnema sylvestre* extract nanoparticles exhibited antihyperglycemic and antihyperlipidemic activities. Gudise et al. discovered that nanotechnology can improve the efficacy of oral therapy in diabetes and hyperlipidemia by testing extracts of *Argyeia pierreana* and *Matelea denticulata* [48]. Taken together, these data indicate that administering a nanoemulsion of *E. pinnatum* leaf extract can improve the effectiveness of oral therapy, including *E. pinnatum* leaf extract. Changing the form of the extract into a nanoemulsion has a greater effect as an antidiabetic and antihyperlipidemic agent. Therefore, changes in herbal preparations in nano form have the potential to be developed into pharmaceutical preparation products in the future.

4. CONCLUSIONS

Nanoemulsions can be generated from *E. pinnatum* leaf extract. The optimal nanoemulsion for antidiabetic and antihyperlipidemic testing had a particle size of 165.70 nm, zeta potential of 22.0 mV, and polydispersity index of 0.562. The administration of nanoemulsion at a dose of 250 mg/kgBW was more effective as an antidiabetic and antihyperlipidemic agent than extracts, which have the same effect as glibenclamide. Therefore, further research on *E. pinnatum* leaf extract has the potential to be used as an active ingredient in antidiabetic and antihyperlipidemic agent products.

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Table 4. Average values for total cholesterol, triglycerides, LDL, and HDL.

Treatments	Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Normal Control	$61.3 \pm 1.81^{\#}$	$54.5 \pm 1.63^{\#}$	$28.37 \pm 4.67^{\#}$	$28.0 \pm 2.50^{\#}$
Diabetic Control	$85.3 \pm 6.81^{*}$	$66.5 \pm 1.53^{*}$	$43.3 \pm 6.67^{*}$	$38.0 \pm 1.50^{*}$
Positive Control	$74.0 \pm 9.54^{*\#}$	$49.7 \pm 10.02^{*\#}$	$35.7 \pm 10.02^{*\#\&}$	$51.3 \pm 4.04^{*\#}$
Treatment I	$79.7 \pm 10.51^{*\#}$	$54.3 \pm 22.28^{\#}$	$37.7 \pm 10.44^{*\#}$	$42.0 \pm 4.58^{*\#}$
Treatment II	$67.0 \pm 0.71^{*\#}$	$45.8 \pm 0.71^{*\#}$	$35.5 \pm 0.71^{*\#\&}$	$44.0 \pm 0.71^{*\#}$

Note : Positive Control: Glibenclamide 0.45mg/kgBW; Treatment I: 250mg/kgBW Extract; Treatment II: 250 mg/kgBW Nanoemulsion Extract; *p<0.01 significant difference compared to the normal control ; #p<0.01 significant difference compared to the diabetic control; $^{\&}$p>0.01 no significant difference compared to the positive control (Glibenclamide 0.45mg/kgBW); All the values are Mean ± SEM. (n=6).

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Author Contributions

All authors contributed to the study's conception and design. F. S. K., H. R., A. G. S., Y. Y., and M. M. performed material preparation, data collection, and analysis. The first draft manuscript was written by F. S. K. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

ETHICS APPROVAL

Ethics approval was obtained from the Ethics Committee of the Faculty of Medicine, Tadulako University (project number 3049/UN/28.1.30/KL).

ACKNOWLEDGEMENT

The author would like to thank two funding this research: the Institute for Research and Community Service, Jambi University, for the innovation research grant from the University's PNBP fund with contract number 200/UN21.11/PT01.05/SPK/2021 and the Faculty of Medicine and Health Sciences for the applied research grant from the Faculty PNBP fund with contract number 350/UN21.11/PT01.05/SPK/2025.

DECLARATION OF GENERATIVE AI

The author team declares that they did not use any AI assistance while compiling this manuscript except for using standard applications, namely Grammarly and Mendeley.

REFERENCES

[1] B. U. Nwosu. (2022). "The Theory of Hyperlipidemic Memory of Type 1 Diabetes". *Frontiers in Endocrinology.* **13**. 10.3389/fendo.2022.819544.

[2] K. Ogurtsova, J. D. da Rocha Fernandes, Y. Huang, U. Linnenkamp, L. Guariguata, N. H. Cho, D. Cavan, J. E. Shaw, and L. E. Makaroff. (2017). "IDF Diabetes Atlas: Global Estimates for the Prevalence of Diabetes for 2015 and 2040". *Diabetes Research and Clinical Practice.* 10.1016/j.diabres.2017.03.024.

[3] V. T. T. Anh, D. T. X. Trang, K. Kamei, T. C. Linh, N. H. Pham-Khanh, N. T. Tuan, and L. T. Danh. (2021). "Phytochemicals, Antioxidant and Antidiabetic Activities of Extracts From Miliusa velutina Flowers". *Horticulturae.* **7** (12). 10.3390/horticulturae7120555.

[4] S. A. S. Jayawardana, J. K. R. R. Samarasekera, G. H. C. M. Hettiarachchi, and M. J. Gooneratne. (2022). "Antidiabetic Properties of Finger Millet (Eleusine coracana (L.) Gaertn.) Varieties Cultivated in Sri Lanka". *Journal of Herbal Medicine.* **32**. 10.1016/j.hermed.2022.100534.

[5] Masfria, U. H. Hap, M. P. Nasution, and S. Ilyas. (2014). "Cytotoxic Activity, Proliferation Inhibition and Apoptosis Induction of Rhaphidophora pinnata (L.F.) Schott Chloroform Fraction to MCF-7 Cell Line". *International Journal of PharmTech Research.*

[6] B. Pascila, A. G. Samudra, F. Sani, and R. Asra. (2020). "Uji Aktivitas Ekstrak Etanol Daun Ekor Naga (Rhaphidophora pinnata (L.f.) Schott.) Sebagai Antihiperurisemia Terhadap Mencit Putih Jantan". *Jurnal Ilmiah Manuntung.* **6** (2): 299-305.

[7] D. Lestari, I. Lestari, and K. F. S. (2021). "Uji Efektivitas Ekstrak Etanol Daun Ekor Naga (Rhaphidophora pinnata (L.f) Schott) Sebagai Antihiperglikemia Terhadap Mencit Putih Jantan yang Diinduksi Sukrosa". *Jurnal*

Ilmiah Manuntung. **7** (1): 100-110. 10.51352/jim.v7i1.433.

[8] B. Anatasya, F. Sanik, and M. Muhaimin. (2021). "Topical Anti-Inflammatory Effect of Ekor Naga (Rhaphidophora pinnata (L.f) Schott) Leaves Extract". *Pharmaciana.* **11** (3): 303. 10.12928/pharmaciana.v11i3.17617.

[9] A. Alam, M. Jha, and S. Faisal. (2022). In: "Medicinal and Aromatic Plants of India Vol. 1, (Medicinal and Aromatic Plants of the World, ch. Chapter 5". 107-174. 10.1007/978-3-030-98701-5_5.

[10] M. A. L. Villar-Cabalhin, M. A. L. C. Aguilar, J. D. A. Renomeron, and R. G. Fuentes. (2025). "Phytochemical Analysis and Pharmacological Properties of Epipremnum pinnatum (L.) Fruit Extracts". *HAYATI Journal of Biosciences.* **32** (4): 1073-1079. 10.4308/hjb.32.4.1073-1079.

[11] Refilda, H. Hamman, and E. Salim. (2019). "Penentuan Kuersetin Pada Ekstrak Aseton Daun Ekor Naga (Epipremnum pinnatum (L.) Engl.) Dengan Metode Kromatografi Cair Kinerja Tinggi (KCKT)". *Jurnal Kimia Unand.* **8** (1): 24-29.

[12] M. Arman, M. Alinaghizadeh, M. Kordi, S. T. Jahromi, H. Amrulloh, and H. Kioumarsi. (2025). "Investigation of the Anti-Diabetic and Anti-Lipid Peroxidative Properties of Gallic Acid in Diabetic Rats". *Journal of Multidisciplinary Applied Natural Science.* **5** (1): 32-41. 10.47352/jmans.2774-3047.226.

[13] R. Dhanya. (2022). "Quercetin for Managing Type 2 Diabetes and Its Complications: An Insight Into Multitarget Therapy". *Biomedicine and Pharmacotherapy.* **146**. 10.1016/j.biopha.2021.112560.

[14] A. S. Farswan, R. Uniyal, R. Sanwal, V. Koul, and A. Kumar. (2022). "Evaluation of Antidiabetic Effect of Epipremnum aureum on Streptozotocin-Induced Diabetic Rats". *Journal of Conventional Knowledge and Holistic Health.* **1**. 10.53517/JCKHH.2581.

[15] S. Bayda, M. Adeel, T. Tuccinardi, M. Cordani, and F. Rizzolio. (2020). "The History of Nanoscience and Nanotechnology: From Chemical-Physical Applications to Nanomedicine". *Molecules.* **25** (1). 10.3390/molecules25010112.

[16] M. Jaiswal, R. Dudhe, and P. K. Sharma. (2015). "Nanoemulsion: An Advanced Mode of Drug Delivery System". *3 Biotech.* **5** (2). 10.1007/s13205-014-0214-0.

[17] T. E. Mungure, S. Roohinejad, A. E. D. Bekhit, R. Greiner, and K. Mallikarjunan. (2018). "Potential Application of Pectin for the Stabilization of Nanoemulsions". *Current Opinion in Food Science.* **19** : 72-76. 10.1016/j.cofs.2018.01.011.

[18] O. R. Alara, N. H. Abdurahman, and O. A. Olalere. (2020). "Ethanolic Extraction of Flavonoids, Phenolics and Antioxidants From Vernonia amygdalina Leaf Using Two-Level Factorial Design". *Journal of King Saud University - Science.* **32** (1). 10.1016/j.jksus.2017.08.001.

[19] A. Safitri, F. Fatchiyah, D. R. T. Sari, and A. Roosdiana. (2020). "Phytochemical Screening, In Vitro Anti-Oxidant Activity, and In Silico Anti-Diabetic Activity of Aqueous Extracts of Ruellia tuberosa L". *Journal of Applied Pharmaceutical Science.* **10** (3). 10.7324/JAPS.2020.103013.

[20] F. Ismed, W. N. Desti, N. Arifa, R. Rustini, and D. P. Putra. (2023). "TLC-Bioautographic and LC-MS/MS Detection of Antimicrobial Compounds From Four Semipolar Extracts of Cladonia Species". *Proceedings of the 2nd International Conference on Contemporary Science and Clinical Pharmacy 2021 (ICCSCP 2021).* 10.2991/ahsr.k.211105.008

[21] A. G. Samudra, N. Ramadhani, R. Pertiwi, D. Fitriani, F. Sanik, and A. Burhan. (2022). "Antihyperglycemic Activity of Nanoemulsion of Brown Algae (Sargassum sp.) Ethanol Extract in Glucose Tolerance Test in Male Mice". *Annales Pharmaceutiques Françaises.* 10.1016/j.pharma.2022.11.011.

[22] T. Suciati, A. Aliyandi, and Satrialdi. (2014). "Development of Transdermal Nanoemulsion Formulation for Simultaneous Delivery of Protein Vaccine and Artin-M Adjuvant". *International Journal of Pharmacy and Pharmaceutical Sciences.* **6** (6): 536-546.

[23] S. Bhatia and S. Bhatia. (2016). In: "Natural Polymer Drug Delivery Systems". 10.1007/978-3-319-41129-3_2.

[24] A. H. Saberi, Y. Fang, and D. J. McClements. (2013). "Effect of Glycerol on Formation, Stability, and Properties of Vitamin-E Enriched Nanoemulsions Produced Using Spontaneous Emulsification". *Journal of Colloid and Interface Science.* **411**. 10.1016/j.jcis.2013.08.041.

[25] I. Kabach, N. Bouchmaa, Z. Zouaoui, A. Ennoury, S. El Asri, A. Laabar, L. Oumeslakht, F. Cacciola, Y. Oulad El Majdoub, L. Mondello, A. Zyad, N. Nhiri, M. Nhiri, and R. Ben Mrid. (2023). "Phytochemical Profile and Antioxidant Capacity, α-Amylase and α-Glucosidase Inhibitory Activities of Oxalis pes-caprae Extracts in Alloxan-Induced Diabetic Mice". *Biomedicine and Pharmacotherapy.* **160**. 10.1016/j.biopha.2023.114393.

[26] J. MacArthur Clark. (2018). "The 3Rs in research: a contemporary approach to replacement, reduction and refinement". *British Journal of Nutrition.* **120** (s1): S1-S7. 10.1017/S0007114517002227.

[27] S. P. Pan, T. Pirker, O. Kunert, N. Kretschmer, S. Hummelbrunner, S. L. Latkolik, J. Rappai, V. M. Dirsch, V. Bochkov, and R. Bauer. (2019). "C13 Megastigmane Derivatives From Epipremnum pinnatum: β-Damascenone Inhibits the Expression of Pro-Inflammatory Cytokines and Leukocyte Adhesion Molecules as Well as NF-κB Signaling". *Frontiers in Pharmacology.* **10**. 10.3389/fphar.2019.01351.

[28] S. Singh, N. Pathak, E. Fatima, and A. S. Negi. (2021). "Plant Isoquinoline Alkaloids: Advances in the Chemistry and Biology of Berberine". *European Journal of Medicinal Chemistry.* **226**. 10.1016/j.ejmech.2021.113839.

[29] D. Wang, L. Qin, C. Jing, G. Wang, H. Zhou, P. Deng, S. Zhang, Y. Wang, Y. Ding, Z. Zhang, Z. Wu, and Y. Liu. (2024). "Biologically Active Isoquinoline Alkaloids Covering 2019–2022". *Bioorganic Chemistry.* **145**. 10.1016/j.bioorg.2024.107252.

[30] T. Taj, R. Sultana, H. D. Shahin, M. Chakraborthy, and M. Gulzar Ahmed. (2021). "Phytol: A Phytoconstituent, Its Chemistry and Pharmacological Actions". *GIS Science Journal.* **8**.

[31] D. Zolotareva, A. Zazybin, A. Dauletbakov, Y. Belyankova, B. Giner Parache, S. Tursynbek, T. Seilkhanov, and A. Kairullinova. (2024). "Morpholine, Piperazine, and Piperidine Derivatives as Antidiabetic Agents". *Molecules.* **29** (13). 10.3390/molecules29133043.

[32] T. N. Doan, T. D. Le, N. A. Ho, T. T. Ho, T. T. Do, H. Hoang, M. H. Nguyen, T. M. Bui, and H. H. Chu. (2024). "Isolation, Anticancer Potency, and Camptothecin-Producing Ability of Endophytic Fungi Isolated From Ixora chinensis". *Science Progress.* **107** (2). 10.1177/00368504241253675.

[33] E. K. Kambale, J. Quetin-Leclercq, P. B. Memvanga, and A. Beloqui. (2022). "An Overview of Herbal-Based Antidiabetic Drug Delivery Systems: Focus on Lipid- and Inorganic-Based Nanoformulations". *Pharmaceutics.* **14** (10). 10.3390/pharmaceutics14102135.

[34] N. H. Hoang, T. Le Thanh, R. Sangpueak, J. Treekoon, C. Saengchan, W. Thepbandit, N. K. Papathoti, A. Kamkaew, and N. Buensanteai. (2022). "Chitosan Nanoparticles -Based Ionic Gelation Method: A Promising Candidate for Plant Disease Management". *Polymers.* **14** (4). 10.3390/polym14040662.

[35] N. Van Bavel, T. Issler, L. Pang, M. Anikovskiy, and E. J. Prenner. (2023). "A Simple Method for Synthesis of Chitosan Nanoparticles With Ionic Gelation and Homogenization". *Molecules.* **28** (11). 10.3390/molecules28114328.

[36] E. Alehosseini, H. Shahiri Tabarestani, M. S. Kharazmi, and S. M. Jafari. (2022). "Physicochemical, Thermal, and Morphological Properties of Chitosan Nanoparticles Produced by Ionic Gelation". *Foods.* **11** (23). 10.3390/foods11233841.

[37] E. V. Popova, I. M. Zorin, N. S. Domnina, I. I. Novikova, and I. L. Krasnobaeva. (2020).

"Chitosan–Tripolyphosphate Nanoparticles: Synthesis by the Ionic Gelation Method, Properties, and Biological Activity". *Russian Journal of General Chemistry.* **90** (7). 10.1134/S1070363220070178.

[38] M. Hajialyani, D. Tewari, E. Sobarzo-Sánchez, S. M. Nabavi, M. H. Farzaei, and M. Abdollahi. (2018). "Natural Product-Based Nanomedicines for Wound Healing Purposes: Therapeutic Targets and Drug Delivery Systems". *International Journal of Nanomedicine.* 10.2147/IJN.S174072.

[39] Y. Poureghbal, M. Rahimi, and M. Akbari. (2022). "Ionic Gelation of Chitosan With Sodium Tripolyphosphate Using a Novel Combined Nebulizer and Falling Film System". *Canadian Journal of Chemical Engineering.* **100** (7). 10.1002/cjce.24234.

[40] R. A. Assi, I. M. Abdulbaqi, and C. S. Yee. (2021). "The Evaluation of Drug Delivery Nanocarrier Development and Pharmacological Briefing for Metabolic-Associated Fatty Liver Disease (MAFLD): An Update". *Pharmaceuticals.* **14** (3). 10.3390/ph14030215.

[41] S. Benamer Oudih, D. Tahtat, A. Nacer Khodja, M. Mahlous, Y. Hammache, A. E. Guittoum, and S. Kebbouche Gana. (2023). "Chitosan Nanoparticles With Controlled Size and Zeta Potential". *Polymer Engineering and Science.* **63** (3). 10.1002/pen.26261.

[42] D. Rahmat, Y. Farida, A. T. Brylianto, R. Sumarny, and S. Kumala. (2020). "Antidiabetic Activity of Nanoparticles Containing Javanese Turmeric Rhizome Extract: The Strategy to Change Particle Size". *International Journal of Applied Pharmaceutics.* **12** (4). 10.22159/jap.2020v12i4.36249.

[43] N. Raval, R. Maheshwari, D. Kalyane, S. R. Youngren-Ortiz, M. B. Chougule, and R. K. Tekade. (2018). In: "Basic Fundamentals of Drug Delivery". 10.1016/B978-0-12-817909-3.00010-8.

[44] S. Soleymanfallah, Z. Khoshkhoo, S. E. Hosseini, and M. H. Azizi. (2022). "Preparation, Physical Properties, and Evaluation of Antioxidant Capacity of Aqueous Grape Extract Loaded in Chitosan–TPP Nanoparticles". *Food Science and Nutrition.* **10** (10). 10.1002/fsn3.2891.

[45] P. Virk, M. A. Awad, S. S. Abdu-llah Alsaif, A. A. Hendi, M. Elobeid, K. Ortashi, R. Qindeel, M. F. El-Khadragy, H. M. Yehia, M. F. Serag El-Din, and H. A. Salama. (2023). "Green Synthesis of Moringa oleifera Leaf Nanoparticles and an Assessment of Their Therapeutic Potential". *Journal of King Saud University - Science.* **35** (3). 10.1016/j.jksus.2023.102576.

[46] R. Gobinath, S. Parasuraman, S. Sreeramanan, B. Enugutti, and S. V. Chinni. (2022). "Antidiabetic and Antihyperlipidemic Effects of Methanolic Extract of Leaves of Spondias mombin in Streptozotocin-Induced Diabetic Rats". *Frontiers in Physiology.* **13**. 10.3389/fphys.2022.870399.

[47] N. Hoshyar, S. Gray, H. Han, and G. Bao. (2016). "The Effect of Nanoparticle Size on In Vivo Pharmacokinetics and Cellular Interaction". *Nanomedicine.* **11** (6). 10.2217/nnm.16.5.

[48] V. Gudise, B. Chowdhury, and A. S. Manjappa. (2021). "Antidiabetic and Antihyperlipidemic Effects of Argyreia pierreana and Matelea denticulata: Higher Activity of the Micellar Nanoformulation Over the Crude Extract". *Journal of Traditional and Complementary Medicine.* **11** (3). 10.1016/j.jtcme.2020.08.001.