

Chemical Content of Sidempuan Salacca (*Salacca sumatrana*) Vinegar and Its Potential as an Antihypercholesterolemia: an in Vitro Assessment

Yusni Atifah^{1,*}, Yuni Ahda¹, Hesty Parbuntari², Jalilah Azizah Lubis³,
Aifa Kurnia¹, Mutiara Ghina¹

¹Department of Biology, Faculty of Mathematics and Sciences, Universitas Negeri Padang, Padang 25131 Indonesia

²Department of Chemistry, Faculty of Mathematics and Sciences, Universitas Negeri Padang, Padang 25131 Indonesia

³Biology Education Study Program, Faculty of Teacher and Training Universitas Muhammadiyah Tapanuli Selatan, Padangsidempuan 22733 Indonesia

Received 25 December 2024; Received in revised form 27 August 2025

Accepted 7 October 2025; Available online 17 December 2025

ABSTRACT

Sidempuan salacca (*Salacca sumatrana* Becc.) is a snakefruit native to Padangsidempuan, South Tapanuli, North Sumatra, Indonesia, known for its sweet-sour taste. Its sourness reduces market appeal and causes economic losses for farmers, while its perishable nature limits shelf life. To enhance its marketability, processing it into vinegar offers a promising alternative, as salacca vinegar has greater functional potential than apple vinegar. This study aimed to identify the chemical composition of Sidempuan salacca vinegar using GC-MS analysis and evaluate its antihypercholesterolemic potential. GC-MS results revealed five dominant bioactive compounds: oleic acid, DI-(9-octadecenoyl)-glycerol 2-hydroxy-1,3-propanediyl ester, borane, 9-octadecenal, and 9-octadecenoic acid (Z). An in vitro cholesterol-lowering assay using the Liebermann–Burchard method and spectrophotometry showed that the vinegar effectively reduced cholesterol, with an LC₅₀ value of 142.232 ppm. In conclusion, Sidempuan salacca vinegar contains active compounds with antioxidant and anti-cholesterol properties, demonstrating potential as a natural functional product for managing cholesterol levels.

Keywords: Antihypercholesterol; Chemical content; GCMS; In vitro; Sidempuan salacca

1. Introduction

Fresh snakefruit (salacca) is a source of fiber, vitamins, minerals, and antioxidants [1]. In a study conducted by Mazum-dara, it was found that salacca is one of the best sources of antioxidants for humans [2]. Recent epidemiological research shows a correlation between the consumption of fruits rich in natural antioxidants and a reduction in the incidence of non-communicable diseases, including cancer, cardiovascular disorders, and diabetes. One plant that can be used as a good source of antioxidants is salacca due to its phytochemical composition, which includes tannins, saponins, flavonoids, and glycosides [3]. The phytochemical content of Sidempuan salacca differs from the phytochemical content of Sidempuan salacca leaf extract, which contains alkaloids and terpenoids [1].

Salacca can not only be consumed fresh but can also be processed into other foods. One alternative processing method for Sidempuan salacca is vinegar production. Vinegar has been known to humans for thousands of years; it is a liquid produced from materials containing starch and sugar that have undergone two stages of fermentation, first alcohol and then acetate fermentation, resulting in a liquid containing at least 4% (v/v) acetic acid. The acetic acid in vinegar has the ability to slow down disaccharidase enzymes involved in the carbohydrate metabolism process, thereby reducing blood glucose levels [32].

Antioxidants function as free radical scavengers that inhibit these free radicals' reactivity and prevent excessive oxidation. In addition to antioxidants, salacca vinegar also contains phenolic compounds, tannins, vitamin C, and flavonoids. The presence particular polyphenol and flavonoid compounds can help in the repair process of

cells and tissues [20].

Flavonoids are polyphenolic compounds that are beneficial as antioxidants; flavonoids in blood cells can act as reservoirs for hydroxyl and superoxide radicals, thereby protecting membrane lipids and preventing damage to red blood cells. In addition, they can enhance erythropoiesis in bone marrow and exhibit immunostimulant effects [7]. Research has shown that 5 varieties of snake fruit from *Salacca zalacca* showed significant differences in the parameters of pH, total phenols, and the antioxidant activity of the respective salacca vinegars [35]. Data regarding the chemical composition of salacca Sidempuan (*Salacca sumatrana*) vinegar is still very limited, so comprehensive testing of this data is necessary.

2. Materials and Methods

The type of research used is qualitative and quantitative. This research was carried out in June-November 2024 at the Biology Laboratory of Universitas Negeri Padang (UNP). The materials used to make salacca vinegar were Sidempuan salacca (*Salacca sumatrana*), sourced from Padangsidempuan. *Saccharomyces cerevisiae* isolate was obtained from the Biology Laboratory of UNP. *Acetobacter aceti*, H_2SO_4 , $(CH_3CO)_2O$, standard cholesterol, 96% ethanol, and chloroform ($CHCl_3$) were obtained from _____. The following tools were used: OHAUS analyzer, Yamato blender, Maspion electric, Hirayama autoclave, laminar air flow (LAF), Binder BD53 incubator, Memmert WNB 14 shaker water bath, LW Scientific vortex, Seward stomacher, Gene-sys 20 spectrophotometer, Hanna pH meter, ATAGO hand refractometer, alcohol meter, Hettich EBA 20 centrifuge, Hettich Zentrifugen cold centrifuge, micro 22R, thermometer, fer-

menter, hose, Amara aerator, Gilson 100-1000 μL non-fixation micropipette, Gilson fixed micropipettes, microtips, autoclaves, pH analysis, total acid analysis [29], GCMS analysis, toxicity test [16], and anticholesterol test [4].

2.1 Making Sidempuan salacca vinegar

The two-phase fermentation process used to make Sidempuan salacca vinegar was as follows:

Fermentation I

Sidempuan salacca extract was transferred into a bottle. Then 50 g of sugar was added and dissolved by stirring. Next, *Saccharomyces cerevisiae* yeast was activated by letting it sit in warm water for 2 minutes. The activated yeast was then added to the bottle, which was then closed tightly for 7 days during which the alcohol was produced.

Fermentation II

The result of fermentation I was then fermented again by adding 90 ml of *Acetobacter aceti* and continuing the fermentation for an additional 14 days to produce salacca vinegar. Pasteurization was performed for 10 minutes using a water bath to stop the activity of bacteria in the salacca vinegar. The salacca vinegar was then harvested by filtering it through a cloth filter [27].

2.2 GCMS analysis

Sample Preparation: 0.1 mL of sample was added to 5 mL of methanol. Extraction was performed using a sonicator for 20 minutes at 40°C. The extraction was pipetted into a vial and tested using a QP-2010 GC-MS plus AOC-20i Autosampler.

GC-MS Operation: GC-MS Instrument Conditions: Injector temperature 250°C, splitless mode, pressure 76.9 kPa, flow rate 14 mL/min, and a ratio of 1:10.

Ion source and interface temperatures 200°C and 280°C, solvent cutoff time 3 minutes, and 400-700 m/z. Column type SH-Rxi-5Sil MS column length 30 m with an inner diameter of 0.25 mm. The initial column temperature was 70°C with a 2-minute holding time, and the temperature was increased to 200°C at a rate of 10°C/min, and the final temperature was 280°C with a 9-minute holding time at a rate of 5°C/min, resulting in a total analysis time of 34 minutes. The obtained chromatogram data were read using the NIST and Wiley 9 libraries.

2.3 Anticholesterol tests

The anticholesterol activity test was carried out using Lieberman-Burchard reagent. The concentration series made based on the orientation was 50; 75; 100; 125; 150 $\mu\text{g/mL}$. The test solution was made by taking 2.0 mL from each concentration series, then adding 2.0 mL of standard cholesterol solution to a tube covered with aluminum foil. Then the solution was incubated at room temperature for 15 minutes, after which it was centrifuged using a vortex for 5 minutes. 2.0 mL of sample solution was taken then 2.0 mL of anhydrous acetic acid and 0.1 mL of concentrated sulfuric acid were added through the wall, forming a green color. The mixture was left for 15 minutes at room temperature and protected from light.

The test solution was then read using a UV-Vis spectrophotometer at a wavelength of 670 nm. Each sample will absorb light at its respective wavelength [15].

2.4 Toxicity analysis

The toxicity of Sidempuan salacca vinegar was tested using the Brine Shrimp Lethality Test (BSLT). A 1000 mL beaker

was prepared as a hatching place for *Artemia salina* Leach larvae. One liter of distilled water was added, along with approximately 30 grams of sea salt, then aerated using an aerator for 10 hours before the *A. salina* eggs were added. The container with the eggs was exposed to light to warm and stimulate the hatching process for 48 hours [26].

Sidempuan salacca vinegar at various concentrations (50%, 75%, 100%, 125%, 150%) was placed in a 25 mL measuring flask. Next, 1 mL of distilled water was added to each salacca vinegar solution and artificial seawater was added to dissolve it. After homogenizing to 25 mL with artificial seawater, it was used as a stock solution with a concentration of 2000 ppm. From the stock solution, concentration variations were prepared in vials at 50 ppm, 100 ppm, 250 ppm, 500 ppm, and 1000 ppm. A blank was prepared as artificial seawater supplemented with 1% distilled water containing larval suspension. For each control sample, the test was repeated three times [15].

Each vial was filled with 10 shrimp larvae aged 48 hours, collected using a 200 μ L micropipette, and 200 μ L of the test solution was added. The larvae were exposed to the test solution at room temperature under light for 24 hours. The mortality of *A. salina* larvae in the vials was observed and recorded [19].

3. Results and Discussion

3.1 GC-MS analysis

Using GC-MS, 16 chemical compounds were identified in the Sidempuan salacca vinegar, including oleic acid, which has been linked to improved cholesterol profiles, and boron compounds, which support lipid metabolism and cholesterol regulation (Fig. 1).

According to the results of the GC-MS analysis, Sidempuan salacca vinegar contained five major constituents. Table 1 displays these compounds.

The two most dominant peak compounds, borane and 9-octadecenal, identified through chromatogram analysis (Figure 1), had an area percentage of 15.24% each. The compounds had retention times of 26.439 and 30.411 minutes, respectively.

Borane compounds are made up of boron and hydrogen and have a large area (%). These compounds can be classified into many types based on the number of boron and hydrogen atoms present. They are typically used in biological systems [31]. Boron plays a role in mineral and hormone metabolism, healthy bone growth, enzyme reactions, and cell membrane damage [12].

Boron also plays a role in lowering cholesterol by increasing high-density lipoprotein (HDL) levels and decreasing low-density lipoprotein (LDL) levels in the blood, demonstrated by the fact that oral administration of boric acid reduced serum TC and LDL-C levels and increased HDL-C in streptozotocin-induced diabetic rats [9].

The administration of two different compounds containing boron at 8mg/kg/day to rats for 14 days was proven to reduce serum LDL-C and TG levels. It was shown that after 1 month of following a boron-rich diet, total serum, LDL-C, and VLDL-C levels significantly decreased, while serum HDL-C levels increased. Research has shown that a boron-rich diet can also help regulate lipid profiles and enhance fatty acid metabolism. Boron-rich foods also happen to be high in fiber content, which can significantly lower serum lipid levels [21].

The compound with the second largest area percentage from the identifica-

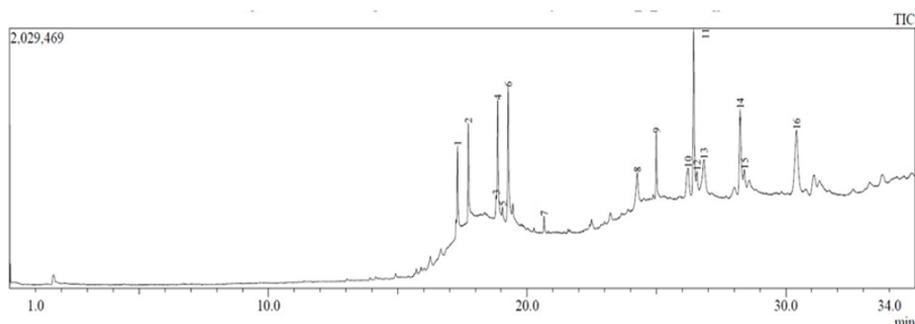
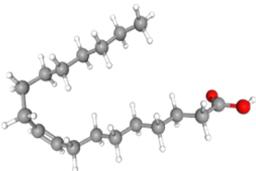
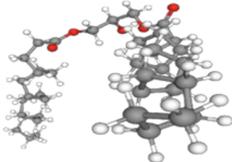
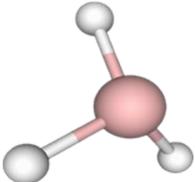
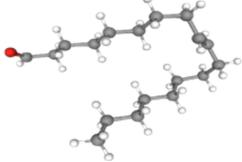
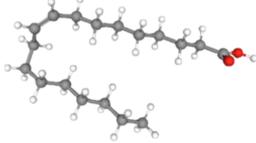


Fig. 1. GC-MS Analysis of Sidempuan salacca vinegar.

Table 1. The main chemical compounds identified from Sidempuan salacca vinegar using GC-MS analysis.

Peak	Retention Time (units)	Compound Name and Structure	Chemical Formula	Peak Area
4	18.862	 Oleic Acid	C ₁₈ H ₃₄ O ₂	10.07%
6	19.269	 DI-(9-octadecenyl)-glycerol/ 2-Hydroxy-1,3-propanediyl ester	C ₃₉ H ₇₂ O ₅	11.48%
11	26.439	 Borane	C ₅ H ₁₃ B	15.24%
14	28.234	 9-Octadecenal	C ₁₈ H ₃₄ O	10.09%
16	30.411	 9-Octadecenoic acid (Z)	C ₂₅ H ₄₄ O ₆	11.89%

tion results was 9-octadecenal. Oleic acid, also known as cis-9-octadecenoic acid, is an unsaturated fatty acid abundant in vegetable oils [28]. Oleic acid is a neutral fatty acid with double bonds that does not increase or decrease LDL cholesterol levels, but can increase HDL cholesterol levels [19]. In addition, long-chain unsaturated fatty acids, especially omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been proven to play an important role in the prevention and treatment of arterial blockage (atherosclerosis), thrombosis, hypertriglyceridemia, and hypertension.

In addition, oleic acid has the potential to prevent and treat various health conditions such as asthma, arthritis, migraines, and various types of cancer, namely prostate, breast, and colon cancer [30]. Oleic acid also has antihypercholesterolemic effects. As an antihypercholesterolemic agent, oleic acid can work through various mechanisms to lower bad cholesterol (LDL) levels and increase good cholesterol (HDL) levels [24]. Vinegar has high oleic acid content, 11.89% according to GCMS results, and can lower LDL cholesterol. One study showed that the double bonds in fatty acids undergo addition reactions and cholesterol binds with fatty acids [10]. This is because unsaturated fatty acids can undergo addition reactions at the sites of their double bonds.

3.2 Toxicity analysis

The toxicity test method used in this study was the Brine Shrimp Lethality Test or BSLT, which aimed to determine the concentration of salacca vinegar needed to kill half of the initial population of test animals, namely *A. salina* larvae, aged 48 hours. The LC₅₀ value can be used to determine the level of toxic effects of a compound and can

also be used to predict its potential as an anticancer agent. By conducting the BSLT, the precise LC₅₀ value of sidempuan vinegar was determined, which is an acute toxicity parameter based on probit analysis [19].

The selection of the larval age (48 h) was based on the fact that the larvae have a fully developed digestive tract, making them sensitive to substances introduced into the water [28].

The percent mortality of *A. salina* larvae increased as the concentration of salacca vinegar increased, indicating the toxicity of the vinegar [16].

Based on the toxicity test of the vinegar, an LC₅₀ value of 142.232 ppm was obtained using the regression equation $y = 2.4596x + 0.2956$ (Fig. 2). In the LC₅₀ calculation, the y-value was 5. The value of 5 represents the LC₅₀ value, in order to determine the LC₅₀ obtained from the results.

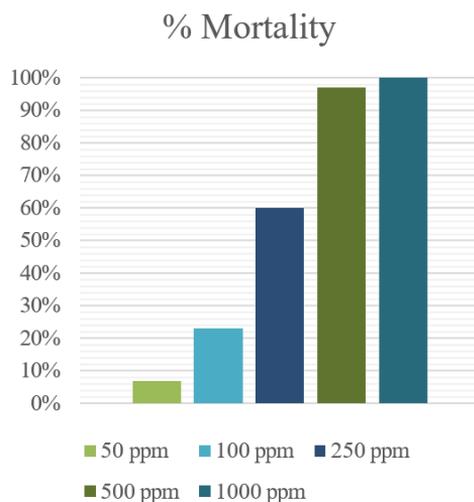


Fig. 2. Percent mortality of *A. salina* larvae.

Table 2. LC₅₀ value of *A. salina* larvae.

Sample	LC ₅₀
Sidempuan Salacca vinegar	142.232 ppm

A substance is considered very toxic if the LC_{50} is ≤ 30 ppm, toxic if it is in the range of 30-100 ppm, and low toxic if it is in the range of 100-1000 ppm [24]. Since the LC_{50} of Sidempuan salacca vinegar was 142.232, it can be categorized as low toxic.

Based on this study, it can be concluded that the administration of salacca vinegar at a concentration of 50-100 ppm is still categorized as safe and should have no toxic effect, while a concentration above 100 ppm is categorized as having a toxic effect but still classified as low. Salacca vinegar has toxic activity due to its high total acid content of 19.5% with a pH of 3.94. This resulted in many *A. salina* larvae dying when exposed to salacca vinegar at concentrations above 100 ppm. This relates to findings from a different study which found that the high acidity of other citrus fruits, namely lemons, can cause irreversible morphological changes, and death [25].

Salacca vinegar contains metabolite compounds such as tannins, saponins, flavonoids, alkaloids, and flavonoids. Secondary metabolite compounds contained in plants can function as a defense against unfavorable environmental conditions, pests, and diseases, as well as a method to attract pollinators, and can also act as signaling molecules. The toxicity of these secondary metabolite compounds can cause death in *A. salina* leach larva [28]. If a compound can interfere with enzyme activity in *A. salina* and result in death, then that compound can be considered toxic and can cause cell death in mammals [25].

In addition, the content of oleic acid in high doses or in certain contexts (such as excess fat accumulation) can contribute to cell damage through mechanisms such as lipotoxicity, where fat accumulation can lead to cell metabolism disorders [8]. Herbal plants high in flavonoids are

commonly used as ingredients in medicine. From this statement, it can be interpreted that the more flavonoids a sample contains, the more toxic it should be towards test organisms [11].

As previously mentioned, Sidempuan salacca vinegar also contains borane compounds, with a high peak area of 15.24%. Borane compounds themselves can be toxic if at a high enough concentration. At low doses, the toxic effects may not be significant, but at higher concentrations they can cause serious damage to cells and tissues [18]. In the case of Sidempuan salacca vinegar, the borane compounds present are harmless if used in the correct concentration.

One requirement for a compound to be developed into a drug is if the compound has an LC_{50} value < 1000 ppm; this indicates that Sidempuan salacca vinegar has the potential to be developed into an antihypercholesterol drug, as it has an LC_{50} value < 1000 ppm [13].

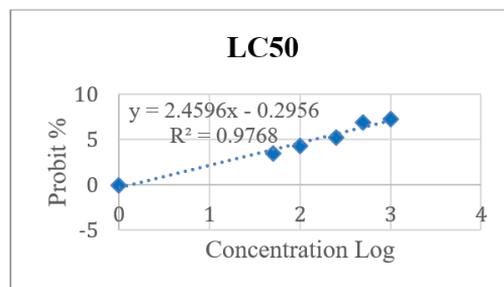


Fig. 3. LC_{50} of Sidempuan Salacca vinegar toward *A. salina* larvae.

3.3 Anticholesterol analysis

3.3.1 Determination of the maximum wavelength

The determination of the maximum wavelength was carried out with the aim of identifying the wavelength region that provides the maximum light absorption value

for cholesterol compounds, which can be determined by measuring the absorbance value of a standard cholesterol solution within the UV-Visible wavelength range. The absorbance measurement is conducted at the maximum wavelength because the absorbance at the maximum wavelength is more stable and has higher sensitivity, thus minimizing errors caused by changes in absorbance values per unit concentration [20].

From the wavelength reading results, it is known that the highest absorbance value is found at the peak of the absorbance curve, namely at wavelengths of 645 nm, 670 nm, and 675 nm. Therefore, in measuring the anti-cholesterol activity using the Liebermann-Burcard method, the absorbance of the sample solution was measured at the wavelength with the maximum absorption value of the cholesterol standard solution, which is 645 nm. The maximum wavelength obtained from the cholesterol standard solution is 645 nm, because at the peak of the curve it forms the maximum absorption.

3.3.2 Determining operating time

Determination of operating time was done to determine the perfect reaction time and the stability of the reaction indicated by no decrease in absorbance. The results of determining the operating time of the cholesterol standard solution at minute 1 to minute 16. From the absorbance results above, it can be seen that from minute 12 to minute 16, the cholesterol standard solution remained stable. So that in measuring anti-cholesterol activity using the Liebermann-Burcard method, the time required for the cholesterol standard solution to react and produce a stable absorbance value is 15 minutes.

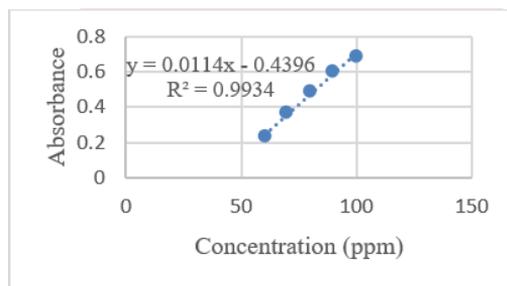


Fig. 4. Standard curves of anticholesterol test.

3.3.3 Creation of standard curves

The standard curve was created by reacting 5 series of cholesterol stock solution concentrations in chloroform with 2 ml of anhydrous acetic acid and 0.1 ml of concentrated sulfuric acid. The correlation coefficient (R^2) of the cholesterol standard solution calibration curve was 0.9934. The linearity result is considered good if the regression coefficient value approaches 1 [30].

3.3.4 Anticholesterol activity test

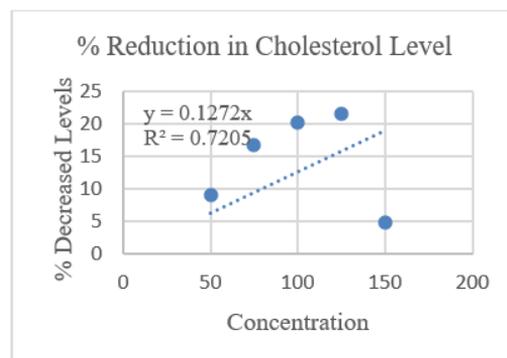


Fig. 5. Cholesterol reduction.

The salacca vinegar at a concentration of 125% showed the most effective cholesterol reduction, with an absorbance value of 0.419. This indicates that the vinegar has activity in lowering cholesterol levels in vitro. At a concentration of 150%, the vinegar showed an increase in absorbance

at 0.547 nm. These results indicate that the salacca vinegar at 125% concentration has the lowest absorbance value but the highest percentage of cholesterol reduction compared to the 150% concentration. This is because at a concentration of 125%, the salacca vinegar binds more cholesterol, resulting in less unbound cholesterol, and when the Lieberman-Burchard reagent is added, it gives a low absorbance result because the color in each test solution fades.

The decrease in cholesterol levels is caused by the presence of secondary metabolites contained in Sidempuan salacca vinegar, which have anti-cholesterol activity, including flavonoids, tannins, saponins, phenols, and alkaloids. Salacca vinegar has higher functional capabilities compared to apple vinegar, because salacca vinegar exhibits higher antioxidant activity due to the presence of phenols and other organic acids [33]. These compounds have been extensively studied and have benefits in reducing metabolic diseases, one of which is high cholesterol. Flavonoids work by scraping cholesterol in the blood [17] and can reduce LDL, reduce triglycerides (TGA), increase HDL, and lower cholesterol levels in blood vessels by exerting effects on particular enzymes. The first enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG Co-A reductase), acts as a catalyst in cholesterol formation. The second enzyme, Lecithin-Cholesterol Acyltransferase (LCAT), converts free cholesterol into more hydrophobic cholesterol esters, allowing the cholesterol esters to bind with lipoprotein core particles to form new HDL, increasing serum HDL levels [6]. Further, saponins exert anti-cholesterol activity by binding cholesterol in the blood. Meanwhile, tannins can inhibit the oxidation of LDL, which can lead to the occur-

rence of atherosclerosis.

Another possible mechanism is the ability of antioxidants to bind LDL, thereby preventing oxidative stress and combating free radicals [20]. The oxidation of LDL cholesterol is a biological process that is suspected to be involved in the initiation and acceleration mechanisms of arterial lesions. Oxidized LDL can cause blood viscosity to increase, thus raising the likelihood of blood vessel blockage (atherosclerosis) [6].

4. Conclusion

From the results of this study, it can be concluded that Sidempuan salacca vinegar contains oleic acid, DI-(9-octadecenoyl)-glycerol 2-Hydroxy-1,3-propanediyl ester, Borane, 9-octadecenal, and 9-Octadecenoic acid (Z), which have activity as anti-cholesterol agents. These compounds exhibit antioxidant properties, which help protect cells from free radical damage and contribute to its cholesterol-lowering effect. The results of the in vitro anti-cholesterol test showed that Sidempuan salacca vinegar can reduce cholesterol levels in vitro, thus demonstrating potential as an anti-cholesterol agent with an LC50 value of 142.232 ppm. These findings place Sidempuan salacca vinegar as a promising natural product with applications in cholesterol management and general health support.

Acknowledgements

The authors would like to thank the research funding from the Directorate of Research, Technology and Community Service, Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research, and Technology in accordance with the Research Implementation Contract number:

069/E5/PG.02.00.PL/2024.

References

- [1] Afra HA, Atifah Y. Effect of Salacca Vinegar on Uric Acid Levels of Rats (*Mus musculus* L.) Fed a High Uric Acid Diet. *Serambi Biologi*. 2022;7(1):82–86.
- [2] Agromedia. *Smart Book: Indonesia's Superior Fruit Plant Cultivation*. Jakarta: Agromedia; 2009.
- [3] Amalina ND, Mursiti S, Marianti A. Uncovering the Anticancer Activity of Citrus Flavonoid Compounds (*Citrus* sp.). Utilization of Indonesia's Natural Resources: Food, Energy and Advanced Materials Security. 2021;1.
- [4] Amin MS. *In vitro* studies: Anticholesterol Effect of Methanol Extract of Parijoto Fruit (*Medinilla speciosa* Blume) on Total Cholesterol [Skripsi]. Jakarta: UIN Syarif Hidayatullah; 2015.
- [5] Anggraini DI, Nabillah LF. Activity Test of Suji Leaf Extract (*Dracaena angustifolia* Roxb.) on *In Vitro* Cholesterol Lowering. *Jurnal Kimia Sains dan Aplikasi*. 2018;21(2):54–58.
- [6] Aprilia F. The activity of black glutinous rice ethanol extract to reduce the level of cholesterol. *Indonesian Journal of Pharmacy*. 2010.
- [7] Atifah Y, Afrilliana F, Azzahra F. Effect of salacca Sidempuan vinegar (sumatrana): *In vivo* study in hypercholesterolemia rats. *Jurnal Biota*. 2023. In press.
- [8] Awaludin Y, Yulma, Kartina. Identification of Secondary Metabolites from Ethanol Extract of Ciplukan (*Physalis angulata*) Leaves and Toxicity Test on Post-Larvae of Tiger Shrimp (*Penaeus monodon*). *JIPK*. 2019;11(2).
- [9] Jiménez SC, Oberhauser L, Maechler P. Role of fatty acids in the pathogenesis of β -cell failure and Type-2 diabetes. *Atherosclerosis*. 2024;398.
- [10] Cahyadi KD, Lestari GAD, Musthika IKT, Esati NK. Analysis of the quality of olive oil obtained from olives (*Olea europaea*) and its application as an anticholesterol. *Jamb J Chem*. 2023;5(1):1–12.
- [11] Cakir S, Eren M, Senturk M, Sarica ZS. The Effect of Boron and Its Physical and Chemical Properties. *Development in Inorganic Chemistry*. 2018;184:165–172.
- [12] Chaves SR, Rego A, Martins VM, Pereira PC, Sousa MJ, Côrte-Real M. Regulation of cell death induced by acetic acid in yeasts. *Front Cell Dev Biol*. 2021;9:6.
- [13] De Caro C, Haller C. *UV-VIS Spectrophotometry: Fundamentals and Applications*. Mettler-Toledo International; 2015. p. 4–14.
- [14] Hashimoto E, Tokushige K, Ludwig J. Diagnosis and classification of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Current concepts and remaining challenges. *Hepato Res*. 2015;45(1):20–8.
- [15] Hernanda M, Yani DF, Wijayanti F. Toxicity Test of Extract and Fraction of Shell of Shrimp (*Caesalpinia bonduc* L.) with the Brine Shrimp Lethality Test Method. *Al Ulum Sains dan Teknologi*. 2021;7(1).
- [16] Ilyas AN, Rahmawati R, Widiastuti H. *In Vitro* Anticholesterol Activity Test of Gedi Leaf Ethanol Extract. *Window of Health: Jurnal Kesehatan*. 2020;57–64.
- [17] Ihsani N, Hernahadini N, Pertiwi L, Kahfi NFM, Fadhilla SN. The effects of key lime (*Citrus aurantifolia*) on concentration and motility of mice (*Mus musculus*) spermatozoa after exposure to cigarette smoke. *Jurnal Kedokteran Yarsi*. 2019;27(1):35–42.
- [18] Jalut LLS, Rupiasih NN, Sardjono Y. Boron dose analysis in BNCT technique with simulation method using PHITS (Particle and Heavy Ion Transport

- Code System) program. *Buletin Fisika*. 2020;21(1).
- [19] Jelita SF, Setyowati GW, Ferdinand M. *Acalypha siamensis* infusion toxicity test using the brine shrimp lethality test (BSLT) method. *Farmaka*. 2020;18(1):14–22.
- [20] Karta IW, Sundari CDWH, Susila LANK, Mastra N. Analysis of active content in “Salacca Vinegar” in Sibatana Village with potential as antidiabetic and anticancer. *Indian J Public Health Res Dev*. 2018;9(5):424. <https://doi.org/10.5958/0976-5506.2018.00480.1>.
- [21] Kelly K, Bell S. Evaluation of the reproducibility and repeatability of GCMS retention indices and mass spectra of novel psychoactive substances. *Forensic Chem*. 2018.
- [22] Nogoy KMC, Kim HJ, Lee Y, Zhang Y, Yu J, Lee DH, Li XZ, Smith SB, Seong HA, Choi SH. *Food Science Nutrition*. Wiley; 2020. DOI:10.1002/fsn3.1644.
- [23] Kuru R, Sahin Y, Gulsah B, Tuzuner BA, Tasli PN, Akyuz S, Ozturk FY, Altuntas Y, Yarat A, Sahin F. Boron-rich diet may regulate blood lipid profile and prevent obesity: A non-drug and self-controlled clinical trial. *J Trace Elem Med Biol*. 2019;54:191–8.
- [24] Lindawati NY, Ningsih DW. Anticholesterol activity of green kiwifruit ethanol extract (*Actinidia deliciosa*). *Jurnal Ilmiah Manuntung*. 2020;6(2):183–91.
- [25] Syahrani R, Umar AH, Asnar NH. Characterization of three antihypercholesterolemia medicinal plants with an approach based on anatomical, histochemistry, and phytochemical profiles. *Pharmakon J Farmasi Indonesia*. 2022;19(2).
- [26] Vitalia N. Toxicity test of Pletekan leaf extract (*Ruellia tuberosa* L.) using the brine shrimp lethality test (BSLT) method. *Jurnal Fitofarmaka Indonesia*. 2018;3(1):146–52.
- [27] Wirmaningsih D, Atifah Y, Helendra. Acetic acid content in Salacca Sidempuan vinegar. *Jurnal Serambi Biologi*. 2023;8(3):280–8.
- [28] Wulandari F. Acute toxicity test of methanol extract of the crown leaf of the god (*Phaleria macrocarpa* [Scheff.] Boerl.) against *Artemia salina* Leach larvae using the brine shrimp lethality test (BSLT) method [Skripsi]. Jakarta: UIN Syarif Hidayatullah; 2014.
- [29] Yang J, Paulino R, Janke-Stedronsky S, Abawi F. Free radical scavenging activity and total phenols of Noni (*Morinda citrifolia* L.) juice and powder in processing and storage. *Food Chem*. 2007;102:302–8.
- [30] Atifah Y, Diana OP. Effects of Sidempuan salacca (*Salacca sumatrana*) vinegar on hyperuricemia: Histopathological assessment. *BIO Web Conf*. 2024;91:01025.
- [31] Zhu Y, Jianghong C, Narayan SH, Hosmane, Yingjun Z. Introduction: Basic concept of boron and its physical and chemical properties. In: *Developments in Inorganic Chemistry*. 2022;2:1–57.
- [32] Zubaidah E. Effect of apple vinegar and salacca vinegar on blood glucose levels of Wistar fed a high-sugar diet. *Jurnal Teknologi Pertanian*. 2011;12(3):163–9.
- [33] Zubaidah E, Austin, Sriherfyna FH. The study of antioxidant activity of snake fruit vinegar from several snake fruit varieties (*Salacca zalacca*). *Jurnal Teknologi Pertanian*. 2016;16(2).