Buletin Kedokteran & Kesehatan Prima
Vol.5 No.1 (2026)
DOI: 10.34012/bkkp.v5i1.7891

ORIGINAL ARTICLE

Antidiabetic potential of andaliman: Histopathological and glycemic improvements in diabetic rats

Boyke Marthin Simbolon[1,2], Juliana Lina[1,2]*, Ayu Theresia Saragih[3]

ABSTRACT

Background: Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia and pancreatic dysfunction, particularly involving the Islets of Langerhans. Andaliman (*Zanthoxylum acanthopodium*) is a plant rich in flavonoids and antioxidants, which are believed to have antidiabetic effects. This study aimed to evaluate the effectiveness of the ethanol extract of Andaliman fruit on pancreatic histopathology in alloxan-induced diabetic rats.
Methods: This study employed a true experimental design with a pre-test and post-test control group structure. Twenty-four male Wistar rats were randomly assigned to six groups: a normal control, a positive control (metformin 9 mg/kg), a diabetic control (alloxan 90 mg/kg), and three treatment groups induced with alloxan followed by administration of Andaliman extract at doses of 150, 250, and 350 mg/kg body weight, respectively. Treatments were administered for 14 days, after which blood glucose analysis (pre- and post-treatment) and pancreatic histopathological assessments were performed.
Results: Statistical analysis revealed a significant reduction in blood glucose levels following treatment ($p = 0.001$). Histopathological examination showed that Andaliman extract ameliorated alloxan-induced damage to the Islets of Langerhans. The 350 mg/kg body weight dose resulted in the most pronounced tissue regeneration compared to the other treatment groups.
Conclusion: The ethanol extract of Andaliman fruit effectively improves pancreatic histopathology and lowers blood glucose levels in diabetic rats.

Keywords: diabetes mellitus, *Zanthoxylum acanthopodium*, pancreatic histopathology, antidiabetic, medicinal plants

Introduction

Diabetes mellitus poses a major global public health challenge, characterized by a steadily growing epidemic with profound socioeconomic and healthcare impacts. In 2017, approximately 8.4% of adults aged 18–99 years worldwide were living with diabetes.[1] Data from Indonesia's Basic Health Research (Riskesdas) 2018 reported a 2% diabetes prevalence among individuals aged 15 years and older based on physician diagnosis. This finding is concerning, as the prevalence determined by blood glucose testing increased from 6.9% in 2013 to 8.5% in 2018, indicating that only about one in four individuals with diabetes are aware of their condition.[2] This "silent epidemic" contributes to the rising incidence of disabling microvascular and macrovascular complications, escalating healthcare costs, and deteriorating quality of life.[3]

The pancreas plays a central role in the pathophysiology of diabetes. It comprises exocrine and endocrine components, the latter organized within the islets of Langerhans, which contain alpha (glucagon-producing), beta (insulin-producing), delta (somatostatin-producing), and F (pancreatic polypeptide-

Affiliation
[1]Department of Clinical Medicine, Universitas Prima Indonesia
[2]Center of Excellence for Phyto Degenerative & Lifestyle Medicine, Universitas Prima Indonesia
[3]Undergraduate Program in Medical Science, Universitas Prima Indonesia

***Corespondence:**
julianalina@unprimdn.ac.id

producing) cells.[4,5] Insulin secretion by beta cells is essential for maintaining blood glucose homeostasis and regulating the metabolism of carbohydrates, lipids, and proteins. Selective destruction of beta cells by chemical agents such as alloxan results in insulin deficiency and subsequent hyperglycemia, thus serving as a well-established experimental model for type 1 diabetes.[6,7] Consequently, exploring agents that can protect or regenerate pancreatic beta cells represents a key therapeutic strategy.

Interest in medicinal plants as complementary or alternative treatments has increased due to their long-standing use, perceived safety, and abundance of bioactive compounds. *Zanthoxylum acanthopodium* DC., commonly known as andaliman or "Batak pepper," is a spice traditionally used by the Batak ethnic group in North Sumatra, Indonesia, to season fish and meat dishes.[8] Phytochemical studies have identified andaliman as a source of alkaloids, flavonoids, triterpenoids, steroids, and saponins.[9] Among these, flavonoids have attracted particular attention for diabetes management due to their strong antioxidant properties and their ability to inhibit carbohydrate-hydrolyzing enzymes such as α-glucosidase, thereby reducing postprandial hyperglycemia.[10]

Emerging preclinical evidence supports the therapeutic potential of andaliman. An in vitro study demonstrated its anti-inflammatory activity by suppressing key mediators such as tumor necrosis factor-alpha, interleukins, and cyclooxygenase.[11] In vivo, ethanol extracts of andaliman have exhibited renoprotective effects in diabetic nephropathy models by improving the histological appearance of renal tubules and glomeruli.[12] Its antioxidant activity is also thought to counteract the oxidative stress that contributes to the progression of diabetes.[13,14] However, studies focusing specifically on its effects on pancreatic histoarchitecture in diabetes remain scarce.

An imbalance between oxidative stress and antioxidant defense mechanisms plays a pivotal role in beta-cell apoptosis and dysfunction in diabetes.[15] Alloxan induces diabetes by generating reactive oxygen species (ROS) that preferentially damage pancreatic beta cells. Therefore, antioxidant-rich agents, such as those derived from andaliman, may help protect against chemical-induced pancreatic injury. This study hypothesizes that the flavonoid-rich extract of andaliman fruit mitigates alloxan-induced pancreatic damage through antioxidant and anti-inflammatory mechanisms, ultimately improving glycemic control and histopathological outcomes.

This research contributes to the scientific validation of a traditional medicinal plant by elucidating its potential mechanism of action at the organ level. It also provides a foundation for developing standardized herbal formulations as adjuncts in diabetes therapy. The study aligns with the growing movement toward integrating evidence-based phytotherapy into comprehensive management strategies for chronic metabolic diseases. Therefore, the primary objective of this study was to evaluate the effects of *Zanthoxylum acanthopodium* (andaliman) ethanol extract on pancreatic histopathological profiles in an alloxan-induced diabetic rat model.

Method

This study employed a true experimental design with a pre-test and post-test control group structure. This design involves measuring outcomes in both control and treatment groups before and after the intervention, allowing for the assessment of changes from baseline and minimizing potential confounders. The study utilized twenty-four male Wistar rats (*Rattus norvegicus*), aged 2 to 3 months and weighing more than 200 grams. The sample size was determined based on the minimum requirement for animal experimental research, with at least four rats per group to allow for statistical analysis and account for potential attrition. Inclusion criteria included male Wistar rats weighing over 200 grams, aged 2 to 3 months, in healthy physical condition, with no anatomical abnormalities, and no previous exposure to experimental studies.

The rats were acclimatized for one week under standard laboratory conditions (12-hour light/dark cycle, 25±2°C, 50±10% humidity) with ad libitum access to standard feed and water. Animals were randomly allocated into six groups (n = 4 per group) using a simple random sampling method to ensure uniform distribution of characteristics. All experimental procedures were approved by the institutional animal ethics committee and conducted in accordance with established guidelines for the care and use of laboratory animals.

Fresh andaliman fruits were obtained from a local market in North Sumatra and botanically authenticated. The ethanol extract was prepared using the maceration method. Briefly, 500 grams of dried crude drug (dried powdered fruit) were macerated in 2 liters of 96% ethanol for 7 days with periodic stirring. The mixture was then filtered using Whatman filter paper No. 1. The filtrate was concentrated using a rotary

vacuum evaporator (Heidolph, Germany) at 50°C to obtain a thick extract, which was subsequently freeze-dried (Lyophilizer, Christ Alpha 1-2 LDplus) to produce a dry, brownish-green powder. The extract was stored at 4°C until use. Alloxan monohydrate (Sigma-Aldrich) and metformin HCl (commercial pharmaceutical grade) were used as diabetic-inducing agent and standard antidiabetic control, respectively.

Following acclimatization, diabetes was induced in all groups except the normal control (Group I) by a single intraperitoneal injection of alloxan at a dose of 90 mg/kg body weight (BW), freshly prepared in cold normal saline.[16] Hyperglycemia (blood glucose >200 mg/dL) was confirmed 72 hours post-injection. The experimental animals were then divided into six groups for a 14-day treatment period. The groups were structured as follows: Group I (Normal Control): Non-diabetic rats receiving only 1% CMC-Na (carboxymethyl cellulose sodium) as vehicle daily for 14 days. Group II (Positive Control): Diabetic rats receiving metformin at 9 mg/kg BW daily via oral gavage for 14 days. Group III (Diabetic Control): Diabetic rats receiving the vehicle (1% CMC-Na) daily for 14 days. Group IV (Treatment 1): Diabetic rats receiving andaliman ethanol extract at 150 mg/kg BW daily for 14 days. Group V (Treatment 2): Diabetic rats receiving andaliman ethanol extract at 250 mg/kg BW daily for 14 days. Group VI (Treatment 3): Diabetic rats receiving andaliman ethanol extract at 350 mg/kg BW daily for 14 days.

Fasting blood glucose levels were measured from the tail vein using a portable glucometer (Accu-Chek Active) on day 0 (baseline, 72 hours post-alloxan injection) and day 14 (after treatment). On day 15, all animals were euthanized under deep anesthesia (ketamine-xylazine combination). The pancreas was immediately harvested, fixed in 10% neutral buffered formalin for 24 hours, processed through graded alcohol and xylene, and embedded in paraffin. Tissue sections (5 µm thick) were stained with hematoxylin and eosin (H&E) for general histopathologic evaluation under a light microscope (Olympus CX23) at 400× magnification. Histopathological analysis focused on the structure, number, and morphology of the islets of Langerhans, as well as evidence of necrosis, inflammation, or vacuolization.

All quantitative data are presented as mean ± standard deviation (SD). Normality of data distribution was assessed using the Shapiro-Wilk test. For normally distributed data, within-group differences (Day 0 vs. Day 14) were analyzed using paired t-tests, while between-group differences were analyzed using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. For non-normally distributed data or ordinal histopathology scores, non-parametric tests (Kruskal-Wallis followed by Mann-Whitney U test) were applied. A p-value of less than 0.05 was considered statistically significant. All analyses were performed using SPSS software version 25.0.

Results

All 24 rats completed the 14-day treatment period without mortality, and data from all subjects were included in the final intent-to-treat analysis. There were no significant differences in baseline body weight or fasting blood glucose levels (pre-alloxan) among the six groups ($p>0.05$), indicating successful randomization. Alloxan induction resulted in a significant elevation of fasting blood glucose levels in all experimental groups except the normal control (Group I), confirming successful diabetes induction.

Table 1. Combined analysis of blood glucose levels and histopathological improvement

Group & Treatment	Baseline blood glucose (day 0) Mean ± SD (mg/dL)	Final blood glucose (day 14) Mean ± SD (mg/dL)	p-value (Within Group)*	Histopathological score (0-3)† Mean ± SD	p-value (vs. diabetic control)‡
I. Normal Control	89.5 ± 7.2	92.3 ± 8.1	0.512	3.0 ± 0.0	<0.001
II. Positive Control (Metformin 9 mg/kg BW)	328.8 ± 32.4	145.5 ± 18.7	<0.001	2.5 ± 0.6	0.002
III. Diabetic Control (Alloxan 90 mg/kg BW)	335.3 ± 28.9	402.0 ± 41.5	0.035	0.8 ± 0.5	—
IV. Andaliman 150 mg/kg BW	321.5 ± 35.1	285.8 ± 30.2	0.019	1.5 ± 0.6	0.041
V. Andaliman 250 mg/kg BW	330.0 ± 29.4	210.3 ± 25.8	<0.001	2.0 ± 0.8	0.008
VI. Andaliman 350 mg/kg BW	324.8 ± 31.6	165.0 ± 20.1	<0.001	2.8 ± 0.5	<0.001

*Note: †Histopathological Scoring: 0 = Severe damage (>75% islet necrosis/vacuolization); 1 = Moderate damage (50-75%); 2 = Mild damage (25-50%); 3 = Normal/Near-normal architecture (<25% damage).
Within-group p-value: From paired t-test comparing blood glucose on Day 0 vs. Day 14 within the same group.
Between-group p-value: From one-way ANOVA followed by Tukey's post-hoc test (or corresponding non-parametric tests) comparing each treated group to the Diabetic Control (Group III) for Final Blood Glucose (Day 14) and Histopathological Score.
SD = Standard Deviation. A dash (—) indicates the reference group for comparison.

The negative control group (Group III) demonstrated a further significant increase in blood glucose from day 0 to day 14 ($p=0.035$), indicating progressive hyperglycemia. In contrast, all treatment groups (II,

IV, V, and VI) exhibited significant reductions in blood glucose levels ($p<0.05$). The highest andaliman extract dose (350 mg/kg BW, Group VI) produced a glucose-lowering effect comparable to that of metformin (Group II), with no significant difference between the two groups on day 14 ($p>0.05$). One-way ANOVA revealed a significant difference in day-14 glucose levels across all groups ($F=85.67$, $p<0.001$). Post hoc analysis indicated significantly lower glucose levels in Groups II, V, and VI compared with the diabetic control (Group III) ($p<0.001$). Furthermore, Group VI exhibited significantly lower glucose levels than Group IV ($p<0.01$).

Representative H&E-stained photomicrographs of pancreatic tissue are shown in Figure 1. The normal control group (Image I) displayed intact and well-defined pancreatic acini and normal, densely packed islets of Langerhans. The diabetic control group (Image III) exhibited severe pathological alterations, including shrunken and degenerative islets, marked vacuolization of beta cells, pyknotic nuclei, reduced islet cell density, and mild peri-islet inflammatory infiltration.

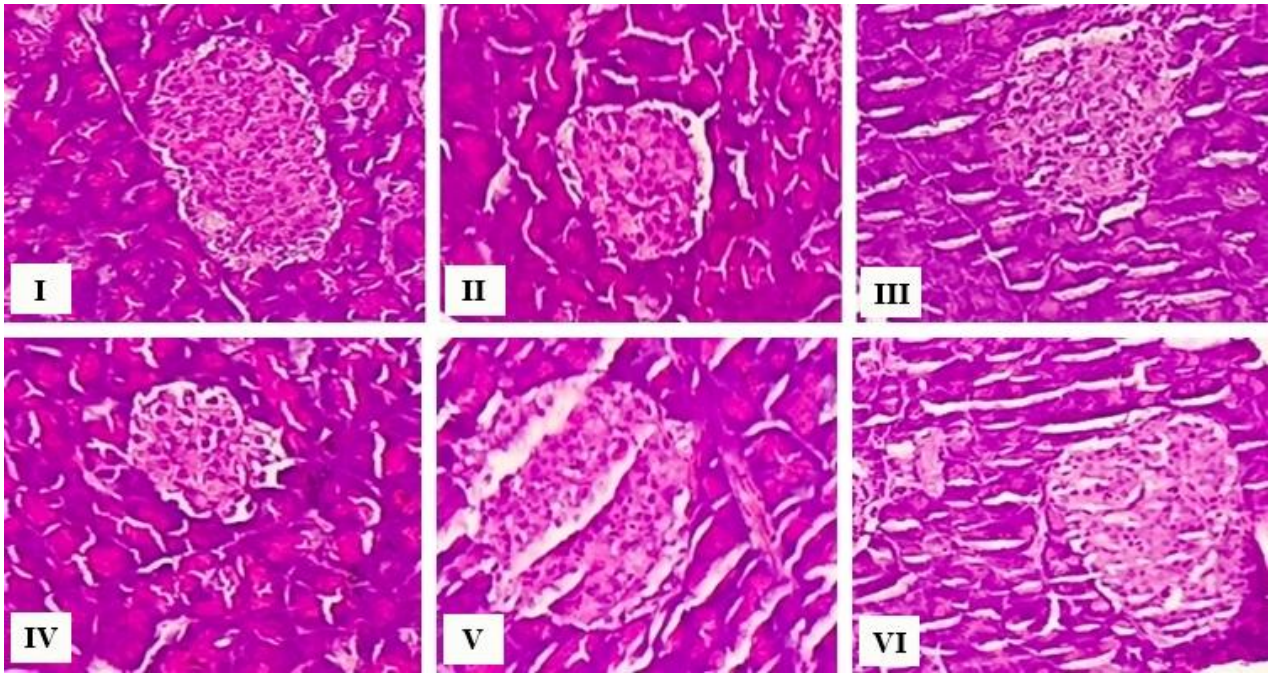

Figure 1. Histological structure of the pancreas (H&E staining, 400× magnification)

Treatment with metformin (Image II) resulted in marked preservation of islet morphology, with only minor vacuolization. Administration of andaliman extract produced a dose-dependent improvement in pancreatic architecture. At 150 mg/kg BW (Image IV), moderate improvement was observed, with reduced vacuolization and clearer islet boundaries compared with the diabetic control. The 250 mg/kg BW dose (Image V) demonstrated further restoration, with larger and more cellular islets. The most pronounced recovery occurred at 350 mg/kg BW (Image VI), where the islets appeared nearly normal in size and cellularity, with minimal cytoplasmic vacuolization, closely resembling the architecture of the metformin and normal control groups.

The semi-quantitative histopathological scores shown in Table 1 support these findings. The Kruskal–Wallis test indicated a significant difference among groups ($p<0.001$), and post hoc Mann–Whitney testing confirmed that all treatment groups (II, IV, V, and VI) had significantly higher scores than the diabetic control (Group III) ($p<0.05$). In addition, Group VI (350 mg/kg BW) scored significantly higher than Group IV (150 mg/kg BW) ($p=0.011$), confirming a dose-dependent effect.

Discussion

The findings of this study demonstrate that the ethanol extract of *Zanthoxylum acanthopodium* fruit exhibits significant antihyperglycemic and pancreatoprotective effects in an alloxan-induced diabetic rat model. The results support the primary hypothesis that the bioactive compounds in andaliman, particularly

flavonoids, can mitigate chemically induced pancreatic damage, likely through mechanisms involving antioxidant activity and anti-inflammatory modulation.

The marked reduction in fasting blood glucose levels among rats treated with andaliman extract, especially at doses of 250 and 350 mg/kg body weight, confirms its antidiabetic potential. This finding is consistent with previous reports on the hypoglycemic activity of andaliman.[17,18] The mechanism appears to be multifactorial. Flavonoids are known inhibitors of intestinal α-glucosidase and α-amylase, the key enzymes responsible for carbohydrate digestion, thereby reducing glucose absorption.[10] In addition, flavonoids can enhance peripheral glucose uptake and utilization by modulating insulin signaling pathways and promoting GLUT4 translocation. The observed efficacy, which is comparable to that of metformin at the highest dose, suggests that andaliman extract may also improve insulin sensitivity.

The most compelling evidence in this study comes from the histopathological analysis. Alloxan induces diabetes through the generation of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide, which are selectively taken up by pancreatic beta cells via the GLUT2 transporter. This uptake leads to DNA fragmentation and cellular necrosis.[15] The severe degeneration, vacuolization, and reduced cellularity observed in the islets of the diabetic control group represent classic histopathological features of oxidative damage. The dose-dependent restoration of islet architecture in the treatment groups strongly indicates a protective effect of andaliman extract against the cytotoxic actions of alloxan.

This pancreatoprotective effect is plausibly mediated by the potent antioxidant constituents of andaliman, including flavonoids and polyphenols.[13,14] These compounds can directly scavenge ROS, chelate metal ions involved in free-radical generation, and upregulate endogenous antioxidant defense systems such as superoxide dismutase and glutathione peroxidase. By neutralizing the oxidative stress triggered by alloxan, the extract may help preserve beta-cell integrity and function. This finding is consistent with the work of Hartanto[19], who reported that andaliman extract promoted pancreatic beta-cell regeneration in a similar experimental model.

The anti-inflammatory properties of andaliman extract, as reported by Kristanty & Suriawati[11], may also contribute to the observed histological improvements. Chronic low-grade inflammation is implicated in the progression of beta-cell dysfunction. By inhibiting pro-inflammatory cytokines such as TNF-α and IL-6, the extract may foster a microenvironment conducive to cell survival and recovery, consistent with the reduced peri-islet inflammatory infiltration observed in this study.

The dose-dependent relationship observed in both glycemic control and histopathological improvement strengthens the causal interpretation. The 350 mg/kg body weight dose produced outcomes statistically comparable to those of metformin, indicating its potential as an effective dose for further investigation. However, while structural improvement is evident, future studies employing immunohistochemical methods for insulin and glucagon are necessary to confirm the functional recovery of specific endocrine cell types within the islets.

This study has several limitations. Although the sample size was sufficient for preliminary conclusions, it remains relatively small. The two-week treatment duration may not fully reflect long-term efficacy or safety, underscoring the need for extended studies. Furthermore, the precise molecular mechanisms and specific flavonoid constituents responsible for these effects warrant further phytochemical and pharmacological characterization. Additional research using alternative diabetic models, such as high-fat diet-induced type 2 diabetes, would broaden understanding of the extract's therapeutic potential.

Despite these limitations, the implications of this research are substantial. It provides scientific validation for the traditional use of andaliman and highlights its potential as a natural source of novel antidiabetic compounds. The dual effects of lowering blood glucose and protecting pancreatic tissue make it a promising candidate for adjunctive therapy that may help preserve endogenous insulin secretion. This work enriches the growing body of evidence supporting the integration of evidence-based phytotherapeutics into the management of chronic metabolic diseases, emphasizing a multidimensional approach to disease modification.

Conclusion

This study concludes that the ethanol extract of *Zanthoxylum acanthopodium* (andaliman) fruit effectively reduces blood glucose levels and ameliorates alloxan-induced pancreatic histopathological damage in a rat model. The effect is dose-dependent, with the 350 mg/kg body weight dose demonstrating results comparable to those of the standard drug metformin. The pancreatoprotective activity, evidenced by

the preservation and restoration of islet architecture, is likely mediated by antioxidant and anti-inflammatory bioactive compounds, particularly flavonoids, present in the extract. These findings validate the traditional use of andaliman and support its potential as a complementary therapeutic agent for diabetes mellitus. Further studies are recommended to isolate the active constituents, elucidate the underlying molecular mechanisms, and conduct clinical trials to evaluate safety and efficacy in humans.

References

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018 Apr;138:271–81.
2. Kementerian Kesehatan Republik Indonesia. Riset Kesehatan Dasar 2018 (2018 Basic Health Research). Jakarta; 2018.
3. Mansour A, Mousa M, Abdelmannan D, Tay G, Hassoun A, Alsafar H. Microvascular and macrovascular complications of type 2 diabetes mellitus: Exome wide association analyses. Front Endocrinol (Lausanne). 2023 Mar 23;14.
4. Kaneto H, Kimura T, Shimoda M, Obata A, Sanada J, Fushimi Y, et al. Molecular Mechanism of Pancreatic β-Cell Failure in Type 2 Diabetes Mellitus. Biomedicines. 2022 Mar 31;10(4):818.
5. Dludla P V, Mabhida SE, Ziqubu K, Nkambule BB, Mazibuko-Mbeje SE, Hanser S, et al. Pancreatic β-cell dysfunction in type 2 diabetes: Implications of inflammation and oxidative stress. World J Diabetes. 2023 Mar 15;14(3):130–46.
6. Rorsman P, Ashcroft FM. Pancreatic β-Cell Electrical Activity and Insulin Secretion: Of Mice and Men. Physiol Rev. 2018 Jan 1;98(1):117–214.
7. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina (B Aires). 2017;53(6):365–74.
8. Silalahi M, Lumbantobing K. Kandungan minyak atsiri andaliman (Zanthoxylum acanthopodium DC) dan bioaktivitasnya. Pro-Life. 2021 Mar 31;8(1):22–31.
9. Anggraeni R. Uji karakteristik simplisia buah andaliman (Zanthoxylum acanthopodium DC.). J Ilm Farm Imelda. 2020 Mar 28;3(2):32–8.
10. Batubara MS, Sabri E, Tanjung M. Pengaruh pemberian ekstrak daun andaliman (Zanthoxylum acanthopodium DC.) terhadap histologis ovarium mencit (Mus musculus L.). BioLink J Biol Lingkung Ind Kesehat. 2020 Jan 22;6(2):196–209.
11. Kristanty R, Suriawati J. Cytotoxic and antioxidant activity of petroleum extract of Andaliman fruits (Zanthoxylum acanthopodium DC.). Int J PharmTech Res. 2014 Jan 1;6:1064–9.
12. Worotikan R V., Tuju EA, Kawuwung F. Analisis efektivitas antidiabetes ekstrak etanol buah andaliman (Zanthoxylum acanthopodium DC) pada histopatologi ginjal tikus putih (Rattus novergicus) yang diinduksi alloksan. J Sains, Mat Edukasi. 2017;5(1).
13. Rienoviar R, Heliawati L, Khoiriyah A. Aktivitas Antioksidan dan Identifikasi Senyawa Aktif dalam Ekstrak Buah Andaliman (Zanthoxylum Acanthopodium DC.). J Ris Ind. 2019;36(2).
14. Gultom S, Suparto IH, Batubara I. Flavonoid Buah Andaliman (Zanthoxylum acanthopodium DC) sebagai Antioksidan dan Inhibitor Enzim α-glukosidase. Institut Pertanian Bogor; 2011.
15. Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative Stress: Pathogenetic Role in Diabetes Mellitus and Its Complications and Therapeutic Approaches to Correction. Bull Exp Biol Med. 2021 May 26;171(2):179–89.
16. Dachi VNO, Rayyan TA, Utami SP, Mutia R, Akbar K, Lumbantobing CJRE, et al. Pengaruh variasi pemberian dosis aloksan terhadap angka kadar gula darah hewan coba. J Prima Med Sains [Internet]. 2022 Jun 7;4(1):32–6. Available from: https://jurnal.unprimdn.ac.id/index.php/jpms/article/view/2460
17. Adnan MC, Albert A, Aprilia GA, Mellenia P, Enda S, Linda C, et al. The effect of andaliman extract (Zanthoxylum acanthopodium DC) on the histology of the STZ-induced diabetes mellitus rats. Jambura J Heal Sci Res. 2021 Nov 30;4(1):334–44.
18. Lontaan F. Analisis efektivitas antidiabetes ekstrak etanol buah andaliman (Zanthoxylum acanthopodium DC) pada kadar glukosa darah tikus putih (Rattus novergicus) yang diinduksi alloksan. Universitas Negeri Manado; 2016.
19. Hartanto TS. Uji Aktivitas Antidiabetes Ekstrak Etanol Buah Andaliman (Zanthoxylum acanthopodium DC.) pada Mencit Swiss Webster. 2018.