Indonesian Journal for Health Sciences
Vol. 7, No. 2, September 2023, pp. 6-13
ISSN 2549-2721 (Print), ISSN 2549-2748 (Online)

EFFECT OF ASHITABA (*Angelica keiskei*) IN LOWERING BLOOD GLUCOSE LEVELS IN MICE (*Mus musculus L.*)

Musparlin Halid[1*], Sri Rahmawati[2]
[1]Department of Medical Record and Health Information, Politeknik Medica Farma Husada Mataram, Mataram, Indonesia
2Department of Pharmacy, Politeknik Medica Farma Husada Mataram, Mataram, Indonesia

Article History:
Submitted:01/12/2023
Accepted:24/08/2023
Published:20/08/2023

Keywords:
Antidiabetes
Angelica keiskei
Ashitaba

ABSTRAK

Abstract:
Ashitaba has been traditionally used for its potential health benefits and is generally considered safe when consumed as part of a balanced diet, it's essential to approach its usage with caution and consult a healthcare professional before starting any new supplement regimen. The study was conducted to determine the activity of ashitaba leaf extract (*Angelica keiskei*) on reducing blood glucose levels in alloxan-induced mice. This research method is pre and post-control group design. Twenty-five mice were divided into five treatment groups. Group I (negative control) was given PEG 4000, group II (positive control) was given glibenclamide, and groups III, IV, and V were given Ashitaba leaf extract at 300, 600, and 1200 mg/kg BW doses. The study results were divided into 2 groups, namely groups of mice with Pre-Diabetes Mellitus and groups of mice with Diabetes Mellitus. The 1200 mg/kg BW dose of Ashitaba (*Angelica keiskei*) leaf extract has the highest activity in reducing blood glucose levels in the group of mice with Pre-Diabetes Mellitus, while the 600 mg/kg BW dose of Ashitaba (*Angelica keiskei*) leaf extract has the highest activity in reducing blood glucose levels in mice with Diabetes Mellitus. Thus, Ashitaba leaf extract can reduce blood glucose in mice.

***Abstrak*:**
Ashitaba telah digunakan secara tradisional untuk manfaat kesehatan yang potensial dan umumnya dianggap aman ketika dikonsumsi sebagai bagian dari diet seimbang, sangat penting untuk mendekati penggunaannya dengan hati-hati dan berkonsultasi dengan profesional perawatan kesehatan sebelum memulai rejimen suplemen baru. Penelitian dilakukan untuk mengetahui aktivitas ekstrak daun Ashitaba (Angelica keiskei) terhadap penurunan kadar glukosa darah pada mencit yang diinduksi aloksan. Metode penelitian ini adalah pre dan post control grup design. Dua puluh lima ekor mencit dibagi dalam 5 kelompok perlakuan. Kelompok I (kontrol negatif) diberi PEG 4000, kelompok II (kontrol positif) diberi glibenklamid, kelompok III, IV, dan V diberi ekstrak daun Ashitaba dengan dosis 300, 600, dan 1200 mg/kg BB. Hasil penelitian dibagi dalam 2 kelompok yaitu kelompok mencit dengan keadaan Pre-Diabetes Mellitus dan kelompok mencit dengan keadaan Diabetes Mellitus. Ekstrak daun Ashitaba (Angelica keiskei) dosis 1200 mg/kg BB mempunyai aktivitas tertinggi dalam menurunkan kadar glukosa darah pada kelompok mencit dengan keadaan Pre-Diabetes Mellitus, sedangkan ekstrak daun ashitaba (Angelica keiskei) dosis 600 mg/kg BB mempunyai aktivitas tertinggi dalam menurunkan kadar glukosa darah pada mencit dengan keadaan Diabetes Mellitus. Dengan demikian, ekstrak daun Ashitaba dapat menurunkan glukosa darah pada mencit.

Corresponding Author:
Musparlin Halid,
Department of Medical Record and Health Information,
Politeknik Medica Farma Husada Mataram,
Mataram, Indonesia.
Email: musparlinhalid@gmail.com

How to Cite:
M. Halid & S. Rahmawati, "Effect of Ashitaba (*Angelica keiskei*) in Lowering Blood Glucose Levels in Mice (*Mus musculus* L.)", Indonesia. J. Heal. Sci., vol. 7, no. 2, pp. 6-13, 2023.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia due to abnormalities in insulin secretion, insulin performance, or both. Diabetes mellitus (DM) has become the world's fourth leading cause of death. Every year there are 3.2 million deaths caused directly by diabetes [1]. There are one person per 10 seconds or six people per minute die from diabetes-related diseases. DM sufferers in Indonesia are 4.5 million, the seventh largest in the world. This figure has increased to 8.4 million and is expected to reach 12.4 million by 2025, fifth in the world [2].

Diabetes is a disease where the patient's body cannot control blood sugar levels. Patients experience metabolic disorders in the process of sugar absorption by the body because the body cannot release or use insulin usually. Insulin is a hormone released by the pancreas, the main substance responsible for maintaining blood sugar levels [3]. The high cost of medical treatment for diabetes mellitus is one of the causes of the high mortality rate of patients, so many people have begun to turn to alternative or traditional medicine. In addition, the use of synthetic drugs poses a risk of permanent organ damage [4].

Ashitaba (*Angelica keiskei*) is an introduced plants, so it is not widely known in Indonesia. Ashibata plants are similar to celery, only ashitaba is taller than celery. The ashitaba plant comes from Hacho Jo Island (Longevity Island). Ashitaba has been traditionally used for its potential health benefits and is generally considered safe when consumed as part of a balanced diet, it's essential to approach its usage with caution and consult a healthcare professional before starting any new supplement regimen. Ashitaba has a long history of use in Japanese folk medicine and is believed to promote longevity and overall health. In Southeast Asia, the ashitaba plant can grow well in East Lombok, located in Sembalun Village. Ashitaba can be called an insulin plant because it can cure diabetes [5].

Ashitaba (*Angelica keiskei*) is one of many plants with high levels of active compounds such as flavonoids, tannins, saponins, and triterpenoids. These active compounds show insulin-like activity, which can control blood sugar levels. This herb is used as a traditional medicine on Izu Island in green juice and promotes healthy food in Japan [6]. Ashitaba contains a yellow concentrated liquid in its stems and leaves called Chalcone. Chalcone is a bright yellow and concentrated liquid in ashitaba that is not found in similar plants. Chalcone contains two flavonoid compounds, Xantoangelol and 4-hydroxyderricin. 4-HD and XA stimulate glucose uptake by skeletal muscle cells through induction of GLUT4 translocation [7]. Mechanistically, both 4-HD and XA stimulate phosphorylation of AMPK and acetyl-CoA carboxylase and increase phosphorylation of liver kinase B1 (LKB1), which acts to stimulate AMPK. Furthermore, RNA interferes with LKB1 expression, and is attenuated by 4-HD and XA and effects a reduction in glucose uptake. With the induction of 4-HD and XA, GLUT4 will help glucose uptake occur through the LKB1 AMPK signaling pathway in 3T3-L1 adipocytes [8].

Similar research has been conducted by Wardani et al. (2020) revealed that Ashitaba leaf extract has an effect as an antibacterial in inhibiting the growth of Staphylococcus epidermidis and is classified as having a moderate inhibition zone response, and the inhibition zone area obtained at 50% concentration, 13.33 ± 2.51, and 100% concentration, 19.66 ± 0.57 can be concluded that the higher the concentration of the extract, the greater the inhibition zone produced [9].

Research on the function of Ashitaba leaf extract (*Angelica keiskei*) which grows a lot in the Sembalun area as an alternative to lowering sugar levels has not been done much, so it is necessary to conduct scientific research to find out. As well as updating this research with previous research, which lies in the sample used, namely previous research

using Staphylococcus epidermidis as a sample. While the research that the researchers conducted used mice. In addition, the previous study tested antibacterial activity against acne, while this study tested antidiabetes using Ashitaba (*Angelica keiskei*) leaf extract. This study aims to determine the effect of Ashitaba (*Angelica keiskei*) leaf extract as antidiabetes in mice.

RESEARCH METHOD

This type of research is true experimental research with a pretest and post-test only control group design approach, on 25 mice divided into 5 groups randomly, each group containing 5 mice, where group 1 is a negative control group, group 2 is a positive control group, groups 3, 4, and 5 are treatment groups with doses of 300 mg/kgBB, 600 mg/kgBB, and 1200 mg/kgBB. In this study, mice were divided into 2 different groups, namely a group of mice with Pre-Diabetes Mellitus conditions and a group of mice with Diabetes Mellitus conditions. This research was conducted in the Chemistry Laboratory and Biology Laboratory, Medica Farma Husada Mataram Polytechnic from January to July 2021. A total of 1000 grams of fresh ashitaba leaves taken from Sembalun, East Lombok Regency which were used as samples.

Workflow

1. Material preparation
   Ashitaba leaves were taken from Sembalun, East Lombok Regency, Ashitaba leaf extract was obtained as much as 144.01 g 1000 grams, then washed with running water until clean, chopped into small pieces, dried, then made into powder with a blender. The finished powder is used for maceration.
2. Maceration
   Pureed Ashitaba leaves were immersed in a maceration container containing 96% ethanol. Simplisia macerated for three days and protected from sunlight. After obtaining the maceration extract of ashitaba leaves, it is then concentrated using a rotary evaporator to a thick extract.
3. Diabetes production in mice
   Making diabetes in mice is done by injecting alloxan monohydrate. Alloxan solution is made by dissolving alloxan monohydrate with saline infusion. Alloxan is a toxic compound used in research to induce diabetes in animals, while saline is a safe solution used for medical purposes, such as hydration and wound cleaning. The two substances serve entirely different roles and have distinct effects on the body. The blood glucose levels of mice were measured with a glucometer three days after alloxan injection. Alloxan was injected intravenously, then the blood glucose levels of the mice were measured again on day 5 to know if there was a decrease in blood glucose levels in the mice. Used doses for inducing diabetes in mice range 100 mg/kg. If mice has increased blood glucose levels to ± 200 mg/dL, then the mice are considered diabetic.
4. Dosage of Ashitaba Leaf Extract
   The doses of ashitaba leaf extract were 300 mg/kg body weight of mice, 600 mg/kg body weight of mice, and 1200 mg/kg body weight of mice given once a day orally.
5. Pharmacological Effect Test
   The test animals used were 25 mice. Mice were first fed for 16-18 hours, the aim is to minimize food factors that can affect blood glucose levels at the time of measurement. Blood collection is done through an intravenous found on mice's tail. Furthermore, 25 mice were treated with alloxan monohydrate. After three days, blood glucose levels were measured again (post-alloxan blood glucose). Furthermore, 25 mice were divided into five treatment groups as follows:
   a. Group I: PEG 4000 was applied for 5 days as a negative control.
   b. Group II: As a positive control, Glibenclamide was given a dose of 0.68 gr/kg BW for 5 days

c. Group III: given a dose of 300 mg / kg BW of ashitaba leaf extract for 5 days
d. Group IV: given a dose of ashitaba leaf extract 600 mg / kg BB for 5 days
e. Group V: given a dose of ashitaba leaf extract 1200 mg / kg BW for 5 days

After treatment, the mice were kept in their respective cages and given food and drink as usual. On the third day, blood samples were taken from all test animals. Furthermore, after five days of treatment, the blood glucose levels of mice were measured again to compare with blood glucose levels after being given alloxan. In this study, mice were divided into two treatment control groups and three treatment control groups, and the number of samples per group was 5, so that a total sample of 25 mice was obtained.

In this study, researchers used a sampling method with a simple random sampling technique, which means a sampling technique that provides equal opportunities for each member of the population to be selected as a sample member and is carried out randomly without regard to the strata in the population.

RESULTS AND ANALYSIS

Yield is the ratio of the amount (quantity) of extract produced from plant extracts.

Table 1. Yield

Ashitaba Leaf Simplisia	Viscous Extract	Yield
286 gr	3.35 gr	1.16 %

Measurement of Blood Glucose Levels of Pre-DM Mice and DM Mice

In this study, mice were divided into two groups: mice with Pre-DM conditions and those with DM conditions. The following is a table of measurements of blood glucose levels of Pre-DM mice and DM mice:

1. Mice with Pre-DM condition

Table 2.
Groups of Mice in Pre-DM Condition

Negative control group (PEG 4000)	U1	192	359
	U2	99	114
Average		145.5	236.5
Ashitaba leaf extract group at a dose of 300 mg/kg BW	U3	192	72
Average		192	72
Group of Ashitaba leaf extract dose of 600 mg/kg BW	U1	162	90
	U3	133	82
	U4	162	87
Average		152.3	86.3
Group of ashitaba leaf extract at a dose of 1200 mg/kg BW	U1	192	52
Average		192	52

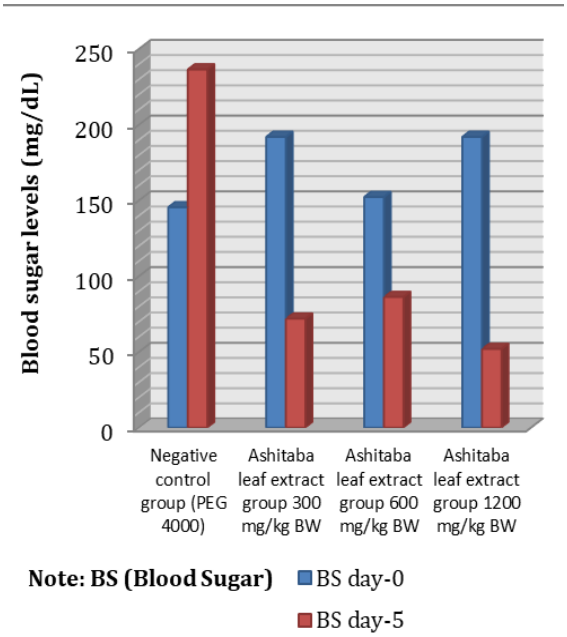

Figure 1. Blood Sugar Condition in Pre-DM Mice after being Injected with Ashitaba leaf

Figure 1 shows the condition of Pre-DM mice with blood glucose levels <200. It can be seen in the graph above there was an increase in blood glucose levels in the negative group mice while the ashitaba leaf extract group of 300 mg/kg BW, 600 mg/kg BW, and 1200 mg/kg BW decreased blood glucose levels.

2. Mice with DM condition

Table 3.
Groups of Mice in DM Condition

Treatment Group	No	H0	H5
Positive control group (glibenclamide)	U2	HI	534
	U3	206	90
	U4	567	486
	U5	HI	473
Average		493.25	395.75
Ashitaba leaf extract group at a dose of 300 mg/kg BW	U2	210	93
	U4	397	312
Average		303.5	202.5
Ashitaba leaf extract group with a dose of 600 mg/kg BW	U2	206	94
	U5	328	105
Average		267	99,5
Ashitaba leaf extract group with a dose of 1200 mg/kg BW	U2	206	139
Average		206	139

Notes: HI blood glucose level >600 mg/dL

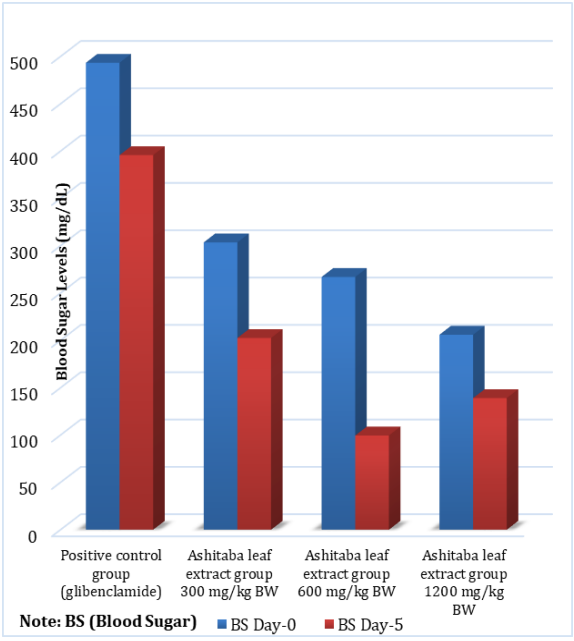

Figure 2. Condition of DM Mice after being injected with Ashitaba leaf

Figure 2 shows the condition of DM mice, namely, with blood glucose levels> 200. It can be seen in the graph above there is a decrease in blood glucose levels in positive group mice, groups of ashitaba leaf extract 300 mg/kg BW, 600 mg/kg BW, and 1200 mg/kg BW.

Ashitaba (*Angelica keiskei*) contains alkaloid, saponin, and glycoside compounds with strong categories in all plant parts. The highest flavonoid, triterpenoid, and tannin content are in ashitaba (*Angelica keiskei*) leaves. These flavonoids are thought to be antidiabetic agents. Flavonoids are natural organic compounds present in plants in general. Natural flavonoids play an important role in preventing diabetes and its complications [10]. Other research suggests that the chalcones found in Ashitaba may help improve insulin sensitivity. Increased insulin sensitivity means that cells are more responsive to the effects of insulin, which can help regulate blood sugar levels more effectively [11].

The use of maceration method in the extraction process was chosen because it is more appropriate than other extraction methods, without using heat, so that the damage factor to the active substance can be minimized, easy workmanship, and simple equipment. Maceration was carried out for 3 days using 96% ethanol solvent as much as 800 ml. The selection of 96% ethanol was chosen because the flavonoid compounds contained in the extract of ashitaba leaves are polar,polar solvent, so a polar solvent is also needed. Flavonoids are polar compounds because they have hydroxyl or sugar groups, so they can dissolve in polar solvents such as ethanol, methanol, butanol, acetone and water. Furthermore, the obtained macerate is then concentrated using a rotary evaporator in order to separate the active substance from the solvent. The results of concentrating the extract obtained ashitaba leaf yield of 1.16% [12].

This antidiabetic test of ethanol extract of ashitaba leaves uses alloxan induction. The test was conducted on 25 mice weighing 20-30 gr, with a correction factor of 5 mice. Mice were grouped into five treatment groups consisting of 2 control groups and 3 test dose groups. The first group is a negative control group where mice are induced with alloxan and then only given PEG 4000. The second group is a positive control where mice are given sulfonylurea antidiabetic

drugs, namely glibenclamide. The third, fourth and fifth groups are dose treatment groups, namely 300 mg/kg BW, 600 mg/kg BW, and 1200 mg/kg BW respectively. Measurement of blood glucose levels of mice was done on the third day.

Alloxan is a diabetogenic agent that is sufficient to be used as an inducer of diabetes in experimental animals. Alloxan has the ability to damage pancreatic beta cells. Before induction with alloxan, the mice were fed for 16 hours. Alloxan is diabetogenic if given parenterally, either by intravenous, intraperitoneal, or subcutaneous routes. Alloxan is injected intravenously into mice which are then checked for blood glucose increase three days later (BS-0). Measurement of mice blood glucose level was measured using Easy Touch biosensor glucometer [13].

In this study, the research data were divided into 2 groups, namely mice with pre-DM conditions (mice with blood glucose levels 110 < Pre-DM < 200 mg/dL) and mice with conditions (mice with blood glucose levels > 200 mg/dL). There are variations in the increase and decrease in blood glucose levels on day 0 and day 5, this is due to differences in the response generated from each individual experimental animal to pancreatic beta cell damage caused by diabetes inducing substances, which in this study used the diabetogenic substance alloxan monohydrate.

During the 5-day treatment there were several mice that dropped out, including 2 mice in the negative group, 1 mice in the positive group, 2 mice in the 300 mg/kg BW dose group, and 3 mice in the 1200 mg/kg BW dose group. From observations up to day 10, mice with the test dose group of ashitaba leaf extract (*Angelica keiskei*) had a stronger and fresher physical condition compared to the state of the positive control group mice given glibenclamide and the negative control group given PEG 4000 [14].

In the negative control group, there was no decrease in blood glucose levels because PEG 4000 is neutral, does not contain any substances so it does not have the effect of lowering blood glucose levels. It can be seen that the final blood glucose level (H5) of the PEG 4000 group has increased from the first day of measurement. In contrast, the positive control group given glibenclamide decreased blood glucose levels because glibenclamide has a mechanism of action to increase insulin secretion [15]. Meanwhile, the groups given 300 mg/kg BW, 600 mg/kg BW, and 1200 mg/kg BW of ashitaba leaf extract experienced a decrease in blood glucose levels due to the active flavonoid content in ashitaba leaves which has a hypoglycemic effect. As a result, bio-flavonoids are now considered as promising natural substances and significantly enrich therapeutic options when fighting diabetes [16].

There are several variations in the increase and decrease in blood glucose levels in mice with Pre-DM conditions, including the PEG 4000 group with an increase in blood glucose levels of 91 mg/dL, the 300 mg/kg BW ashitaba leaf extract group with a decrease in blood glucose levels of 120 mg/dL, the 600 mg/kg BW ashitaba leaf extract group with a decrease in blood glucose levels of 66 mg/dL and the 1200 mg/kg BW ashitaba leaf extract group with a decrease in blood glucose levels of 140 mg/dL [17].

Furthermore, in mice with DM conditions, there are several groups that can reduce blood glucose levels, including the glibenclamide group with a decrease in blood glucose levels of 97 mg/dL, the 300 mg/kg BW ashitaba leaf extract group with a decrease in blood glucose levels of 101 mg/dL, the 600 mg/kg BW ashitaba leaf extract group with a decrease in blood glucose levels of 167 mg/dL, the 1200 mg/kg BW ashitaba leaf extract group with a decrease in blood glucose levels of 67 mg/dL [18]. The highest decrease in blood glucose levels in mice with Pre-DM conditions is with a group of 1200 mg/kg BB ashitaba leaves with a decrease of 140 mg/dL, while in mice with DM conditions, namely with a group of 600 mg/kg BB ashitaba leaves with a decrease of 167 mg/dL [19].

In the test dose group, ethanol extract of ashitaba leaves (*Angelica keiskei*) has one of the compounds including flavonoid compounds. A number of studies have been conducted to demonstrate the hypoglycemic effect of flavonoids using different experimental models, the results of which plants containing flavonoids have been shown to have beneficial effects in fighting diabetes mellitus [20], either through the ability to reduce glucose absorption or by increasing glucose tolerance and here flavonoids have the same mechanism as sulfonylurea oral hypoglycemic drugs in reducing blood glucose levels in mice by increasing insulin secretion in the pancreas [21].

CONCLUSION

Ashitaba (*Angelica keiskei*) contains alkaloid, saponin, and glycoside compounds with strong categories in all parts of the plant. The highest flavonoid, triterpenoid, and tannin content is in ashitaba (*Angelica keiskei*) leaves. These flavonoids are thought to be antidiabetic agents. Flavonoids are natural organic compounds present in plants in general. Natural flavonoids play an important role in preventing diabetes and its complications.

REFERENCES

[1] R. I. Putri, "Determinants of diabetic nephropathy in patients with diabetes mellitus at RSUD DR. M. Soewandhie Surabaya," *J. Berk. Epidemiol.*, vol. 3, pp. 109–121, 2015, [Online]. Available: https://media.neliti.com/media/publications/76507-ID-none.pdf

[2] N. N. Veridiana and M. A. Nurjana, "Hubungan Perilaku Konsumsi dan Aktivitas Fisik dengan Diabetes Mellitus di Indonesia (The Correlation Consumption Behavior and Physical Activity with Diabetes Mellitus in Indonesia)," *Bul. Penelit. Kesehat.*, vol. 47, no. 2, pp. 97–106, 2019.

[3] P. S. Nugroho and A. C. Wijayanti, "Body Mass Index and Its Relation to Diabetes Mellitus at the Age of > 15 Years in Indonesia, Study of Indonesian Family Life Survey Data V," *J. Publ. Kesehat. Masy. Indones.*, vol. 5, no. 1, p. 12, 2018, doi: 10.20527/jpkmi.v5i1.4998.

[4] M. J. Lede, T. Hariyanto, and V. M. Ardiyani, "The Effect of Blood Sugar Levels on Diabetes Mellitus Wound Healing at Dinoyo Health Center Malang," *Nurs. News (Meriden).*, vol. 3, no. 1, p. 547, 2018.

[5] N. Ohkura, G. Atsumi, K. Ohnishi, K. Baba, and M. Taniguchi, "Possible antithrombotic effects of *Angelica keiskei* (Ashitaba)," *Pharmazie*, vol. 73, no. 6, pp. 315–317, 2018, doi: 10.1691/ph.2018.8370.

[6] M. Kweon, H. Lee, C. Park, Y. H. Choi, and J. H. Ryu, "A chalcone from ashitaba (*Angelica keiskei*) stimulates myoblast differentiation and inhibits dexamethasone-induced muscle atrophy," *Nutrients*, vol. 11, no. 10, pp. 1–13, 2019, doi: 10.3390/nu11102419.

[7] D. L. Aulifa, I. K. Adnyana, J. Levita, and S. Sukrasno, "4-Hydroxyderricin isolated from the sap of *Angelica keiskei* koidzumi: Evaluation of its inhibitory activity towards dipeptidyl peptidase-iv," *Sci. Pharm.*, vol. 87, no. 4, pp. 3–12, 2019, doi: 10.3390/scipharm87040030.

[8] M. Ohta, A. Fujinami, K. Oishi, N. Kobayashi, K. Ohnishi, and N. Ohkura, "Ashitaba (*Angelica keiskei*) Exudate Prevents Increases in Plasminogen Activator Inhibitor-1 Induced by Obesity in Tsumura Suzuki Obese Diabetic Mice," *J. Diet. Suppl.*, vol. 16, no. 3, pp. 331–344, May 2018, doi: 10.1080/19390211.2018.1458366.

[9] A. K. Wardani, S. Malfadinata, and Y. Fitriana, "Test of Antibacterial Activity of Acne Causes of Staphylococcus epidermidis Using Ashitaba Leaf Extract (*Angelica keiskei*).," *Lumbung Farm. J. Ilmu Kefarmasian*, vol. 1, no. 1, p. 14, 2020, doi: 10.31764/lf.v1i1.1206.

[10] L. K. Caesar and N. B. Cech, "A review of the medicinal uses and pharmacology of ashitaba," *Planta Med.*, vol. 82, no.

14, pp. 1236–1245, 2016, doi: 10.1055/s-0042-110496.
[11] I. P. A. Wiralaga, I. W. Sudira, I. M. Kardena, and A. A. G. O. Dharmayudha, "The effect of ethanol extract of ashitaba leaves on the histopathology of the stomach of male mice," *Bul. Vet. Udayana*, vol. 7, no. 1, pp. 26–33, 2015.
[12] H. T. C. Fuentebella *et al.*, "Comparative study: Banana-polyester fiber with guava extract (GuaBaNIKA sock) as an alternative to cotton in prosthetic socks," *UERM Heal. Sci. J.*, vol. 8, no. 2, pp. 108–114, 2019.
[13] N. Ohkura *et al.*, "Anti-platelet effects of chalcones from *Angelica keiskei* Koidzumi (Ashitaba) in vivo," *Pharmazie*, vol. 71, no. 11, pp. 651–654, 2016, doi: 10.1691/ph.2016.6678.
[14] N. Ohkura, G. Atsumi, S. Uehara, M. Ohta, and M. Taniguchi, "Ashitaba (*Angelica keiskei*) Exerts Possible Beneficial Effects on Metabolic Syndrome," *OBM Integr. Complement. Med.*, vol. 4, no. 1, pp. 1–1, 2018, doi: 10.21926/obm.icm.1901005.
[15] L. Zhang *et al.*, "Simultaneous Optimization of Ultrasound-Assisted Extraction for Flavonoids and Antioxidant Activity of *Angelica keiskei* Using Response Surface Methodology (RSM)," *Molecules*, vol. 24, no. 19, 2019, doi: 10.3390/molecules24193461.
[16] T. Zhang *et al.*, "The Ashitaba (*Angelica keiskei*) Chalcones 4-hydroxyderricin and xanthoangelol suppress melanomagenesis by targeting BRAF and PI3K," *Cancer Prev. Res.*, vol. 11, no. 10, pp. 607–619, 2018, doi: 10.1158/1940-6207.CAPR-18-0092.
[17] K. Iimura, J. I. Hattan, N. Misawa, and K. Shindo, "CDNA cloning and functional analyses of ashitaba (*Angelica keiskei*) sesquiterpene synthase genes," *J. Oleo Sci.*, vol. 69, no. 7, pp. 711–718, 2020, doi: 10.5650/jos.ess19275.
[18] A. A. Devanadera, C. G. M. Peña, and J. L. R. Perez, "Anti-hyperglycemic and Anti-hypercholesterolemic Effects of," *Lyceum Philipp. Cabrini Coll. Allied Med. Res.*, vol. 2, no. 2, pp. 29–38, 2017.
[19] J. A. M. Tattao, R. R. Velasco, and J. S. Doctolero, "Potential of ashitaba (*Angelica keiskei* Koidzumi) leaves as a feed additive on the diet of Nile tilapia fry (Oreochromis niloticus L .)," *Int. J. Fish. Aquat. Stud.*, vol. 8, no. 1, pp. 16–19, 2020.
[20] Y. S. Kil, S. T. Pham, E. K. Seo, and M. Jafari, "*Angelica keiskei*, an emerging medicinal herb with various bioactive constituents and biological activities," *Arch. Pharm. Res.*, vol. 40, no. 6, pp. 655–675, 2017, doi: 10.1007/s12272-017-0892-3.
[21] S. Rahmi and A. Kartini, "The Effect of Extract (*Angelica keiskei*) on Reducing Blood Pressure Level among Post-Partum Period with Hypertension," *Int. J. Nurs. Heal. Serv.*, vol. 3, no. 1, pp. 192–199, 2020, doi: 10.35654/ijnhs.v3i1.272.