e-ISSN 2621-8445 | p-ISSN 2621-8437

Potential Effects of Javanese Chili (*Piper retrofractum* Vahl.) Kombucha to Reduce Hyperglycemic and Maintain Spatial Memory: In Vitro and In Vivo Studies

Eri Dwi Suyanti[a,1], Salma Mutiara Tsani[a,2], Wafiq Hanifah[b,3], Fithratun Nastiti[c,4], Marcellino Maatita[b,5,*], Arta Farmawati[d,6]

[a]Program Studi Biologi, Fakultas Biologi, Universitas Gadjah Mada, Indonesia
[b]Program Studi Kedokteran, Fakultas Kedokteran Kesehatan Masyarakat dan Keperawatan, Universitas Gadjah Mada, Indonesia
[c]Program Studi Farmasi, Fakultas Farmasi, Universitas Gadjah Mada, Indonesia
[d]Departemen Biokimia, Fakultas Kedokteran Kesehatan Masyarakat dan Keperawatan, Universitas Gadjah Mada, Indonesia

[1]eri.dwi.suyanti@mail.ugm.ac.id; [2]salma.m.t@mail.ugm.ac.id; [3]wafiqhanifah@mail.ugm.ac.id;
[4]fithratun.nastiti@mail.ugm.ac.id; [5]marcellinomaatita@mail.ugm.ac.id*;
6a.farmawati@ugm.ac.id
*penulis korespondensi

ABSTRACT

Type 2 Diabetes Mellitus (T2DM) and Alzheimer's Disease (AD) are diseases which incidence and prevalence increase with age. Diabetic patients have 50-75% higher risk of developing AD compared to patients without diabetes. This study aimed to determine the potential effect of Javanese chili kombucha to reduce hyperglycemia and to act as a neuroprotective in diabetes patients with a risk of AD complications through in vitro and in vivo studies. The experiment carried out was fermentation of Javanese chili kombucha for 3, 6, 9, 12 and 15 days. Organoleptic tests, pH measurements, total titratable acidity (TTA), antioxidant tests and α-amylase inhibition tests were conducted to determine the best duration for kombucha fermentation. Phytochemical screening and in vivo tests using 5 groups of rats included measurements of fasting blood glucose (FBG), malondialdehyde (MDA) assay and antidementia (T-maze). The 9th day fermentation preparation was the best preparation, with organoleptic test results of brown color, sour smell with a pH of 3.43 (safe), an antioxidant IC_{50} value of 10.5 (very strong), an α-amylase inhibition IC_{50} value of 117.14 (moderate) and TTA 1.44 (normal). Phytochemical screening results show that Javanese chili kombucha contains alkaloids, flavonoids, terpenoids/steroids, saponins, and phenolics. Based on in vivo tests, Javanese chili kombucha can reduce FBG levels and can maintain the spatial memory of experimental animals with a statistically significant T-maze duration time between groups. The MDA level test showed oxidative stress results that were not significantly different between groups.

Keywords: Alzheimer, diabetes, javanese chili, kombucha

ABSTRAK

Diabetes Melitus Tipe 2 (DMT2) dan Alzheimer's Disease (AD) merupakan penyakit yang insidensi dan prevalensinya meningkat seiring bertambahnya usia. Pasien diabetes memiliki risiko 50-75% lebih tinggi untuk mengalami AD dibandingkan dengan pasien tanpa diabetes. Penelitian ini bertujuan untuk mengetahui potensi kombucha cabai jawa dalam menurunkan hiperglikemia dan sebagai agen neuroprotektif pada pasien diabetes yang berisiko mengalami komplikasi AD melalui studi in vitro dan in vivo. Percobaan yang dilakukan meliputi fermentasi kombucha cabai jawa selama 3, 6, 9, 12, dan 15 hari. Uji organoleptik, pengukuran pH, total titratable acidity (TTA), uji antioksidan, dan uji inhibisi α-amilase dilakukan untuk menentukan lama fermentasi kombucha terbaik. Skrining fitokimia dan uji in vivo menggunakan 5 kelompok tikus meliputi pengukuran kadar

glukosa darah puasa (GDP), uji malondialdehida (MDA), dan antidementia (T-maze). Preparasi fermentasi hari ke-9 merupakan preparasi terbaik, dengan hasil uji organoleptik berupa warna coklat, aroma asam dengan pH 3,43 (aman), nilai IC_{50} antioksidan sebesar 10,5 (sangat kuat), nilai IC_{50} inhibisi α-amilase sebesar 117,14 (sedang), dan TTA 1,44 (normal). Hasil skrining fitokimia menunjukkan bahwa kombucha cabai jawa mengandung alkaloid, flavonoid, terpenoid/steroid, saponin, dan fenolik. Berdasarkan uji in vivo, kombucha cabai jawa dapat menurunkan kadar GDP dan dapat mempertahankan memori spasial hewan percobaan dengan perbedaan waktu durasi T-maze yang bermakna secara statistik antar kelompok. Uji kadar MDA menunjukkan hasil stres oksidatif yang tidak berbeda signifikan antar kelompok.

Kata Kunci: *Alzheimer, cabai jawa, diabetes, kombucha*

1. Introduction

Alzheimer's disease (AD) and Diabetes Mellitus (DM) are diseases which incidence and prevalence continue to increase with age (León et al., 2021). In Indonesia there has been an increase in the number of elderly people from 18 million people in 2010 to 25.9 million people in 2019. Meanwhile, Type 2 Diabetes Mellitus (T2DM) is currently still a major health problem throughout the world, reaching 7.4% population (León et al., 2021). The latest data in 2021 states that diabetes patients in Indonesia have increased by 81.8% compared to 2019, namely around 19.46 million people (Magliano & Boyko, 2021). Meanwhile, AD itself is the most common type of dementia in the world (León et al., 2021). AD patients in Indonesia in 2019 were 987,673 people and it is estimated that this will increase to 3,399,285 people in 2050 (Nichols et al., 2022). AD has many risk factors, one of which is T2DM (Nisa & Lisiswanti, 2016). Studies show that diabetes patients have a 50–75% higher risk of suffering from AD compared to patients without diabetes (León et al., 2021). Seeing the trend of increasing AD and the high risk of diabetes patients developing AD, it is crucial to reduce the severity of the T2DM condition and prevent its complications (Nisa & Lisiswanti, 2016).

Nowadays, therapy for T2DM patients is carried out by medications, such as pioglitazone and metformin. However, long-term use of pioglitazone has the potential to cause more chronic side effects such as the risk of heart failure, edema, and the risk of fractures due to bone loss (Liao et al., 2017). Similarly, consumption of metformin causes side effects of gastrointestinal disorders such as nausea and vomiting (Herawati et al., 2021). Therefore, adjuvant therapies are needed for diabetes patients to minimize side effects that arise due to consumption of commercial antidiabetic drugs. One of the natural ingredients rich in bioactive compounds found in Indonesia is Javanese chili. Javanese chili (*Piper retrofractum* Vahl.) is a spice native to Indonesia which has been proven to have antidiabetic pharmacological effects. Apart from that, this plant is rich in piperine compounds which have been identified as having various pharmacological activities such as analgesic, hepatoprotective, lipid peroxidation inhibitor, and anti-inflammatory (Coman et al., 2012). However, the use of Javanese chili is currently still limited to traditional drinks (*jamu*) so it is not very popular with the public.

One of the beverage products currently popular is kombucha. Kombucha is a drink produced by bacterial and fungal fermentation in symbiosis with various bioactive

compounds such as antioxidants, polyphenols, flavonoids, catechins, fiber, vitamins and minerals. Kombucha has also been shown to have antihyperglycemic effects by reducing glycosylated hemoglobin (HbA1c) and increasing plasma insulin, hemoglobin, and glycogen levels in streptozotocin and alloxan-induced diabetic Wistar rats (Srihari et al., 2013). Previous studies showed kombucha beverage has been reported to be beneficial to human health, including its potential for diabetic treatment. However, this remains to be proved in Alzheimer's Disease.

The combination of Javanese chilies and kombucha has the potential to support diabetes therapy and prevent the development of AD with minimal side effects. However, the efficacy of Javanese chili kombucha drinks has not been studied. Therefore, this study aims to determine the potential effect of Javanese chili kombucha to reduce hyperglycemia and to act as a neuroprotective in diabetes patients with a risk of AD through in vitro and in vivo studies which includes organoleptic tests and measuring pH values, antioxidants, α-amylase inhibition test, total titratable acidity (TTA), phytochemical test, spatial working memory test, and malondialdehyde (MDA) assay. Additionally, this research could be a new approach to the potential of Javanese chili kombucha as a functional drink for the development food and beverages industry

2. Methodology

Collection, Determination, and Preparation of Javanese Chilies

Javanese chilies are collected from local farmers in Special Region of Yogyakarta, Indonesia. Furthermore, plant determination was carried out based on observations of the morphophysiological characteristics of plants (Sari & Nugraheni, 2013) which were carried out by the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada.

The obtained Javanese chilies were then washed and drained. Next, the sample was dried at 60°C using a drying oven until the weight was constant. Dried Javanese chili simplicial was stored in a glass bottle with a tight lid and placed in a desiccator as an ingredient for making dried Javanese chili tea.

Kombucha Fermentation

The sugar solution was made by heating water and granulated sugar in a pan (150 g of granulated sugar in 1 L of water). While stirring, dried Javanese chilies (50 g) was added into the mixture. The mixture was heated for 10 minutes and cooled. The cooled mixture was filtered to separate the dregs to produce 750 mL of Javanese chili tea filtrate. The filtrate was then poured into a glass jar, the SCOBY starter was added then covered with a white cloth and tied with a rubber band (Purnami et al., 2018). Kombucha fermentation was carried out for 3, 6, 9, 12, and 15 days (Kusuma & Fibrianto, 2018). Organoleptic properties and antioxidant activity were evaluated during fermentation to determine the best fermentation time.

Organoleptic Tests and pH Measurements

Organoleptic tests included observing the color, smell, and form of Javanese chili kombucha (Febriella, V., Nisa, A., & Lukman, 2021). Generally, the Javanese chili kombucha had a brown to dark brown color, formed a liquid with a sour smell, and the pH value ranged from 4 to 3. The pH value test was carried out using a pH meter (PHS-3C). The pH meter was calibrated using calibration solutions (pH 4.0, 7.0, and 10.0). The calibration process should be performed every time we use the instrument. Measurement pH value of Javanese chili kombucha was done by keeping the electrode submerged in 10 mL of liquid kombucha. When the pH value measured, take the electrode out and wash it in deionized water, blot it with paper then put it in the storage solution (KCl).

Total Titratable Acidity (TTA) Levels

Total titratable acidity (TTA) analyze was carried out using the acid-base titration method. A total of 20 mL of Javanese chili kombucha was put into an Erlenmeyer flask then dissolved in 2 times the volume of distilled water. Next, 2-3 drops of 1% PP indicator were added and titrated with 0.1 N NaOH until the solution color appeared pink. Total acid was calculated using the formula (Puspaningrum et al., 2022):

Total titratable acidity (%) =

$$\frac{V\ NaOH\ x\ Normalitas\ NaOH\ 0{,}1\ N\ x\ Berat\ \ CH3COOH\ x\ Faktor\ pengenceran}{Berat\ sampel} x\ 100\%$$

Antioxidant Test

The antioxidant test of Javanese chili kombucha was carried out using the diphenylpicrylhydrazyl (DPPH) method modified from Puspaningrum et al. (2022). DPPH reagent was prepared by dissolving 0.0394 g of 1,1 diphenyl-2-picrylhydrazyl in 250 mL of 97% ethanol. A sample of 10 µL in a test tube was added to 1 mL of DPPH solution then 97% ethanol was added to 5 mL. Furthermore, the solution was vortexed and the absorbance was measured at λ=517 nm using a UV-Vis spectrophotometer. Absorbance measurements were also carried out on a blank solution (DPPH solution without test material) and a positive control (piperine). The piperine control solution (1000 ppm) was prepared by dissolving 10 mg of piperine in 97% ethanol (10 mL), then the solution was diluted gradually to 100 ppm. The inhibition value of the solution is calculated by the formula:

% Inhibition= Blank Absorbance − Sample Absorbance / Blank Absorbance x100%

In Vitro Test

Analysis of a-Amylase Inhibition

Analysis of α-amylase inhibition was carried out by preparing a solution of 0.1 g of potato starch in 100 mL of sodium acetate buffer solution (0.1% w/v). A total of 27.5 mg of α-amylase was dissolved in 100 mL of distilled water as an enzyme solution. The colorimetric reagent was obtained by mixing 3.5 dinitrosalicylic acid 96 mM with sodium potassium tartrate. The kombucha drink was mixed with a starch solution and α-amylase

then incubated at 25℃ for 3 minutes. The percentage of inhibition was calculated from the absorption value at λ=540 nm with a UV-Vis spectrophotometer (Khan et al., 2022).

Phytochemical Screening

The phytochemical test of Javanese chili kombucha was carried out using thin layer chromatography (TLC) on F254 silica gel plates and a reference standard of 10 ppm piperine. Kombucha and piperine standards were spotted at 12 points and eluted using hexane-ethyl acetate-dichloromethane (5:3:2) v/v solvent. The reagents used were Dragendorff, AlCl3, SbCl3, Anisaldehyde-sulfuric acid, KOH, and FeCl3. The elution results were observed in visible light, UV light 254 nm and 365 nm.

In Vivo Test

Preparation and Acclimatization of Experimental Animals

Male Sprague Dawley rats weighing 150-220 g were divided into 5 groups: normal control group (F1) and diabetic groups (F2, F3, F4, F5). The number of samples for each group was determined using the Federer formula (Lestari, 2021). Each treatment group consisted of 5 rats. Rats were acclimatized in the laboratory for 14 days and were given free access to standard diet and distilled water. All research protocols were approved by the Medical and Health Research Ethics Committee, Universitas Gadjah Mada (approval no. KE/FK/1494/EC/2023) and were carried out in accordance with Indonesian code of practice for the care and use of animals for scientific purposes.

Diabetes Induction and FBG Analysis

Rats were fasted for 8 hours and then their initial fasting blood glucose (FBG) levels were measured using a glucometer by taking blood samples through the lateral vein. Rats were injected with streptozotocin (STZ) at a dose of 60 mg/kgBW and nicotinamide (NA) 100 mg/kgBW intraperitoneally (Firdaus et al., 2016). Later, FBG levels were evaluated to confirm the T2DM condition. Rats with FBG levels over 200 mg/dL were declared to have T2DM (Khan et al., 2022).

Treatment Groups

Each group of rats was given different treatment. F1 group was a normal control without treatment, F2 group was injected by STZ-NA (untreated diabetic group), F3 were diabetic group treated with metformin, F4 were diabetic group treated with the optimal kombucha, and F5 were diabetic group treated with the optimal combination of metformin+kombucha.

Providing Treatment

Treatment for each group of rats was carried out for 14 days in the morning (09.00 WIB). Rat's body weight was measured every 3 days (Lestari et al., 2016). The unconsumed feed was weighed every day and the rat's blood glucose was measured every 5 days.

Spatial Working Memory Evaluation

To evaluate the spatial working memory, the T-maze test was performed. Each rat was placed in a T-shaped apparatus and allowed to explore the maze. The travel time was recorded to assess spatial memory performance (Moeliono et al., 2014).

Malondialdehyde (MDA) Assay.

Rat blood plasma from the orbital sinus of the eye and heart was taken using a syringe. Blood was collected in labeled centrifuge tubes containing anticoagulant. Blood was centrifuged at 3000 rpm for 10 minutes and stored at 20°C. Rat MDA levels were tested using the thiobarbituric acid reactive substances (TBARS) reaction method with a spectrophotometer (Jozwik et al., 1999).

Data Analysis

The results of organoleptic tests of kombucha, pH values, total acids, and phytochemical tests was analyzed descriptively. Antioxidant levels and α-amylase inhibition were analyzed statistically using IC_{50} value calculations. Kruskal Wallis test was performed to analyze the MDA levels among groups. ANOVA test was performed to analyze blood glucose levels and spatial memory among groups.

3. Results and Discussion

Sample Collection, Determination, and Preparation

Javanese chili samples were obtained from Mertosanan Kulon, Potorono, Banguntapan, Bantul, Special Region of Yogyakarta. The samples were then analyzed by the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada to determine the samples' taxonomy. The results of the determination were completed on July 28 2023 with number 0392/5.Tb./VII/2023, namely Javanese chili with the following details:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Piperales
Family: Piperaceae
Genus: Flute player
Species: *Piper retrofractum* Vahl.
Local Name: Javanese Chili

A total of 800 g of wet samples of fresh Javanese chilies were dried using an oven for 2 weeks until a constant dry weight of 240 g was obtained. The dried sample was then ground with a grinder to obtain 238.36 g of Javanese chili powder.

Kombucha Fermentation

Kombucha fermentation produced kombucha drinks with fermentation variations of 3, 6, 9, 12 and 15 days. Based on research conducted by Kusuma & Fibrianto (2018),

kombucha fermentation was carried out for 3, 6, 9, 12, and 15 days to optimize the phytochemical compounds contained in kombucha.

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

Figure 1. Results of Javanese chili kombucha fermentation based on fermentation duration: (a) 3 days; (b) 6 days; (c) 9 days; (d) 12 days; (e) 15 days.

Organoleptic Test and pH Test

Based on the organoleptic tests (Table 1), the color of the kombucha preparation tended to be brown, had a sour smell, and was liquid with a decreasing pH value. The longer the fermentation time, the darker the brown color of the kombucha. In addition, the increased number of microbes makes kombucha look cloudy and dark (Febriella et al., 2021).

Table 1. Organoleptic Properties of Different Fermentation Duration of Javanese Chili Kombucha

Fermentation Duration (Day)	Color	Smell	Form	pH
3	Brown	Sour, sweet	Liquid	3.66
6	Slightly dark brown	Sour, sweet	Liquid	3.62
9	Dark brown	Sour	Liquid and contains sediment	3.43
12	Dark brown	Sour, alcoholic	Liquid and contains sediment	3.26
15	Dark brown	Sour, alcoholic	Liquid and contains sediment	3.14

The activity of bacteria and fungi (SCOBY) in sugar metabolism produces organic acids and alcohol, giving kombucha a low pH and a distinctive sour aroma (Febriella et al., 2021). According to Naland (2008), the acidity level of kombucha that is safe to consume is in the pH range of 2.5-4.6. It indicates that all fermentation time of Javanese chili kombucha investigated in this study resulted in kombucha which is safe to consume.

Antioxidant Test

The antioxidant test results are presented in the form of a table of IC_{50} values shown in Table 2. Based on the antioxidant test, the IC_{50} value of Javanese chili kombucha was ranging between 10.5 and 287.5 µg/mL which indicated a moderate to very strong antioxidant activity. Specifically, the strongest IC_{50} value was found on the kombucha which was fermented for 9 days (IC_{50} value of 10.5 µg/mL). The lower IC_{50} value indicates higher activity of a substance in reducing free radicals (Suratno et al., 2019).

Antioxidants play a role in improving cognitive function by protecting neurons from oxidative damage (Hemamalini & Rao, 2013). This shows that the Javanese chili kombucha has the potential to be a neuroprotective agent to prevent neuronal damage caused by diabetes.

Table 2. IC_{50} Value of Processed Javanese Chili Kombucha

Fermentation Duration (day)	IC_{50} (µg/mL) Value	Antioxidant Categories
3	287.5	Very week
6	347.5	Very week
9	10.5	Very strong
12	14.5	Very strong
15	101.7	Moderate
Vitamin C (positive control)	14.79	Very strong

α-amylase Inhibition Test

α-amylase inhibitors are compounds that inhibit the activity of the α-amylase enzyme which plays a role in breaking down starch into maltose or alpha configuration glucose (Prahesti et al., 2018). The breakdown of starch into glucose causes high blood glucose levels. This inhibitory ability prevents increased glucose absorption from the digestive tract into the blood. Acarbose is a conventional drug which works by inhibiting α-amylase activity in carbohydrate catabolism (Fauzi et al., 2018). Thus, the antidiabetic potential of Javanese chili kombucha can be determined by testing the α-amylase inhibitory activity in vitro with acarbose as a comparison.

Table 3. IC_{50} Value of Processed Javanese Chili Kombucha

Fermentation Duration (day)	IC_{50} (µg/mL) Value	Inhibition Categories
3	162,21	Moderate
6	140,65	Moderate
9	117,14	Moderate
12	120,79	Moderate
15	125,42	Moderate
Acarbose (positive control)	127,17	Moderate

Based on the results of the α-amylase inhibition test (Table 3), the IC_{50} value of all kombucha preparations showed moderate α-amylase inhibitory activity. Specifically, kombucha which fermented for 9 days had the best inhibitory activity with an IC50 value of 117.14 µg/mL, stronger than acarbose. This shows that the Javanese chili kombucha is potential to be used as an antidiabetic drug.

Total Titratable Acidity

The results of the total titratable acidity content titration test are shown in Figure 1. Based on the TTA test, the acid content in the kombucha preparation increases with the length of fermentation time. During fermentation, the SCOBY metabolizes sucrose and

produces organic acids so that the longer it lasts, the more acidic the kombucha preparation will be (Febriella et al., 2021).

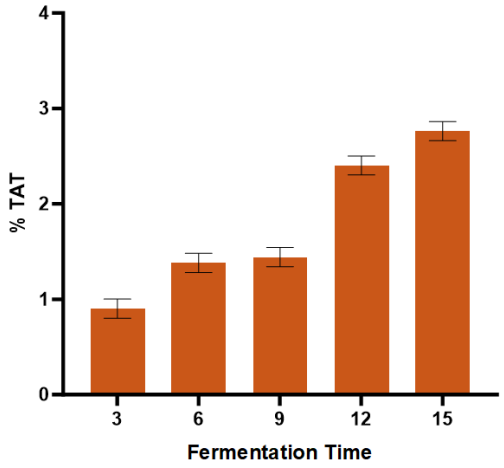


Figure 2. Total titratable acidity test for kombucha preparations

Phytochemical Screening

Based on various previous tests, it could be concluded that the best preparation of kombucha was the 9-days fermented kombucha. Thus, the kombucha fermented for 9 days was used in the phytochemical test using thin layer chromatography (Table 4).

Table 4. Results of Phytochemical Screening of Javanese Chili Kombucha

Class	Kombucha
Alkaloids	+
Flavonoids	+
Terpenoids/Steroids	+
Coumarin	-
Saponin	+
Phenolic	+

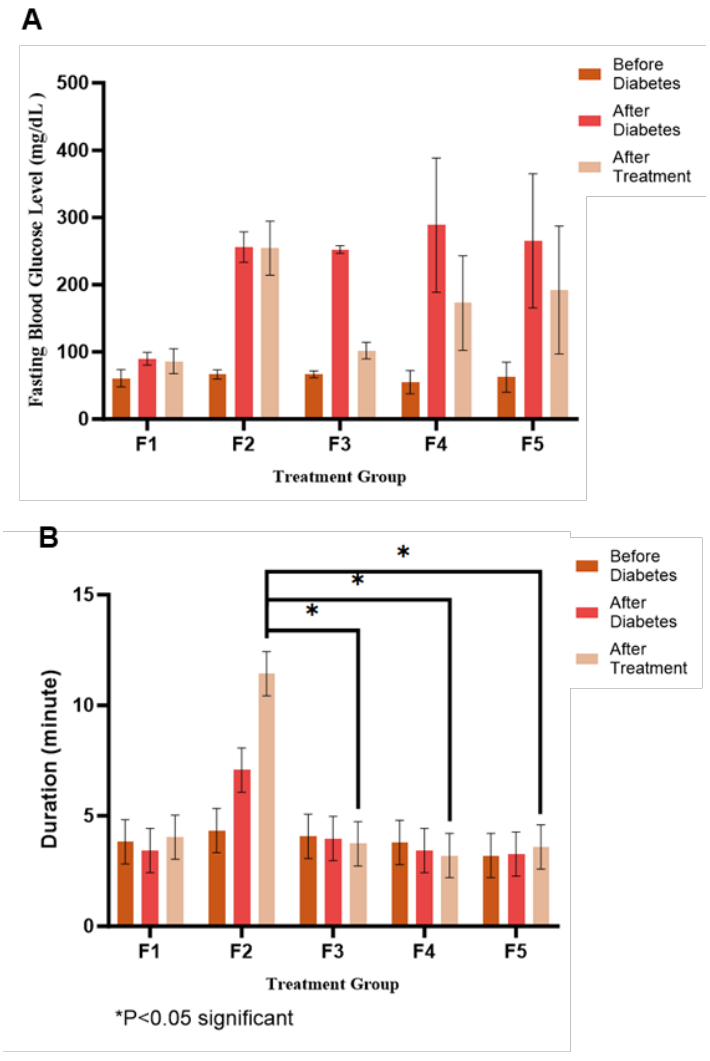
Based on the phytochemical tests carried out and presented in Table IV, there were alkaloids, flavonoids, terpenoids, saponins and phenolic compounds that were successfully detected in the 9-days kombucha fermentation. According to previous study, Javanese chilies contain flavonoids, alkaloids and saponins (Primadiamanti *et al.*, 2023). Secondary metabolites produced by plants have different benefits for the metabolism of living things. According to Afifah *et al.* (2021), secondary metabolites of saponins, flavonoids, polyphenols and alkaloids can repair pancreatic β cells so that they can increase insulin production.

In Vivo

Based on the analysis of FBG levels in rats (Figure 2A), it can be seen that the F4 group experienced a decrease compared to the F2 group. This shows that the Javanese chili kombucha had the activity of reducing FBG levels in rats. According to a research by Atal et al. (2012), the piperine compound which found in Javanese chili can reduce

blood glucose levels in rat because it acts as a β3-adrenergic receptor agonist thereby increasing levels of thermogenesis, lipolysis and insulin receptors. As a result, there is an increase of intracellular metabolism, thereby reducing glucose levels in the blood (Atal et al., 2012).

Based on the T-maze travel time test (Figure 2B), it can be seen that group F4, namely the group of rats treated with Javanese chili kombucha, was able to maintain spatial memory significantly compared to group F2, which was the negative control diabetic rat. In addition, the F4 treatment group had the lowest travel time compared to other treatment groups. This is in line with the research results of Roshanbakhsh et al. (2020) which shows that the piperine compound can be an effective therapy for spatial memory disorders in rats by reducing the extent of demyelination and increasing the myelin repair process.



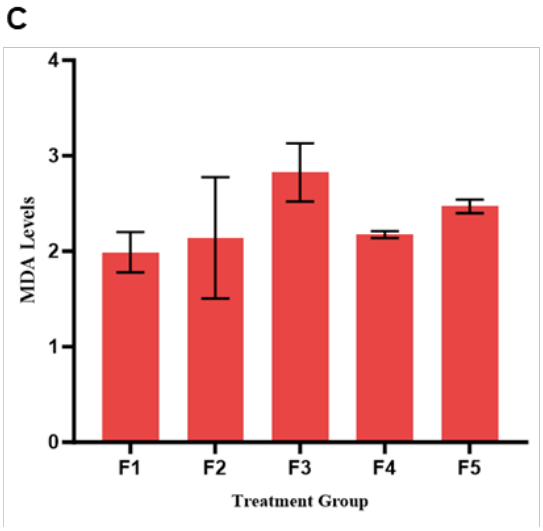


Figure 3. Results of In Vivo Study. A) The fasting blood glucose (FBG) levels of rats. There is no significant difference between each treatment group; B) The T-Maze travel time for each group of rats. A significant difference was shown between F1 to F3, F4, and F5 after treatment C) MDA levels analysis. F1 group was a normal control without treatment, F2 group was injected with STZ-NA (untreated diabetic group), F3 was the diabetic group treated with metformin, F4 was the diabetic group treated with the optimal kombucha, and F5 was the diabetic group treated with the optimal combination of metformin+kombucha.)

Based on the MDA analysis of rats (Figure 2C), it appears that there were no significant differences among treatments. MDA levels indicate the level of oxidative stress experienced by rats. The analysis results showed that oxidative stress was still at normal levels in all groups. The significance among treatment groups might be achieved if the duration of the treatments were extended. In previous studies, it took more than 14 days of treatment to show a significant reduction in MDA levels in diabetic mouse models (He et al., 2022; Wang et al., 2019).

Although this study presents important findings on the potential of Javanese chili kombucha for type 2 diabetes treatment and its preventive effect on Alzheimer's Disease, certain methodological issues should be acknowledged. We considered extending the duration of treatments in mice to minimize bias for the next research. Additionally, while in vivo tests provide valuable insights into the potential neuroprotective effect of Javanese chili kombucha, they may not fully capture the quantitative expression of the molecular marker related to type 2 diabetes and Alzheimer's Disease, it remains unidentified. Incorporating complementary techniques such as quantitative PCR in future studies required to enhance the depth of these research.

4. Conclusion

In vitro study revealed that the bioactive compound of Javanese chili kombucha had the α-amylase inhibitory activity. The kombucha preparation that resulted in the highest α-amylase activity was that fermented for 9 days with an IC_{50} value of 117.14 µg/mL.

The compound profile contained in Javanese chili kombucha is alkaloids, flavonoids, terpenoids/steroids, and saponins. Based on in vivo tests, Javanese chili kombucha can reduce FBG levels and can maintain the spatial memory of experimental animals with a significantly lower T-maze duration time than that of the untreated diabetic group. The MDA level test showed oxidative stress results that were not significantly different among groups. Javanese chili kombucha has been proven to have a potential effect in reducing hyperglycemia and maintaining spatial memory so that it can provide a therapeutic effect for patients of type 2 diabetes and a protective effect against its complication risk with Alzheimer's Disease.

5. Acknowledgment

This study was funded by the Ministry of Education, Culture, Research and Technology of Republic of Indonesia through the Student Creativity Program 2023. Authors are grateful for the support from Universitas Gadjah Mada for facilitating this research.

References

Afifah, A. R., Wahyu, S., Gayatri, S. W., Daeng Kanang, I. L., & Indarwati Abdullah, R. P. (2021). Pengaruh pemberian ekstrak buah pare terhadap penurunan kadar glukosa darah pada mencit (Mus musculus). *Indonesian Journal of Health*, 1(03). https://doi.org/10.33368/inajoh.v1i03.66

Atal, S., Agrawal, R. P., Vyas, S., Phadnis, P., & Rai, N. (2012). Evaluation of the effect of piperine per se on blood glucose level in alloxan-induced diabetic mice. *Acta Poloniae Pharmaceutica - Drug Research*, 69(5).

Coman, C., Rugină, O. D., & Socaciu, C. (2012). Plants and natural compounds with antidiabetic action. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 40(1). https://doi.org/10.15835/nbha4017205

Fauzi, N. I., Ardiansyah, S. A., & Hidayat, S. (2018). Skrining mekanisme kerja daun malaka (Phyllanthus emblica L.) sebagai antidiabetes. *Talenta Conference Series: Tropical Medicine* (TM), 1(3). https://doi.org/10.32734/tm.v1i3.271

Febriella, V., Nisa, A., & Lukman, A. (2021). Inovasi minuman herbal yang difermentasi dengan starter kombucha dan pengaruhnya terhadap mutu organoleptik, pH, dan nilai antioksidan. *Food and Agroindustryy Journal*, 2(2), 33-40. https://jurnal.uts.ac.id/index.php/JTP/article/view/1227

Firdaus, F., Rimbawan, R., Marliyati, S. A., & Roosita, Katrin. (2016). Model tikus diabetes yang diinduksi streptozotocin-sukrosa untuk pendekatan penelitian diabetes. *Jurnal Media Kesehatan Masyarakat Indonesia*, 12(1). https://doi.org/10.30597/mkmi.v12i1.550

He, Q., Xu, J. Y., Gu, J., Tong, X., Wan, Z., Gu, Y., Fang, C., & Qin, L. Q. (2022). Piperine is capable of improving pancreatic β-cell apoptosis in high fat diet and streptozotocin induced diabetic mice. *Journal of Functional Foods*, 88. https://doi.org/10.1016/j.jff.2021.104890

Hemamalini, & Rao, M. S. (2013). Anti stress effect of Centella asiatica leaf extract on hippocampal CA3 neurons – a quantitative study. *International Journal of Pharmacology and Clinical Sciences*, 2(1), 25-32.

Herawati, L. W., Himawan, N. S. S., & Kusmini. (2021). Penggunaan metformin terhadap kejadian efek samping mual muntah pada pasien diabetes melitus. *Jurnal Pendidikan Tambusai*, 5(3), 11328-11332.

Isabel Lira-De León, K., Delia Bertadillo-Jilote, A., Gustavo García-Gutiérrez, D., & Antonio Meraz-Ríos, M. (2021). Alzheimer's Disease and Type 2 Diabetes Mellitus: Molecular Mechanisms and Similarities. *In Neurodegenerative Diseases - Molecular Mechanisms and Current Therapeutic Approaches*. https://doi.org/10.5772/intechopen.92581

Purnami, K. Ita, Anom Jambe, AAGN., & Wisaniyasa, N. W. (2018). Pengaruh jenis teh terhadap karakteristik teh kombucha. *Jurnal Ilmu dan Teknologi Pangan* (ITEPA), 7(2). https://doi.org/10.24843/itepa.2018.v07.i02.p01

Jozwik, M., Wolczynski, S., Jozwik, M., & Szamatowicz, M. (1999). Oxidative stress markers in preovulatory follicular fluid in humans. *Molecular Human Reproduction*, 5(5). https://doi.org/10.1093/molehr/5.5.409

Khan, S., Khan, H. U., Khan, F. A., Shah, A., Wadood, A., Ahmad, S., Almehmadi, M., Alsaiari, A. A., Shah, F. U., & Kamran, N. (2022). Anti-Alzheimer and antioxidant effects of nelumbo nucifera l. alkaloids, nuciferine and norcoclaurine in alloxan-induced diabetic albino rats. *Pharmaceuticals*, 15(10). https://doi.org/10.3390/ph15101205

Kusuma, G. S. P., & Fibrianto, K. (2018). Pengaruh optimasi lama fermentasi terhadap karakteristik kombucha daun tua kopi robusta dampit metode oksidatif dan non-oksidatif. *Jurnal Pangan dan Agroindustri*, 6(4), 87–97.

Lestari, A. D. (2021). *Pengaruh pemberian ekstrak etanol daun mangga (Mangifera indica L.) terhadap kadar glukosa darah mencit diabetes dengan induksi aloksan*. Indonesia: Universitas dr. Soebandi.

Lestari, W. T., Tana, S., & Isdadiyanto, S. (2016). Indeks kuning telur dan nilai haugh unit telur puyuh (Coturnix coturnix japonica L.) hasil pemeliharaan dengan penambahan cahaya monokromatik. *Buletin Anatomi Dan Fisiologi*, 24(1), 42–49.

Liao, H. W., Saver, J. L., Wu, Y. L., Chen, T. H., Lee, M., & Ovbiagele, B. (2017). Pioglitazone and cardiovascular outcomes in patients with insulin resistance, pre-diabetes and type 2 diabetes: A systematic review and meta-analysis. *BMJ Open* 7(1). https://doi.org/10.1136/bmjopen-2016-013927

Magliano, D. J., & Boyko, E. J. (2021). IDF Diabetes Atlas 10th edition scientific committee. *IDF DIABETES ATLAS* [Internet]. Brussels.

Moeliono, A. P., Maryadhi, N. M. D. D. , Cahyadi, M. F., Irmayanti, N. M. F., & Leliqia, N. P. E. (2014). Uji aktivitas antidementia minuman gambir dan minuman gambir kombucha lokal bali secara in vivo. *Jurnal Farmasi Udayana*, 3(1), 1–3.

Naland, H. (2008). *Kombucha: Teh dengan seribu khasiat*. Indonesia: AgroMedia Pustaka.

Nichols, E., Steinmetz, J. D., Vollset, S. E., Fukutaki, K., Chalek, J., Abd-Allah, F., Abdoli, A., Abualhasan, A., Abu-Gharbieh, E., Akram, T. T., Al Hamad, H., Alahdab, F., Alanezi, F. M., Alipour, V., Almustanyir, S., Amu, H., Ansari, I., Arabloo, J., Ashraf, T., et al. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: An analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health*, 7(2). https://doi.org/10.1016/S2468-2667(21)00249-8

Nisa, K., & Lisiswanti, R. (2016). Faktor risiko demensia Alzheimer. *Medical Journal Of Lampung University*, 5(4).

Prahesti, D. A., Pujiyanti, S., & Rukmi, M. I. (2018). Isolasi, uji aktivitas, dan optimasi inhibitor a-amilase isolat kapang endofit tanaman binahong (Anredera cordifolia (Ten.) Steenis). *Jurnal Biologi*, 7(1).

Primadiamanti, A., Saputri, G. A. R., & Nurhasanah, N. (2023). Uji aktivitas kombinasi ekstrak daun tapak dara (Catharanthus roseus l.g.don) dan ekstrak cabai jawa (piper retrofractum vahl) sebagai antioksidan secara in vivo. *Jurnal Ilmu Kedokteran Dan Kesehatan*, 10(1). https://doi.org/10.33024/jikk.v10i1.8717

Puspaningrum, D. H. D., Sumadewi, N. L. U., & Sari, N. K. Y. (2022). Karakteristik kimia dan aktivitas antioksidan selama fermentasi kombucha cascara kopi arabika (Coffea arabika L.) Desa Catur Kabupaten Bangli. *Jurnal Sains Dan Edukasi Sains*, 5(2). https://doi.org/10.24246/juses.v5i2p44-51

Roshanbakhsh, H., Elahdadi Salmani, M., Dehghan, S., Nazari, A., Javan, M., & Pourabdolhossein, F. (2020). Piperine ameliorated memory impairment and myelin damage in lysolecethin induced hippocampal demyelination. *Life Sciences*, 253. https://doi.org/10.1016/j.lfs.2020.117671

Sari, E. R., & Nugraheni, E. R. (2013). Uji aktivitas antifungi ekstrak etanol daun cabai jawa (Piper retrofractum) terhadap pertumbuhan candida albicans. *Biofarmasi Journal of Natural Product Biochemistry*, 11(2). https://doi.org/10.13057/biofar/f110202

Srihari, T., Karthikesan, K., Ashokkumar, N., & Satyanarayana, U. (2013). Antihyperglycaemic efficacy of kombucha in streptozotocin-induced rats. *Journal of Functional Foods*, 5(4). https://doi.org/10.1016/j.jff.2013.08.008

Suratno, S., Rizki, M. I., & Pratama, M. R. F. (2019). In-vitro study of antioxidant activities from ethanol extracts of akar kuning (Arcangelisia flava). *Jurnal Surya Medika*, 4(2). https://doi.org/10.33084/jsm.v4i2.594

Wang, C., Cai, Z., Wang, W., Wei, M., Kou, D., Li, T., Yang, Z., Guo, H., Le, W., & Li, S. (2019). Piperine attenuates cognitive impairment in an experimental mouse model of sporadic Alzheimer's disease. *Journal of Nutritional Biochemistry*, 70. https://doi.org/10.1016/j.jnutbio.2019.05.009