

# Assessment of the Physicochemical, Nutritional Composition, and Biological Properties of a Novel Vinegar Derived from a Local Thai Champuling Fruit (*Baccaurea polyneura hook.f*)

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## Abstract

Fruit vinegar is globally recognized for its bioactive compounds and is associated with a variety of health-promoting properties. This study investigates the physicochemical and nutritional composition and the biological properties of novel vinegar produced from a local Thai wild fruit called “champuling” (*Baccaurea polyneura Hook.f.*). The total soluble solids (TSS), peak acetic acid, pH, phenolics, flavonoids, and carotenoids, along with the biological properties, including cytotoxicity, anti-adipogenesis, and antibacterial and antifungal activity of champuling vinegar (JCV), were measured. Throughout the fermentation process, characteristic changes were observed, including a reduction in total soluble solids, increased alcohol and acetic acid levels, and a decline in pH. Nutritionally, 100 mL of JCV contained 0.78 g of protein, 18.04 g of carbohydrates, 75.39 kcal of total energy, and significant amounts of vitamin B, predominantly niacin (B3), pantothenic acid (B5), and biotin (B7). JCV also exhibited notable phenolic, flavonoid, and carotenoid compounds, contributing to strong antioxidant capacity as shown by 69.84 and 84.65% inhibition by DPPH and ABTS assays, respectively. In addition to its antioxidant potential, JCV demonstrated antibacterial activity against several pathogenic bacteria by inhibiting *P. aeruginosa*, *E. coli*, and *B. subtilis*. Cytotoxicity tests showed that JCV was non-toxic at a dilution ratio of 1:100 to human proximal tubular cells (HK-2) and mouse fibroblast cells (3T3-L1). At this same dilution, JCV significantly inhibited adipocyte differentiation, suggesting anti-adipogenic potential. However, under conditions of glutamate-induced neurotoxicity using neuroblastoma cells (SH-SY5Y) as a model, JCV did not exhibit neuroprotective effects. This study demonstrated the inclusive evaluation of champuling vinegar (JCV), highlighting its potent antioxidant, antibacterial, and anti-adipogenic effects. These multifunctional bioactivities position JCV as a promising functional food ingredient or nutraceutical for managing metabolic disorders and microbial control. Further studies in animal models and clinical trials are essential to validate its efficacy, safety, and mechanisms, paving the way for its therapeutic development and future applications.

**Keywords:** *Baccaurea polyneura Hook.f.*, Champuling, Fruit vinegar, Functional food, Antioxidant, Antibacterial, Antifungal, Anti-adipogenesis

## Introduction

Vinegar is a functional food produced through the fermentation of fruits and vegetables. It primarily contains acetic acid and other bioactive components,

which vary depending on the base ingredients [1-3]. Vinegar production involves two main processes. First, alcohol is generated through the fermentation of sugar

by yeast. Second, acetic acid is produced by acetic acid bacteria [1,2]. According to the U.S. Food and Drug Administration (FDA), vinegar must contain at least 4% acetic acid, but it can be as high as 8% [4]. Similarly, the acetic acid concentration in fermented and distilled vinegar, as set by the Ministry of Public Health of Thailand, is required to be no less than 4% w/v [5]. Additionally, there can be significant differences in the sensory properties of each type of vinegar, including taste, aroma, and color, which depend on the raw materials used [1-3].

In addition to its widespread culinary applications, vinegar has recently gained attention for its potential health benefits. Research, both *in vitro* and *in vivo*, has shown that fruit vinegar, such as that derived from apples, tomatoes, persimmons, rosehips, nipa palm, and ginkgo, possesses health-promoting properties. These benefits include antioxidant, antiinflammation, antimicrobial effects, inhibition of adipogenesis, and mitigation of LPS-induced neurobehavioral changes [6-10]. Moreover, clinical studies conducted in 2023 demonstrated that, in insulin-resistant diabetes patients, daily consumption of apple cider vinegar (30 mL/day) for more than eight weeks significantly improved fasting blood glucose and lipid profiles [11]. Additionally, daily vinegar ingestion in overweight individuals was associated with improvements in depressive symptoms [12]. There is also emerging evidence suggesting that vinegar could exert a neuroprotective effect against neurodegenerative conditions like Alzheimer's disease [13]. These findings highlight the potential of vinegar to promote metabolic health and contribute to the prevention of chronic diseases [14].

*Baccaurea polyneura* Hook.f. is considered one of the rarest plants within the *Baccaurea* genus [15]. The distribution of this species extends across Malaysia, Sumatra, and parts of Thailand. The fruit of this plant is known by several names, including "cham ri" (Pattani), "cham rai" (Nakhon Si Thammarat), "mafailing", "ka cham pu ling", "champuling" (Trang, Yala, and Narathiwat), and "Jentik-Jentik" (Malaysia) [16-18]. For the purpose of this study, we use the name "champuling". Previous findings on the biological properties of Malaysian *Baccaurea polyneura* demonstrated its high levels of total flavonoids and total phenolics, indicating its strong antioxidant properties

[18]. This is consistent with a study using crude extracts of Thai *Baccaurea polyneura*, which demonstrated the presence of phenolic compounds and beta-carotene, both of which are well-documented for their antioxidant activities [19]. Despite the distribution of the plant and the antioxidant properties of its fruit, research on champuling remains limited. Moreover, no known study has explored the physicochemical, biological properties, and pharmacological effects of vinegar derived from the local Thai champuling (*Baccaurea polyneura*).

As there are currently no known studies on champuling vinegar (JCV), this research was designed to evaluate its physicochemical and nutritional composition, as well as its bioactive properties, including antioxidant, antimicrobial, antifungal, anti-adipogenic, cytotoxic, and neuroprotective effects. The findings from this study may support the potential development of JCV as a functional food and nutraceutical product. Furthermore, the production and application of JCV could provide economic opportunities for rural communities in Thailand, particularly in Narathiwat province.

## Materials and methods

### Source of materials

The champuling fruit was first purchased randomly from a local market in Narathiwat Province, and then subsequently transported to the laboratory at the Faculty of Science and Technology, Princess of Naradhiwas University, Narathiwat, Thailand to be immediately stored in refrigerators for subsequent vinegar preparation.

### Champuling vinegar preparation

The champuling fruit was thoroughly washed, and the pulp, including seeds, was separated and weighed to a total of 500 g, which was then mixed with 500 mL of dH<sub>2</sub>O, maintaining a 1:1 ratio. The mixture was pressed using a hydraulic press (CMC Hydraulic Press Ltd., Thailand). To achieve a total soluble solids (TSS) content of 20 °Brix, sucrose was then added to the mixture, which was then sterilized by heating it at 100 °C for 30 min. The sterilized juice (250 mL) was transferred to a 500-mL sterile flask and allowed to cool to ambient temperature. Subsequently, 5% v/v inoculum of *Saccharomyces cerevisiae* (10<sup>6</sup> CFU/mL) was added to the mixture, and the first phase of fermentation was

carried out at 25 °C until its TSS decreased to 5 °Brix (10 days), after which, the fermentation was stopped using 0.2 g/L potassium metabisulfite. The alcohol content in the mixture was analyzed using an Ebulliometer. In the second phase of fermentation, on day 10, the solution from step 1, with approximately 12% alcohol content, was adjusted to pH 5.5 using a sodium hydroxide solution. A 5% (v/v) inoculum of *Acetobacter aceti* was then added, and fermentation continued in a rotary incubator shaker at 120 rpm and 25 °C. The percentage of acetic acid was analyzed, and the fermentation was terminated when the alcohol content was less than 0.5% (20 days). The final champuling vinegar (JCV) was then used in subsequent experiments. The overall experimental workflow is presented in **Figure 1(C)**.

#### Physicochemical evaluation

The vinegar's pH was measured using a pH meter. The total soluble solids (TSS) were determined using a handheld refractometer, and the alcohol content (alcohol%, v/v) was assessed using an ebulliometer. The percentage of acetic acid was determined by the titrating technique with 0.1 M sodium hydroxide solution. Each experiment was conducted in triplicate, and a Completely Randomized Design (CRD) was applied for the analysis of physicochemical data.

#### Determination of moisture, protein, total fat, carbohydrate, total sugar, total energy, vitamin A, total carotenoid, vitamin C, and vitamin B complex

The proteins, fats, carbohydrates, total sugar, total energy, and moisture content in JCV were determined by the relevant methods of analysis of AOAC International [20]. The content of moisture was determined using AOAC (Loss on Drying at 105 ± 3 °C), total protein was measured by using AOAC (Kjeldahl Method), while the total Fat was determined using AOAC (Acid hydrolysis Method), and the total sugar content was analyzed using the AOAC (2023) 925.35 (B) method. The total carbohydrate and total energy were obtained from the calculation. The contents of all the above components were measured and expressed as weight per 100 mL of JCV. Vitamin A, total carotenoid, vitamin C, and vitamin B complex in JCV were determined by using the in-house method based on chemical and technical assessment (2004),

spectrophotometric method, in-house method TE-CH-177, and in-house method based on Analytica Chimica Acta 569 (2006), respectively.

#### Determination of total phenolic content (TPC) and total flavonoid content (TFC)

The quantification of the phenolic content was determined using the Folin-Ciocalteu phenol reagent/spectrophotometer modified from Yingngam *et al.* [21]. The result was expressed as mg gallic acid equivalent (mgGAE) per 100 g of sample. The total flavonoid content of the vinegar samples was determined using a colorimetric assay modified from Makanjuola [22]. The result was expressed as mg quercetin equivalent (mg QE) per 100 g of vinegar.

#### Analysis of antioxidant activity

##### DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant assay

The antioxidant activity of the JCV was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant assay kit (D678; DOJINDO (Kumamoto, Japan)) according to the manufacturer's protocol. Briefly, a volume of 20 µL of JCV was mixed with 100 µL of DPPH working solution and 80 µL of assay buffer. The reaction was incubated in the dark at 25 °C for 30 min. The absorbance was measured at 517 nm using a microplate reader (CLARIO star plus, BMG Labtech, Ortenberg, Germany). Distilled water was used as a blank, and the DPPH solution with ethanol was used as a control. The percentage of inhibition was calculated using the Eq. (1):

$$\% \text{ Inhibition} = [(A_{cs} - A_s) / A_{cs}] \times 100\% \quad (1)$$

where

$A_{cs}$  = the absorbance of the control-blank and

$A_s$  = the absorbance of the sample-blank

##### (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity assay (ABTS)

For the (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity assay (ABTS), the ABTS<sup>+</sup> radical solution was prepared from potassium persulfate (2.45 mmol/L) and ABTS salt (7 mmol/L) at a ratio of 1:2, then incubated in the dark for

12 h. Each 20  $\mu\text{L}$  of JCV was mixed with 180  $\mu\text{L}$  of ABTS<sup>+</sup> radical solution, and the reaction mixture was incubated in the dark at room temperature for 30 min. The absorbance at 734 nm (CLARIO star plus, BMG Labtech, Ortenberg, Germany) was recorded. Distilled water served as the blank, and the percentage of inhibition was calculated using Eq. (2) as follows:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

where

$A_0$  = the absorbance of the control,

$A_1$  = the absorbance of the sample

### Assessment of the antibacterial and antifungal activity

Pathogenic microorganisms, including *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, were cultured in Nutrient Broth (NB) and Sabouraud Dextrose Broth (SDB) (Himedia™, India). The cultures were incubated at 35 °C for approximately 24 h under aerobic conditions. A tenfold serial dilution ( $10^{-1}$  to  $10^{-6}$ ) was performed to adjust the microbial concentration to 0.5 McFarland standard. The prepared pathogenic microorganisms were then swabbed onto Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (Himedia™, India). The plates were left undisturbed for 20 min to allow microbial absorption into the medium. One milliliter of non-diluted JCV was applied to a sterilized disc filter (6 mm in diameter) and left for 2 min to ensure complete absorption. The disc was then placed on the surface of MHA and SDA plates containing the test microorganisms. Sterile distilled water was used as a negative control. The plates were incubated at 35 °C for 24 h, and the antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (in mm) surrounding the test microorganisms.

### Evaluation of cellular toxicity

Two normal cell lines were employed in this study, namely human kidney proximal tubular epithelial cells (HK-2) and mouse fibroblast cells (3T3-L1). The HK-2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with low glucose, 10% fetal bovine serum (FBS), and antibiotics (penicillin and

streptomycin, 100 mg/mL, 1%), while the 3T3-L1 cells were maintained in DMEM high glucose with 10% FBS, and 1% antibiotics. Both cell lines were incubated at 37 °C with 5% CO<sub>2</sub> until they were used. To evaluate the cytotoxic effects of JCV, cell viability was assessed using the assessment of cell metabolic activity (MTT). In brief, cells were seeded at  $1 \times 10^4$  cells/well in 96-well plates for 24 h, followed by treatment with various dilution ratios of JCV: medium (1:800, 1:400, 1:200, 1:100, 1:40, 1:20 and 1:10). After incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution was added and the cells were incubated in the dark for 4 h. Next, 100  $\mu\text{L}$  of DMSO was used to dissolve formazan crystals. The absorbance was recorded at 570 nm using a CLARIO star plus microplate reader (BMG Labtech, Ortenberg, Germany). Cell viability was calculated using the equation:

$$\% \text{ Cell viability} = [\text{Absorbance sample}/\text{Absorbance control}] \times 100 \quad (3)$$

### Determination of adipogenic differentiation

The 3T3-L1 cells were seeded at a density of  $1 \times 10^5$  cells/mL in a 6-well plate and incubated for 48 h. When the cells reached confluence, they were incubated for an additional 48 h to allow contact inhibition. Next, the cells were stimulated with a differentiation cocktail (MDI) composed of 1  $\mu\text{g}/\text{mL}$  dexamethasone, 1  $\mu\text{g}/\text{mL}$  insulin, and 0.5 mM IBMX, and then treated with JCV at a dilution ratio of 1:100 for 48 h. After incubation, the MDI stimulation was replaced with a medium containing insulin and JCV for another 2 days. The medium and JCV were then changed every 2 consecutive days for up to 10 days. On day 10, differentiated adipocytes, which were identified by the presence of large lipid droplets in the cytoplasm, were observed, stained with Oil Red O, and imaged under a microscope. The amount of lipid content was quantified by dissolving the stained lipid droplets in isopropanol and measuring the absorbance at 500 nm using a microplate reader.

### Glutamate induced neurotoxicity in SH-SY5Y cells

Neuroblastoma cells, SH-SY5Y (ATCC), were maintained in DMEM/ F12 medium (Gibco) supplemented with 10% fetal bovine serum and 1%

penicillin-streptomycin, and incubated at 37 °C with 5% CO<sub>2</sub>. To induce glutamate-mediated neurotoxicity, cells at a density of 1×10<sup>4</sup> cells per well were seeded in 96-well plates and cultured for 24 h. Neurotoxicity was then triggered by treating the cells with 20 mM L- glutamate (Sigma-Aldrich) in serum-free medium for 24 h, with or without JCV at various concentrations. The viability was measured by the MTT assay, and the absorbance was recorded at 570 nm. Data from 3 independent experiments were presented as the mean percentage of the treated groups relative to their respective untreated controls.

### Statistical analysis

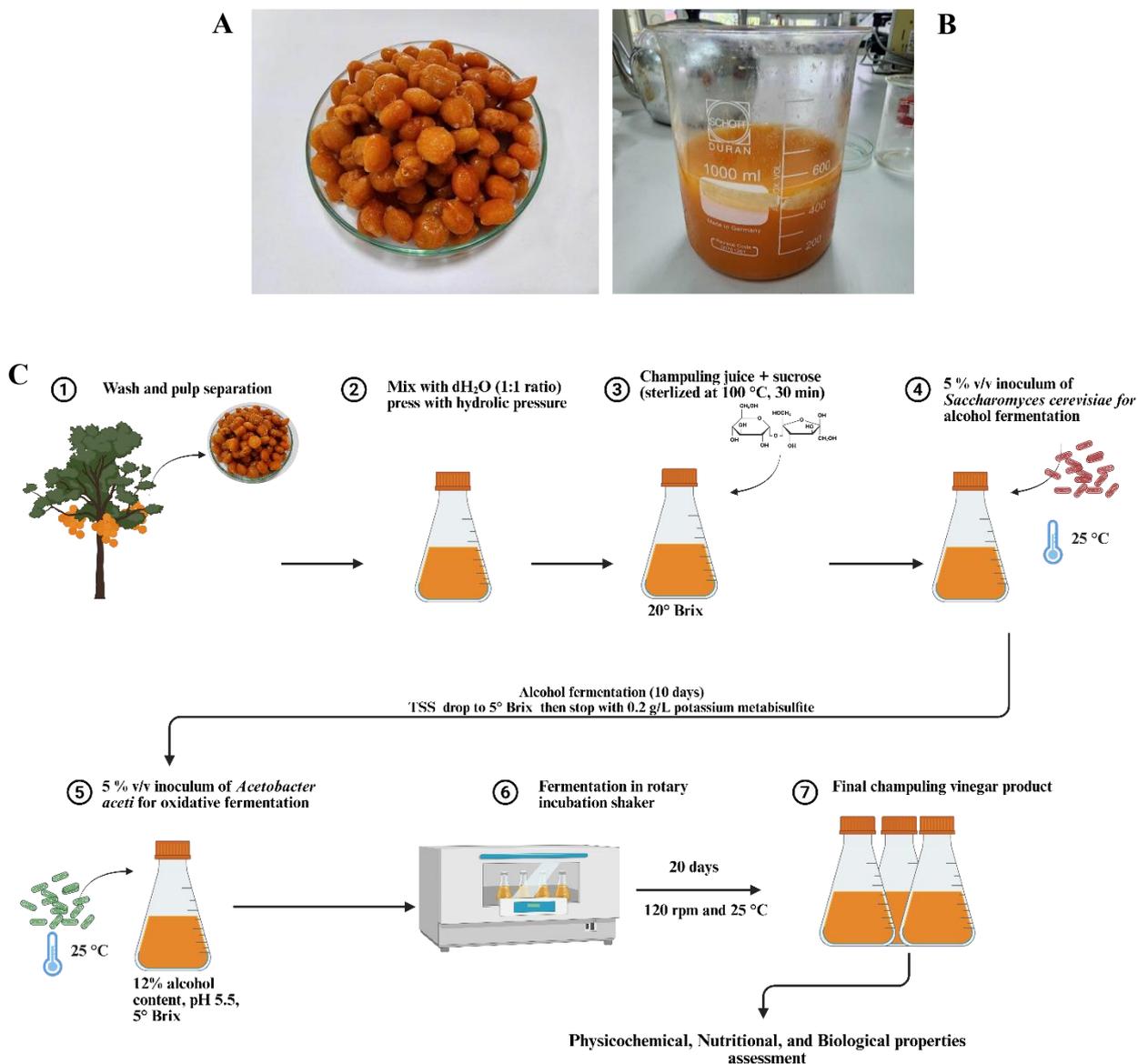
The experimental results were expressed as mean ± standard deviation (S.D). Data analysis was performed using One-way analysis of variance (ANOVA) followed by post hoc comparisons with Duncan's multiple range test (DMRT). A *p*-value less than 0.05 was considered significant. All statistical analyses were conducted using GraphPad Prism software (version 8.0; GraphPad Software, Inc., San Diego, CA, USA).

## Results and discussion

### Physicochemical screening of champuling vinegar (JCV)

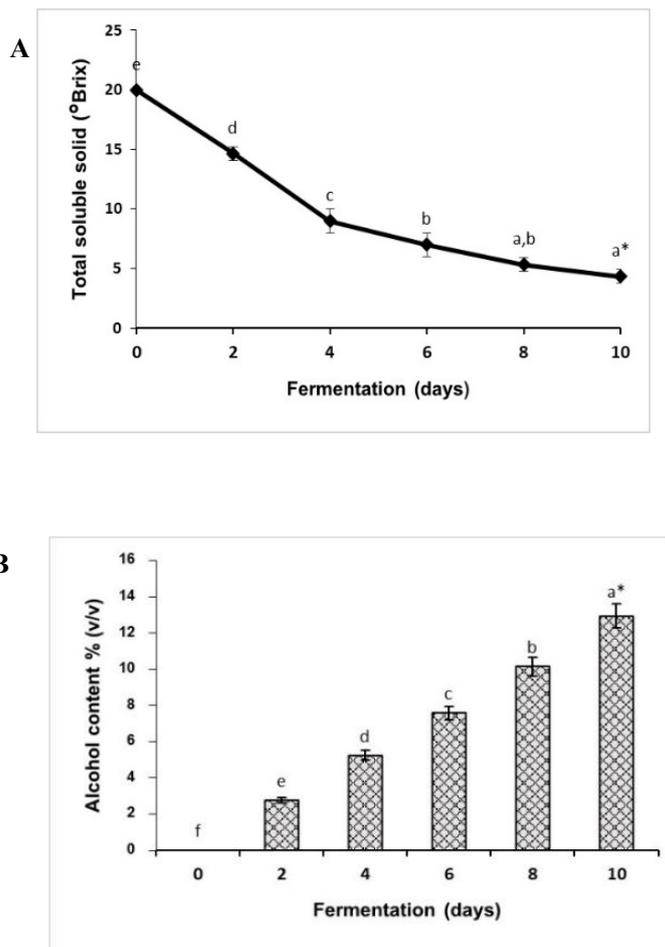
The production of champuling vinegar in this study lasted for 30 days. This process involved fermentation with *Saccharomyces cerevisiae* for an initial 10 days, followed by acetic acid production by *Acetobacter aceti* for an additional 20 days. The overall fermentation process is shown in **Figures 1(A) - 1(C)**. From day 0 to day 2 of the fermentation phase I, no alcohol was detected. As fermentation progressed from day 2 until the end of the 10 days, the alcohol content increased from 2.77 ± 0.17 to 12.94 ± 0.04% (**Figure 2(B)**) corresponding with a significant decrease in total soluble solids, which dropped from 20 to 5.33 ± 0.58 °Brix (*p*-value < 0.05, **Figure 2(A)**). This is because, during alcohol production, the yeast utilizes the sugars in the fermentation medium as substrates for energy metabolism. Next, optimal conditions for acetic acid production were created using a mixture obtained from the initial fermentation phase with 12% alcohol content

and a pH of 5.5, and this was then inoculated with *Acetobacter aceti*. It was found that the bacteria began converting alcohol into acetic acid on day 3 of the fermentation phase II, and this value significantly increased in a time-dependent manner. The maximum acetic acid content was 6.52 ± 0.19%, which was observed on day 18, and this value was maintained until day 20 (*p* < 0.05, **Figure 3**). Typically, peak acetic acid concentrations in vinegar vary depending on the type of vinegar and the raw material used. However, all vinegar must contain a minimum of 4.0% (w/v) according to the Thai fermented vinegar standard (TIS 326/2547), and this percentage can range from 4.0 to 8.0% (w/v) in fruit vinegar [4,5]. For instance, Thai rice vinegar exhibits an acetic acid concentration greater than 4% (w/v), balsamic vinegar 5 - 7% (w/v), wild date vinegar 4.61% (w/v), tomato vinegar 5.14% (w/v), and apple vinegar 5.0% (w/v) [6,23-26]. In this study, the peak acetic acid production of JCV was found to exceed the Thai standard (TIS 326/2547) and fall within normal ranges compared to other fruit vinegars. However, multiple factors may influence the concentration of acetic acid in vinegar, including the ethanol concentration produced by *Saccharomyces cerevisiae*, the evaporation process, temperature alteration, light exposure, and storage procedure [27]. At the end of fermentation (day 30), the pH value of champuling vinegar (JCV) obtained from this study was evaluated for acidity profile, compared with 4 commercial fruit vinegars. The JCV exhibited a pH of approximately 2.42 ± 0.02 (**Table 1**), representing the lowest acidity level among all samples tested. In contrast, the commercial coconut vinegar (CCV) displayed the highest pH at 3.21 ± 0.03. Typically, the pH of fruit vinegars can range from 2.40 to 3.90, influenced by the type of raw material and production methods employed [27]. The notably low pH of JCV, coupled with its high acetic acid content, suggests pronounced acidic characteristics. These properties are advantageous. The acetic acid and low pH not only increased membrane permeability in Gram-negative bacteria, leading to cell death [28-29] but also improved preservative qualities, and more efficient extraction of bioactive compounds during the fermentation process [28-29].

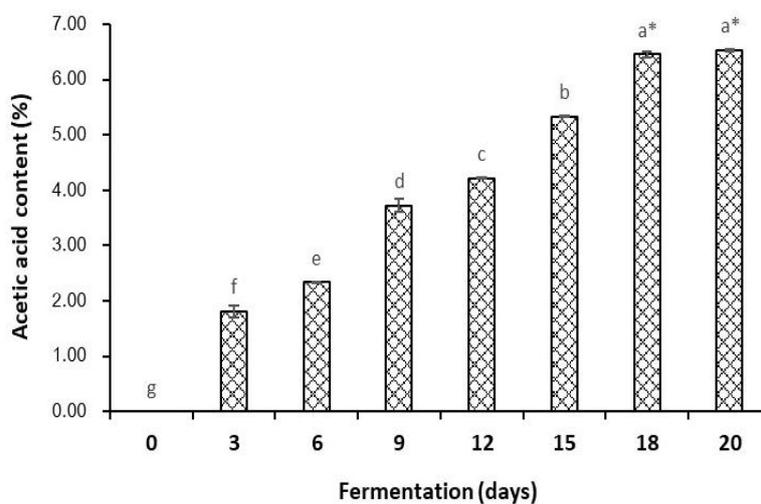


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**Figure 1** Experimental workflow for champuling vinegar (JCV) preparation. The flowchart illustrates the step-by-step procedure. De-shelled champuling fruit (A), champuling juice (B), schematic flowchart for the vinegar fermentation process (C).



**Figure 2** Changes in total soluble solids (A) and alcohol content (%) (B) of champuling liquid after 10 days of fermentation. Data were presented as mean  $\pm$  standard deviation ( $n = 3$ ).  $p$ -value  $< 0.05$  was considered statistically significant. a\* - f = mean for groups in homogeneous subsets was displayed (subset for  $\alpha = 0.05$ ).



**Figure 3** Percentage of acetic acid production in JCV after 20 days of fermentation. Data were expressed as mean standard deviation ( $n = 3$ ).  $p$ -value  $< 0.05$  was considered statistically significant. a\* - f = mean for groups in homogeneous subsets.

**Table 1** Comparative analysis of pH value among JCV and the 4 commercial vinegars.

Sample	pH	<i>p</i> -value (Compared to JCV)
JCV	2.42 ± 0.02	-
MCV	2.83 ± 0.01***	< 0.0001
ACV	2.80 ± 0.02***	< 0.0001
PCV	2.80 ± 0.04***	< 0.0001
CCV	3.21 ± 0.03***	< 0.0001

Data were presented as mean ± SD (n = 5). JCV = Champuling vinegar; MCV = Mulberry vinegar; ACV = Apple cider vinegar; CCV = Coconut vinegar; PCV = Pineapple vinegar. Statistical differences were considered at *p*-value < 0.05. \*\*\* *p*-value < 0.0001.

### Nutritional, total carotenoid, and vitamin composition in champuling vinegar (JCV)

Vinegar is widely used in food consumption and culinary applications. Understanding its nutritional content provides essential information regarding its composition and potential health benefits. Vinegar has been known to contain a range of nutritional constituents, including amino acids, sugars, vitamins, and microelements [30]. As shown in **Table 2**, the nutritional composition of 100 mL of JCV was found to include 0.78 g of total protein, 0.01 g of total fat, 13.20 g of total sugar, 18.04 g of total carbohydrate, 81.02 g of moisture, and 75.39 kcal of total energy. Previous findings have indicated that the sugar constituents of vinegar may exert immunomodulatory, antioxidant, and anticoagulant effects. Polysaccharides in buckwheat vinegar exhibit strong DPPH radical scavenging activity, while the polysaccharides in Korean persimmon vinegar stimulate immune responses in macrophage cells by increasing the production of proinflammatory cytokines and nitric oxide. Additionally, sorghum vinegar can reduce platelet aggregation by inhibiting the synthesis of cyclooxygenase-1 (COX-1) and thromboxane A<sub>2</sub> (TXA<sub>2</sub>) [31]. As reported in **Table 2**, JCV contains 0.40 mg of total carotenoids and 26.67 µgRE of vitamin A, while ascorbic acid (vitamin C) was undetectable. Additionally, JCV contains 131.63 mg of the vitamin B complex, with the highest concentrations of B3 (77.76 mg), followed by B5 (52.25 mg), B7 (1.33 mg), B9 (0.282 mg), and B6 (0.0089 mg). Notably, vitamins B1, B2, and B12 were not detected. These findings align with other studies, which reported the presence of B vitamins in various types of vinegar, including sorghum

vinegar (B1, B2 and B3), oat vinegar (B1, B2 and B3), palm vinegar (B3 and B5), and balsamic vinegar (B3) [30]. The high levels of vitamins B3 (niacin) and B5 (pantothenic acid) in JCV may offer several clinical benefits, as both vitamins play crucial roles in various physiological processes. B3 could lower total cholesterol, LDL-C, apoB, and TG while raising HDL. B3 and B5 could support skin and nerve health, wound healing, and skin repair [32]. According to the Thai Dietary Reference Intakes (DRIs), based on a 2,000-kcal diet, JCV contributes a small portion of daily energy (approximately 3.77%), protein (about 1.56% of the recommended 50 g/day), and carbohydrate and sugar content (6.01% of the recommended 300 g/day). In the context of vitamins, JCV contributes a large portion of the B3, B5, and B7 needed by the body, with approximately 518.4% of the recommended 15 mg NE, 1,045% of the recommended 5 mg, and 4,433% of the recommended 30 µg, respectively [33]. Thus, incorporating JCV with high vitamin B3, B5, and B7 into people's diets or cosmetic products may provide these potential clinical benefits. However, more clinical research is needed to fully understand the impact of JCV and optimize its therapeutic use.

### Determination of total phenolic content (TPC) and total flavonoid content (TFC)

Phenolic compounds and flavonoid compounds are widely recognized for their role in antioxidant activity. The presence of flavonoids and phenolic compounds can contribute to antioxidant activity, anti-inflammatory effects, and other bioactive properties. The screening of total phenolic content and flavonoids in the JCV in this study demonstrated that JCV contains

8.55 ± 0.25 mg GAE/100 g and 30.33 ± 4.74 mg QE/100 g, respectively (**Table 3**). When compared with Liu *et al.* [34]'s findings, whereby 23 fruit vinegars had TPCs of 29.64 - 3,216.60 mg GAE/L and TFCs of 2.22 - 753.19 mg QE/L, JCV was found to fall within the same range, indicating its comparable phenolic and flavonoid content to some commonly consumed fruit vinegars. These results suggest that JCV possesses a promising antioxidant profile. However, it is essential to

investigate the concentrations of individual phenolic compounds as well as the flavonoid compounds within JCV, as different compounds exhibit distinct pharmacological properties. In addition, the synergy between different compounds should also be investigated since the overall potency of a substance would depend not only on the concentration but also on its activity.

**Table 2** Nutritional composition and vitamin content of champuling vinegar (JCV), including vitamin A, total carotenoids, vitamin C, and B-complex vitamins.

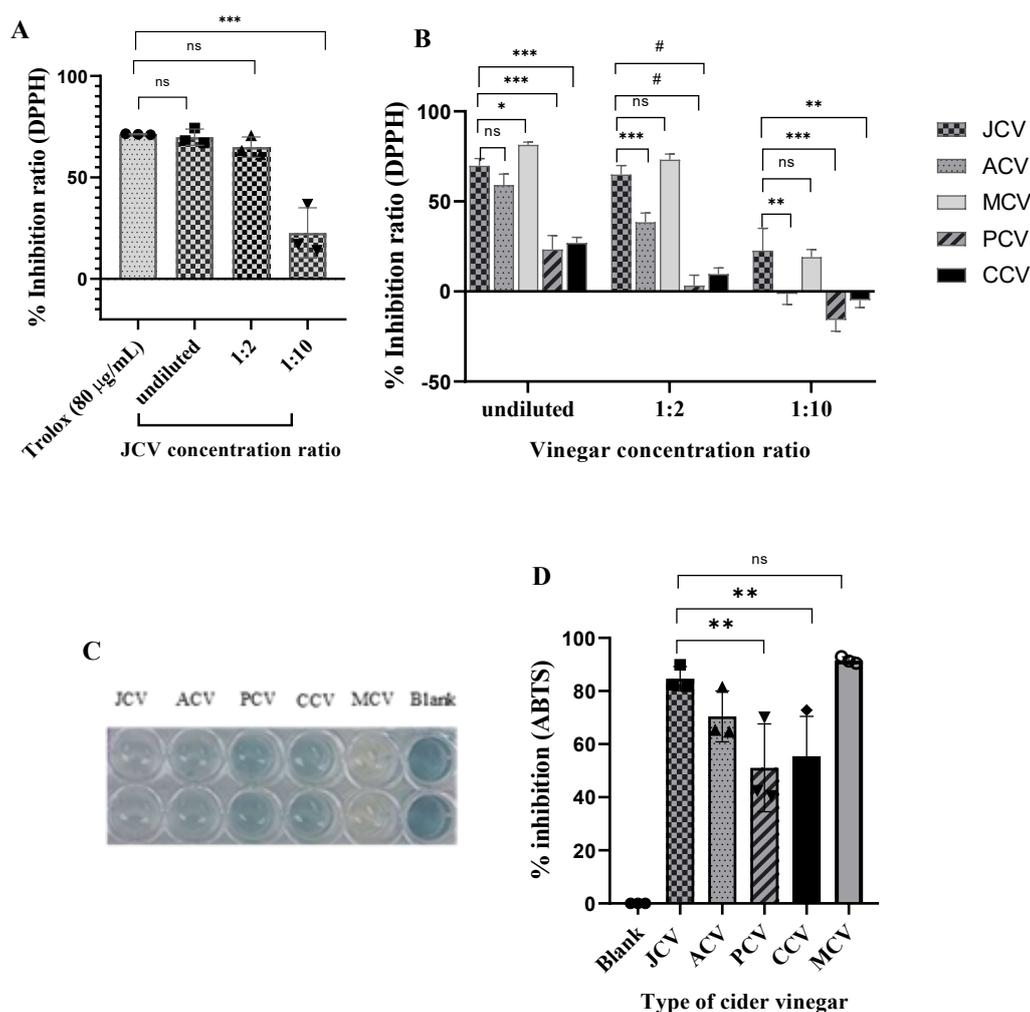
Parameters	JCV	Thai FDA RDI <sup>§</sup> [33]	% Thai FDA RDI
Total protein (g/100 mL)	0.78	50 g	1.56
Total Fat (g/100 mL)	0.01	65 g	0.00024
Total sugar (g/100 mL)	13.20	-	-
Carbohydrate <sup>#</sup> (g/100 mL)	18.04	300 g	6.01
Moisture (g/100 mL)	81.02	-	-
Total Energy <sup>#</sup> (kcal/100 mL)	75.39	2,000 kcal	3.77
Total carotenoid (mg/100 mL)	0.40	-	-
Vitamin A (µgRE/100 mL)	26.67	800 µgRAE	3.33
Vitamin C (mg/100 mL)	Not detect	-	-
B complex (mg/100 mL)	131.63	-	-
B1 (mg/100 mL)	Not detect	1.2 mg	-
B2 (mg/100 mL)	Not detect	1.2 mg	-
B3 (mg/100 mL)	77.76	15 mg NE	518.4
B5 (mg/100 mL)	52.25	5 mg	1045
B6 (mg/100 mL)	0.0089	1.3 mg	0.68
B7 (mg/100 mL)	1.33	30 µg	4433
B9 (mg/100 mL)	0.282	400 µg DFE	70.5
B12 (mg/100 mL)	Not detect	2.4 µg	-

Note: \$ = Recommend amount for total energy of 2,000 kcal, # = The data obtained from the Calculation. Protein conversion factor = 6.25, Thai FDA RDI = Thai Reference Daily Intakes [33]. All data were derived from a single screening measurement of one vinegar sample. The data was obtained from a single screening measurement of the vinegar sample.

**Table 3** Total phenolic content and total flavonoid content in champuling vinegar (JCV).

Sample	Total phenolic (mgGAE/100 g)	Total flavonoid (mgQE/100 g)
JCV	8.55 ± 0.25	30.33 ± 4.74

Note: Data were expressed as mean ± SD (n = 3).



**Figure 4** Antioxidant activity levels of JCV compared to 4 other commercial fruit-based vinegars, assessed by DPPH (A,B) and ABTS (C,D) assays. Data were expressed as mean  $\pm$  SD ( $n = 3$ ).  $p$ -value  $< 0.05$  was considered statistically significant. \*  $p$ -value  $< 0.05$ , \*\*  $p$ -value  $< 0.001$ , \*\*\*  $p$ -value  $< 0.0001$ . JCV = champuling vinegar; MCV = mulberry vinegar; ACV = apple vinegar; CCV = coconut vinegar; PCV = pineapple vinegar.

### Antioxidant analysis

Oxidative stress plays an important role in the development of several diseases, and targeted antioxidant strategies, thereby alleviating oxidative stress and minimizing cellular damage, may improve therapeutic outcomes [35]. As depicted in **Figures 4(A)** and **4(B)**, the antioxidant activity of JCV and the other 4 commercial fruit vinegars was evaluated. The results indicated that undiluted JCV exhibited an inhibition of DPPH radical scavenging activity of approximately  $69.84 \pm 3.97\%$ , reflecting strong antioxidant potential. When compared to other commercial fruit vinegars, specifically mulberry (MCV), apple (ACV), pineapple (PCV), and coconut (CCV), JCV demonstrated a

significantly higher percent inhibition than both coconut and pineapple vinegars. However, no significant difference was observed between JCV and either mulberry or apple vinegar (**Figure 4(B)**). Notably, the antioxidant activity of JCV was also not significantly different from that of trolox at a concentration of  $80 \mu\text{g/mL}$ , suggesting comparable efficacy to this standard antioxidant compound. Regarding the ABTS results, as illustrated in **Figures 4(C)** and **4(D)**, around  $84.65\%$  inhibition was found in JCV treatment, which is slightly lower than the % inhibition of mulberry vinegar, while higher than that of apple, pineapple, and coconut vinegar. The strong antioxidant properties of JCV, linked to its phenolic and flavonoid content, are

consistent with the findings of Duan *et al.* [36], who reported a strong positive correlation between TPC, TFC, and antioxidant activity. Similar effects were observed in hawthorn and apple cider vinegars [37, 38], while persimmon vinegar polyphenol (PVP) protected HepG2 cells from H<sub>2</sub>O<sub>2</sub>-induced damage by modulating the Nrf2 pathway [39]. In line with these findings, JCV demonstrated strong antioxidant activity in both DPPH and ABTS assays, highlighting its potential in mitigating oxidative stress.

#### Screening of the antimicrobial and antifungal activity

The antimicrobial and antifungal activity of JCV was tested against pathogenic microorganisms, including *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. As shown in **Table 4**, the JCV exhibited the highest inhibition against *P. aeruginosa*, with a clear zone of  $12.33 \pm 0.58$  mm. It also showed

significant inhibition of *E. coli* and *B. subtilis*, with inhibition zones of  $9.0 \pm 1.00$  and  $8.33 \pm 0.58$  mm, respectively. These inhibitions indicate broad-spectrum efficacy. However, no inhibition was observed against *S. aureus* and *C. albicans*. Acetic acid, vinegar's main bioactive component, exerts antimicrobial effects by disrupting microbial membranes and intracellular pH, leading to cell death [8,40]. It is effective against drug-resistant pathogens such as *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* at 2 - 6% concentrations [41,42], and inhibits biofilm formation and virulence in colistin-resistant *P. aeruginosa* via membrane damage and oxidative stress [28]. Other organic acids and phenolics also enhance vinegar's antimicrobial activity [43,44]. This study revealed that JCV possesses potent antibacterial activity, especially against *P. aeruginosa*, *E. coli*, and *B. subtilis*. The mechanistic effects on its antibacterial activity are likely due to the presence of high acetic acid content, low pH level, and phenolic compounds.

**Table 4** Clear zone on the inhibition of pathogenic microorganisms after JCV treatment.

Sample	Inhibition zone (mm)				
	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
Negative control	-	-	-	-	-
JCV	$12.33 \pm 0.58$	$9.00 \pm 1.00$	$8.33 \pm 0.58$	-	-

Note: Data were expressed as mean  $\pm$  SD (n = 3); - indicates no inhibitory effect.

#### Assessment of the cytotoxic effect of JCV on mouse fibroblast cells (3T3-L1) and human proximal tubular cells (HK-2)

The cytotoxic effects of JCV on 2 types of normal cell lines, the 3T3-L1 (mouse fibroblast cells) and HK-2 (human proximal tubular cells), were tested. The toxicity of JCV at various concentrations over 24 h was investigated by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay). As indicated in **Figures 5(A)** and **5(B)**, the JCV at concentration ratios of 1:40, 1:20 and 1:10, which were over 1% vol/vol, significantly affected the survival of both cell types. In contrast, the concentration ratios of 1:800, 1:400, 1:200 and 1:100 were not toxic to the cells. These results align with a previous study, which used apple cider vinegar as a test compound and found that

concentrations of apple cider vinegar at 5, 2.5 and 1.25% were toxic to fibroblast (3T3 BALB/C) cells, reducing cell viability below 70% [45].

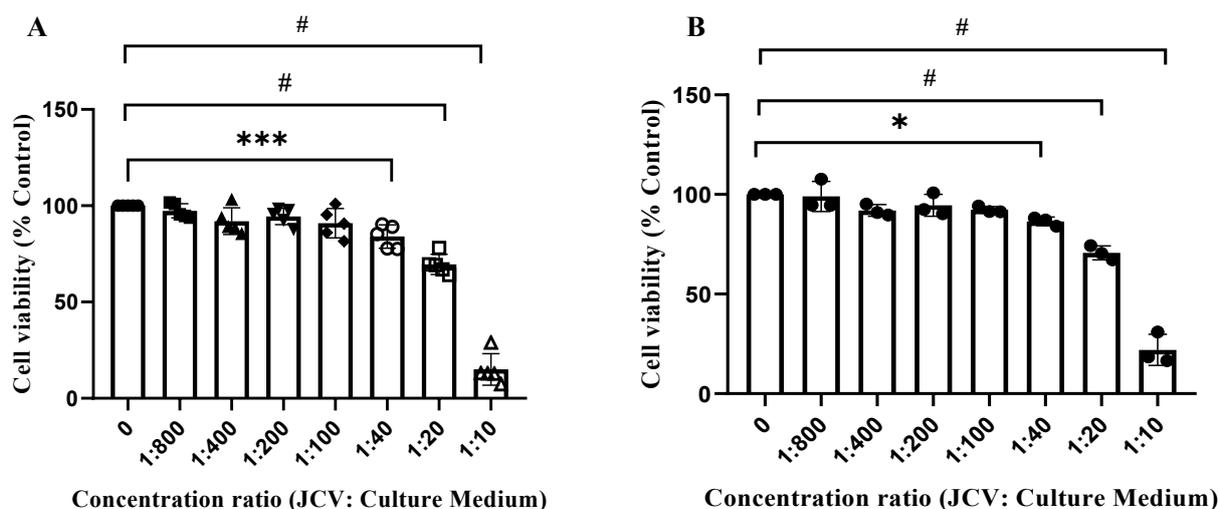
#### Effects of JCV on adipogenesis

Adipogenesis is the process by which preadipocytes differentiate into mature adipocytes, playing a key role in fat storage and adipose tissue development. This complex process is regulated by transcription factors, signaling pathways, and enzymes. Impaired adipose tissue function is associated with metabolic disorders, cardiovascular diseases, and cancers [46]. Interestingly, after 10 days of treatment, the control cells (without JCV) in this study showed a significant increase in mature adipocytes, as indicated by abundant intracellular lipid droplets (**Figure 6(A)**).

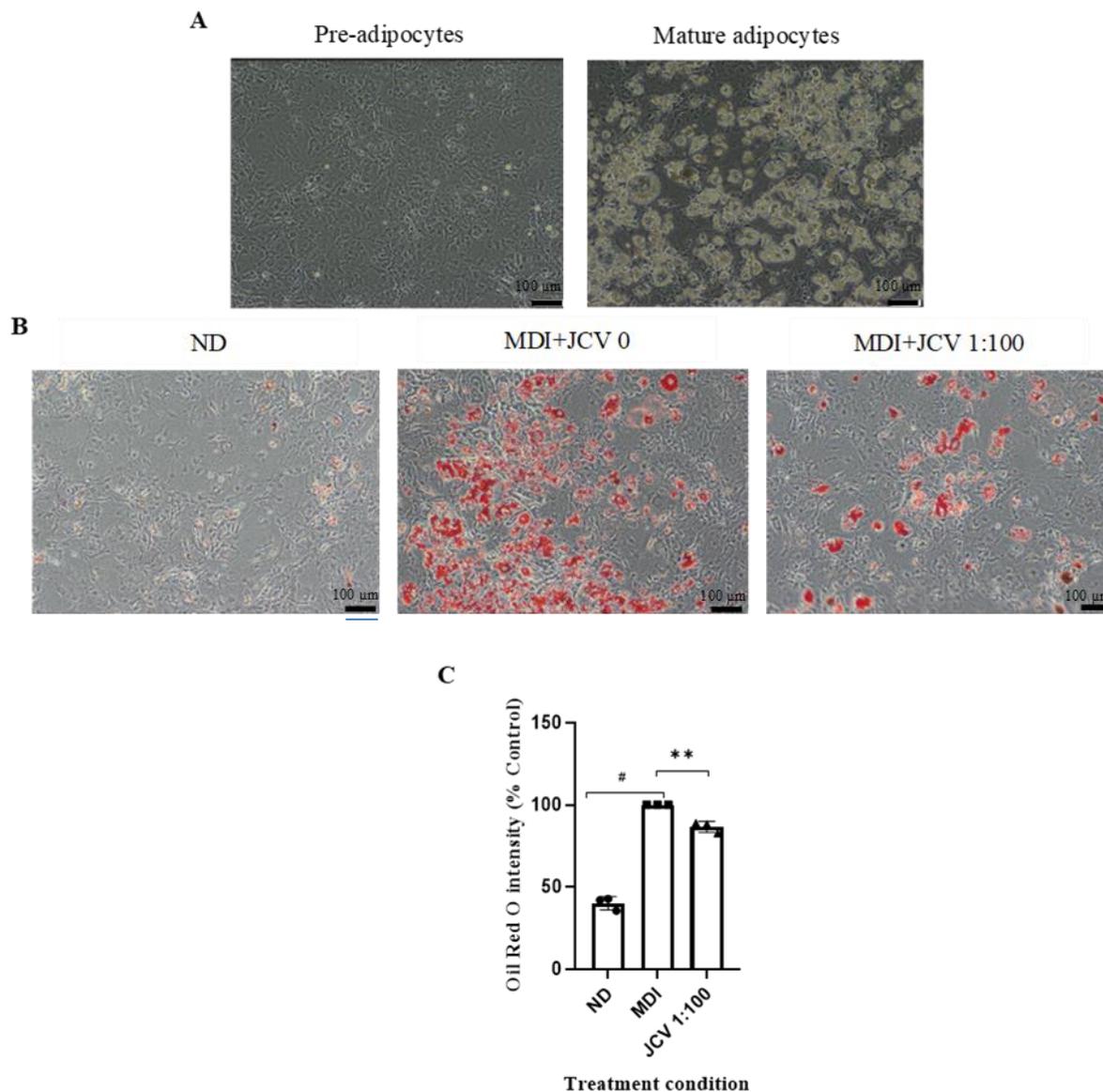
In contrast, the JCV-treated cells exhibited markedly reduced adipogenesis. Oil Red O staining (**Figure 6(B)**) and absorbance quantification (**Figure 6(C)**) confirmed decreased lipid accumulation in the JCV-treated cells. These results suggest that JCV effectively inhibits adipogenesis. This finding aligned with the study by Hosoda *et al.* [9], who reported that ginkgo vinegar suppressed adipogenesis in 3T3-L1 cells and high-fat diet-fed mice by downregulating C/EBP and PPAR expression and reducing lipid accumulation. In 2022, Lim and colleagues demonstrated that treatment with 3% Aronia vinegar (AV) not only reduced lipid accumulation and downregulated PPAR- $\gamma$  and C/EBP $\alpha$  expression in 3T3-L1 cells but also decreased fat mass and serum lipid levels in an animal model [47]. Thus, JCV's anti-adipogenic effect may involve targeting and inhibiting key transcription factors like PPAR $\gamma$  and C/EBP $\alpha$ , highlighting a mechanism that requires further study.

### Neuroprotective potential against glutamate-induced toxicity in SH-SY5Y cells

Glutamate is the main excitatory neurotransmitter in the CNS, but excess levels cause excitotoxicity, leading to neuronal death in conditions like Alzheimer's, Parkinson's, stroke, and trauma [48]. Targeting excitotoxicity offers neuroprotection by reducing inflammation and restoring neurotransmitter balance, making it a promising therapeutic approach [48]. As shown in **Table 5**, exposure to 20 mM glutamate significantly decreased cell viability to  $64.48 \pm 0.03$ . Although treatment with JCV at dilution ratios of 1:100, 1:500 and 1:1,000 showed statistically significant differences in cell viability compared to glutamate treatment, the improvement was less than 25% and thus did not qualify as a neuroprotective effect under these conditions.



**Figure 5** Effect of champuling vinegar (JCV) at different concentrations on cell viability of 3T3-L1 cells (A,  $n = 5$ ) and HK-2 cells (B,  $n = 3$ ) after 24-hour exposure. Data were expressed as mean  $\pm$  SD.  $p$ -value  $< 0.05$  was considered statistically significant. \*  $p$ -value  $< 0.05$ , \*\*\*  $p$ -value  $< 0.001$  and #  $p$ -value  $< 0.0001$ .



**Figure 6** Adipocyte formation in 3T3-L1 cells after treatment with JCV, visualized by Oil Red O staining. (A) Representative images showing lipid accumulation in control cells after 10 days of differentiation. (B) Comparison of lipid content between groups. (C) Quantification of intracellular fat based on Oil Red O staining intensity. Data were expressed as mean ± SD (n = 3). \* *p*-value < 0.05, \*\* *p*-value < 0.01, \*\*\* *p*-value < 0.001, # *p*-value < 0.0001. Note: MDI = adipogenic induction medium; ND = no adipogenic induction medium.

**Table 5** Glutamate-induced neurotoxicity assay and neuroprotective effect of JCV.

Treatment groups	% Cell viability	<i>p</i> -value
Control	100	< 0.001
Glutamate (20 mM)	64.48 ± 0.03	-
Glutamate (20 mM) + JCV 1:100	69.83 ± 1.50	0.0364
Glutamate (20 mM) + JCV 1:500	71.42 ± 1.87	0.0080
Glutamate (20 mM) + JCV 1:1,000	79.03 ± 3.77	< 0.0001

All data are represented as Mean ± S.D. (n = 3). *p*-value < 0.05 is a significant difference compared to the glutamate (20 mM) treatment condition.

### Limitations of the study

While this study provides valuable insights into the physicochemical characteristics, nutritional content, and bioactive properties of champuling vinegar (JCV), several limitations should be noted. First, the biological evaluations including antioxidant, antibacterial, and anti-adipogenic effects, were conducted primarily *in vitro*. Although promising, these results may not directly translate to *in vivo* conditions due to the complexity of metabolic processes and interactions within living organisms. Additionally, the lack of observed neuroprotective effects in excitotoxicity assays using a single concentration of glutamate and limited treatment ratios may not fully capture the potential of JCV in other neural models or pathways. Future studies should explore a wider range of concentrations and time points, and include *in vivo* models to validate these findings. Moreover, the specific active compounds responsible for each biological effect were not isolated or identified, which limits our understanding of the mechanisms underlying JCV's functionality. In addition, optimizing the fermentation process and conducting in-depth studies on JCV's flavor profile, aroma, sensory evaluation, shelf-life, fermentation scalability, and consumer acceptance are crucial for enhancing its market potential.

### Conclusions

This study demonstrated that champuling vinegar (JCV) significantly enhances physicochemical properties, phenolic content, antioxidant activity, antimicrobial effects, and anti-adipogenic potential, indicating its promise as a high-quality functional food product. Collaborating with local community cooperatives for JCV production could promote sustainable agriculture, generate additional income for rural communities in Narathiwat, and position JCV as a valuable product in both local and global vinegar markets.

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### Declaration of Generative AI in Scientific Writing

The author (s) hereby declare that the language used in this document has been assisted by Grammarly and ChatGPT. The AI was utilized to provide linguistic support, including grammar correction and vocabulary suggestions. However, the authors confirm that the final content reflects their understanding and contributions.

### CRedit Author Statement

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