Model Aren Vinegar (Arenga pinnata Merr.) Phytochemical Analysis and Hypoglycemic Effects in Streptozotocin-Niacinamide-Induced Rats

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Keywords: Streptozotocin, Niacinamide, Hypoglycaemic Agent, Fermented Food.

Abstract: Fermented foods or drinks have potentials to prevent type 2 diabetes. Vinegar is a fermented product that has been used empirically for treating various diseases, including DM. The use of aren vinegar is rarely investigated. Meanwhile, aren vinegar as a hypoglycaemic agent has not been widely explored. This study aimed to determine the phytochemical content and the effect of aren vinegar on hyperglycaemia conditions. A total of 30 rats, divided into three treatment groups, were used in this study. The hyperglycaemia model was obtained by the intraperitoneal induction of Streptozotocin-Niacinamide in male Wistar rats. Phytochemical analysis shows that aren vinegar contains flavonoid compounds (1.03 mg/100 g QE) and phenolic compounds (111.62 mg/100 g GAE). After receiving aren vinegar for four weeks, blood glucose levels in hyperglycaemic mice dropped by 3.21%. One-way ANOVA and post hoc LSD tests used in the statistical analysis of blood glucose levels show a significant difference between the aren-vinegar group and the control group ($p < 0.001$). Aren vinegar has a hypoglycaemic effect as it produces flavonoid and phenol compounds.

1 INTRODUCTION

Diabetes mellitus (DM) is a global health issue causing a rapidly increasing prevalence. The International Diabetes Federation (IDF) organization estimated that at least 463 million people aged 20–79 years in the world suffered from diabetes in 2019, equivalent to a prevalence rate of 9.3% of the total population at the same age. Indonesia itself is ranked 7th among the ten countries with the highest number of diabetes prevalence (Pangribowo, 2020). DM is a metabolic disorder resulting from interrupted insulin secretion, insulin action, or both. Diabetes is the state of high plasma glucose levels (fasting plasma glucose (FPG) of 126 mg/dL) (Fakhruddin et al., 2017) or blood sugar levels two hours after eating (2-h PG) of 200 mg/dL during an OGTT (oral glucose tolerance test) or an A1C of 6.5% (48 mmol/mol). Diabetes is a condition where a hyperglycemic crisis occurs with randomized plasma glucose of 200 mg/dL (11.1 mmol/L) (American Diabetes Association, 2020). Insulin deficiency triggers chronic hyperglycemia with disturbances in carbohydrate, fat, and protein metabolism (Pizzino et al., 2017). Factors that contribute to hyperglycemia are decreased insulin secretion, decreased glucose utilization, and increased glucose production. Lifestyle interventions for risk prevention of type 2 diabetes have been explored in several studies. One of the lifestyle interventions, such as the use of fermented foods, is known to have a good effect on health.

Vinegar is a fermented food product that contains sugar. One of the plants that produces vinegar is the Aren plant (Arenga pinnata Merr.). Aren contains various secondary metabolites and has an antioxidant activity. Aren roots contain flavonoids, alkaloids,

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Indrayoni, P., Ananta, I. and Padmiswari, A.
Model Aren Vinegar (Arenga pinnata Merr.) Phytochemical Analysis and Hypoglycemic Effects in Streptozotocin-Niacinamide-Induced Rats.
DOI: 10.5220/0011939200003576
In *Proceedings of the 2nd Bali Biennial International Conference on Health Sciences (Bali BICHS 2022)*, pages 64-68
ISBN: 978-989-758-625-5

steroids, tannins, saponins, anthraquinones, and terpenoids (Zainudin et al., 2015). The ethanol extract of aren seeds is known to contain flavonoids, triterpenoids, saponins, and tannins (Arief et al., 2017).

Tapping male flower sap of aren plants can produce sugar, which can turn into vinegar, drink and alcohol (Lempang, 2012). The fermentation of sap into aren wine generally lasts for a day with a mix of yeast (Saccharomyces) and bacteria such as Lactobacillus (Mussa, 2014). In aren wine, lactic alcohol acetate fermentation occurs spontaneously by involving lactic acid bacteria, yeast, and acetic acid bacteria (Pradnyandari et al., 2017). Vinegar is produced by the spontaneous fermentation of aren sap over time.

Vinegar has been proven to lower blood glucose. Types of vinegar used for diabetes treatment are produced by salak, apple, grape, pineapple skin, sugar cane, coconut, and palm (Soltan & Shehata, 2012; Gheflati et al., 2019; Hermawati, Sitasiwi, Jannah, 2020). Vinegar has a glucose-lowering effect on patients with glucose abnormalities, but the mechanism of this effect is unclear. Although some studies confirm that aren vinegar is practically useful, further investigation on whether aren vinegar is potential as a hypoglycemic agent has not received much attention. Therefore, this study aimed to ascertain the phytochemical composition, antioxidant capacity, and hyperglycemic effects of aren vinegar. The chemical content of aren vinegar was determined through phytochemical screening. The DPPH method was used to identify antioxidant activity in aren vinegar, while the effect of hypoglycemia was determined by measuring the blood glucose of experimental animals. This study used hyperglycemic albino Wistar rats that were given aren vinegar for 28 days. The last day of the experiment was the period of measuring the rats' blood glucose levels.

2 MATERIALS AND METHODS

2.1 Materials and Chemicals

Aren sap is obtained from Buleleng district, Bali. The fermentation of sap into vinegar was carried out spontaneously at a room temperature. The reagents used included Folin-Ciocalteau's, methanol (E. Merck), $CaCO_3$, $AlCl_3$, acetate buffer, gallic acid (E. Merck), quercetin, Streptozotocin (Sigma-Aldrich), niacinamide (Sigma-Aldrich), citrate buffer, and aqueous solution NaCl 0.9%.

2.2 Phytochemical Screening

Alkaloids, saponins, flavonoids, phenols, and amino acids are the phytochemicals in aren vinegar under investigation. Using Mayer's reagent, the alkaloid content was determined by looking at the appearance of a white precipitate which indicated the presence of alkaloids. When the sample was shaken for 15 minutes, the procedure produces 2-cm foam that contained saponins. The presence of flavonoid content was determined using the alkaline reagent assay. While the presence of protein and amino acid content was assessed using the ninhydrin test, the phenol content test was conducted by administering $FeCl_3$ solution (De Silva et al., 2017).

The overall phenol and flavonoid in the vinegar were measured using Widodo's method with slight modifications (Widodo, Sismindari, Asmara, & Rohman, 2019). A 40 µl of vinegar (1 mg/ml; 1 mg dissolved in 1 ml methanol) was mixed with 360 µl of distilled water; 100 µl of Folin-Ciocalteau and the solution were shaken and left for two minutes. The reaction was neutralized using 500 µl of 10% $CaCO_3$ and mixed until the solution was homogeneous. The mixture was incubated for 20 minutes at 40°C. A 150-µl test solution was included in the microplate, and the absorbance was measured at a wavelength of 732 nm. The total phenol content was expressed as mg of gallic acid equivalent to g of the extract through linear regression prepared from gallic acid at various concentrations (0, 5, 10, 15, 20, and 25 µg/ml).

The total flavonoid content was measured by a mixture of 100 µl vinegar, 150 µl solution of 0.1 M $AlCl_3$ (blank without $AlCl_3$ and replaced with methanol 150 µl), 350 µl of aquadest, 250 µl acetate buffer (pH 3.8), and added with methanol up to a total volume of 1,250 µl. The test solution was incubated at 35°C for 30 minutes, and the absorbance was measured with a UV-Vis spectrophotometer at 435 nm. Total flavonoid content was expressed as quercetin equivalents per g extract by generating a standard curve with a series of concentrations from 0 to 100 µg/ml of quercetin.

2.3 Antioxidant Activity

The 0.4 mM DPPH solution was obtained by dissolving 15.8 mg of DPPH in 100 mL of methanol, and 1 mL of the solution was taken. It was mixed with 4 mL of the extract. The standard blank solution used methanol and quercetin. The test solution was shaken and remained to stand at a room temperature for 30 minutes (Permatasari et al., 2019). The absorbance was observed at a wavelength of 515.5 nm using a

UV-visible spectrophotometer (Biochrome SN 133467).

2.4 Hyperglycemia Induction

The hyperglycaemia model was obtained through the induction of streptozotocin-niacinamide according to the Furman method (2015). Niacinamide was dissolved in a 0.9% NaCl solution to a concentration of 230 mg/ml. As much as 32.5 mg streptozotocin (STZ) was put in a microcentrifuge tube and covered with aluminum foil. Next, inject niacinamide i.p at a dose of 230 mg/kg (1 ml/kg). Niacinamide injection was performed for 15 minutes before STZ administration. After niacinamide injection, STZ solution was immediately dissolved in 50 mM sodium citrate buffer (pH 4.5) at a concentration of 32.5 mg/ml. Then the STZ solution was injected i.v. at a dose of 65 mg/kg (2 ml/kg).

2.5 Determination of Rat Blood Glucose Levels

This study used 24 male albino Wistar rats weighing 150–200 grams. The inclusion criteria of the experimental animals were rats with blood glucose levels of > 150 mg/dl and rats without any anatomical abnormalities. Sick mice, those with passive movement, and those died during treatment were excluded from the samples. The blood glucose levels of experimental animals were determined by the glucose oxidase biosensor method using a commercial glucometer kit. Measurements were made after the rats had fasted for 12 hours. The rats' blood samples were taken from the lateral tail vein and dripped into a glucometer strip.

3 RESULTS

The results of the phytochemical screening of aren vinegar are shown in Table 1 and 2.

Table 1: Phytochemical screening of aren vinegar.

Content Test	Reagent	Results
Fenol	$FeCl_3$	+
Amino acid/protein	Ninhydrin	-
Flavonoid	Alkaline reagent	+
Alkaloid	Mayer	-
Saponin		-

Table 2: Total flavonoid, total phenol and DPPH test of aren vinegar.

Average Flavonoid Content (mg/100g QE)	Average Phenol Content (mg/100 g GAE)	IC 50	Antioxidant Activity
1,03	111,62	7.879,43	Weak

The hypoglicemic activity of aren vinegar is shown in table 3.

Table 3: Blood glucose levels.

		Group		
		I	II	III
Average Blood Glucose Levels (mg/dL)	Day 0	267.5 ± 26.30	233.75 ± 34	99 ± 16.59
	Day 7	255.75 ± 38.46	230.75 ± 29.81	101.75 ± 15.88
	Day 14	250.75 ± 36.18	230.25 ± 30.58	103.25 ± 16.5
	Day 21	245.75 ± 35.76	229 ± 28.90	105.5 ± 16.30
	Day 28	235.75 ± 30.48	226.25 ± 30.99	105.75 ± 15.84
Differences in Blood Glucose Levels (%)		11.87	3.21	6.82

Group I: group of hyperglycemic rats given glibenclamide
Group II: group of hyperglycemic rats given 1 mL of aren vinegar
Group III : a group of normal rats given aquadest

4 DISCUSSION

Phytochemical screening shows the presence of phenolic and flavonoid compounds in aren vinegar (Table 1). An alkaline reagent test was used to determine the presence or absence of flavonoids in aren vinegar. Aren vinegar dripped with NaOH became a solution with an intense yellow color. Then, it became colorless when added with dilute acid, indicating the presence of flavonoids. The phenol content test was carried out by dripping FeCl3 solution, and the appearance of a bluish-black color indicated the presence of phenol. The continuation of the phytochemical screening was aimed to determine the levels of total flavonoids and phenols in aren vinegar. In addition to determining the total levels of

flavonoids and phenols, the antioxidant activity in aren vinegar was weak according to the IC50 test results.

Aren contains various secondary metabolites and have antioxidant activity. Aren sap contains water, carbohydrates, ash, protein, fat, and organic acids (citric, tartaric, malic, succinic, lactic, fumaric, and pyroglutamic acids) (Karouw and Lay, 2006). Spontaneous fermentation of aren sap from time to time will produce vinegar, with acetic acid as the main component.

The aren-vinegar group experienced a decrease in blood glucose levels. Vinegar might serve as a protective measure to avoid excessive body weight gains and high plasma concentrations of glucose, triglycerides and cholesterol (Dios Lozano et al., 2012). Vinegar ingestion may enhance satiety (Darzi et al., 2013).

Glucose metabolism was likely affected by the consumption of vinegar. Many studies on vinegar consumption support this finding. For instance, the study of Hu et al. (2020) found that butyric acid and acetic acid can increase islet and beta cell viability. At a concentration of 1 mM, these two short-chain fatty acids can stop apoptosis, decreased viability, mitochondrial dysfunction, and the overproduction of ROS and NO caused by streptozotocin. According to Gheflati et al. (2019), individuals with diabetes and dyslipidaemia who took apple cider vinegar had a lower glycaemic index and less oxidative stress. According to a study by Soltan and Shehata (2012), diabetic rats were benefited from receiving several vinegars for six weeks, including apple cider vinegar, wine vinegar, cane vinegar, coconut vinegar, palm vinegar, and artificial vinegar. These types of vinegar also have a hhypocholesterolaemia effect.

5 CONCLUSIONS

In conclusion, aren vinegar contains flavonoid and phenolic compounds, while its antioxidant activity is classified as weak. Aren vinegar also has a hypoglycaemic activity. Further testing is needed to determine the chemical content of aren vinegar and its mechanism in lowering blood glucose levels.

ACKNOWLEDGEMENTS

The authors would like to thank the Institute Technology and Health Science Bali (ITEKES Bali) for giving financial support.

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