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Antidiabetic Activity of Asam Paya (Eleiodoxa conferta) Fruit Vinegar through α-Amylase Inhibition: A Spectrophotometric Study

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Abstract
Diabetes mellitus remains a growing global health burden, affecting an estimated 537 million adults in 2021 and projected to reach 783 million by 2045. Controlling postprandial hyperglycemia through inhibition of the α-amylase enzyme is one accessible non-pharmacological strategy. Asam paya (Eleiodoxa conferta), a swamp palm fruit native to West Kalimantan, Indonesia, is traditionally processed into fruit vinegar and contains acetic acid and secondary metabolites with potential enzyme-inhibitory activity. This study determined the antidiabetic activity of asam paya fruit vinegar as an α-amylase inhibitor using a spectrophotometric method. A quasi-experimental design with purposive sampling was used. Vinegar was produced by sequential alcoholic and acetic fermentation (11 days) of asam paya fruit juice, then evaluated at five concentrations (1000–5000 ppm) with five replicates each (n = 25). Vinegar quality (sugar, acetic acid, pH) and phytochemical content (flavonoid, tannin, alkaloid, saponin) were assessed. α-Amylase inhibition was measured by the starch–iodine (Fuwa) method at λmax 580 nm using UV-Vis spectrophotometry, with acarbose as positive control; IC50 was determined by linear regression.The vinegar contained 5% sugar, 4.56% acetic acid, and pH 5.20, meeting SNI 01-3711-1995 vinegar quality standards. Phytochemical screening detected tannin and saponin but not flavonoid or alkaloid. The regression equation for vinegar was y = 0.0014x + 20.833 (R^2 = 0.9852), giving IC50 = 20,833.6 ppm, while acarbose showed y = 0.0094x + 49.707 (R^2 = 0.9824), IC50 = 32 ppm. Asam paya fruit vinegar met national vinegar quality standards but showed only very weak α-amylase inhibitory activity (IC50 > 200 ppm) relative to acarbose, most plausibly reflecting its low acetic acid content and the limited secondary metabolite profile retained after acetic fermentation.

Keywords: Diabetes Mellitus; Eleiodoxa conferta; Fruit Vinegar; α-Amylase; IC50

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder in which insulin cannot be used effectively to regulate blood glucose, resulting in hyperglycemia. The International Diabetes Federation estimated that 537 million adults were living with diabetes worldwide in 2021, a figure projected to reach 783 million by 2045, placing Indonesia among the countries with the largest burden of cases [1]. Type 2 DM accounts for roughly 90–95% of cases in Indonesia and is characterized by impaired insulin production, impaired insulin action, or both, and is closely linked to unhealthy diets and sedentary lifestyles [2].

One accessible strategy to manage postprandial hyperglycemia is inhibition of α-amylase, the enzyme that hydrolyzes starch into simple sugars in the digestive tract; slowing this process blunts the post-meal glucose spike [3]. Acarbose, a synthetic α-amylase/α-glucosidase inhibitor, is widely used for this purpose but is associated with gastrointestinal side effects, motivating interest in herbal alternatives with milder profiles. Vinegar, a fermented product rich in acetic acid, has repeatedly been shown to attenuate postprandial glucose and insulin responses in clinical studies, and a systematic review of dietary acetic acid supplementation reported measurable reductions in fasting blood glucose in adults with type 2 diabetes [4]. At the enzymatic level, organic acids identified in commercial vinegars — including acetic, citric, and tartaric acid — have been shown to inhibit α-amylase and α-glucosidase in vitro, although acetic acid itself was the weakest inhibitor among the acids tested [5].

Asam paya (Eleiodoxa conferta), locally known as asam kelubi, is a swamp-dwelling palm fruit endemic to peat and freshwater swamp forests of Kalimantan and Sumatra. It is traditionally consumed fresh, made into sweets, or used as a folk remedy for mouth ulcers. A recent nutritional and bioactivity assessment reported that E. conferta fruit extracts possess antioxidant capacity together with inhibitory activity against α-amylase and α-glucosidase [6], and the fruit is a source of potassium, calcium, iron, and vitamin C [7]. Other Kalimantan studies have reported strong DPPH radical-

scavenging activity in asam paya fruit extracts [8], supporting its traditional standing as a functional food.

Because the carbohydrate content of asam paya makes it suitable for vinegar production, and because fruit vinegars from other locally consumed sources have shown antidiabetic promise — salak and apple vinegar lowered blood glucose in alloxan-induced diabetic rats by 38.4% and 33.1%, respectively [9], while nipa palm vinegar inhibited α-glucosidase and α-amylase with IC50 values of 144.5 and 90.3 mg/mL [10] — it is of interest to determine whether asam paya fruit vinegar exhibits comparable antidiabetic potential. This study therefore aimed to evaluate the antidiabetic activity of asam paya fruit vinegar as an α-amylase inhibitor at concentrations of 1000–5000 ppm using a spectrophotometric method.

Materials and methods

Study design and sample

This study used a quasi-experimental design with a purposive sampling technique [12]. The study population was asam paya fruit vinegar prepared from fruit meeting predefined ripeness criteria (intact skin, reddish-brown color). The sample consisted of the vinegar tested at five concentrations — 1000, 2000, 3000, 4000, and 5000 ppm — each with five replicates, giving 25 experimental units. The number of replicates was calculated using the Federer formula, $(r - 1)(t - 1) \geq 15$, where t is the number of treatment groups (5); this yielded $r \geq 4.75$, rounded up to 5 replicates per group. The study was conducted from August 2024 to July 2025 at the Chemistry Laboratory and Clinical Laboratory of Poltekkes Kemenkes Pontianak, and at the Chemistry and Research-Biotechnology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, Pontianak.

Preparation of asam paya fruit vinegar

Fresh asam paya fruit (5 kg) was collected from Dusun Sungai Kantuk, Sepauk, Sintang Regency, West Kalimantan, and identified at the Biology Laboratory, FMIPA Universitas Tanjungpura. The pulp was separated from skin and seeds, blended with water, and filtered to obtain the juice. The juice (2 L) was mixed with 20% sucrose and heated until dissolved, cooled to room temperature, inoculated with 15 g of tape (fermented cassava) yeast, and fermented anaerobically in sealed glass bottles covered with cotton and gauze for 11 days to yield the fruit vinegar.

Vinegar quality tests

Sugar content was measured with a hand refractometer. Acetic acid content was determined by acid–base titration with 0.1 N NaOH (standardized against oxalic acid) using phenolphthalein indicator, following SNI 01-3711-1995 for fermented vinegar [11]. pH was measured with a calibrated pH meter. Phytochemical screening for flavonoid, tannin, alkaloid, and saponin content was performed using standard colorimetric and precipitation reactions [14].

α-Amylase inhibition assay

Inhibitory activity against α-amylase was assessed by the starch–iodine (Fuwa) method [13]. Briefly, 1% starch substrate was incubated with α-amylase enzyme and phosphate buffer (pH 6.9) in the presence of the test sample (vinegar or acarbose at 1000–5000 ppm), the reaction was halted with 1 N HCl, and residual starch was visualized by adding iodine–iodide indicator to form a blue–purple complex. Absorbance was read at the wavelength of maximum absorbance (determined to be 580 nm) on a UV-Vis spectrophotometer. A blank (substrate and enzyme without inhibitor) was used as reference. Percentage inhibition was calculated as: $\%\text{inhibition} = [1 - (\text{Abs sample} / \text{Abs blank})] \times 100\%$. A calibration curve of starch standards (0.1–0.5%) was constructed to confirm assay linearity. For each sample type, percentage inhibition was plotted against concentration and fitted by linear regression ($y = a + bx$); IC50 was calculated as $(50 - a)/b$ [15]. Acarbose (1000–5000 ppm) was tested in parallel as the positive control.

Data analysis

Data were analyzed univariately using descriptive statistics (mean, range) to characterize inhibitory activity across concentrations, and linear regression was used to estimate the IC50 of the vinegar and of acarbose. IC50 values were interpreted using the categorical inhibitor-strength scale of Mardawati et al. (very strong, IC50 < 50 ppm; strong, 50–100 ppm; moderate, 100–150 ppm; weak, 150–200 ppm; very weak, > 200 ppm).

Results

Botanical determination confirmed the collected fruit as Eleiodoxa conferta (Griff.) Burret. The vinegar quality assessment showed a sugar content of 5%, an acetic acid content of 4.56%, and a pH of 5.20 — values consistent with the SNI 01-3711-1995 requirements for fermented table vinegar [11].

Phytochemical screening

Phytochemical screening detected tannin and saponin in the vinegar, while flavonoid and alkaloid tests were negative (Table 1).

No.	Metabolite	Reagent / Result	Conclusion
1	Flavonoid	Mg+HCl: colorless	Negative (–)
2	Alkaloid	Mayer: orange	Negative (–)
3	Tannin	FeCl3: dark blue	Positive (+)
4	Saponin	Stable foam	Positive (+)

Table 1: Phytochemical screening of asam paya fruit vinegar.

α-Amylase inhibitory activity

The wavelength of maximum absorbance for the starch–iodine complex was 580 nm. The starch calibration curve showed good linearity ($y = 11.2x + 0.0698$, $R^2 = 0.9567$; Figure 1), confirming assay reliability.

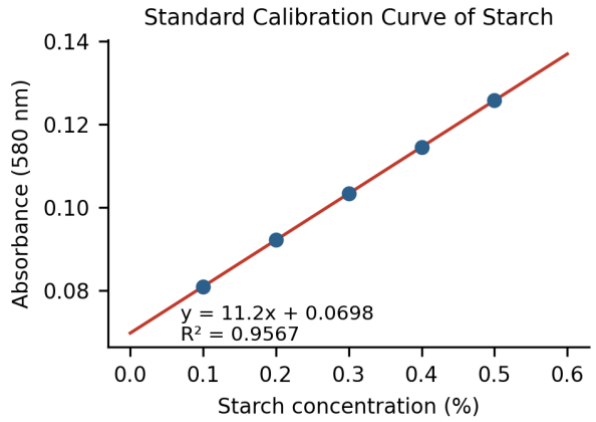

Figure 1: Standard calibration curve of starch absorbance at 580 nm (R^2 = 0.9567).

Inhibitory activity of asam paya vinegar against α-amylase increased linearly with concentration across the 1000–5000 ppm range (y = 0.0014x + 20.833, R^2 = 0.9852; Figure 2), giving an IC50 of 20,833.6 ppm. Based on the classification of Mardawati et al. [16], an IC50 above 200 ppm places asam paya vinegar in the very-weak inhibitor category.

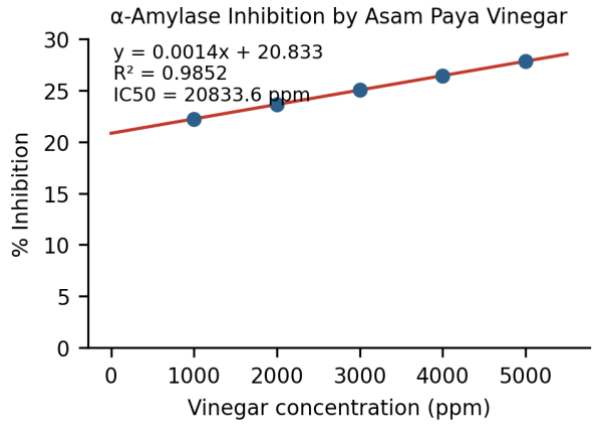

Figure 2: Relationship between asam paya vinegar concentration and % α-amylase inhibition (IC50 = 20,833.6 ppm).

Acarbose, used as the positive control, showed a much steeper inhibition response (y = 0.0094x + 49.707, R^2 = 0.9824; Figure 3), with an IC50 of 32 ppm, corresponding to the very-strong inhibitor category (IC50 < 50 ppm) [16].

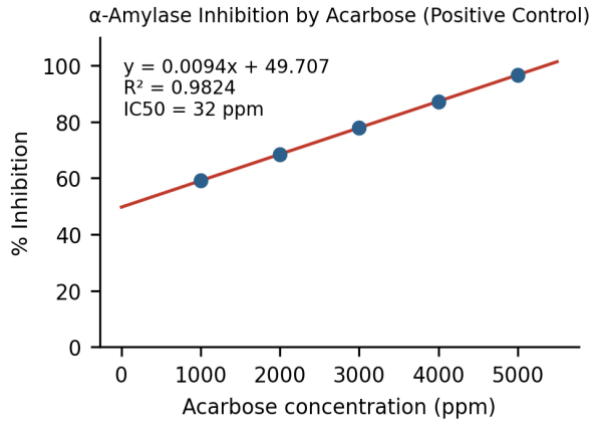

Figure 3: Relationship between acarbose concentration and % α-amylase inhibition (IC50 = 32 ppm).

Discussion

The sugar, acetic acid, and pH values obtained confirm that asam paya vinegar meets the Indonesian National Standard for fermented table vinegar (SNI 01-3711-1995) [11], indicating that the fermentation process itself was successful even though the resulting product showed weak antidiabetic activity. This distinction — between meeting food-quality standards and possessing pharmacological activity — is an important one for functional-food claims about fermented products.

The very weak α-amylase inhibition observed for asam paya vinegar (IC50 = 20,833.6 ppm) contrasts sharply with acarbose (IC50 = 32 ppm) and can be explained largely by its low acetic acid concentration. In a comparative in vitro study of organic acids identified in commercial vinegars, acetic acid was consistently the weakest α-amylase and α-glucosidase inhibitor among the six organic acids tested, whereas citric, tartaric, and malic acids were considerably more potent; vinegars with higher total organic-acid content, rather than acetic acid concentration alone, showed the strongest enzyme-inhibitory activity [5]. Because asam paya vinegar's acetic acid content (4.56%) sits at the lower end of the typical fermented-vinegar range, and its dominant organic acid is acetic acid rather than a mixture rich in citric or tartaric acid, a weak inhibitory response is consistent with this literature.

The comparatively low sugar content of the fruit juice (5%) likely limited the extent of the alcoholic-to-acetic fermentation, since adequate substrate sugar (commonly cited in the 10–25% range for vinegar fermentation) is needed to generate sufficient ethanol for subsequent conversion to acetic acid. This is consistent with reviews of fruit-vinegar biotechnology showing that the composition of the starting juice strongly determines the acid and bioactive-compound profile of the finished vinegar [19]. The resulting acetic acid level in turn kept the measured pH relatively close to neutral (pH 5.20); because α-amylase retains substantial catalytic activity across a broad pH range spanning roughly 5 to 8, with optimal activity typically reported between pH 5 and 8 depending on the enzyme source [17], the pH of asam paya vinegar was not low enough to itself suppress enzyme activity, further limiting the inhibitory effect observed.

Only tannin and saponin were detected in the phytochemical screening, while flavonoid and alkaloid were absent. The inhibitory contribution of tannin toward digestive enzymes depends on its ability to form complexes with enzyme proteins, which is influenced by tannin structure, degree of polymerization, and the reaction conditions; depending on the specific tannin subtype present, such interactions can either decrease or, in some cases, paradoxically increase measured enzyme activity [18]. This structure-dependence may partly explain why the tannin present in asam paya

vinegar did not translate into meaningful α-amylase inhibition. The absence of flavonoid and alkaloid is plausibly related to the acetic fermentation process itself: reviews of fruit-vinegar production report substantial losses of polyphenolic compounds, including flavonoids and anthocyanins, during acetic-acid fermentation — with reported losses as high as 60–91% relative to the starting juice in some fruit vinegars — because acetic acid bacteria metabolize and oxidize these compounds during acetification [19]. Given that raw asam paya fruit extracts have previously been reported to retain measurable α-amylase and α-glucosidase inhibitory activity together with antioxidant capacity [6,8], the comparatively weak activity of the fermented vinegar in the present study suggests that the fermentation process itself, rather than an inherent lack of bioactivity in the fruit, is the more likely driver of the reduced antidiabetic potential.

This interpretation is further supported by comparison with other regionally consumed fruit vinegars. Nipa palm vinegar, tested under a comparable spectrophotometric assay, inhibited α-amylase with an IC50 of 90.3 mg/mL — several orders of magnitude more potent than asam paya vinegar in the present study [10] — while salak and apple vinegar produced measurable blood-glucose reductions in vivo [9]. Broader clinical evidence also indicates that dietary acetic acid, delivered at sufficient and sustained doses, can modestly improve fasting glucose in adults with type 2 diabetes even when it's in vitro enzyme-inhibitory potency is limited [4]. Collectively, these findings suggest that any antidiabetic benefit of asam paya vinegar, if present, is more likely to operate through acetic acid's broader metabolic effects on glucose handling than through direct α-amylase inhibition at the concentrations tested here.

Conclusion

Asam paya fruit vinegar produced by 11-day fermentation met the Indonesian national quality standard for fermented vinegar (5% sugar, 4.56% acetic acid, pH 5.20) and contained tannin and saponin as its detectable secondary metabolites. However, its α-amylase inhibitory activity was classified as very weak (IC50 = 20,833.6 ppm), far below that of acarbose (IC50 = 32 ppm), most plausibly reflecting its comparatively low acetic acid content, near-neutral pH, and loss of flavonoid/alkaloid compounds during acetic fermentation. Future work should examine whether adjusting fermentation time, starter type, or initial sugar content improves the acid and bioactive-compound profile of asam paya vinegar, and should compare the vinegar with unfermented fruit extracts to directly test whether fermentation itself attenuates antidiabetic activity.

Conflict of interest

Authors state no conflict of interest.

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