Quantitative analysis of total flavonoids in wedang uwuh and its effect on fasting blood glucose and malondialdehyde levels in diabetes mellitus model rats

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Abstract. *Wedang uwuh*, a traditional Indonesian herbal beverage, contains bioactive compounds with potential antioxidant properties. This study evaluated its total flavonoid content (TFC) and therapeutic effects on lipid peroxidation and glycemic profiles of diabetic rats. TFC was quantified using spectrophotometric and LC-MS methods. For the *in vivo* assay, streptozotocin-nicotinamide-induced diabetic male rats were divided into five groups: normal control (N), negative control (KN), positive control (KP), traditional infusion (F1), and standardized extract (F2) administered over 21 days. Hepatic malondialdehyde (MDA) and fasting blood glucose (FBG) levels were monitored. The extract exhibited a TFC of 42.37±1.91 mg QE/g, identifying key flavonoids like Procyanidin B2 and Hesperetin. Despite these antioxidants, ANOVA showed no statistically significant reduction in hepatic MDA levels across the groups ($p=0.179$). Concurrently, FBG levels remained significantly elevated in all diabetic cohorts ($p<0.05$), with F2 paradoxically accelerating glycemic levels by 21.95%. Although *Wedang uwuh* possesses distinct flavonoid profiles, the current dosages failed to alleviate lipid peroxidation or improve fasting hyperglycemia. Future studies must optimize the dose-response relationship for therapeutic standardization.

1 Introduction

Wedang uwuh is a traditional Indonesian herbal beverage recognized for its abundance of bioactive compounds, particularly flavonoids, which have garnered significant scientific interest due to their potent antioxidant properties [1].

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Flavonoids belong to the polyphenol class, exerting antioxidant effects by scavenging free radicals and modulating cellular antioxidant defenses, which subsequently aids in mitigating oxidative stress associated with various chronic diseases [1,2]. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, leading to cellular damage that contributes to aging and the progression of various diseases [3]. MDA serves as a key biomarker for oxidative stress, reflecting lipid peroxidation and cellular membrane damage [2].

The antioxidant capacity of Wedang Uwuh stems from the synergistic interaction of its botanical constituents, primarily Sappan wood (*Caesalpinia sappan*) containing brazilin, along with ginger, cloves, cinnamon, and cardamom [4]. These bioactive compounds are typically liberated via thermal decoction, a process essential for the aqueous extraction of polar flavonoids to facilitate systemic absorption and inhibit lipid peroxidation pathways [5]. Quantifying the total flavonoid content and evaluating its impact on MDA levels are essential for assessing this *in vivo* antioxidant potential, as flavonoids protect cells by scavenging reactive oxygen species (ROS) and enhancing enzymatic defenses [6]. Furthermore, mitigating this oxidative stress is closely linked to metabolic regulation in diabetes mellitus, where chronic hyperglycemia accelerates ROS production, leading to pancreatic β-cell dysfunction and insulin resistance [7]. Consequently, assessing the efficacy of *Wedang Uwuh* in reducing fasting blood glucose (FBG) levels in diabetic rat models is critical to understanding its broader therapeutic potential in managing metabolic disorders. Despite its traditional use, a significant research gap in relation to the integration of high-resolution LC-MS metabolite profiling with *in vivo* assessments to validate the synergistic effects remains. This study addresses this gap by providing a detailed phytochemical characterization and evaluating its influence on FBG and MDA levels, thereby establishing a crucial scientific baseline for future dose optimization and the standardization of Wedang Uwuh as a functional herbal beverage [4,8].

2 Method

2.1 Ethical Statement and Research Setting

The study was conducted at the Experimental Animal Housing Unit (UKHP - Unit Kandang Hewan Percobaan), IPB University. All experimental procedures were approved by the Institutional Animal Care and Use Committee with ethical clearance number 339-2025 IPB. Strict adherence to laboratory ethics and data integrity protocols was maintained throughout the study.

2.2 In Vivo Experimental Design and Induction

Twenty-five Male Sprague-Dawley (SD) rats were utilized in this study. Diabetes was induced through the administration of Nicotinamide (NA) at a dose of 110 mg/kg BW, followed by Streptozotocin (STZ) at 65 mg/kg BW. The rats were divided into five experimental groups: normal control (N), negative control (KN), positive control (KP), and two treatment groups (F1 and F2). The intervention lasted for a duration of 21 days, during which F1 received a traditional Wedang Uwuh infusion (13.5 mL/kg BW) and F2 received the standardized extract (100 mg/kg BW). The dosages for F1 and F2 were determined by converting the traditional human daily consumption of *Wedang Uwuh* to an equivalent animal dose and aligning with established pharmacological ranges for herbal extracts to ensure physiological relevance and therapeutic efficacy.

2.3 Biochemical and Molecular Analysis

FBG levels were monitored weekly via tail vein blood sampling using an Accu-Chek® Active glucometer. At the end of the treatment period, MDA levels in liver tissue were quantified using the TBARS method to assess oxidative stress, with results expressed as mean ± SD. Additionally, total flavonoid content was evaluated using spectrophotometric UV-VIS compared to rutin/quercetin. The identification of specific flavonoid compounds in *Wedang Uwuh* was performed using Liquid Chromatography-Mass Spectrometry (LC-MS).

2.4 Statistical analysis

Data collected from the biochemical assays, including FBG, MDA levels, and total flavonoid content, were expressed as mean ± standard deviation (SD) to represent the precision of the triplicated measurements. Statistical significance between the experimental groups (N, KN, KP, F1, and F2) was determined using a one-way analysis of variance (ANOVA). Post-hoc testing, such as Tukey's Honestly Significant Difference (HSD), was subsequently applied to identify specific differences between the treatment means, particularly comparing the 21-day intervention effects of the traditional infusion (F1) and the standardized extract (F2). All statistical analyses were performed using professional software (SPSS 26), with a p-value of less than 0.05 ($p<0.05$) considered statistically significant to ensure the integrity and reliability of the findings.

3 Result and Discussion

3.1 Total Flavonoid Content of *Wedang Uwuh*

The quantitative analysis of *Wedang Uwuh* revealed a consistent total flavonoid content (TFC) with a mean value of 42.37 mg QE/g. Based on triplicate measurements, the individual samples yielded TFC values ranging from 41.03 to 44.56 mg QE/g. The analytical method demonstrated high precision and reproducibility, as evidenced by a standard deviation (SD) of 1.91 and a Relative Standard Deviation (RSD) of 4.51%.

Table 1. Identification of Flavonoid Compounds in *Wedang Uwuh* via LC-MS Analysis.

Retention Time (RT)	Measured ion Mass (m/z)	Ion Formula $[M-H]^+$	Compound and Structure	Group
3.567	579.1511	$[C30H27O12]^+$	Procyanidin B2; (2R,2′R,3R,3′R,4R)-2,2′-Bis(3,4-dihydroxyphenyl)-3,3′,4,4′-tetrahydro-2H,2′H-4,8′-bichromene-3,3′,5,5′,7,7′-hexol	Flavonoid (dimer catechin-(4α→8 catechin).
3.567	303.0849	$[C16H15O6]^+$	Hesperetin; 3′,5,7-Trihydroxy-4′-methoxyflavanone	Flavonoid
4.249	303.0873	$[C16H15O6]^+$	Hesperetin; 3′,5,7-Trihydroxy-4′-methoxyflavanone	Flavonoid
4.550	269.0820	C16H13O4	Isoformononetin; 3-(4-Hydroxyphenyl)-7-methoxy-4H-chromen-4-one	Flavonoid
4.973	285.0771	C16H13O5	Glycitein; 4′,7-Dihydroxy-6-methoxyisoflavone	Flavonoid

The flavonoid compounds in *Wedang Uwuh* (Table 1) possess the biological capacity to protect liver tissue from oxidative stress [9]. At a retention time of 3.567 min, co-elution was observed for Procyanidin B2 and Hesperetin, which were clearly resolved and identified by the high-resolution mass spectrometer via their respective measured ion masses of 579.1511 and 303.0849 m/z. These findings demonstrate the potential bioactivity of flavonoid compounds in *Wedang Uwuh* to mitigate oxidative hepatic damage; however, the dosage and formulation utilized have not yet reached the effective therapeutic threshold required to counteract oxidative stress induced by diabetogenic agents [1]. This is consistent with the understanding that the efficacy of flavonoids is highly dependent on dosage, bioavailability, and the extraction and formulation methods, which influence the stability and bioactivity of the compounds [10,11].

3.2 Fasting Blood Glucose

The profile of FBG levels across all experimental groups demonstrated significant baseline variations on Day 0 ($p \leq 0.001$), confirming successful hyperglycemia induction in the diabetic models (KN, KP, F1, and F2) compared to the normoglycemic status of Group N (96.20±9.52 mg/dL), a statistically significant variance that consistently persisted on Days 7, 14, and 21 (Table 2). Over the 21-day period, Group N maintained a relatively stable glycemic profile with a minor final increase (Δ = +8.60; +8.94%), whereas the diabetic cohorts exhibited distinct fluctuations; Group KN showed a transient drop on Day 7 before progressively rebounding to 344.00 ± 135.28 mg/dL on Day 21 (Δ = +7.60; +2.26%), while Group KP displayed a steady upward trend toward the end of the study (Δ = +38.20; +11.43%).

Tabel 2. Fasting blood glucose (FBG) levels (mg/dL) among all experimental groups at various time intervals.

Group	Day 0 Mean ± SD	Day 7 Mean ± SD	Day 14 Mean ± SD	Day 21 Mean ± SD	Δ	% Change
N	96.20 ± 9.52	97.80 ± 3.56	97.20 ± 6.91	104.80 ± 7.53	+8.60	+8.94%
KN	336.40 ± 86.48	277.40 ± 141.31	321.00 ± 149.88	344.00 ± 135.28	+7.60	+2.26%
KP	334.20 ± 71.49	325.60 ± 137.45	338.00 ± 69.88	372.40 ± 118.88	+38.20	+11.43%
F1	322.00 ± 104.60	310.20 ± 88.64	362.60 ± 69.99	340.00 ± 103.34	+18.00	+5.59%
F2	334.40 ± 62.52	365.60 ± 73.83	343.80 ± 172.05	407.80 ± 193.63	+73.40	+21.95%
p	0.000**	0.005**	0.006**	0.022*	-	-

Data are presented as mean ± SD; *) Normally distributed data: p1) using One-way ANOVA test: Test for differences between groups using LSD test. **) Non-normally distributed data p1) using Kruskal-Wallis test: Test for differences between groups using Mann Whitney. N = Normal; KN = Negative Control; KP = Positive Control; F1 = Uwuh infusion intervention; F2 = Uwuh extract intervention

Following the successful induction of chronic oxidative stress and baseline hyperglycemia via the STZ-NA regimen, the 21-day intervention revealed distinct systemic responses across all experimental groups. FBG profiles remained elevated in all diabetic cohorts, with the traditional infusion (F1) demonstrating a more stabilized glycemic

trajectory, whereas the standardized extract (F2) conversely accelerated progressive metabolic impairment by the end of the study (Table 2).

3.3 MDA levels

Flavonoids are recognized as bioactive compounds capable of enhancing the capacity of the body's antioxidant defense system and reducing oxidative biomarkers such as MDA, a hallmark of lipid damage [1]. Furthermore, *in vivo* metabolic factors and interactions with other biomolecules also influence the clinical effectiveness of flavonoids in reducing oxidative stress biomarkers [12].

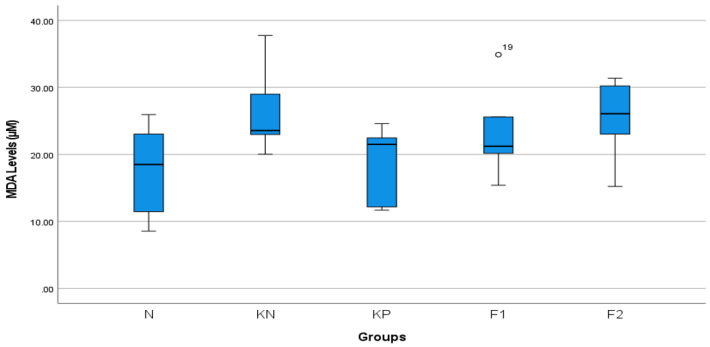

Fig. 1. MDA levels

MDA levels across all experimental groups after a 21-day intervention. Data are presented as medians with interquartile ranges (IQR) represented by the boxplots, where horizontal lines within the boxes indicate the median values. Statistical analysis via one-way ANOVA followed by Tukey's post-hoc test. N = Normal Control; KN = Negative Control; KP = Positive Control; F1 = Traditional *Wedang Uwuh* infusion intervention; F2 = Standardized *Wedang Uwuh* extract intervention

The elevation of hepatic MDA in the negative control group confirms that STZ-NA induction triggers chronic oxidative stress, where severe hyperglycemia accelerates reactive oxygen species (ROS) production, leading to the peroxidation of polyunsaturated fatty acids (PUFAs) in cell membranes. Although high-resolution LC-MS profiling successfully identified potent antioxidant flavonoids within *Wedang Uwuh*—such as Procyanidin B2, Hesperetin, Isoformononetin, and Glycitein—neither the traditional infusion [F1] nor the standardized extract [F2] significantly suppressed this lipid peroxidation pathway. This lack of statistical significance indicates that the tested doses fell below the required therapeutic threshold to neutralize the overwhelming diabetogenic ROS load, or that advanced membrane damage restricted the bioaccessibility of these polar metabolites. Consequently, unmitigated lipid peroxidation structurally disrupted cellular integrity, forming a pathological loop that worsened systemic glycemic control (Figure 1).

4 Conclusion

In conclusion, *Wedang Uwuh* exhibits a substantial total flavonoid content (42.37±1.91 mg QE/g) with prominent bioactive compounds identified via high-resolution LC-MS, including Procyanidin B2, Hesperetin, Isoformononetin, and Glycitein. However, a 21-day administration of either the traditional formulation (F1) or the standardized extract (F2) did not significantly reduce hepatic MDA levels ($p=0.179$) or ameliorate FBG in STZ-NA-induced diabetic rats, with F2 conversely accelerating glycemic elevation. These findings indicate that while *Wedang Uwuh* possesses strong descriptive antioxidant profiles, the current dosages and preparation methods remain insufficient to overcome severe

diabetogenic-induced oxidative and metabolic distress. Consequently, future investigations should focus on optimizing dose-response relationships, evaluating long-term safety, and enhancing the bioavailability of its specific phytoconstituents to effectively standardize *Wedang Uwuh* as a therapeutic functional beverage.

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