The effect of Indonesian herbal drink “Mbah Jayus” on hyperglycemic rats

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Abstract. Diabetes mellitus is a metabolic disorder disease caused by disorder of insulin secretion, insulin action or both. Unlike the synthetic compounds, herbal of natural origin are safer and have less side effects. One of Indonesian herbal drink called “jamu” have many health effects, one of them is antidiabetic. Indonesian herbal drink “Mbah Jayus” is made by *Zingiber officinale* Rosc, *Cymbopogon citrate*, *Nigella sativa*, *Cinnamomum chinense* BL, and *Amomum cardamomum* Willd which has highly antioxidant. In this study, antioxidant property was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging method (IC\(_{50}\)), total phenolic content and total flavonoid content. The antidiabetic effect was conducted on *Rattus norvegicus* rats. Diabetes was induced with intraperitonial injection of alloxan monohydrate (150 mg/kg body weight). The result showed that Indonesian herbal drink “Mbah Jayus” can decrease significantly the blood glucose level (P<0.05) in treated rats. So, the antioxidants property of phenolic and flavonoid content might be responsible for the antidiabetic efficacy Indonesian herbal drink “Mbah Jayus”.

1. Introduction
Diabetes mellitus is a metabolic disorder that involves the inability to produce insulin or use it properly caused by destruction of T-cell-mediated pancreatic \(\beta\)-cells [1, 2]. The World Health Organization (WHO) has estimated that 439 million people will be diabetic in 2030 [3]. In Indonesia it is predicted that by the year 2030 it may increase to 21.3 million [4].

Treatment of diabetes seemed imperative from time to time. Unlike chemical drugs or synthetic ones, medicinal plants are quite effective, cheaper and easily available. Indonesian herbal drink “Mbah Jayus” made by medicinal plants such as *Zingiber officinale* Rosc, *Cymbopogon citrate*, *Nigella sativa*, *Cinnamomum chinense* BL, and *Amomum cardamomum* Wild. Medicinal plants in Indonesian herbal drink “Mbah Jayus” contain of phytoactive constituents like phenolic and flavonoid compound that have shown potential in health.

Phenolics are heterogeneous compound derived from plant secondary metabolism. According to Bravo [5] phenolic compound can be classified into flavonoid and non-flavonoid. Phenolic and flavonoid
compound are potent antioxidant, anti-inflammation, and antidiabetic agent. This compound helps to suppress the glucose level by enhancing hepatic glucokinase activity, probably by enhancing the insulin release from pancreatic islets [6] also scavenge the generated ROS [7]. Therefore, phenolics can be utilized as a standard for evaluating the antidiabetic potential of Indonesian herbal drink “Mbah Jayus”. The present study was designed to evaluate the in vivo antidiabetic potential of Indonesian herbal drink “Mbah Jayus” in alloxan-induced diabetic rats.

2. Materials and Methods

2.1. Materials
Material in this study was Indonesian herbal drink “Mbah Jayus” from small-medium enterprise (SME) Barokah Hijrah Malang, jamu Vitadar from PT Sidomuncul, and mineral water was used as solvent. Alloxan, aquadest sterile, PAP pellet diet-PT JAPFA COMFEED, sucrose, alcohol, aquadest, chloroform and male wistar rats (Rattus norvegicus).

2.2. Quantitative analyses of phytochemical constituents

2.2.1. Total phenolic content
Total phenolic contents were measured with Folin-Ciocalteu (FC) reagent. An aliquot of 0.2 ml sample was mixed with 0.8 ml Na2CO3 (7.5%) and 1 ml of FC reagent (10%). The solution was incubated at room temperature for 30 minutes, and the absorbance was measured at 765 nm with a spectrophotometer. Total phenols were calculated from standard curve and are expressed as milligrams of gallic acid equivalents (GAE) per g of extract. This method was modified from Widyaningsih et al. [8].

2.2.2. Total flavonoid content
Using modified Atanassova method [9], total flavonoid contents were measured with 1 ml sample mixed with 0.3 ml AlCl3 (10%), 0.3 ml NaNO2 (5%), 2 ml NaOH and 2.4 ml aquadest. The solution was incubated at room temperature for 5 minutes, and the absorbance was measured at 510 nm with a spectrophotometer. Total flavonoid content was calculated as quercetin using the following equation based on the calibration curve, and expressed as milligrams of quercetin equivalent (QE) (mg/g).

2.2.3. DPPH radical scavenging activity
DPPH is a commercial oxidizing radical which can be reduced by antioxidants. In this study, DPPH was dissolved in ethanol. The Indonesian herbal drink “Mbah Jayus” and jamu “Vitadar” were diluted with ethanol at various concentrations to get sample solution. 1 ml of the DPPH was added to each sample solution. After that, incubated at room temperature for 30 minutes and the absorbance were measured at 517 nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of blank solution (no sample). Phenolics and flavonoids reduced DPPH radical by their hydrogen donating ability. The ability to scavenge the DPPH radical was expressed as IC50. This method was modified from Widyaningsih et al. [8].

2.2.4. Evaluation of in vivo antidiabetic activity
Male wistar rats (Rattus norvegicus) (n= 20, approximately 2 month old, weighing 150-180 g) were housed in cages under environmentally controlled condition (temperature 27±2°C) with a 12 h light/dark cycle. After acclimatization, the rats were divided into 5 experimental groups, with each group consisting 4 rats. According to Akuodor et al. [10] diabetes was induced by intraperitoneal injection of 150 mg/kg b.w alloxan monohydrate in the group II,III,IV, and V. Rats with blood glucose ≥126 mg/dL after 3 day were considered diabetic [11]. Rats in group I and II received only normal mineral water orally and served as negative and positive control. Diabetic rats in group III were treated with 63 mg/200 g body weight of
IHB “Mbah Jayus”, whereas those in group IV were treated with 126 mg/200 g body weight of IHB “Mbah Jayus”, and group V were treated with 126 mg/200 g body weight of jamu “Vitadar”. Oral treatment was treatment was provided daily for 28 days after diabetic induction. Baseline blood glucose values were obtained using an Easy Touch GCU at week 0, 2 and 4. The experimental design was approved by The Institutional Animal Ethics Committee (932-KEP-UB).

3. Results and Discussion

3.1. Results of total phenolic, total flavonoid, and IC50 analysis

Table 1 shows that total phenolic content in the IHD “Mbah Jayus” concentration 1 and 2, and jamu “Vitadar” were 2.0717, 7.2017, and 8.2857 mg GAE/g. The flavonoid content in the IHD “Mbah Jayus” concentration 1 and 2, and jamu “Vitadar” were 0.8707, 4.0563, and 1.698 mg/QE. Jamu “Vitadar” had highest concentration of phenol but total flavonoid was greater in the IHD concentration 2. In DPPH assay, the IC50 of jamu “Vitadar” was 720.782 ppm while IHD “Mbah Jayus” concentration 1 and 2 was 807.627 ppm and 856.136 ppm. A high absorbance value indicates that the sample possesses high antioxidant activity. The antioxidant activity of IHD and JV extract may be attributed to their chemical composition and phenolic acid content. Based on Jayaprakasa et al. [12] some bioactive compounds present in citrus possessed high total antioxidant activity, which was due to the presence of flavonoid, phenolic and carotenoid.

| Sample | Phenol mg GAE/g | Flavonoid mg/QE | IC50 ppm |
|--------|----------------|----------------|-----------|
| IHD 1  | 2.0717 ± 0.0280 c | 0.8707 ± 0.0490 c | 807.627 ± 10.79 a |
| IHD 2  | 7.2017 ± 0.1181 b | 4.0563 ± 0.0938 a | 856.136 ± 86.5 a |
| JV     | 8.2857 ± 0.0155 a | 1.698 ± 0.330 b | 720.782 ± 0.320 a |

*The data is expressed as mean ± S.E.M of three separate experiments.
Total phenolic content mg/g, gallic acid equivalents
Flavonoid content mg/g, quercetin equivalent
Means within a column followed by different letters were significantly different at P<0.05

Plants have diverse groups of phenolic compound. Zingiber officinale Rosc contained phenolic content such as 6-gingerol and 6-shogaol [13], Nigella sativa seed contained kaempferol and quercitrin [14] and phenolic acid and vanillic acid [15], Amomum cardamomum Willd contained flavonoid and polyphenol [16], Cinnamomum chinense BL contained safrole, eugenol and cinnamaldehyde, Cymbopogun citrate contained luteolin, isoorientin-2'-O-rhamnose, quercetin, kaempferol and apigenin [17] and elimicin, catecol, chlorogenic acid, caffeic acid and hydroquinone [18]. Many spices contained high levels of phenolics and demonstrated high antioxidant capacity. Our results showed that the JV was the most powerful phenolic antioxidant and exhibited the strongest radical scavenging activity among three extracts.

3.2. Antidiabetic activity

Free radicals have been claimed to play an important role in affecting human health. Free radical can caused several diseases including cancer, hypertension, heart attack, and diabetes [19]. Free radicals are capable of damaging cellular molecules, DNA, proteins, and lipid leading to altered cellular functions [6]
and oxidative stress [20]. Oxidative stress causes membrane damage leading finally to membrane rupture in different cellular types and can increase the production of oxygenated free radical also decreased antioxidant enzyme activities [20].

Phenolic have received considerable attention because of their potential antioxidant activities. Phenolic content has physiological functions, including free radical scavenging, anti-diabetic, and anti-inflammatory [21]. According to Pietta [22] the antioxidant activity of phenolic is largely due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quencher and potential metal chelator. Many recent studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally-induced diabetes in animal models, as well as reducing the severity of diabetic complications [15].

In this study, a total of 20 rats were administered with two different doses of IHD (63 mg/200 g and 126 mg/200 g), 126 mg/200 g of JV, mineral water was given in the positive and negative control. A total of 3 blood collections were done to quantify blood glucose levels (mg/dL) during 4 weeks. During week 0, the blood glucose was high (>126 mg/dl) in all groups except negative control. During week 2, blood glucose's treated group was lower than the positive control. This indicated that IHD and JV extract exhibited anti-hyperglycemic activity. During week 4, blood glucose’s treated group also lower than positive control. This indicated that IHD and JV extract at high levels provided hypoglycemic activity along with the natural ability of the rats to retain its normal blood glucose level. Throughout the whole test, the negative control was utilized to show the normalization of the blood glucose level during the whole treatment to guarantee that the varying decrease of blood glucose of rats was mainly because of the IHD and JV extract.

As shown in Figure 1, a significant decrease (P>0.05) was noted in the level of glucose in diabetic mice compared with the negative control. However, IHD 1 can decrease the blood glucose until 55.7156 %, IHD 2 can decrease the blood glucose until 44.0144 %, and JV can decrease until 41.8209 %. The ability of IHD 1 and 2, and also JV to decrease the blood glucose may cause by phenolic compound and antioxidant activity. The potential mechanism of the protective effects of phenolic compounds, including flavonoids, is thought to be due to their scavenging of free radicals. A study was done by da Silva [21] cinnamon contained phenolic compound such as cinnamaldehyde, cinnamic acid, coumarin and cynamyl alcohol can reducing blood glucose.

![Figure 1](image1.png)

**Figure 1.** Time course change in fasting blood glucose level in negative control (C-), positive control (C+), Indonesian herbal drink concentration 1 (IHD 1), Indonesian herbal drink concentration 2 (IHD 2), jamu Vitadar (JV). Data are mean ± SEM; n=4 rats for each group. ANOVA test was used to compared normal rats, diabetic untreated and treated with extract (P<0.05).
4. Conclusion
In the present study, Indonesian herbal drink “Mbah Jayus” and jamu “Vitadar” has ability to decreased blood glucose. The antioxidant activity and phenolic content analysis revealed the highest concentration was from jamu “Vitadar”, and IHD 1 has the highest ability to decreased blood glucose until 55.7156 %.

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