



Preliminary Analysis of the Antidiabetic Activity of Indonesian Polyherbal Formulation

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Abstract

This study aims to prepare polyherbal formulations and also analyze the antidiabetic potentials of the prepared polyherbal in animals. The ethanol extracts of the leaves of *Smallanthus sonchifolius*, *Stevia rebaudiana*, *Syzygium polyanthum*, and *Camellia sinensis* were used in this study. The extracts of the aforementioned plants were prepared in the ratio of 2:1:1:1. The formulation was tested for antidiabetic activity *in vitro* through the inhibition of alpha-glucosidase and *in vivo* using alloxan-induced diabetes in mice. The polyherbal has an inhibitory effect with the IC₅₀ value of 26.23 µg/mL compared to acarbose (control) was 17.02 µg/mL. The diabetic animals were observed to show an obvious decline in glucose level when compared with control (P < 0.001) after treatment.

Keywords: Antidiabetic, Extract, Formulation, Glucose, Herb, Inhibition

1. Introduction

Diabetes Mellitus (DM) is a metabolic disorder attributed to the pancreas inability to produce enough insulin or the ineffective insulin utilization by the body. There are two main categories, type 1 and 2. The first is insulin-dependent, juvenile, or childhood-onset diabetes, and is based on the inadequate production of insulin¹. While the other one is non-insulin-dependent or adult-onset diabetes (Type 2) because of the less efficiency of insulin hormone. According to WHO, the number of DM patient in Indonesia may increase by 21.3 million in 2030, while International Diabetes Federation predicted about 14.1 million raise in 2035 with type 2 diabetes constituting 90% of all cases².

Based on characterization results, the extracts of yakon (*Smallanthus sonchifolius*), stevia (*Stevia rebaudiana*), and bay (*Syzygium polyanthum*) were subjected to further pharmacological screening. Polyherbal formulations

contain multiple ingredients of different plants origin. Also, these products were used to enhance the effect or check the harmfulness of other ingested medications. They may also produce a synergetic effect due to the presence of the various constituents³. Polyherbal products were observed to be better with the extended therapeutic potentials when compared to single herbal medicines. Hence, this research aimed to carry out the medicinal preparation in form of polyherbal formulation using plants with known antidiabetic activity.

2. Materials and Methods

2.1 Collection of Plants

The proposed herbal drug (*S. sonchifolius*, *S. rebaudiana*, *S. polyanthum*, and *Camellia sinensis*) was collected from Balitro, Bogor, West Java. All of the used leaves were shade-dried, made into powder with moderately coarse texture,

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and then stored in an airtight container. The powder was used for extraction later on. The plant specimens were identified and authenticated in Herbarium Bogoriense by Dr. Atik Retnowati, with the voucher number No 455/IPH.1.01.

2.2 Preparation of Polyherbal Formulation

The dried leaves were ground into a powder and extracted using absolute ethanol of 80% for about 6 hours with

a Soxhlet apparatus. These leaves were then dried at 60°C and were preserved at 4°C. Furthermore, five formulations (F1-F5) were produced by mixing different ratios of pulverized plant materials. The constituents of the polyherbal formula included the leaves of yakon (*S. sonchifolius*), bay (*S. polyanthum*), stevia (*S. rebaudiana*), and tea (*C. sinensis*) plants with ratios as shown in Table 1.

Table 1. Composition of polyherbal formulation

Formulations	<i>S. sonchifolius</i> leaves (g)	<i>S. polyanthum</i> leaves (g)	<i>S. rebaudiana</i> leaves (g)	<i>C. sinensis</i> leaves (g)
F1	400	-	100	100
F2	200	200	100	100
F3	100	300	100	100
F4	300	100	100	100
F5	-	400	100	100

2.3 Alpha-Glucosidase Inhibitory Activity of Polyherbal Formulation (PF)

The plant extracts (50 µL) at various concentrations levels (10 to 1000 µg/mL) were incubated with 10 µL of α-glucosidase (maltase), yeast (Sisco Research Laboratories Pvt. Ltd.), and enzyme solution (1 U/mL) for 20 min at 37°C and 125 µL of 0.1M phosphate buffer (pH 6.8). After 20 min of incubation, the reaction was initiated by adding 20 µL of 1M pNPG (substrate) and then incubated for 30 min. The reaction was terminated by adding 0.1N of Na₂CO₃ (50 µL) and the final absorbance was calculated at 405 nm using Biotek multi-well plate reader⁵. Besides, acarbose drug was used as a positive control at different dilutions levels (10 to 1000 µg/mL). The results of this analysis were expressed as percentage inhibition, calculated with the formula below:

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100$$

where, A_s represents the absorbance of the test substance while A_c is the absorbance of control. The polyherbal combination with the IC₅₀ value closer to the acarbose (control) was chosen for the animal testing^{4,5}.

2.4 Animals and Experimental Design

2.4.1 Induction of Experimental Diabetes

The pancreatic β-cells of the experimental animals were damaged by inducing them with 150 mg/kg BW of alloxan injected intraperitoneally for 3 consecutive days. This process can result in reduced insulin production and hyperglycemia. The blood glucose level of the samples was examined before and after 72 hours of injection to observe the diabetes development. Furthermore, they were sustained for 5 days and the experimentation began on day six. This study, however, comprised only animals with a glucose concentration of less than 250 mg/dL. The methods in this study were approved by the ethical committee of the Medicine Department of Universitas Indonesia with the certificate number: 0251/UN2.F1/Etik/2018.

2.4.2 Research and Development

The diabetic samples were then grouped into five, comprising of six per unit, and treated with the chosen polyherbal formulation once daily for 22 days as presented below:

Group I (Normal healthy control): given only vehicle (CMC Na 0,5%)

Group II diabetic control: received vehicle only

Group III diabetic mice: received acarbose 6.5 mg/kg BW

Group IV diabetic mice: received PF 100 mg/kg BW

Group V diabetic mice: received PF 200 mg/kg BW

Group VI diabetic mice: received PF 400 mg/kg BW

And, then the blood glucose level of these animals was recorded on days 3, 14, and 22 of this experiment^{6,7}.

3. Results

The plant formulation and their combined extracts are used as a drug of choice rather than individual herbs. The results of a preliminary test using in vitro alpha-glucosidase enzyme inhibition method are shown in Table 2.

The results of antidiabetic activity test of combined herbal formula indicated that the IC₅₀ value of F1 was 278.43 µg/mL; 290.87 µg/mL for F2, 274.05 µg/mL for F3, 26.23 µg/mL for F4, and 216.14 µg/mL for F5. F4 has the closest IC₅₀ to the control (acarbose), thus used in the next analysis.

Table 2. *α*-Glucosidase inhibitory effects of Polyherbal Formulation

Formulations	IC ₅₀ values of <i>α</i> -glucosidase inhibitory effects of Polyherbal Formulation (µg/mL)
Acarbose	17.25
F1	278.43
F2	290.87
F3	274.05
F4	26.23
F5	216.14

In vivo antidiabetic activity test of F4 was performed on alloxan-induced mice. The results of this study are shown in Table 3.

Based on this experiment, the polyherbal products developed a hypoglycemic effect at 400 mg/kg in a dose-related manner. This occurs mostly in normal and glucose-fasted loaded rats.

Table 3. Antidiabetic activity of Polyherbal Formulation in alloxan-induced type 2 diabetic mice

Groups	Blood Glucose Levels (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
Normal Control	87.23±2.24	89.56±3.23	86.67±3.45	84.68±6.50
Diabetic Control	258.50±2.50	260.35±5.60 ^a	287.50±5.80 ^a	293.20±6.75 ^a
Acarbose (6.5 mg/kg)	260.45±3.45	215.10±6.50 ^b	196.60±6.50 ^b	99.40±4.5 ^b
Polyherbal (PF) 100 mg/kg BW	250.10±5.60	212.20±5.60 ^b	176.20±3.45 ^b	160.45±5.60 ^b
Polyherbal (PF) 200 mg/kg BW	256.20±9.20	204.60±6.45 ^b	169.20±5.60 ^b	120.30±4.50 ^b
Polyherbal (PF) 400 mg/kg BW	260.45±10.23	205.40±7.50 ^b	150.80±7.60 ^b	100.80±6.75 ^b

The values are mean ± SEM, n=5, ^ap<0.001 compared to a normal control group, and ^bp<0.001 compared to the diabetic control group

4. Discussion

Although most bioactive compounds can work on one target, they could also work on multi-targets. The pathology of diabetes mellitus is related to many metabolic pathways, multiple genes, and multiple functional proteins so it is imperative to discover drugs that come in the form of combination⁸. Various constituents in a formula or herb may enhance the bioavailability or the function of

several targets instead of one to produce the synergistic effects⁹. These polyherbal formulations are produced with an herbal origin and are used to strengthen or check the effects of other herbal compounds. This product may have a wide spectrum of biological functions. Furthermore, these polyherbal products may produce synergetic action due to multiple ingredients and contain potentially agonistic/antagonistic pharmacological agents within themselves⁷.

The study by Mahajan *et al* showed the polyherbal of *C. caesia*, *E. alsinoide*, *C. lanatus*, *G. sylvestre* and *T. cordifolia*, *W. coagulans*, and *C. bonduct* antidiabetic effect to rats induced by alloxan at 400 mg/kg of body weight. Our result is similar to that study, which showed a decrease of blood glucose level in the dose of 400 mg/kg of body weight compared to positive control. Sabu *et al* evaluated the methanolic extract (75%) of *Terminalia chebula*, *Terminalia bellerica*, *Embolica officinalis*, and their combination named 'Triphala.' Oral administration of the extracts (100 mg/kg body weight) reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats significantly within 4 h and the daily administration of the drug produced a sustained effect¹⁰.

The research conducted by Aziz *et al.* using 95% ethanol extract of Yakon leave (*S. sonchifolius* (Poepp. & Endl.) succeeded in isolating α -glucosidase inhibitor compounds, which were the FOS group, nystose, with the IC₅₀ value of 33.62 ppm, while the positive control for acarbose had 21.36 ppm⁵. Habib *et al.* conducted an *in vivo* study of standardized yakon extract (*S. sonchifolius*) against 340 mg/kg BW and 6800 mg/kg BW doses of compounds in the FOS group. Furthermore, this was administered for 90 days in streptozotocin-induced rats and showed a decreased blood glucose and insulin levels with improved pancreatic function to near normal¹¹. Genta *et al.* used this same extract, which was administered orally to diabetic rats similar to the aforementioned; also reduced the blood glucose level¹². This extract equally causes the blood glucose level to decrease in diabetogenic subjects due to the presence of polyphenol compounds that act as antioxidants¹³. Other compounds found in the Yakon leaves were quercetin, ferulic, p-coumaric, caffeic, chlorogenic, and protocatic acids.

The research conducted by Widyawati *et al.* in Health Community Center, Medan, North Sumatra, Indonesia from 2010–2011 reported *S. polyanthum* (Wight) as the most commonly used herb in the management of diabetes mellitus traditionally¹⁴. The results showed the alpha-glucosidase enzyme inhibitory activity from the leaf extract, which was taken from several regions in Java Island. It showed that the water obtained had a better IC₅₀ value of 51.10 μ g/mL compared to those from West and East Java. Furthermore, the conducted isolation found several compounds in the flavonoid class, including quercetin, coniferin, juncusol, and retusine¹⁵. The medium-polar extract of *S. rebaudiana* leaves produced an obvious ($P < 0.01$) dose-dependent decline in blood glucose level and body weight when administered orally (200 and 400 mg/kg) for 10 days. However, the rats treated with glibenclamide and Stevia was later observed

to gradually regain weight. The treatments done with Stevia extract was failed to cause hypoglycemia and loss of weight, yet the restoration of the pancreas' β -cells with the antagonizing β -necrotic action of alloxan was observed.

Previous studies reported the improvement of blood sugar level after feeding the green tea extract or the polyphenols, flavonoids, and catechins to chemically-induced hyperglycemia animals¹⁶. According to Shimizu *et al.* the hypoglycemic activity of Japanese tea in normal and streptozotocin-induced diabetic rats was identified as a polysaccharide. The bioactive constituents of tea, especially catechins, chlorogenic acid, caffeine, and the flavins, showed an inhibitory effect on α -glucosidase and α -amylase activity¹⁷. Therefore, it has resulted in the decreased levels of glucose, lipid, metabolites, and albuminuria. Furthermore, the pronounced anti-hyperglycemic effect was observed based on synergistic actions of various effective ingredients in Polyherbal formulation. The declined glucose is due to elevation of glycogenesis or the increased entry of glucose molecules to various skeletal muscles¹⁸.

5. Conclusion

The polyherbal formulation is a potential antidiabetic agent; therefore, it becomes interesting to investigate the underlying action molecular mechanism(s) and long-term toxicity studies of different animal species. Besides, after the completion of preclinical studies, this herbal product needs to be tested on human DM patients to ascertain the therapeutic efficacy and safety.

6. Acknowledgement

This work was funded by the INSINAS grant (G1/118, 2020) of the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (KEMENRISTEK DIKTI)

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