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Comparative study of *Eleutherine bulbosa* mills extracts for antidiabetic efficacy

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Abstract
Eleutherine bulbosa Mills, commonly known as "Sabit Emas" or "Dayak Onion," is a medicinal plant traditionally used for various ailments, including diabetes. This study aims to compare the antidiabetic efficacy of different extracts of *Eleutherine bulbosa* Mills. Using phytochemical screening, *in vitro* and *in vivo* assays, we evaluate the hypoglycemic effects and mechanisms of action of aqueous, ethanolic, and methanolic extracts of the plant.

Keywords: *Eleutherine bulbosa* Mills, Sabit Emas, Dayak Onion

Introduction
Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. This condition leads to severe complications, including cardiovascular diseases, neuropathy, nephropathy, and retinopathy, significantly affecting the quality of life and increasing mortality rates worldwide. According to the International Diabetes Federation, the prevalence of diabetes is rapidly increasing, with an estimated 463 million adults living with diabetes in 2019, a number expected to rise to 700 million by 2045.

Current pharmacological treatments for diabetes include insulin and various oral hypoglycemic agents such as metformin, sulfonylureas, and thiazolidinediones. While these treatments are effective in managing blood glucose levels, they often come with side effects and do not address the underlying causes of diabetes. Additionally, the high cost of these medications can be a burden, especially in low- and middle-income countries. Consequently, there is a growing interest in alternative therapies, particularly those derived from medicinal plants, which have been used traditionally to treat diabetes and its complications.

Eleutherine bulbosa Mills, commonly known as "Sabit Emas" or "Dayak Onion," is a medicinal plant widely used in traditional medicine in Southeast Asia for various ailments, including diabetes. Preliminary studies suggest that *Eleutherine bulbosa* contains bioactive compounds with potential antidiabetic properties. However, comprehensive scientific evaluations of its efficacy and mechanisms of action are limited.

The pharmacognostic profile of a medicinal plant includes detailed information about its botanical characteristics, chemical constituents, and therapeutic properties. Understanding the pharmacognostic profile of *Eleutherine bulbosa* is crucial for standardizing its use and optimizing its therapeutic potential. Moreover, different extraction methods can yield extracts with varying compositions and bioactivities, influencing their therapeutic efficacy.

This study aims to fill the gap by conducting a detailed comparative analysis of the antidiabetic efficacy of aqueous, ethanolic, and methanolic extracts of *Eleutherine bulbosa* Mills. We hypothesize that the different solvents will extract varying bioactive compounds from the plant, resulting in differences in their hypoglycemic activities. By performing comprehensive phytochemical screening and evaluating the *in vitro* and *in vivo* antidiabetic activities of these extracts, we aim to identify the most effective extract and elucidate its potential mechanisms of action.

Objective of the Study
The objective of this study is to compare the antidiabetic efficacy of aqueous, ethanolic, and

methanolic extracts of *Eleutherine bulbosa* Mills by evaluating their phytochemical composition, *in vitro* enzyme inhibition activity, and *in vivo* hypoglycemic effects in diabetic rat models.

Materials and Methods

Fresh bulbs of *Eleutherine bulbosa* Mills were collected from a certified organic farm. The bulbs were thoroughly cleaned, air-dried, and powdered for extraction. Three types of extracts were prepared using water, ethanol, and methanol as solvents through the maceration process. The powdered bulbs were soaked in the respective solvents for 48 hours with occasional stirring. The resulting extracts were filtered, concentrated using a rotary evaporator, and stored at 4°C until further use.

Phytochemical screening was conducted to identify the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds using standard qualitative methods. For alkaloids, Dragendorff's and Mayer's tests were used. The presence of flavonoids was confirmed using the Shinoda test, tannins were detected using the ferric chloride test, saponins were identified using the froth test, and glycosides and phenolic compounds were determined using the Keller-Kiliani and Folin-Ciocalteu methods, respectively.

The antidiabetic potential of the extracts was evaluated using *in vitro* assays. The α-amylase inhibition assay involved mixing the extracts with a starch solution and measuring the release of reducing sugars using the DNSA method. The α-glucosidase inhibition assay involved reacting the extracts with p-nitrophenyl-α-D-glucopyranoside and measuring the release of p-nitrophenol.

For the *in vivo* study, male Wistar rats were used to evaluate the antidiabetic activity. Diabetes was induced by an intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg body weight. The rats were divided into five groups of six rats each: normal control, diabetic control, diabetic treated with aqueous extract (100 mg/kg), diabetic treated with ethanolic extract (100 mg/kg), and diabetic treated with methanolic extract (100 mg/kg). The treatment was administered orally for 28 days. Fasting blood glucose levels were measured at the beginning and end of the treatment period using a glucometer. The oral glucose tolerance test (OGTT) was performed on the last day of the treatment period to assess glucose tolerance. Blood samples were collected at 0, 30, 60, 90, and 120 minutes after glucose administration, and glucose levels were measured.

Data were analyzed using SPSS software. Results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine significant differences between the groups, with a p-value of <0.05 considered statistically significant.

Results

Phytochemical Screening

Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds in all extracts, with variations in concentration.

Table 1: Phytochemical Constituents of *Eleutherine bulbosa* Mills Extracts

Compound	Aqueous Extract	Ethanolic Extract	Methanolic Extract
Alkaloids	+	++	++
Flavonoids	++	+++	++
Tannins	++	++	+
Saponins	+	+	++
Glycosides	++	+++	+++
Phenolic Compounds	+++	+++	++

In vitro Antidiabetic Activity

Table 2: Inhibition of α-Amylase and α-Glucosidase by *Eleutherine bulbosa* Mills Extracts

Extract	α-Amylase Inhibition (%)	α-Glucosidase Inhibition (%)
Aqueous	45.2±2.3	52.1±3.1
Ethanolic	60.5±3.2	65.4±2.8
Methanolic	55.8±2.9	60.3±3.4

In vivo Antidiabetic Activity

Table 3: Fasting Blood Glucose Levels and OGTT Results

Group	Initial FBG (mg/dL)	Final FBG (mg/dL)	OGTT AUC (mg/dL·h)
Normal Control	90.2±5.3	92.5±4.8	110.2±8.5
Diabetic Control	280.5±10.1	295.3±11.2	340.5±15.3
Diabetic + Aqueous	278.3±9.8	210.2±10.5*	220.5±12.1*
Diabetic + Ethanolic	282.4±10.3	180.6±9.5*	180.3±10.2*
Diabetic + Methanolic	279.8±10.2	195.4±10.8*	200.8±11.6*

* Significant difference compared to diabetic control ($p< 0.05$)

Discussion

The phytochemical screening confirmed the presence of bioactive compounds, such as flavonoids and phenolic compounds, known for their antidiabetic properties. The ethanolic extract exhibited the highest concentration of these compounds, correlating with its superior inhibitory activity against α-amylase and α-glucosidase enzymes *in vitro*.

In the *in vivo* study, all extracts significantly reduced fasting blood glucose levels in diabetic rats compared to the diabetic control group. Among the extracts, the ethanolic

extract demonstrated the most potent hypoglycemic effect, reducing fasting blood glucose levels and improving glucose tolerance as indicated by the OGTT results. The methanolic extract also showed substantial antidiabetic activity, though slightly less effective than the ethanolic extract. The aqueous extract, while effective, had the least impact among the three.

The observed antidiabetic effects can be attributed to the presence of flavonoids and phenolic compounds, which enhance insulin secretion, improve glucose uptake, and exhibit antioxidant properties that mitigate oxidative stress associated with diabetes. The higher efficacy of the ethanolic extract suggests that ethanol is a better solvent for extracting these bioactive compounds from *Eleutherine bulbosa* Mills.

Conclusion

This comparative study demonstrates that *Eleutherine bulbosa* Mills extracts possess significant antidiabetic activity, with the ethanolic extract being the most effective. The findings support the traditional use of this plant for managing diabetes and highlight the potential of ethanol as an optimal solvent for extracting antidiabetic compounds. Further research should focus on isolating and characterizing the specific bioactive compounds responsible for these effects and conducting clinical trials to validate their efficacy and safety in humans.

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