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Research Article

Pharmacognostic Profile and Antidiabetic Activity of *Eleutherine bulbosa* Mills. Bulbs from East Kalimantan, Indonesia

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ABSTRACT

Background: *Eleutherine bulbosa* (Mill.) Urb., known as Bawang Dayak, is commonly found growing in Kalimantan, Indonesia. The characteristics of the environment in which the plant grows greatly influence the characteristics of the plant and its biological activity. **Objective:** This study aimed to determine the pharmacognostic profile and evaluate the antidiabetic activity of *E. bulbosa* bulb extract obtained from Kota Bangun, Kutai Kertanegara, Indonesia. **Methods:** The dried powdered of *E. bulbosa* bulbs was extracted using two different methods (maceration and microwave-assisted extraction). The evaluation of the sample fluorescence and non-specific parameters was done. The antidiabetic activity of the extracts was conducted using streptozocin-induced mice and histopathology analysis. **Results:** The fluorescence evaluation indicated various secondary metabolites contained in *E. bulbosa* simplicia. Non-specific parameters include drying loss (8.83±0.2611%), water content in simplicia (8.04±0.7481%), specific gravity (1.04±0.0058%), total ash content (20.3±0.1583%), acid insoluble ash content (1.70±0.0608%), ethanol content in extract (0.99±0.0017%), lead content (0.010 mg/g), cadmium (0.091 mg/g), Arsenic (0.0003 mg/g), aflatoxin (not detected), and mold/yeast (0.8 × 105 colonies/g) meet the established regulations. Meanwhile, the antidiabetic assay showed that both extracts decreased the blood glucose levels in mice during the 14 days of treatments in a dose-dependent manner. This activity is correlated with the increased repair of pancreatic beta cells as observed by histopathological analysis. **Conclusion:** The dried bulbs of *E. bulbosa* obtained from Kota Bangun, Kutai Kertanegara, East Kalimantan, Indonesia meet the standards set as raw materials for antidiabetic herbal medicines.

Keywords: Antidiabetic activity, Bawang Dayak, contaminant levels evaluation, *Eleutherine bulbosa* (Mill.) Urb, pharmacognostic profile.

INTRODUCTION

Eleutherine bulbosa (Mill.) Urb., known by the local name "Bawang Dayak," comes from the Iridaceae family. This plant is easy to find growing in the forests of Kalimantan, Indonesia, spread throughout the Kalimantan region, and some people have cultivated this plant, although most people still harvest it from its natural habitat to meet market demand[1]. This plant is also found on other islands in Indonesia, such as Sulawesi and Sumatera.

Traditionally, this plant is trusted and used by local people to treat various diseases such as diabetes mellitus, hypertension, stroke, and sexual disorders, and it is also used to treat cancer such as breast, prostate, and cyst cancer[2]. In additon, it is also used to increase breast milk production. Consistent with its traditional uses, *E. bulbosa* bulbs has been scientifically proven to exhibit biological activities such as antihyperglycemic[3,4], antioxidant[5], antidiabetic (including α-glucosidase inhibitor, oral glucose tolerance activity, and antidiabetic activity)[3,6,7], immunomodulator[8], antihypercholesterol[9], antihypertension[10], anticancer[11,12], and antiinflammation[13]. It has also been reported that this plant contains secondary metabolites such as flavonoids, alkaloids, saponins, quinones, tannins[5,14,15], and polyphenols[16].

To be accepted as a raw material for herbal medicine, a plant must meet the requirements, especially the content of active substances and the contaminants related to safety. Meanwhile, its presence in natural habitats with various conditions causes differences in the quality and quantity of the active components contained therein, so the pharmacological activity of each region cannot be confirmed correctly. Apart from that, it also affects contamination in each growing area. Muthia et al. (2021) have succeeded in standardizing the *E. bulbosa* bulb in various regions in Kalimantan (including Banjarbaru, South Kalimantan; Palangkaraya City, Central Kalimantan; and Balikpapan, East Kalimantan)[17]. However, this region is not yet sufficiently representative of the entire region's growth of *E. bulbosa*. Nonetheless, it can be used as a reference/standard for making medicinal raw materials from other regions. For instance, *E. bulbosa* which grows naturally in Kota Bangun, Kutai Kertanegara, East Kalimantan has not been stardardized. Therefore, it is necessary to determine a pharmacognostic profile as a basis for guaranteeing the quality of raw materials using a pharmacognosy approach.

This research aimed to determine the pharmacognostic profile and evaluate the antidiabetic activity of *E. bulbosa* bulb extract obtained from Kota Bangun, Kutai Kertanegara, Indonesia. Determination of the pharmacognostic profile is limited and focuses on fluorescence, non-specific character, and contamination evaluation. Meanwhile, the antidiabetic evaluation focuses on

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measuring the blood glucose levels of mice induced with streptozotocin (STZ) and treated with extract samples. In addition histopathological tests were carried out to see the changes in improvement/ increase in pancreatic beta cells.

MATERIALS AND METHODS

Plant Materials

The fresh sample of *E. bulbosa* bulbs was collected from Kota Bangun, Kutai Kertanegara, East Kalimantan, Indonesia. The voucher specimen (010/PTUP-LP/FFUNMUL/VI/2022) was identified by a plant taxonomist from the Laboratory of Dendrology, Faculty of Forestry, Universitas Mulawarman.

Extraction Process

The extraction process was conducted with two different extraction methods, namely maceration and microwave-assisted extraction (MAE). The first method, the dried sample (200 g), was macerated using ethanol as a solvent for 3x24 hours[6]. Meanwhile, in the second method, the dried sample (200 g) was extracted using the MAE method with water as a solvent under specific extraction conditions[18]. The extract solution and residue were separated using filter paper and then evaporated using a rotary evaporator. The extract obtained was stored in a tightly closed container until ready to use.

Fluorescence analysis

Fluorescence analysis was performed using the standard method to analyze dried powdered bulbs of *E. bulbosa* for fluorescence characteristics under visible light, long and short-UV light to detect fluorescence secondary metabolite content in the sample before and after adding specific reagents according to literature[19,20,21].

Non-Specific Character of Dried Powder

Non-specific character analysis was used to evaluate the levels of drying loss, water content, specific gravity, total ash content, acid insoluble ash content, and ethanol content in extract in dried powder of *E. bulbosa* bulb using standard method according to literature[22–25].

Contaminant analysis

Contaminant analysis evaluated the heavy metal and microbial contaminants (aflatoxin and molt/yeast) of *E. bulbosa* bulb samples. The heavy metal content of dried powder of *E. bulbosa* bulb was analyzed using an atomic absorption spectrometer (AAS) based on the literature [17,21], with argon as the carrier gas and a flow rate of 1 ml/2 min. Meanwhile, aflatoxin contamination was analyzed using LC-MS/MS, and Molt/Yeast was analyzed using the total plate number method based on the standard literature[17,26,27].

In vivo Antidiabetes Activity Assay

Forty-five Swiss albino mice were used in this study under approval by the ethics committee of the Faculty of Pharmacy, Universitas Mulawarman (55/KEPK-FFUNMUL/EC/EXE/07/2022). Experimental animals were divided into nine groups: normal control, positive control (metformin), negative control, ethanol, and water extract with doses of 0.13, 0.52, and 0.91 mg/20 kg BW, respectively. Before being treated with the extract, diabetes was induced in mice by injecting STZ solution at 0.2 ml/20 g BW into mice intraperitoneally. STZ was dissolved in a pH 4.4 buffer solution. 150 mg of STZ was dissolved in 4.95 ml of citric acid and 5.05 ml of sodium nitrate. Blood glucose was checked using a glucose strip test 3 days after STZ induction. After blood sugar rose from normal, the mice were given extract treatment for 14 days[7,10,28]. On the 15th day, blood sugar levels were measured again, and data analysis was carried out to see changes in blood glucose levels before and after giving the sample.

Histopathology Analysis

Histopathological analysis was carried out after treatment and observation on day 15 by isolating the pancreas of mice to make hematoxylin-eosin (HE) preparations. The isolated tissue is placed in a formaldehyde solution to be made into a paraffin block. After making a paraffin block, an incision is made using a microtome. The incision was then subjected to HE staining. HE staining aims to see the morphology and histology of pancreatic cells. The first step of HE staining begins with tissue deparaffinization using graded xylol for five minutes, followed by graded alcohol (absolute alcohol, 90%, 70%, 50%) for two minutes each. Next, wash with running water for two minutes. After washing, the tissue is dipped in Mayer's Hematoxylin for five minutes, followed by 1% lithium carbonate for three minutes. After processing with lithium carbonate, the tissue is dipped in eosin for five minutes and washed with running water for two minutes. Next, a dehydration process was carried out using graded alcohol (70%, 90%, 100% alcohol) for two minutes each, followed by a clearing process using graded xylol for three minutes each. The final stage of the HE staining process is mounting with Entelan and covering with cover glass. The results are observed under a microscope to see the histology of the cells in the islets of Langerhans and the surrounding tissue, whether necrosis has occurred or not. Data was analyzed qualitatively[29].

RESULTS

Fluorescences Evaluation

The fluorescences characteristic of dried bulb powder of *E. bulbosa* was evaluated under short and long ultraviolet (UV) and visible light before and after treatment with specific reagents (shown in Table 1). Plant materials exhibit fluorescence due to the presence of many chemical components. Certain wavelengths of visible light, short ultraviolet light (254 nm wavelength), and long ultraviolet light (366 nm wavelength) would cause the chemical components treated with various reagents to fluoresce.

Non-Specific Characters Evaluation

The non-specific character of *E. bulbosa* simplicial bulb powder was evaluated, including drying loss, water content, specific gravity, total ash content, acid insoluble ash content, and ethanol content in extract (Table 2). The simplicial characterization of *E. bulbosa* bulbs was assessed to establish requirements and clarify the material under investigation since the origin of the growth environment influenced the active compound content.

Contamination Evaluation

The contamination evaluation included mold/yeast, aflatoxin, and heavy metals (lead, cadmium, and arsenic). The test results in Table 3 show that *E. bulbosa* bulbs were safe from contamination from heavy metals, aflatoxins, and mold/yeast (calculated using the total plate number method) following those stipulated in the Indonesian National Standard regulations (SNI).

Antidiabetic Evaluation

The effects of various doses of both extracts from *E. bulbosa* bulb on blood glucose levels in mice are shown in Table 4. The blood glucose levels from each extract were compared with the control (normal, positive, and negative). At three different doses, each extract showed potential as an antidiabetic compared to the positive control. Increasing the dose shows a tendency to increase the percent decrease in blood glucose levels. Meanwhile, the ethanol extract showed a more significant percent reduction than the water extract.

Based on Table 4 above, it shows that both extracts have potential anti-diabetic activity in mice. The effect also shows an improvement

Table 1: Fluorescence Results of Dried Bulbs Powder of *E. bulbosa*.

Sample + Reagent	Color Observed		
	Visible Light	UV Short (254 nm)	UV Long (366 nm)
Powder as such	Pink		
Powder + FeCl3	Brown	Fluorescence green	Fluorescence green
Powder + HCl	Yellow	Slightly yellowish green Fluorescence	Light green
Powder + Acetic acid	Yellow	Fluorescence light green	Fluorescent yellowish green
Powder + 0.1 N NaOH	Dark purple	Dark green	Fluorescence Green
Powder + Ammonia	Red	Purplish light green	Fluorescence Light Green
Powder + liebermann-burchard	Brownish-yellow	Yellowish green fluorescence	Fluorescence yellowish green

Table 2: Non-Specific Character of *E. bulbosa* bulbs simplicial.

No	Test	Replication (%)			Results (%)	Requirement
		R1	R2	R3		
1	Drying Loss	8.54	8.92	9.04	8.83 ± 0.2611	≤ 10.0%
2	Water Content in simplicial	8.12	8.75	7.26	8.04 ± 0.7481	≤ 15.0%
3	Specific Gravity	1.04	1.04	1.05	1.04 ± 0.0058	-
4	Total Ash Content	20.35	20.14	20.45	20.3 ± 0.1583	≤ 37.8%
5	Acid Insoluble Ash Content	1.67	1.57	1.56	1.70 ± 0.0608	≤ 26.2%
6	Ethanol Content in Extract	0.99	0.99	0.99	0.99 ± 0.0017	≤ 1.0%

Table 3: Heavy Metal and Microbial Contamination.

No	Contamination Test		Units	Result	Requirement
1	Heavy Metal	Lead (Pb)	mg/g	0.010	≤ 10 mg/kg
		Cadmium (Cd)	mg/g	0.091	≤ 0.3 mg/kg
		Arsenic (As)	mg/g	< 0.0003	≤ 5 mg/kg
2	Aflatoxin	B1, B2, G1, G2	µg/Kg	ND	≤ 5 µg/kg
3	Mold/Yeast	Total plate number	Colonies/g	$0.8x10^5$	≤ $5x10^5$ Colonies/g

Table 4: Antidiabetic Activity of *E. bulbosa* Extract.

Test Group	Doses (mg/20 g BW)	Average blood glucose levels (mg/dL)			Percent Decrease (%)
		Before Induction	After Induction	14 days after treatment	
Control	Normal (without treatment)	109	109	111	-1.90
	Negative (0.5% NaCMC)	124	272	271	-0.42
	Positive (Metformin)	112	229	143	36.84
Ethanol Extract	0.13	123	215	160	24.17
	0.52	112	274	186	30.50
	0.91	98	224	151	31.24
Water Extract	0.13	113	229	190	17.79
	0.52	109	214	172	19.13
	0.91	137.2	256	197	22.23

in pancreatic beta cells, as shown in the histopathological test results in Figure 1. Improvement in pancreatic beta cells increases with increasing dose compared to the negative and normal control.

DISCUSSION/CONCLUSION

A pharmacognostic profile studies the inherent characteristics, constant parameters, and definitive qualitative and quantitative values that lead to a particular medicinal plant's characteristics. This profile plays a crucial role in the authentication and standardization of medicinal plants, especially to guarantee the quality and quality of the ingredients, as well as avoiding adulteration of ingredients to seek personal gain[17].

In the present study, we used the bulb of *E. bulbosa* from Kota Bangun, Kutai Kertanegara, East Kalimantan, Indonesia, to determine its pharmacognostic profile and antidiabetic evaluation. Pharmacognostic evaluations are the first stage in defining criteria for evaluating the authenticity and quality of every crude drug medicine. Fluorescence, non-specific character, and contaminant evaluation are some pharmacognostic parameters that are standard values that should be met to obtain herbal medicine raw materials that the commercial market can accept. The test results of several pharmacognostic parameters show that the *E. bulbosa* bulbs obtained in the area of Kota Bangun, Kutai Kertanegara, East Kalimantan, Indonesia, meet standardization values following established regulations [26,27] and previous studies [17,30]. Meanwhile, both extracts' antidiabetic activity assay results (obtained from different extraction methods) show consistency in potential activity as reported in previous studies [3,6,7,10,28,31,32]. Therefore, *E. bulbosa* bulbs can meet pharmacognostic criteria and have the antidiabetic activity to be used as a source of raw materials for antidiabetic herbal medicines.

Based on the evaluation of pharmacognostic profile and its antidiabetic activity, the dried powdered sample of *E. bulbosa* bulbs from Kota Bangun, Kutai Kertanegara, East Kalimantan, Indonesia meets the established standardization requirements to be used as raw material for antidiabetic herbal medicine.

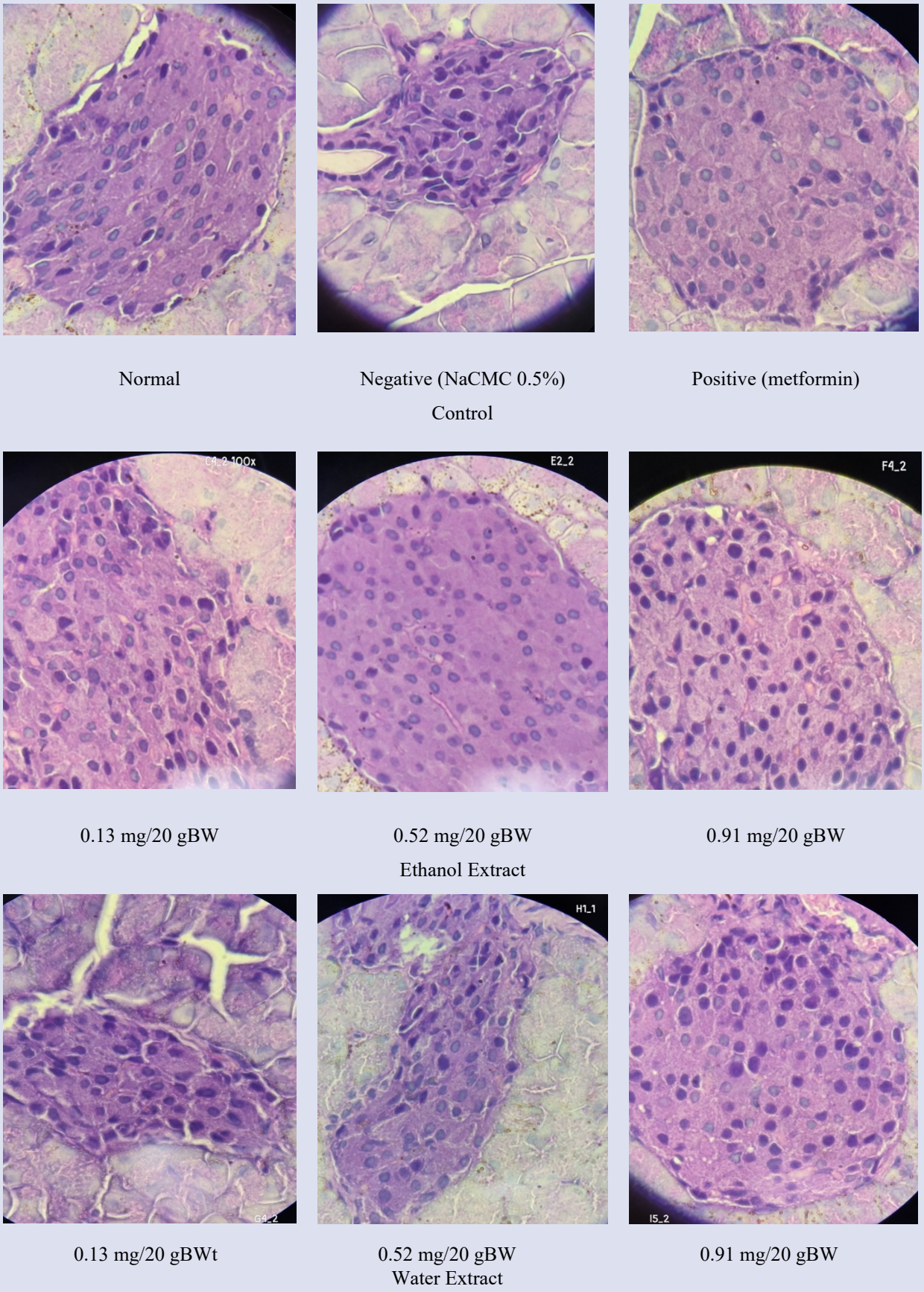

Figure 1: Histopathological picture of pancreatic beta cells in experimental animals.

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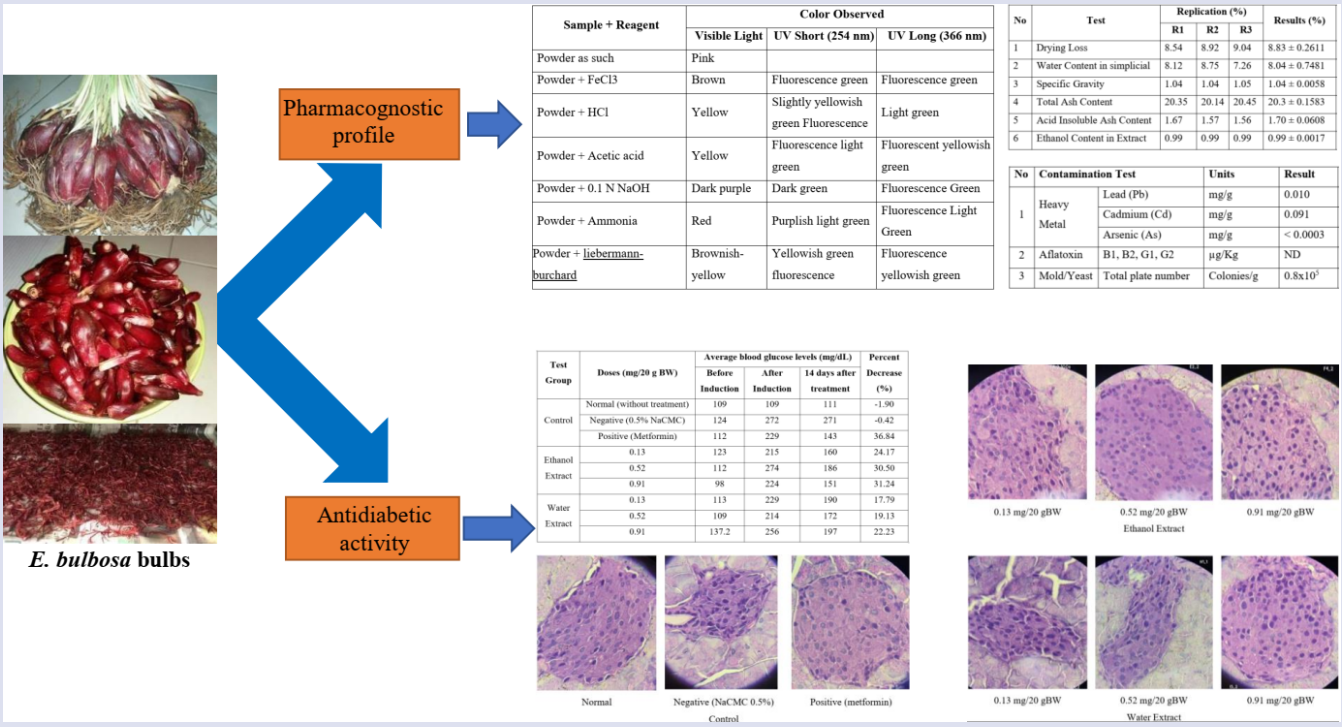
GRAPHICAL ABSTRACT



Sample + Reagent	Color Observed		
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Powder + FeCl3	Brown	Fluorescence green	Fluorescence green
Powder + HCl	Yellow	Slightly yellowish green Fluorescence	Light green
Powder + Acetic acid	Yellow	Fluorescence light green	Fluorescent yellowish green
Powder + 0.1 N NaOH	Dark purple	Dark green	Fluorescence Green
Powder + Ammonia	Red	Purplish light green	Fluorescence Light Green
Powder + liebermann-burchard	Brownish-yellow	Yellowish green fluorescence	Fluorescence yellowish green

No	Test	Replication (%)			Results (%)
		R1	R2	R3	
1	Drying Loss	8.54	8.92	9.04	8.83 ± 0.2611
2	Water Content in simplicial	8.12	8.75	7.26	8.04 ± 0.7481
3	Specific Gravity	1.04	1.04	1.05	1.04 ± 0.0058
4	Total Ash Content	20.35	20.14	20.45	20.3 ± 0.1583
5	Acid Insoluble Ash Content	1.67	1.57	1.56	1.70 ± 0.0608
6	Ethanol Content in Extract	0.99	0.99	0.99	0.99 ± 0.0017

No	Contamination Test		Units	Result
1	Heavy Metal	Lead (Pb)	mg/g	0.010
		Cadmium (Cd)	mg/g	0.091
		Arsenic (As)	mg/g	< 0.0003
2	Aflatoxin	B1, B2, G1, G2	µg/Kg	ND
3	Mold/Yeast	Total plate number	Colonies/g	$0.8x10^5$

Test Group	Doses (mg/20 g BW)	Average blood glucose levels (mg/dL)			Percent Decrease (%)
		Before Induction	After Induction	14 days after treatment	
Control	Normal (without treatment)	109	109	111	-1.90
	Negative (0.5% NaCMC)	124	272	271	-0.42
	Positive (Metformin)	112	229	143	36.84
Ethanol Extract	0.13	123	215	160	24.17
	0.52	112	274	186	30.50
	0.91	98	224	151	31.24
Water Extract	0.13	113	229	190	17.79
	0.52	109	214	172	19.13
	0.91	137.2	256	197	22.23

SUMMARY

1. The fluorescence evaluation indicated various secondary metabolites contained in *E. bulbosa* simplicia.
2. Non-specific parameters include drying loss (8.83±0.2611%), water content in simplicia (8.04±0.7481%), specific gravity (1.04±0.0058%), total ash content (20.3±0.1583%), acid insoluble ash content (1.70±0.0608%), ethanol content in extract (0.99±0.0017%), lead content (0.010 mg/g), cadmium (0.091 mg/g), Arsenic (0.0003 mg/g), aflatoxin (not detected), and mold/yeast (0.8 × 105 colonies/g) meet the established regulations.
3. The antidiabetic assay showed that both extracts decreased the blood glucose levels in mice during the 14 days of treatments in a dose-dependent manner.

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