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Research Article

PHYSICOCHEMICAL PROPERTIES, PHYTOCHEMICAL SCREENING, AND ANTIHYPERGLYCEMIC ACTIVITY OF INAI (*LAWSONIA INERMIS* L.) LEAVES ACTIVE FRACTION ON STREPTOZOTOCIN-INDUCED DIABETIC MICE

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ABSTRACT

Objective: This study was designed to explore the preliminary phytochemical and physicochemical analysis of Inai (*Lawsonia inermis* L.) leaves and further evaluation of its antihyperglycemic effect on diabetic rats from its active fraction.

Methods: The study begins with making the powder of Inai (*L. inermis* L.) leaves and then evaluates the physicochemical characterization. The powder of Inai leaves was fractionated and performed by the standard phytochemical screening method and streptozotocin-induced diabetic mice for evaluated the antihyperglycemic effect of Inai leaves.

Result: The physicochemical evaluation showed that the powder of Inai leaves has a good and high purity level; while the phytochemical screening showed that Inai leaves fractions have various phytoconstituents. The active fraction which was obtained from ethyl acetate fraction showed a significant reduction of the blood glucose.

Conclusion: The leaves of Inai (*L. inermis* L.) have the antihyperglycemic activity.

Keywords: Antihyperglycemic, Diabetic, Inai, Streptozotocin, Mice.

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INTRODUCTION

Diabetes mellitus (DM) is a disease or disorder of chronic metabolism with various etiologies characterized by high blood sugar (hyperglycemia) with carbohydrate, lipid, and protein metabolism due to insulin insufficiency function [1]. The state of chronic hyperglycemia will cause tissue damage such as kidney damage, retinal nerves, and cardiovascular vessels [2]. It is associated to long-term damage of various organs such as eyes, liver, kidneys, nerves, and blood vessels and it may cause degenerative diseases in central nervous system [3]. The prevalence of diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [4].

Nowadays, there is a global increase in the prevalence of DM related to the lifestyles and the resulting surge from in obesity [5]. Reasons for this rise are sedentary lifestyle, obesity, consumption of high energy rich diet, higher lifespan, etc. [6]. The most majority of these cases will be Type 2 DM (non-insulin-dependent DM) [7]. In spite of the availability of various antidiabetic agents and its secondary complications continue to become a major problem in the world population, medicinal plants and their extracts or fractions have an ancient background in this issue and used as an alternative method to treat the diabetes patient throughout the world and popular as nutraceutical [8,9].

One plant that is widely used as a traditional medicine is Inai (*Lawsonia inermis* L.). The leaves, flowers, seeds, stem bark, and plant roots have the potential to cure headaches, arthritis, diarrhea, and fever [10]. This plant is known throughout the world as a cosmetic ingredient used for hair dye, nails, and leather [11]. Among the people, stew leaves used as a medicine to relieve itching and ulcers that are suspected due to increased blood sugar levels. In addition, the leaf is also used by certain rural communities in Indonesia as a skin wound healer [12,13].

Based on various research results, Inai plant exhibits various pharmacological activities such as antibacterial, anti-irritant, antioxidant, antihyperglycemia, anti-inflammatory, analgesic, antipyretic, anticoagulant, and inotropic activity [14,15]. The antihyperglycemic effect of Inai leaves has been previously described for its leaves ethanolic extract [12]; nevertheless, the available information regarding the leaves is incomplete. The present study was designed to perform physicochemical and phytochemical analyses of Inai (*L. inermis* L.) and to evaluate its active fraction for antihyperglycemic activity in streptozotocin (STZ)-induced diabetic mice.

METHODS

Plant collection and identification

Fresh Inai (*L. inermis* L.) was collected from the city of Langsa (Aceh, Indonesia) and authenticated by the Indonesian Institute of Sciences: Research Center For Biology (No: 2534/IPH.1.01/If.07/XII/2015). The dried leaves samples were crushed and grounded to obtain a finely divided powder.

Preparation of fractions

Ethanolic extract of the powder was obtained by maceration method for 7 days followed by filtration. The ethanolic solvent was evaporated on rotary evaporator to obtain crude ethanolic extract and dried using freeze dryer to get dried crude ethanolic extract. The ethanolic extract was suspended in distilled water and partitioned with hexane, ethyl acetate, and water to obtain fractions of these solvents. The solvents were removed on rotary evaporator to obtain dried fractions [16,17].

Physicochemical evaluation

Analysis of physicochemical constants of the Inai leaves powder and ethanolic extracts has been done to evaluate the quality and purity of the herbal drugs. Various physicochemical parameters such as total ash value, acid-insoluble ash value, moisture content, alcohol, and water-soluble extractive value were calculated as per the World Health Organization (WHO) and Indonesia Materia Medika Guidelines [18,19].

Preliminary phytochemical screening
Phytochemical screening carried out on Inai leaves hexane fraction, ethyl acetate fraction, and water fraction, including examing the chemical metabolites constituent of alkaloids, flavonoids, glycosides, saponins, tannins, triterpenoids, and steroids [20,21].

Preparation of animals
Healthy adult male mice (20–30 g body weight) from animal house of Faculty of Pharmacy, University of Sumatera Utara, were used for the study. The use of animals was approved by "Animal Research Ethics Committees (ARECs) of University of Sumatera Utara (AREC Reg. No: 415/KEPH-FMIPA/2017)." Mice were housed in polycarbonate cages under room temperature (20 ± 2°C), relative humidity (60–70%) and were exposed to 12 h day-night circle. They were fed on a standard pellet diet and water *ad libitum.*

Oral glucose tolerance test
The oral glucose tolerance test was performed in overnight fasted (18 h) normal mice. Healthy mice were randomly selected and distributed into five groups (n=6). One of those groups was administered carboxymethyl cellulose (CMC) 5% and the four groups were given orally glibenclamide (0.65 mg/kg bw), hexane fraction, ethyl acetate fraction, and water fraction of Inai (*L. inermis* L.) with 600 mg/kg bw, respectively. 1% glucose was fed 1 h after the administration of fractions and glibenclamide. Blood was withdrawn from the tail vein at 30, 60, 90, and 120 min of glucose administration and glucose levels were estimated using a blood glucose monitoring kit. The most active fraction decreases blood glucose level which obtained from analyzed result will be used for further experiment.

Experimental design for antihyperglycemic activity
Experimental diabetes was induced by single intraperitoneal injection of 55 mg/kg of STZ, freshly dissolved in citrate buffer (pH 4.5). After 3 days of STZ injection, mice with fasting glucose above 200 mg/dl were considered as diabetic and included in the study (Fazrina).

The animals were divided into five groups of three animals for each group was used in this experiment:
Group I: Normal control animals given normal pellet and CMC 0.5% b/v.
Group II: STZ-induced mice.
Group III: Mice were induced by STZ and treated with glybenclamide (0.65 mg/kg).
Group IV: Mice were induced by STZ and treated with ethyl acetate fraction (600 mg/kg).

The extract was given daily through oral way for 15 days.

Statistical analysis
All the values were determined by triplicates and expressed as mean ± standard error of mean. The significant difference of data between different groups was compared by ANOVA followed by Duncan's test.

RESULTS

Physicochemical evaluation
Table 1 summarizes the result of physicochemical evaluation from Inai (*L. inermis* L.) leaves powder and ethanol extract.

Phytochemical screening of Inai (*L. inermis* L.) fractions
Screening results of Inai (*L. inermis* L.) leaves fractions showed different chemical compound in different fraction. The results can be seen in Table 2.

Oral glucose tolerance test
The oral glucose tolerance test was comparable to that of the standard antidiabetic drug, glibenclamide. Maximum effect was observed for ethyl acetate fraction. The results can be seen in Table 3 and Fig. 1.

Effect of Inai leaves ethyl acetate fraction on diabetic mice
STZ-induced diabetic mice significantly increased the blood glucose level as shown in Table 4. The antihyperglycemic effect of Inai leaves ethyl acetate fraction at dose 600 mg/kg was studied in diabetic mice. Table 4 and Fig. 2 show the level of blood glucose at various intervals to observe the effect of different treatment using pumpkin flesh and seeds extracts and metformin.

DISCUSSION

The result of physicochemical properties evaluation shows that the powder and the ethanolic extracts were made with a good quality and high purity level, which accepted by the WHO. The most important parameter of physicochemical is the value of moisture. The less value of moisture content could prevent bacterial, fungal, and yeast growth so the quality will be good for a long of time [22].

The phytochemical screening investigation of different fractions of Inai (*L. inermis* L.) results shows that Inai leaves fractions revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, steroids, and terpenoids (Table 2). Many of these phytochemical

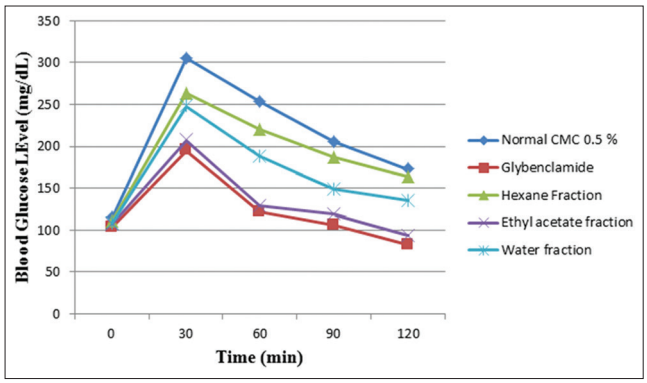

Fig. 1: The oral glucose tolerance test result

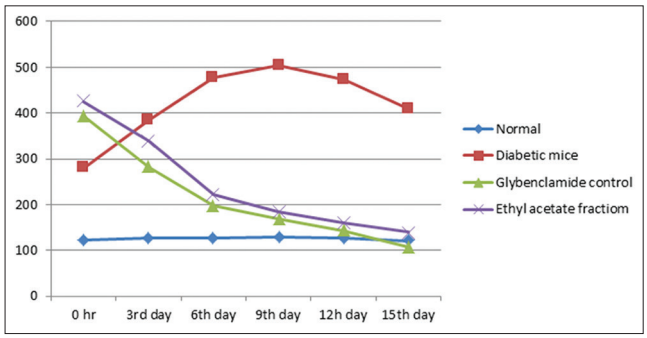

Fig. 2: Effect of Inai leaves ethyl acetate fraction on blood glucose level in diabetic mice (mg/dl)

Table 1: Physicochemical evaluation result of Inai leaves powder and ethanolic extract

Inai leaves	Physicochemical evaluation				
	Moisture content (%)	Total Ash value (%)	Acid-insoluble Ash (%)	Extractive soluble values	
				Alcohol (%)	Water (%)
Powder	7.3	5.49	0.82	14.30	21.96
Ethanolic extract	6.98	2.31	0.59	27.61	23.61

Table 2: Phytochemical screening result of Inai *(L. inermis* L.) fractions

No	Screening	Inai (*L. inermis* L.) leaves fractions		
		Hexane	Ethyl acetate	Water
1	Alkaloids	−	+	+
2	Flavonoids	−	+	+
3	Glycosides	−	−	+
3	Saponins	−	−	+
4	Tannins	−	+	+
5	Triterpenoid/steroids	+	−	−

+Sign indicates secondary metabolite is present, −sign indicates secondary metabolite is not present. *L. inermis: Lawsonia inermis*

Table 3: Effect of different fractions of Inai leaves and glibenclamide on oral glucose tolerance of mice

Groups	Blood glucose level (mg/dL)				
	Normal	30 min	60 min	90 min	120 min
Control CMC 5%	115.00±3.785	305.33±20.744*	254.66±10.962*	206±17.691*	173.66±10.503*
Glybenclamide 0.35 mg/kg	103.66±3.785	195.66±6.11**	122.66±9.609**	106±6.524**	83±4.35**
Hexane fraction 600 mg/kg	111.33±11.718	263.66±40.079**	220.33±15.631**	187.33±23.501**	164.66±5.859**
Ethyl acetate fraction 600 mg/kg	107.33±6.11	207.33±10.066**	129.33±1.154**	119.66±5.033**	94.66±6.658**
Water fraction 600 mg/kg	109.00±2.645	248.66±22.120**	188.33±23.459**	149.66±11.150**	136.66±4.163**

Values are given as mean±SEM for five mice in each group. *p<0.001; Control CMC was compared with the normal; fractions and standard treated groups were compared with the control CMC diabetic group was compared with normal group. SEM: Standard error of mean, CMC: Carboxymethyl cellulose

Table 4: Effect of Inai leaves ethyl acetate fraction on blood glucose level in diabetic mice

Group	Blood glucose level (mg/dL)					
	0 h	3rd day	6th day	9th day	12th day	15th day
I	123.8±4.91	126±11.11	127.6±6.22	129.8±4.08	127.6±5.17	121.4±5.128
II	280±26.18[a]	384.4±12.9[a]	478.4±15.7[a]	504.2±11.7[a]	472.8±12.53 [a]	410.6±17.12[a]
III	394±31.44[b]	282.4±49.86[b]	197±8.86[b]	168.4±7.16[b]	143.8±9.7[b]	107.8±3.27[b]
IV	425.2±71.09[b]	338±4.56[b]	221.4±7.98[b]	185.6±15.24[b]	161.2±15.77[b]	140.6±11.82[b]

Values are given as mean±SEM for three mice in each group. [a]p<0.001 Diabetic group was compared with normal group. [b]p<0.001 was compared with diabetic group. SEM: Standard error of mean

compounds have been shown to produce potent antihyperglycemic activity.

The evaluation of the effect of three fractions (hexane, ethyl acetate, and water) from Inai leaves revealed the result that the most potential antihyperglycemic activity. The ethyl acetate fraction from Inai leaves showed a significant decrease in the blood glucose levels of the mice. This potential effect of ethyl acetate fraction from Inai leaves might be achieved by facilitating insulin release in pancreatic β-cells, inhibiting glucose absorption in gut, stimulating glycogenesis, and protect the DNA from the oxidative damage, so it can resist the problem in β-cell [23-25].

CONCLUSIONS

These findings suggest that Inai (*L. inermis* L.) leaves have a potent antidiabetic activity in STZ-induced diabetic mice.

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AUTHORS CONTRIBUTION

All the author have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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