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Antioxidant and Antidiabetic Potential of Galing Stem Extract (*Cayratia trifolia* Domin)

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ABSTRACT

Galing (*Cayratia trifolia* Domin.) have been used empirically to treat various diseases, one of them is antidiabetic. **Objective:** To determine the phytochemical content and the activity of the ethanol extract of the galing stem as antioxidant and antidiabetic. **Method:** The galing stem extract was investigated for phytochemical by Thin Layer Chromatography (TLC) and its antioxidant activity using DPPH scavenging activity assay. *In vivo*, antidiabetic test was conducted by animal diabetes modeling that has given streptozotocin 150 g/kg BW and 10% of sucrose solution intraperitoneally. The average level of fasting blood glucose at 307 mg/dL. Mice were divided into 6 groups, normal control group, diabetes induction group, positive group (treated by glibenclamide) and treatment group consisted of three groups were treated by ethanol extract of galing stem in dose 400, 500, and 600 mg/kg BW, respectively. Mice treated with appropriate doses of each treatment once a day for 7 days. The measuring blood glucose level was using a photometer 5010V5+. **Results:** The ethanol extract of galing stem contained the alkaloid, flavonoid, saponin, tannin, and triterpene. It is potential as an antioxidant with the IC_{50} 61,52 mg/L which indicated that the extract had strong antioxidant. The antidiabetic activity showed that the effect of extract 400 and 500 mg/kg BW are not different significantly with glibenclamide in reducing blood glucose levels subset of the statistics ANOVA ($p > 0.05$). **Conclusion:** The ethanol extract of galing stem is potent as an antioxidant due to the phytochemical content inside particularly the flavonoid compound.

Key words: Antioxidant, Antidiabetic, Galing stem, Mice, Phytochemical.

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by hyperglycemia and abnormal metabolism of carbohydrates, fats, and proteins that caused by decreasing insulin secretion, decreasing insulin sensitivity or both.^{1,2} Diabetes mellitus is a disease that causes a risk mortality/morbidity and decreasing of patients life.² Treatments of diabetes have been conducted in various ways, such as regular exercise and diet. The treatment can also by giving of insulin or commercial antidiabetic medicines which are commonly known as synthetic drugs. This treatment is expensive and causes side effects. Some symptoms of the side effects are included bloating and diarrhea. The other side effects that may result are the increasing risk of myocardial infarction and the increased risk of cardiovascular side effects.^{2,3}

The using of traditional medicines is safer than the modern medicines because traditional medicines have less relatively side effects than modern medicines.^{4,5}

The number of synthetic organic chemical compounds have been available for diseases treatment, but very important to look for the other alternatives that allow for better effectiveness and therapy which are expected to have minimal side effects; the medicines extracted from plants.⁶ One of the plants that have been used by

Indonesian people as traditional medicine is galing (*Cayratia trifolia* Domin). The roots of galing have been used empirically to treat various diseases, such as poultice on boil surface. The seeds infuse and galing bulbs extract traditionally been used for diabetic patients to lower the blood sugar levels, galing also has efficacious as anti-diuretic, anti-tumor, neuralgia, and splenopathy. The bulbs commonly are used in snakebite treatment.⁷ Research conducted by Batra *et al.*⁸ showed that flavonoids contained by the galing roots have antidiabetic effect which improves pancreatic β -cells that have been damaged.⁸ However, the activity of galing stem as antidiabetic has not been tested scientifically. Therefore we are interested in conducting the antioxidant activity and antidiabetic effects of stem galing ethanol extract in mice by of streptozotocin inducing method.

MATERIALS AND METHODS

Chemicals

Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), streptozotocin from Sigma Chemie GmbH Aldrich. Pro analysis grades of ethanol, n-hexane, ethyl acetate, methanol, Dragendorff reagents, ammonia vapor reagent, Lieberman-Buchard reagent, H_2SO_4

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0.1 M, FeCl₃ 1%, sucrose 10%, citrate buffer and TLC silica gel plate of 60 GF254 was purchased from Merck. Distilled water was obtained through a Millipore-Q50 Ultrapure water system (Sartorius). The stock solution of DPPH ($c = 100$ mg/L) was prepared by dissolving 5 μ g of DPPH with 20 ml ethanol and diluted to 50 ml. The stock solution of ascorbic acid ($c = 100$ mg/L) was prepared by dissolving 5 mg with 10 ml ethanol and diluted to 50 ml in the flask.

Extraction

The sample of galing stem was macerated with 96% ethanol for 3x24 h at room temperature. Remacerated was conducted by stirring and replacing the solvent after first maserat filtering for 3 times. The filtrate was collected then concentrated by rotary vacuum evaporator at 50 °C to obtain a viscous extract.⁸

Phytochemical screening

Phytochemical screening was conducted by using TLC silica gel plate of 60 GF254. The Extracts were spotted on ± 1 cm from the bottom edge of the plate with the capillary tube, then dried and eluted with the eluent n-hexane: ethyl acetate: methanol. Alkaloids detected by Dragendorff reagent, flavonoids detected by ammonia vapor reagent, saponin detected by H₂SO₄ 0.1 M reagent, tannins identified by FeCl₃ 1% reagent, and terpenoids detected by a Lieberman-Buchard reagent.⁹

DPPH radical scavenging assay

The antioxidant activity determined by DPPH radical scavenging was using the method as described by Phang *et al.* The extracts with various concentrations were mixed with 1 mL of DPPH (100 μ g/L), 2 mL of ethanol, and incubated at room temperature (37°C) for 30 min. Absorbance was read at 520 nm using spectrophotometer (UV-2450 Shimadzu). Methanol was used as blank, and DPPH solution without addition of extract was used as a control. The sample was diluted in 1000 mg/L to provide 50, 100, 200, 400 and 800 mg/L of sample solution. Ascorbic acid was used as a standard comparison, divided into five concentrations (1, 2, 3, 4, and 5 mg/L). The percentage inhibition activity was calculated as $[(A^0 - A^1)/A^0] \times 100$, where A^0 was the absorbance of the control, and A^1 was the absorbance of the extract/standard. The Inhibition Concentration 50% (IC₅₀) value was determined by interpolation from non-linear regression of plot of percentage of inhibition against the concentration of extracts, which is defined as the amount of extract needed to scavenge 50% of DPPH radicals.¹⁰

Animals modeling

Animals test used are male mice in 2-3 month and 20-30 g which are healthy and behaving normally. Male mice were adapted for one week to create diabetes by streptozotocin-induced based on Body Weight (BW) with of 150 mg/kg BW for a dose that diluted in citrate buffer pH 4.5 by intraperitoneal (i.p). This test has been approved by the ethical research committee of Faculty of Medicine, Universitas Haluoleo Kendari, No: 737/UN29.20/PPM/2016.

In vivo, antidiabetic assay

Mice were acclimated to the cage for 7 days by given food twice a day and ad libitum drink, then the mice fasted for 10-12 h, and the fasted normal glucose blood level was measured by the photometer. The 30 mice were intraperitoneally given 150 mg/kg BW of streptozotocin and 10% of sucrose solution to obtain mice diabetic modeling. After 2 days of the inducing, fasting blood glucose levels (glucose diabetes) of the mice were measured. If blood glucose levels of mice were increased in >62-175 mg/dL, the mice were considered to have diabetes. The mice randomly were divided into 6 groups which were consisted of normal control group, diabetes induction group, positive group, and treatment

group. The treatment group consisted of stem galing ethanol extract in 400, 500, and 600 mg/kg BW.

Data analysis

The results of the research were analyzed by using one-way ANOVA test and LSD by Statistical Product and Service Solution (SPSS) 17.0 version for Windows 8. The statistical analysis aims to determine the differential effect as antidiabetic among three concentration of the extract and to provide clear data about the comparison effect between positive control and extract.

RESULTS AND DISCUSSION

Phytochemical screening

The extract was obtained by maceration method on galing stem. The maceration process uses room temperature to minimize the damage of bioactive compounds in the extract. The analysis done on the sample is qualitative phytochemical test by TLC method with a specific reagent. The phytochemical test aims to determine the existence of bioactive compounds that are expected to act as antioxidants or antidiabetics. The results of phytochemical tests can be seen in Table 1.

Antioxidant activity

The parameter used for antioxidant activity is IC₅₀, which was defined as the concentration of antioxidant that caused loss of 50% of DPPH activity by comparison to ascorbic acid. The antioxidant activity of the extract and ascorbic acid can be seen in Table 1.

The IC₅₀ of the extract was 61,52 mg/L which indicated that the extract had strong antioxidant activity. The range strong antioxidant activity were ranging 50-100 mg/L. The IC₅₀ of ascorbic acid was 3.97 mg/L which indicated that it has very strong of antioxidant activity category because of its IC₅₀ values less than 50 mg/L. The strong category as an antioxidant of *C. trifolia* Domin, most likely due to high levels of flavonoids. It contains kaempferol, myricetin, and quercetin which is included in the group of flavonoids.¹¹ Flavonoids can act as an antioxidant by reacting with free radicals which it is essential for maintaining the balance of oxidants and antioxidants in bodies.^{7,11}

Antidiabetic effect

The effectiveness of ethanol extract of galing stem as antidiabetic was conducted to determine the effectiveness in decreasing blood glucose levels fastly of diabetic male mice. The 36 of male mice which 6 of the mice, as the normal group was not given treatment and 30 mice were induced by streptozotocin. The number 150 mg/kg BW of streptozotocin was injected intraperitoneally which is based on the research of Mega (2012). Streptozotocin has been successful as inducers with 50 mg/kg BW and 150 mg/kg BW; diabetic mice were obtained in 2 days after

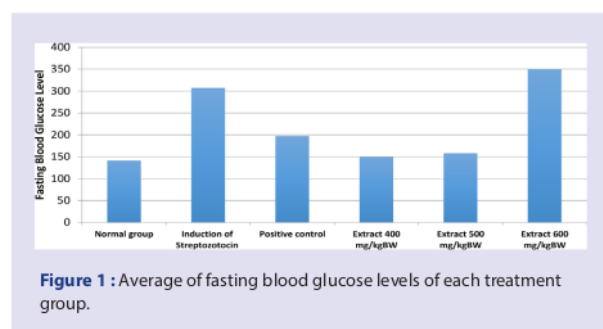
Table 1 : Results of phytochemical screening and antioxidant activity.

Sample	Phytochemical components	Reagent	Results	IC ₅₀ (mg/L)
C. trifolia L. extract	Alkaloids	Dragendorff	+	61.52
	Flavonoids	Ammonia	+	
	Saponin	H ₂ SO ₄	+	
	Tanin	FeCl ₃ 1%	+	
	Triterpen	Lieberman-burchard	+	
Ascorbic acid				3.97

(+): contain secondary metabolites

Table 2 : Average of decreasing fasting blood glucose levels of mice based on group treatment.

Group	Blood glucose level (mg/dL) $\pm$ SD	
	Before treatment	After treatment
Normal	141.25 $\pm$ 9.53	141.25 $\pm$ 9.53
Negative control	307 $\pm$ 124.05	307 $\pm$ 124.05
Positive control (Glibenclamide)	196.75 $\pm$ 28.41	110.25 $\pm$ 28.41
Extract 400 mg/kg BW	149.75 $\pm$ 41.26	157.25 $\pm$ 41.26
Extract 500 mg/kg BW	158 $\pm$ 9.01	149 $\pm$ 9.01
Extract 600 mg/kg BW	350 $\pm$ 36.57	-43 $\pm$ 31.43



induction with 150 mg/kg BW and at least 28 days after streptozotocin induction with 50 mg/kg BW.¹²

Streptozotocin is used as inducer because it is selective β cell damage, so induction with streptozotocin is enough success with 90%. Streptozotocin enters into the pancreatic β cells via GLUT2 and causes DNA alkylation. DNA damage that induced by streptozotocin will activate the poly ADP-ribosylation. This process leads to the depletion of NAD⁺ and ATP reduction which will inhibit the secretion and decrease the synthesis of insulin. Streptozotocin will release a number of nitric oxide by inhibiting the activity of aconitase that effect in DNA damage which causing the Krebs cycle inhibition thereby reducing the ingress of oxygen in mitochondria which these effects severely limit mitochondria to produce ATP and cause the depletion of nucleotides in β pancreas cell.^{8,12}

Blood glucose levels Increasing after inducing streptozotocin were observed in 2 $\times$ 24 h, and the mice were given sucrose 10% solution to avoid hypoglycemia post-injection. Fasting blood glucose levels of mice were measured after 18-24 h. Before measurement of glucose blood levels, the mice fasted for 10-12 h to maintain the stability of blood glucose levels and to avoid the changes in blood glucose levels due to food intake. Measurement of the first day (24 h) showed that 26.6% of the mice were in diabetic which is caused by the different response of each mouse in streptozotocin. Measurement of the second day (48 h) showed that all mice were in diabetic.

The average of fasting blood glucose levels of mice increased to 307 mg/dL which was characterized by increasing glucose persistent blood levels. The thirty diabetic mice were randomly divided into five groups: induction streptozotocin group, the treatment groups which were consisted of positive control (treated glibenclamide), and three groups for extract treatment. Each group was orally given galing stem extract once a day for seven days. Measurement of fasting blood glucose levels was conducted on the eight days. Average of fasting blood glucose levels could be seen in Table 2.

The comparison effect as antidiabetic can be seen in Figure 1. The graph shows that the highest concentration of fasting blood glucose level is treatment by extract 600 mg/kg BW, then followed by negative control. It means there is no influence of therapy for extract 600 mg/kg BW. According to Pasaribu *et al.* (2012), increasing of concentration should be improving the responses, but the increasing of concentration of galing stem extract eventually declined the increasing of responsiveness, it was caused that accomplished concentration was not able to improve the response again.¹³ It often occurs in natural medicine because natural medicine contains not only a single compound but also contains a wide variety of chemical compounds. Increasing concentration, the number of chemical compounds will be increasing, so the interaction among the components can cause the effects decreasing.¹³ It can be concluded that the third dose (600 mg/kg) BW does not affect as antidiabetic.

Different with the concentration of extract 400 mg/kg BW show the significant effect of decreasing fasting blood glucose level, as well as 500 mg/kg BW. Both of these concentrations can decrease the level of blood glucose twice than negative control, and the ability of them little bit effective than positive control that treated by glibenclamide, although not significant based on statistical data ANOVA and post hoc LSD test. The average of decreasing fasting blood glucose level clearly seen in Table 2.

The decreasing of fasting blood glucose levels due to the containing of flavonoids, alkaloids, and saponins. Mechanism action of saponin that could help reducing blood sugar levels by inhibiting the activity of the enzyme alpha-glucosidase (the enzyme that responsible for the conversion of carbohydrates into glucose).¹⁴ Alkaloids were proved significantly to have regeneration ability for damaged β -pancreas cell. The insulin secretion increase was caused by the effect of sympathetic nerve stimulation (sympathomimetic) of alkaloid which affected the increase of insulin secretion.^{6,15}

Study of Prameswari and Widjanarko reported that flavonoid could reduce blood glucose levels by its ability as an antioxidant,¹⁶ it could scavenge the free radicals, thereby reducing insulin resistance.^{12,17} Another mechanism of flavonoids, especially quercetin was inhibiting GLUT2 of intestinal mucosa which could decrease the absorption of glucose.⁸ These mechanisms caused the decreasing in glucose and fructose uptake from the gut, so the blood glucose levels were decreasing.^{13,15,16}

Glibenclamide therapy could decrease the blood glucose levels of diabetic mice by average reduction were 110.25 mg/dL. The decreasing of blood glucose levels were caused by glibenclamide (class of sulfonylureas) which worked by increasing insulin sensitivity and increasing the insulin secretion of pancreas beta cells.¹² Glibenclamide is only effective in diabetes mellitus type 2 which the diabetic condition was not complicated, and the beta cells are still in normal function.¹³

This study proves again that the amount of natural wealth that can be utilized in improving the quality of health, not only in the form of plants but also animals, such as the use of shrimp shells as a source of glucosamine¹⁸ and many more that can be explored, to produce a safer and high quality product.

CONCLUSION

Ethanol extract of galing stem (*C. trifolia* Domin.) contains secondary metabolites which are alkaloids, saponins, terpenoids, tannins, and flavonoids. Galing stem extract has antioxidant activity with a strong category. It also has antidiabetic effects at 400 mg/kg BW and 500 mg/kg BW which was equal to the positive control group (glibenclamide).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

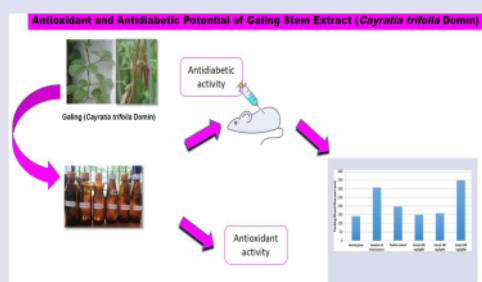
ABBREVIATIONS

ANOVA: Analysis of Variance; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **IC₅₀:** The concentration required to achieve 50% effect; **LSD:** Least Significant Difference.

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GRAPHICAL ABSTRACT



SUMMARY

- Galing stem (*C. trifolia* Domin.) extract contain secondary metabolites like alkaloids, saponins, terpenoids, tannins, and flavonoids.
- Ethanol extract of galing stem has antioxidant activity with IC₅₀ 61,52 mg/L which indicated has strong activity.
- The ethanol extract also has high potential as antidiabetic. The extract 400 and 500 mg/kg BW are not different significantly with glibenclamide in reducing blood glucose levels subset of the statistics ANOVA ($p > 0.05$)

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