Antidiabetic Activity of Extract and Fractions of Castanopsis costata Leaves on Alloxan-induced Diabetic Mice

Maulana Yusuf Alkandahri1,*, Dani Sujana2, Dadang Muhammad Hasiyim2, Mareetha Zarah Shafirany3, Lela Sulastri4, Maya Arfania1, Dedy Frianto1, Farhamzah1, Anggun Hari Kusumawati1, Nia Yuniarsih1

1Faculty of Pharmacy, Buana Perjuangan Karawang University, Karawang, West Java, INDONESIA.
2Diploma Program of Pharmacy, Karsa Husada Garut College of Health Sciences, Garut, West Java, INDONESIA.
3Department of Pharmaceutical Biology, School of Pharmacy Muhammadiyah Cirebon, Cirebon, West Java, INDONESIA.
4Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy Muhammadiyah Cirebon, Cirebon, West Java, INDONESIA.

Correspondence
Maulana Yusuf Alkandahri
Faculty of Pharmacy, Buana Perjuangan Karawang University, Karawang, West Java, INDONESIA.
E-mail: alkandahri@gmail.com

ABSTRACT
Background: In North Sumatra, Castanopsis costata is commonly used by traditional practitioners for the treatment of diabetes mellitus, however, no studies have been carried out to substantiate this practice. Therefore, this study aims to evaluate the anti-diabetic activity of extracts and fractions of C. costata leaves in mice with alloxan-induced diabetes. Methods: Swiss albino mice with alloxan-induced diabetes were acquired and subjected to the experimental evaluations. Subsequently, the fasting blood glucose levels of the diabetic mice groups treated with glibenclamide, extract and fractions, as well as the untreated group, were evaluated after two weeks of therapy. Results: Based on the results, the ethanolic extract of C. costata considerably reduced the mice’s blood glucose levels in a dose-dependent manner, at dosages of 25, 50, 100, and 200 mg/kgBW (% DBGL: 21.10%, 46.36%, 58.94%, and 60.93%, respectively). In addition, the water fraction of C. costata leaves produced a greater reduction in blood glucose levels (% DBGL: 78.93%), compared to the ethyl acetate and n-hexane fractions (% DBGL: 67.06% and 58.83%), respectively. Meanwhile, treatment with the antidiabetic drug, glibenclamide (5 mg/kgBW) produced a 50.75% reduction in blood glucose levels. Conclusion: Based on the findings, the extract and fractions of C. costata leaves were concluded to exhibit significant anti-diabetic activity. This supported the claim that traditional practitioners in North Sumatra use the plant extract for diabetes treatment.

Key words: Castanopsis costata, Antidiabetic activity, Diabetes mellitus, North Sumatra.

INTRODUCTION
Diabetes mellitus is one of the world’s most prevalent diseases and is characterized by a variety of causes that impair insulin production and/or activity, resulting in a loss of glucose homeostasis, as well as associated metabolic abnormalities of carbohydrate, fat, and protein.1,2 According to the International Diabetes Federation (IDF), increased blood glucose is the world’s third-leading risk factor for early death, after high blood pressure and cigarette use.2 Therefore, diabetes mellitus has become a major cause of illness and mortality, globally, with the number of cases estimated to be 642 million by 2040, the majority of whom live in low- and middle-income nations.3 Despite the availability of natural and synthetic numerous anti-diabetic medicines on the market, the condition remains a significant concern.4 Currently, the commonly used medicines for diabetes treatment are limited by several factors, including limited effectiveness, excessive cost, and various adverse effects.5 Alternatively, medicinal herbs with purported anti-diabetic properties are often used, particularly in underdeveloped nations. In comparison to traditional medications, these plants provide numerous advantages, including cost-effectiveness, ease of accessibility, broad cultural acceptance, and lesser side effects.6 A study by Piero et al., highlighted over 1,200 species of medicinal plants used by ethnic groups worldwide as traditional anti-diabetic medicines.6 These traditional medicines are believed to contain active ingredients with anti-diabetic activity, although the geographic conditions, climate, and extraction techniques used are also believed to have a possible influence on the potency.7-8 Indonesia has the world’s country second largest forest biodiversity, with 28,000 plant species, of which 2,500 species, including Castanopsis costata, commonly known as “Cep-cepan”, are used as medicine.9,10 C. costata belongs to the Fagaceae family and has been known to exhibit therapeutic activities, for instance, anti-fever, digestive issue alleviation, and analgesic properties.11 According to previous studies, C. costata exhibits numerous biological properties, including antioxidant activity, anti-inflammatory activity,12 and antimalaria activity.13 Furthermore, in North Sumatra, the plant is frequently used to treat diabetes, however, there are no studies confirming this practice. Therefore, this study aims to evaluate the anti-diabetic activity of the extract and fractions of C. costata leaves.

MATERIALS AND METHODS
Plant material collection and preparation
About 10 kg of fresh C. costata leaves were collected in Pancur Batu Districts, North Sumatra, and transported to the Central Laboratory, Buana Perjuangan Karawang University for cleaning, air drying, milling, and extraction. The Herbarium Unit of the Department of Biology, Faculty of Mathematics and Science, Universitas Padjadjaran later recognized the plant as C. costata (Code: 219/KB/04/2017).

Reagents and instruments
This study induced diabetes in Swiss albino mice using alloxan monohydrate (Sigma Chemical
Company, USA), while glibenclamide (PT Indofarma Tbk, Indonesia) was used as a conventional hypoglycemic agent. In addition, the plant components were extracted using ethanol (EMSURE® ACS Merck, Darmstadt, Germany), and the solvents were evaporated using a BUCHI Rotavapor® R-100 (PT. BUCHI Tangerang, Indonesia), while the blood glucose levels were determined using a Sinocare Safe-Accu Chek Glucometer (Sinocare Healthcare, Indonesia).

**Extraction and fractionation of C. costata**

About 1 kg of C. costata leaf powder was macerated in 70% ethanol for 72 hours to obtain a liquid extract. The liquid extract was then concentrated using a rotary evaporator at 50°C to reach a concentration of about 15.00% (fixed weights of the extracts were divided by simplicia weights multiplied by 100%) and diluted in distilled water to create varied dosages as required. Subsequently, the ethanolic extract of C. costata leaves was diluted in a 1:3 combination of ethanol and water and about 100 g of the extract was separated by liquid-liquid partitioning using ethyl acetate (EA) (4 x 150 ml) and n-hexane (4 x 150 ml), to obtain 3 fractions: n-hexane (30.20 g, 30.20%), ethyl acetate (EA) (43.00 g, 43.00%), and water (26.80 g, 26.80%).

**Experimental animals**

A total of 36 adult male Swiss albino mice aged 2-3 months old with the body weights of 25-30 g, were obtained from the Animal House, School of Pharmacy, Institut Teknologi Bandung, and housed under normal conditions with 12 hour-12 hour light-dark cycles. The mice were caged in groups of four, with free access to food, as well as water, then moved to the laboratory 7 days before the clinical studies acclimate to the new habitat. This study protocol was approved by the Faculty of Medicine Ethics Committee, Universitas Padjadjaran, Indonesia (No. 359/UN6.KEP/EC/2021).

**Anti-diabetic assay**

For this assay, male Swiss albino mice were subjected for fasting throughout 12-14 hours overnight, then their weight and fasting blood glucose levels were determined using a glucometer. Only mice with a fasting blood glucose level above 200 mg/dl were included in the study.14 To induce diabetes, each mouse was administered a single intraperitoneal injection of freshly prepared alloxan monohydrate solution (150 mg/kgBW) (volume 1 ml/kg) made based on each mouse’s weight and solubilized with 0.5 ml natrium citrate at a pH of 4.5 before injection.15-16 The mice were given food and water 30 minutes after the injection and the plasma blood glucose level of each mouse was evaluated 72 hours, using blood drawn from the tail.

**Experimental design**

To determine the fasting blood glucose levels, the mice were separated into nine groups of four mice each. The plant extract were administered three days after the injection to all the groups, except the diabetes control group. Subsequently, blood samples were drawn from each group on days 0, 3, 9, 12, and 15, to determine the blood glucose levels. Group 1 served as diabetic control and group 2 was administered standard drugs (glibenclamide, 5 mg/kgBW orally, daily). Also, groups 3, 4, 5, and 6 were administered the ethanol extract at doses of 25, 50, 100, and 200 mg/kgBW, respectively, while groups 7, 8, and 9 received the water fraction, ethyl acetate fraction, and n-hexane fraction (100 mg/kgBW each day) respectively, for two weeks.

**Data analysis**

The results are displayed as mean ± SEM values. In addition, One-way analysis of variance (ANOVA) was performed to determine the differences in mean values between treatment groups. Subsequently, a Tukey’s test was performed to determine whether the group mean values were significantly different at p<0.05. Meanwhile, the data were analyzed statistically using SPSS version 22, and converted into bar charts and tables, using Excel 2019.

**RESULTS AND DISCUSSION**

**Anti-diabetic activity of extract and fractions of C. costata leaves**

For two weeks, alloxan-induced diabetic mice were orally administered ethanolic extract and fractions of C. costata. Alloxan induces build up through the glucose transporter 2 (GLUT2) and tends to destroy certain types of insulin secreted by pancreatic cells, resulting in decreased glucose absorption by peripheral organs, consequently, inducing diabetes. This is because alloxan induces the production of free radicals through redox processes, causing tissue damage, as well as cell degeneration, and ultimately, cell degeneration.15-17 This was corroborated by the increased fasting blood glucose level measured 72 hours after injection.

Based on the results, there was a significant reduction in the glucose levels of blood samples from the mice treated for two weeks with C. costata extract and fractions. However, after 9 days of treatment, no significant changes in glucose levels were observed. In comparison to the glibenclamide (5 mg/kg) group, there were significant (p<0.05) hypoglycemic effects between day 9 and day 15. In addition, a significant (p<0.05) reduction in glucose levels was observed in the blood samples from the groups treated with C. costata extract and fractions, from day 12 to 15, compared to the diabetic control group. Table 1 shows the effect of various dosages of C. costata extract and fractions on the fasting blood glucose levels of the mice. These findings show the extract and fractions of C. costata leaves have the capacity to decrease blood glucose levels in mice. The ethanolic extract of C. costata leaves significantly (p<0.05) decreased blood glucose level in a dose-dependent manner with the inhibitory activity observed in the C. costata-treated mice at doses of 25 mg/kgBW (% DBGL: 21.10±3.02%), 50 mg/kgBW (% DBGL: 46.36±3.90%), 100 mg/kgBW (% DBGL: 58.9±1.58%) and 200 mg/kgBW (% DBGL: 60.9±1.79%). However, the water fraction of C. costata leaves produced a higher reduction of blood glucose level (% DBGL: 78.93±5.62%), compared to the ethyl acetate fraction (% DBGL: 67.06±1.82%) and n-hexane fraction (% DBGL: 58.83±4.00%) (Figure 1).

Therefore, the extract and fractions of C. costata leaves were concluded to be able to reduce the elevated fasting blood glucose level. This ability is possibly due to the presence of antioxidant phytochemicals, for instance, flavonoids, polyphenols, and tannins, which act as free radical scavengers.12,17-18 The antioxidants’ mechanism of action is believed to be an insulin-like effect on peripheral tissues, through stimulation of the regeneration process or the release of pancreatic insulin synthesis from existing cells. This increases the rate of glucose removal from the circulation by increasing filtration, as well as renal excretion, and improving metabolism, as well as fat deposit integration, a process involving insulin synthesis by the pancreas.19

**CONCLUSION**

The extract and fractions of C. costata leaves exhibited a considerable anti-diabetic effect, as demonstrated in this study. These findings corroborate the plant’s use for diabetes treatment by traditional practitioners in North Sumatra. However, further studies are required to determine the exact mechanism of the anti-diabetic effect of C. costata leaves.
Table 1: Effect of extract and fractions of C. costata on fasting blood glucose level.

| Trial Group | Day 0       | Day 3       | Day 6       | Day 9       | Day 12      | Day 15      |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| DC          | 273.75±48.85| 274.50±64.02| 205.25±10.06| 229.25±26.10| 234.75±18.13| 249.00±12.85|
| PC          | 216.75±8.20 | 201.75±7.09 | 189.25±3.35 | 170.75±6.18**| 124.25±4.01**| 106.75±4.05**|
| CCE 25      | 226.25±3.47 | 221.50±2.25 | 229.50±7.77 | 191.50±3.87 | 183.50±12.12**| 178.50±4.44**|
| CCE 50      | 250.75±14.74| 219.50±1.44 | 215.50±2.90 | 189.25±2.95 | 159.25±3.30**| 134.50±4.91**|
| CCE 100     | 250.25±19.20| 217.50±1.04 | 206.00±1.73 | 187.00±2.86 | 148.75±4.11**| 102.75±1.55**|
| CCE 200     | 225.25±8.71 | 215.50±1.71 | 192.00±2.38 | 184.50±2.66 | 158.75±7.73**| 88.00±0.90**|
| WFCC 100    | 303.75±33.64| 249.50±28.29| 221.00±10.90| 189.75±2.39| 142.75±1.60**| 64.00±12.25**|
| EAFCC 100   | 294.50±23.97| 261.25±18.38| 237.25±26.63| 195.75±6.92| 163.25±10.23**| 97.00±6.92**|
| nHFCC 100   | 300.00±37.73| 270.25±32.39| 229.75±15.71| 202.75±12.81| 160.50±3.43**| 123.50±6.54**|

Information:
DC = Diabetic control (PGA 1% suspension).
PC = Positive control (glibenclamide dose 5 mg/kgBW).
CCE 25 = Administration of ethanol extract of the C. costata leaves dose 25 mg/kgBW.
CCE 50 = Administration of ethanol extract of the C. costata leaves dose 50 mg/kgBW.
CCE 100 = Administration of ethanol extract of the C. costata leaves dose 100 mg/kgBW.
CCE 200 = Administration of ethanol extract of the C. costata leaves dose 200 mg/kgBW.
WFCC 100 = Administration of water fraction of the C. costata leaves dose 100 mg/kgBW.
EAFCC 100 = Administration of ethyl acetate fraction of the C. costata leaves dose 100 mg/kgBW.
nHFCC 100 = Administration of n-hexane fraction of the C. costata leaves dose 100 mg/kgBW.

Data are presented as mean±SEM of four animals in each group. ** shows p<0.05 compared to the diabetic control group. PGA: Pulvis Gummi Arabicum.

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**Graphical Abstract**

**About Authors**

**Maulana Yusuf Alkandahri:** He is a pharmacist, lecturer and researcher at the Faculty of Pharmacy, Buana Perjuangan Karawang University, Karawang, West Java, Indonesia. He is completed Master of Pharmacology from the Faculty of Pharmacy, Padjadjaran University, Bandung, West Java, Indonesia in 2018, after which he is continued his Ph.D program at the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University. Currently, he is focused on conducting research on local Indonesian medicinal plants that have pharmacological activity.

**Dani Sujana:** He is a pharmacist, lecturer and researcher at the Diploma Program of Pharmacy, Karsa Husada Garut College of Health Sciences, Garut, West Java, Indonesia. He is completed Master of Pharmacology from the Faculty of Pharmacy, Padjadjaran University, Bandung, West Java, Indonesia in 2019, after which he is continued his Ph.D program at the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University. Research interests are pharmacological activity, especially from natural product.
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