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Antihyperglycemic Effects of Active Fraction of Ethanol Extract from *Castanopsis costata* Leaves and *Channa striata* Extract on Rats

Yanski Darmanto[1], Razoki[2*], Elfia Neswita[3]

[1,2,3]Bachelor's Program In Clinical Pharmacy, Faculity Of Medicine, Dentistry, Heatlh Sciences, Universitas Prima Indonesia, Medan

[*]Corresponding Author: Razoki

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ABSTRACT

Anti-hyperglycemic experiments were conducted on male Wistar rats divided into six groups: Group 1 (negative control), Group 2 (active fraction of Castanopsis costata ethanol extract at 200 mg/kg body weight), Group 3 (Channa striata extract at 250 mg/kg body weight), Group 4 (active fraction of Castanopsis costata ethanol extract at 50 mg/kg body weight + Channa striata extract at 250 mg/kg body weight), Group 5 (active fraction of Castanopsis costata ethanol extract at 100 mg/kg body weight + Channa striata extract at 250 mg/kg body weight), and Group 6 (active fraction of Castanopsis costata ethanol extract at 200 mg/kg body weight + Channa striata extract at 250 mg/kg body weight). The most notable reduction in blood glucose levels was observed in Group 4, with an average decrease of -55.40%, followed by Group 6 (-55.19%), Group 5 (-52.80%), Group 2 (-52.01%), and Group 3 (-52.88%). These findings indicate that combining the active fraction of Castanopsis costata ethanol extract with Channa striata extract effectively lowers blood glucose levels in Wistar rats.

Keywords: Antihyperglycemia, Active fraction of ethanol extract, Blood glucose level

INTRODUCTION

Diabetes mellitus is a persistent metabolic condition marked by heightened blood glucose levels (hyperglycemia) due to deficiencies in insulin secretion, insulin action, or both. It includes various forms such as type 1 diabetes, type 2 diabetes, and gestational diabetes, with type 2 being the most common and frequently associated with obesity and a sedentary way of life [1].

The International Diabetes Federation (IDF) reports a significant global rise in diabetes prevalence. In 2019, an estimated 463 million people worldwide had diabetes, projected to increase to 700 million by 2045. In Indonesia, diabetes prevalence is also on the rise, posing a significant public health challenge [2].

Diabetes mellitus (DM), classified as a non-communicable disease, is a major systemic health concern in Indonesia due to its high incidence and mortality rates. It manifests as chronic hyperglycemia caused by inadequate insulin production or ineffective insulin action [3].

The use of medicinal plants has been a longstanding practice globally. In Indonesia, traditional herbal medicines known as "jamu" have been culturally significant since ancient times and continue to be valued for their perceived health benefits. In recent years, there has been increasing interest in traditional medicine alongside scientific advancements in healthcare.

In the community around Tangkahan Forest, part of Mount Leuser National Park in North Sumatra's Langkat Regency, primarily inhabited by the Karo ethnic group, Cep-cepan leaves (Castanopsis costata) are traditionally used to manage various conditions including diabetes, hypertension, abdominal pain, digestive issues, and external wounds. There is potential interest in exploring these leaves as a possible analgesic, although scientific research on their efficacy is lacking [4].

Indonesia boasts the world's second-largest forest biodiversity, with approximately 28,000 plant species, including 2,500 medicinal plants. Castanopsis costata, locally known as "Cep cepan," is among those utilized for medicinal purposes [5].

Snakehead fish (Channa striata) is recognized for its high protein content compared to other freshwater species. Its protein acts as an antioxidant, reportedly inhibiting the α-glucosidase enzyme responsible for converting carbohydrates into glucose, thereby assisting in regulating blood glucose levels [6].

MATERIALS & METHODS

Materials

The materials that will be used in the research are *Castanopsis costata* leaves, Snakehead Fish Extract, Glucose, Distilled Water, 96% Ethanol, n-Hexane, Test Animals, Test Animal Feed, Distilled Water, Concentrated HCl, Magnesium Powder, Chloroform, H2SO4, Anhydrous Acetic Acid, and FeCl3.

Tools

The tools that will be used in the research include Beaker Glass, Glass Cups, Measuring Glasses, Erlenmeyer Flasks, Graduated Pipettes (pyrex), Separatory Funnels (pyrex), Rotary Vacuum Evaporator, Test Tubes, Analytical Balance, Blender, Beaker Glass, Stopwatch, Stirring Rod, Mortar and Pestle, Dropper Pipettes, Filter Paper, Syringes, Scissors, Water Baths, Refrigerator, Feeding Tube, Cotton, Gloves, Glucometer Strips, and Glucometer.

Plant Identification

The plant identification procedure took place at the "Herbarium Medanense" botanical laboratory, situated in the Faculty of Mathematics and Natural Sciences, Department of Biology, at the University of Sumatera Utara in Medan. The approval number for this process is 2210/MEDA/2024

Preparation of Dried *Castanopsis Costata Leaves* Leaf Samples

The leaves of *Castanopsis Costata* were gathered and rinsed extensively with flowing water, then dried and measured for their wet weighdt. Subsequently, the material is subjected to a aired process in a specialized drying cabinet at a controlled temperature of 57°C. Once completely dried, the product is then measured for its dry weight and subsequently pulverized using a blender [10].

Extraction of *Castanopsis Costata Leaves*

The maceration process was employed to produce an ethanol extract of Leaves *Castanopsis Costata* leaves, at a ratio of 1:10. A portion of ethanol extract of *Castanopsis Costata* leaves leaf simplicia powder was placed in a container and mixed with 10 parts of 96% ethanol solvent. Immerse for a duration of 6 hours, intermittently agitating, followed by a subsequent period of 24 hours of inactivity. Subsequently, it was filtered. The filtration procedure was repeated once, and then all the macerate was gathered and subjected to evaporation using a rotary evaporator until a concentrated extract was obtained and thickened using a water bath [10]

Phytochemical Screening

Phytochemical screening was conducted on dried leaves and ethanol extracts of *Castanopsis Costata* Leaves, specifically analyzing alkaloids, glycosides, saponins, flavonoids, tannins, and steroids/ triterpenoids [1,2,10,11,].

Fractionation of *Castanopsis Costata* Leaves

The fractionation of Castanopsis costata leaves extract was conducted using a separatory funnel method. Initially, ten grams of the extract were weighed and dissolved in n-hexane at a 1:10 ratio. This step was repeated three times, with a waiting period of 15-20 minutes each time, resulting in the separation of two layers: an upper n-hexane layer and a lower layer. The lower layer was then isolated, and 96% ethanol was added at a 1:10 ratio. This process was also repeated three times with a 15-20 minute waiting period each time, leading to the formation of polar and non-polar layers. The upper layer, representing the active fraction of the ethanol extract of Castanopsis costata leaves, was collected. After obtaining all fractions, the extracts were evaporated using a rotary vacuum evaporator at 40°C. Finally, the remaining solution was further evaporated using a water bath, resulting in thick n-hexane and thick 96% ethanol fractions

Animal Preparation

Thirty male rats were distributed into six treatment groups, with each group comprising five rats. The rats underwent a 7-day acclimation period, after which their fasting blood sugar levels were measured following an 8-hour fast to establish baseline values. Subsequently, the rats were induced with 40% glucose and received their respective treatments. Blood sugar levels were monitored every 30 minutes for a total duration of 120 minutes, starting 30 minutes post-treatment.

The groups were categorized as follows: Group 1: Control group, received no treatment. Group 2: Administered with an active fraction of ethanol extract from Castanopsis costata leaves at a dosage of 200 mg/kg body weight (BW). Group 3: Given Channa striata extract at a dosage of 250 mg/kg BW. Group 4: Provided with a combination of an active fraction of ethanol extract from Castanopsis costata leaves at 50 mg/kg BW and Channa striata extract at 250 mg/kg BW. Group 5: Administered with a combination of an active fraction of ethanol extract from Castanopsis costata leaves at 100 mg/kg BW and Channa striata extract at 250 mg/kg BW. Group 6: Received a combination of an active fraction of ethanol extract from Castanopsis costata leaves at 200 mg/kg BW and Channa striata extract at 250 mg/kg BW.

Blood sugar levels examination

Observe the reduction in blood sugar levels at 0 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes.

STATISTICAL ANALYSIS

The in vivo results underwent analysis using ANOVA with Tukey's Multiple Comparison Test, with the significance level set at $p < 0.05$. All measurements are reported as mean ± standard deviation (SD).

RESULT AND DISCUSION

Phytochemical Screening

The results of the phytochemical screening of simplicia powder and ethanol extract contained secondary metabolite compounds in the alkaloids, flavonoids, glycosides, saponins, tannins and steroids/triterpenoids. The results of phytochemical screening can be seen in Table 1.

Table 1. Secondary metabolite compounds found in ethanol extract of *Castanopsis Costata* leaves

Phytochemical Constituent	Extract
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	+
Tannin	+
Steroids / Triterpenoids	+

Yield Calculation

Table 2. Yield Calculation

Sample	Weight of Fresh Simplisia (g)	Weight of Dried Simplisia (g)	Weight of Extract (g)	% Yield
Castanopsis costata Leaves	3000 g	1000 g	220 g	33,33%

$$\%\text{Yield} = \left(\frac{Weight\ of\ Dried\ Simplisia}{Weight\ of\ Extract}\right) \times 100$$

Channa Striata Extract

The *Channa striata* extract in this study was purchased from PT. Akar Rimba Nusantara, located at Jl. Setiabudi No.2 Dusun IV, Galuh City, Perbaungan, Serdang Bedagai Regency, North Sumatra.

Antihyperglycemia Test

The antihyperglycemic activity test of the active fraction of ethanol extract from *Castanopsis costata* leaves and *Channa striata* extract in male white rats induced with 40% glucose involves the following steps:

First, the rats are fasted for approximately 8 hours and then induced with 40% glucose. This is because glucose can rapidly increase the blood sugar levels of the rats without damaging the insulin-producing cells in the pancreas. The 40% dosage is administered orally using an oral gavage.

Fasting blood glucose measurement is chosen to obtain accurate data because, in this state, the rats' blood sugar levels are not influenced by the food provided to them, which would significantly impact the blood sugar levels. The results of this measurement are recorded as the initial blood sugar levels (H0).

Rats are considered hyperglycemic if their fasting blood glucose levels reach ≥ 150 mg/dL. The negative control group is given distilled water (G-) to compare and observe if there is any effect of the treatment on blood sugar reduction since the distilled water in the extract group serves as a suspending agent.

Group 2 received the active ethanol extract fraction from Castanopsis costata leaves. Group 3 was administered Channa striata extract. Group 4 received a combination of 50 mg/kg body weight of the ethanol extract fraction from Castanopsis costata leaves and 250 mg/kg body weight of Channa striata extract. Group 5 was treated with a combination of 100 mg/kg body weight of the ethanol extract fraction from Castanopsis costata leaves and 250 mg/kg body weight of Channa striata extract. Group 6 was provided with a combination of 250 mg/kg body weight of the active ethanol extract fraction from Castanopsis costata leaves and 200 mg/kg body weight of Channa striata extract. These dosage selections were based on previous research indicating potential antidiabetic properties of these plants. Each treatment was administered once daily, and blood sugar levels were monitored at 30, 60, 90, and 120 minutes after confirming hyperglycemia in the rats.

Data Analysis

Data Distribution Test (Normal Distribution)

To test whether the research data follows a normal distribution, the Kolmogorov-Smirnov Goodness of Fit Test is used.

Table 3. Results of normality test

Minutes	G1	G2	G3	G4	G5	G6
0	,200*	,200	,200*	,200*	,200*	,200*
30	,200*	,009	,200*	,076*	,200*	,200*
60	,200*	,021	,200*	,132*	,200*	,100*
90	,200*	,016	,200*	,200*	,200*	,200*
120	,200*	,005	,200*	,200*	,052*	,200*

Information : G1 : Group 1, G2 : Group 2, G3 : Group 3, G4 : Group 4, G5 : Group 5, G6 : Group 6

Based on the normality test results using the Kolmogorov-Smirnov test, it is observed that the significance values for the research data from 30 minutes to 120 minutes are greater than 0.05 (p >0.05). Therefore, it is concluded that the research data follows a normal distribution. In other words, the assumption of normality is fulfilled.

Homogeneity of Variance Test

The homogeneity (equality) of variance test can be conducted using the Levene test (Levene Test Homogeneity of Variance).

Table 4. Results of homogeneity test

Minutes	Levene Statistic	df1	df2	Sig.
0	1,618	5	24	,194
30	2,061	5	24	,106
60	3,010	5	24	,030
90	3,821	5	24	,011
120	2,381	5	24	,069

Based on the analysis results, for the observations at 30, 60, 90, and 120 minutes, the significance values obtained are greater than 0.05 (p > 0.05) at 30 and 120 minutes. Therefore, it is concluded that the variance of the data in these observation results is homogeneous.

One-way ANOVA Analysis

In this study, the initial hypothesis (H0) posits that there is no significant difference in the decrease in blood sugar levels among the groups (treatments). Conversely, the alternative hypothesis (Ha) proposes that there is a significant difference in the decrease in blood sugar levels among the groups (treatments). Conclusions are drawn by comparing the obtained significance value (p-value) with the researcher's predetermined alpha level, which is set at 0.05 (5%).

If the significance value (p-value) obtained from the statistical analysis is greater than 0.05 (p > 0.05), then H0 is accepted, suggesting that there is no significant difference in the decrease in blood sugar levels between the groups (treatments). In contrast, if the significance value is less than 0.05 (p < 0.05), then H0 is rejected, indicating that there is a significant difference in the decrease in blood sugar levels between the groups (treatments), and Ha is accepted.

Therefore, the acceptance or rejection of H0 and Ha is based on whether the obtained p-value is greater than or less than 0.05, respectively, as determined by the statistical analysis.

Variabel	The mean decrease in blood sugar levels						Sig.
	G1	G2	G3	G4	G5	G6	
0 Minutes	147,6	148,6	147,8	131,8	124	123,6	,002
30 Minutes	9,8	-45,2	-32,4	-37,2	-26	-31,2	,000
60 Minutes	20,2	-77,2	-61,4	-72,4	-55,8	-60,6	,000
90 Minutes	177,4	-106	-101	-105,2	-94	-100,9	,000
120 Minutes	184	-129	-123	-129	-118,4	-125,3	,000

Information : G1 : Group 1, G2 : Group 2, G3 : Group 3, G4 : Group 4, G5 : Group 5, G6 : Group 6

Based on the results of the One-way ANOVA test, which consistently showed significance values below 0.05 (p < 0.05) at each time point (30 to 120 minutes), it can be concluded that there is a statistically significant difference in the reduction of blood sugar levels among the treatment groups across all observation times. This indicates that the treatments administered to Groups 2, 3, 4, 5, and 6 have distinct effects in lowering blood sugar levels compared to the control group (Group 1) at each time

interval measured. These findings support rejecting the null hypothesis (H0) that there is no significant difference between the groups, in favor of the alternative hypothesis (Ha) that there is indeed a significant difference in the effectiveness of treatments across the groups.

Post Hoc LSD

The next step involves conducting the Least Significant Difference (LSD) Post Hoc test to identify which treatment group shows a significant difference in blood sugar level reduction compared to the others. The initial hypothesis (H0) in this study states that there is no significant difference in blood sugar level reduction among the treatment groups. Conversely, the alternative hypothesis (Ha) suggests that there is a significant difference in blood sugar level reduction among the treatment groups. Conclusions are drawn by comparing the obtained significance level (p-value) with the predetermined alpha level of 0.05 (5%). If the p-value is greater than 0.05 ($p > 0.05$), H0 is accepted, indicating no significant difference among groups, and Ha is rejected. Conversely, if the p-value is less than 0.05 ($p < 0.05$), H0 is rejected, suggesting a significant difference among groups, and Ha is accepted.

Observation time	Treatment	G1	G2	G3	G4	G5	G6
30 Minutes	G1		,000	,000	,000	,000	,000
	G2	,000		1.000	.343	.006	.329
	G3	,000	1,000		.343	.006	.329
	G4	,000	,343	,343		.001	.978
	G5	,000	,006	,006	,001		.000
	G6	,000	,329	,329	,978	,000	
60 Minutes	G1		,000	,000	,000	,000	,000
	G2	,000		,699	,204	,002	,472
	G3	,000	,699		,103	,004	,737
	G4	,000	,204	,103		,000	,053
	G5	,000	,002	,004	,000		,009
	G6	,000	,472	,737	,053	,009	
90 Minutes	G1		,000	,000	,000	,000	,000
	G2	,000		,471	,201	,021	,941
	G3	,000	,471		,566	,004	,517
	G4	,000	,201	,566		,001	,227
	G5	,000	,021	,004	,001		,000
	G6	,000	,941	,517	,227	,017	
120 Minutes	G1		,000	,000	,000	,000	,000
	G2	,000		,374	,057	,149	,232
	G3	,000	,374		,285	,025	,752
	G4	,000	,057	,285		,002	,447
	G5	,000	,149	,025	,002		,012
	G6	,000	,232	,752	,447	,012	

Information : G1 : Group 1, G2 : Group 2, G3 : Group 3, G4 : Group 4, G5 : Group 5, G6 : Group 6

The results of the post hoc LSD analysis revealed significant differences between all treatment groups receiving the extracts and the negative control group at minutes 30, 60, 90, and 120. This strongly indicates that both the ethanol extract fraction from Castanopsis costata leaves and Channa striata extract possess anti-hyperglycemic properties.

In this context, the potential blood glucose-lowering effects of Castanopsis costata leaves are attributed to antioxidant phytochemicals such as flavonoids, polyphenols, and tannins. These compounds are known for their ability to scavenge free radicals. The mechanism of action of antioxidants is believed to mimic insulin in peripheral tissues, promoting insulin synthesis or release from pancreatic cells. This process enhances glucose disposal from the bloodstream by improving filtration and

renal excretion, increasing metabolism, and facilitating fat storage, which involves insulin synthesis by the pancreas, as discussed [5].

Therefore, several hypotheses posited in this study are supported, indicating that the administration of the ethanol extract fraction from Castanopsis costata leaves at 200 mg/kg body weight, Channa striata extract at 250 mg/kg body weight, as well as combinations thereof (50 mg/kg + 250 mg/kg, 100 mg/kg + 250 mg/kg, and 200 mg/kg + 200 mg/kg), led to reduced blood glucose levels in male Wistar rats induced with 40% glucose compared to those not treated with these extracts (negative control). Notably, the combination of the ethanol extract fraction from Castanopsis costata leaves at 200 mg/kg body weight with Channa striata extract at 250 mg/kg body weight demonstrated optimal effectiveness as an anti-hyperglycemic agent over a 120-minute period.

CONCLUSION

This study examines the effects of ethanol extract fractions from Castanopsis costata leaves and Channa striata extract on reducing blood sugar levels in male Wistar rats induced with 40% glucose. The results demonstrate significant decreases in blood sugar levels across all observation times in the treatment groups compared to the negative control group, indicating that both extracts possess anti-hyperglycemic properties.

Further analysis using the Post Hoc LSD test confirms significant differences in blood sugar reduction between the treatment groups themselves, supporting the rejection of the null hypothesis and indicating varying degrees of anti-hyperglycemic effects among the treatments.

The study underscores the potential of Castanopsis costata leaves and Channa striata extract as effective anti-hyperglycemic agents. The presence of antioxidant phytochemicals like flavonoids, polyphenols, and tannins in Castanopsis costata leaves is likely responsible for their anti-hyperglycemic properties by potentially mimicking insulin actions in peripheral tissues. Optimal doses for achieving anti-hyperglycemic efficacy are identified as 200 mg/kg body weight of ethanol extract from Castanopsis costata leaves and 250 mg/kg body weight of Channa striata extract administered over a 120-minute period.

These findings provide valuable insights for future research and the development of alternative therapies for managing hyperglycemia.

Declaration by Authors

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