

ANTIHYPERLIPIDEMIC ACTIVITY OF EXTRACT AND FRACTIONS OF *Castanopsis costata* LEAVES ON RATS FED WITH HIGH CHOLESTEROL DIET

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ABSTRACT

Castanopsis costata is a widely distributed medicinal plant species in North Sumatra, Indonesia. Historically, the Karo people used it to treat various ailments. Therefore, this study aims to determine the extract and fractions of *C. costata* leaves' anti-hyperlipidemic efficacy. This was evaluated in a rat model of hyperlipidemia induced by a high cholesterol diet. The results showed that the extract and fractions of *C. costata* leaves had significant anti-hyperlipidemic and anti-atherogenic effects in a rat model of hyperlipidemia induced by a high cholesterol diet. Meanwhile, this is inextricably linked to the flavonoid and phenolic compounds levels. These compounds bind to free radicals, preventing lipid peroxidation in the process. The extract and fractions of *C. costata* leaves also contain steroid compounds functioning as cholesterol in the synthesis of lipoproteins and chylomicrons. Therefore, it is an important source of natural compounds used to develop new treatments for hyperlipidemia and atherosclerosis, but further study is needed to determine the exact mechanism of the anti-hyperlipidemic effect of *C. costata* leaves.

Keywords: *Castanopsis costata*, Anti-hyperlipidemic, Atherosclerosis, North Sumatra.

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INTRODUCTION

Hyperlipidemia relates to an increased risk of atherosclerotic cardiovascular diseases (ASCVDs). The prevalence of this disease is quite high and increasing in both developed and developing countries in the world. The prevalence of this disease is very high and increasing in both developed and developing countries.¹ It is an important risk factor in the initiation and progression of atherosclerosis. The main manifestations of this disorder include increased plasma concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and low concentrations of high-density lipoprotein cholesterol (HDL-C).² Therefore, the primary aim of treating hyperlipidemia and arteriosclerosis is to reduce the increase in plasma lipid levels.³ Currently, the available hypolipidemic drugs have been reported to have several concerning side effects, including hyperuricemia, muscle damage, impotence, memory loss, peripheral neuropathy, body aches, gynecomastia, and skin rashes. There is an increased risk of myopathy and rhabdomyolysis, which is common when used in combination with other drugs.⁴ Most hypolipidemic drugs are effective when used for several weeks but, as a result, exacerbate side effects such as liver damage.⁵ Therefore, there is a need to search for new anti-hyperlipidemic agents derived from natural ingredients in herbal plants. Compared with conventional medicine, these plants offer

many benefits, including cost-effectiveness, broad cultural acceptance, easy accessibility, and fewer side effects.⁶ Indonesia, the second-largest country in the world with forest biodiversity, has 28,000 plant species, out of which 2,500 are medicinal plants.⁷ One such medical plant is *Castanopsis costata*, commonly known as the "Cep-cepan". Empirically, it is used for anti-fever, relieves indigestion, and is analgesic.⁸ Based on previous studies, *C. costata* was reported to have several pharmacological activities, including antioxidant,⁹ anti-inflammatories,¹⁰ antimalarial,¹¹ and antidiabetic.¹² In North Sumatra, this plant is often used to treat hyperlipidemia, but there are no studies that confirm this effect. Therefore, this study aimed to investigate the hypolipidemic effect of *C. costata* leaf extract and fractions in reducing blood plasma lipid levels.

EXPERIMENTAL

Plant Material

Fresh *C. costata* leaves were procured from the Pancur Batu traditional market in North Sumatra, Indonesia. A botanist from the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, authorized it as *Castanopsis costata* (Code: 219/HB/04/2017). Afterward, 10 kg of leaves were transferred to the central laboratory, Universitas Buana Perjuangan Karawang, for extraction and fractionation.

Extraction and Fractionation

About 1.5 kg of *C. costata* leaf powder was macerated in 70% ethanol for 72 hours to get a liquid extract, which was then concentrated at 11.60% using a rotary evaporator at 40-50°C to a degree of concentration of 11.60% (fixed weight of extract divided by the weight of Simplicia multiplied by 100%). It had been diluted in water to form dosages varied as required. Subsequently, the ethanolic extract of the leaves (CCE) was diluted during a 1:3 combination of ethanol and water. About 100 g CCE of it had been separated by liquid-liquid partitioning using ethyl acetate (EA) (4 x 150 ml) and *n*-hexane (4 x 150 ml) to get 3 fractions, like *n*-hexane (nHFCC) (27.00 g, 27.00%), ethyl acetate (EAFCC) (22.00 g, 22.00%), and water (WFCC) (11.00 g, 11.00%).

Phytochemical Screening

The phytochemical screening of *C. costata* leaves extract and fractions was performed to determine the presence of secondary metabolites such as alkaloids, flavonoids, polyphenols, saponins, tannins, terpenoids, and glycosides.¹³

Determination of Total Phenolics

Total phenolics were determined by using Folin–Ciocalteu reagent.¹⁴ 100 milligrams of powdered samples was extracted for 15 min with 10 mL of 70% methanol. For total phenolics assay 0.1 mL of extract was dissolved into 0.4 mL methanol and 2.5 ml of Folin–Ciocalteu reagent and kept at 25 °C for 3-5 min; 0.8 ml NaHCO₃ (75 g L⁻¹) solution was added to the mixture. After 60 min at 25 °C, absorbance was measured at 765 nm with a Shimadzu UV-1601 spectrophotometer (Merk, Japan). The results were expressed as gallic acid equivalents (GAE).

Determination of Total Flavonoids

100 milligrams of powdered samples were extracted for 15 min with 10 mL of 70% methanol. For total flavonoids assay 0.1 mL of extract was dissolved into 2.4 mL methanol, 0.1 mL aluminum chloride (10%), 0.1 mL sodium acetate (1 M) and 2.3 mL aquadest. After 30 min at 25 °C, absorbance was measured at 432 nm with a Shimadzu UV-1601 spectrophotometer (Merk, Japan). The results were reported in quercetin equivalents (QE).¹⁵

Analysis of Rutin and Quercetin

100 milligrams of powdered samples were extracted for 15 min with 10 mL of 70% methanol (HPLC-Gradient grade, VWR chemicals), then filtered using a PTFE syringe filter with a pore of 0.45 µm. The chromatography was performed using a Shimadzu LC-20AT HPLC system with a detector (UV-VIS SPD20A) with a SIL-20HT autosampler (Merk, Japan). Extracts (20 µL) were analyzed at a flow rate of 0.7 ml/min and a column temperature of 35 °C. The column used is a C18-column nonpolar (150 x 4.6 mm), a particle size of 5 µm (CNW, USA). A 40-min gradient program was used with 1% v/v acetic acid

in ultrapure water (eluent A) and acetonitrile (eluent B) as follows: 0-5 min: 10% B, 5-15 min: 40% B, 15-20 min: 60% B, 20-30 min: 90% B, 30-40 min: 10% B. Absorbance was measured at 272 nm.

Experimental Animal

Male albino Wistar rats weighing 150-200 g were obtained from The Central Animal House, School of Pharmacy, Institut Teknologi Bandung, and West Java, Indonesia. They were maintained in the experimental chamber under 12 hour-12 hour light-dark cycle conditions. In addition, rats were randomized into experimental and control groups with 4 rats in each cage. Standard pellets were used as a base feed during the experiment period. Control and experimental animals were then provided with pure drinking water ad libitum. The standard oral gastric cannula was used for drug administration in experimental animals. This experimental protocol was approved by the Research Ethics Commission, Universitas Padjadjaran, Bandung, Indonesia (No. 369/UN6.KEP/EC/2021).

Induction of Hyperlipidemia

A high cholesterol diet was prepared by mixing 1.8 mg/200 g BW of propylthiouracil suspended in 1% pulvis gummi arabicum and 10 ml/kg BW egg yolk administered orally for 15 days.¹⁶

Protocol for Antihyperlipidemic Activity

Experimental animals were divided into 9 groups, with 4 animals in each. Group 1 was served as control of a high cholesterol diet (HC), and group 2 was given standard medication (simvastatin, 10 mg/kg BW orally, every day). Meanwhile, groups 3, 4, 5, and 6 were administered CCE in doses of 25, 50, 100, and 200 mg/kg BW, respectively, while groups 7, 8, and 9 were administered WFCC, EAFCC, and nHFCC (100 mg/kg BW daily) each, for 15 days after induction of a high cholesterol diet.

Blood Sampling and Biochemical Parameter Assay

On day 16, the rat's blood was collected by a retro-orbital puncture technique, under mild ether anesthesia, and allowed to clot at room temperature for 30 min. Subsequent to this, the blood samples were centrifuged at 3000 rpm for 20 minutes. The serum was separated and stored at -20°C until biochemical evaluation. It was then analyzed using a HumaLyzer 2000 spectrophotometer (Human Diagnostics) for TC, TG, and HDL-C which were determined using a diagnostic kit (Human Diagnostics Worldwide) obtained from the Lab-Mark company, Czech. Very low-density lipoprotein (VLDL), low-density lipoprotein cholesterol (LDL-C), atherogenic index (AI), and coronary risk index (CRI) were calculated using Modi *et al.*'s formula.^{17,18}

- $VLDL = \frac{TG}{5}$
- $LDL-C = \text{Total Cholesterol} - (HDL-C + VLDL)$
- $Atherogenic\ Index\ (AI) = \frac{LDL-C}{HDL-C}$
- $Coronary\ Risk\ Index\ (CRI) = \frac{TC}{HDL-C}$

Statistical Evaluation

Data were analyzed using SPSS version 22, while one-way analysis of variance (ANOVA) was used for statistical analysis, followed by the Tukey HSD post-hoc test. The test results are shown as mean $\pm$ S.E.M with $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

Phytochemical Screening

Table-1 shows the phytochemical screening of the extract and fractions of *C. costata* leaves, indicating the presence of chemical elements such as alkaloids, flavonoids, polyphenols, saponins, tannins, triterpenoids, and steroids, as well as anthraquinone glycosides.

Total Phenolics and Flavonoids of *C. costata* Extract

In our experimental work investigated, ethanolic extract of *C. costata* leaves has been shown to have the highest total phenolic and total flavonoid contents (Table-2).

Table-1: Phytochemical Screening of Extract and Fractions of *C. costata* Leaves

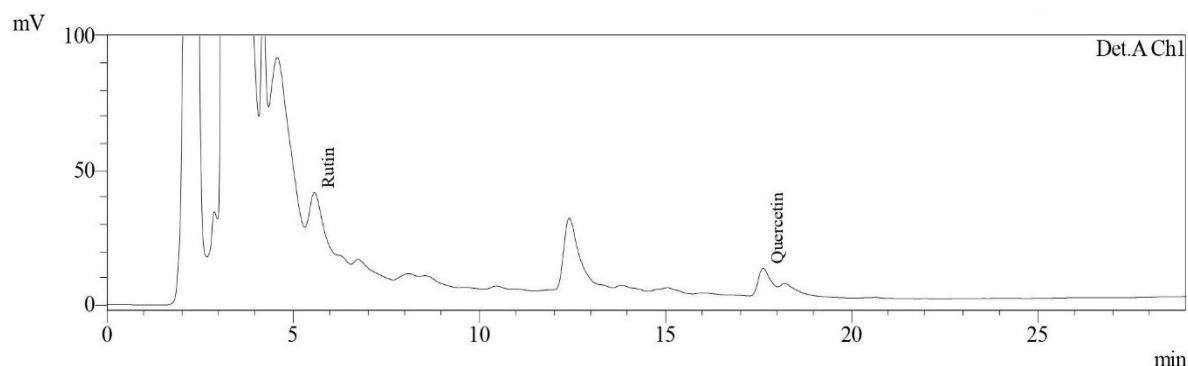
Phytochemical Compounds	Reagents	Observation	Results			
			CCE	WFCC	EAFCC	nHFCC
Alkaloids	Dragendorff	(+) Light brown	(+)	(-)	(-)	(+)
	Bouchardat	(+) Dark brown				
	Mayer	(+) Muddy and white sediment				
Flavonoids	Zn + HCl (p)	(+) Red	(+)	(+)	(+)	(-)
	Mg + HCl (p)					
Polyphenols	1% FeCl ₃	(+) Dark blue	(+)	(+)	(+)	(-)
Saponins	Hot water + HCl	(-) Bubble	(+)	(+)	(+)	(-)
Tannins	1% Gelatin	(-) White sediment	(+)	(+)	(+)	(-)
Triterpenoids and Steroids	Liebermann-Burchard	(+) Purple	(+)	(-)	(+)	(+)
Anthraquinone glycosides	EtOH + H ₂ SO ₄ + Benzene + NaOH	(+) Red in NaOH layer	(+)	(-)	(-)	(-)

(+) = Contained, (-) = Not contained.

Table-2: Total Phenolic and Flavonoid Contents of *C. costata* Extract

Sample	Total Phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
CCE	80.22 ± 0.92	18.96 ± 0.39

The values are means ± SEM of three replicates.

Fig.-1: HPLC Chromatogram of *C. costata* Extract

Effect of Extract and Fractions of *C. costata* Leaves on Total Cholesterol and Triglycerides

The total cholesterol levels increased in the hyperlipidemia-induced (HC) group compared to the normal group (NC). The value increased to 241.75 ± 19.68 mg/dL compared to the normal group, 77.78 ± 2.50 mg/dL. Therefore, it indicates hypercholesterolemia. In the group treated with CCE (25, 50, 100, and 200 mg/kg) the values decreased to 210.16 ± 7.00 , 203.85 ± 4.76 , 197.93 ± 2.96 , and 166.40 ± 6.09 mg/dL, respectively. Meanwhile, in the treatment group of WFCC, EAFCC, and nHFCC (100 mg/kg), the values decreased to 218.05 ± 2.83 , 231.25 ± 1.71 , and 155.24 ± 2.16 mg/dL, respectively. There was a significant decrease in total cholesterol values in the CCE 100 and 200 mg/kg and nHFCC 100 mg/kg treatment groups. The positive control group (PC) also significantly reduced the total serum cholesterol level to 153.82 ± 14.51 mg/dL (Fig.-2).

The triglycerides level had reached 124.02 ± 6.18 mg/dL in the group induced by hyperlipidemia (HC) compared to the normal group (NC), 66.13 ± 2.79 mg/dL, and indicating hypertriglyceridemia. In the group treated with CCE (25, 50, 100, and 200 mg/kg), the values decreased significantly to 106.03 ± 3.32 , 101.79 ± 1.99 , 98.49 ± 2.96 , and 95.67 ± 1.86 mg/dL. Meanwhile, in the treatment group of WFCC, EAFCC and

nHFCC (100 mg/kg), the values decreased to 114.39 ± 4.67 , 119.72 ± 2.34 , and 93.34 ± 2.06 mg/dL, respectively. There was a significant decrease in total values of cholesterol in the nHFCC 100 mg/kg treatment group. In addition, the positive control group (PC) also significantly reduced serum triglyceride levels to 90.58 ± 2.19 mg/dL (Fig.-3).

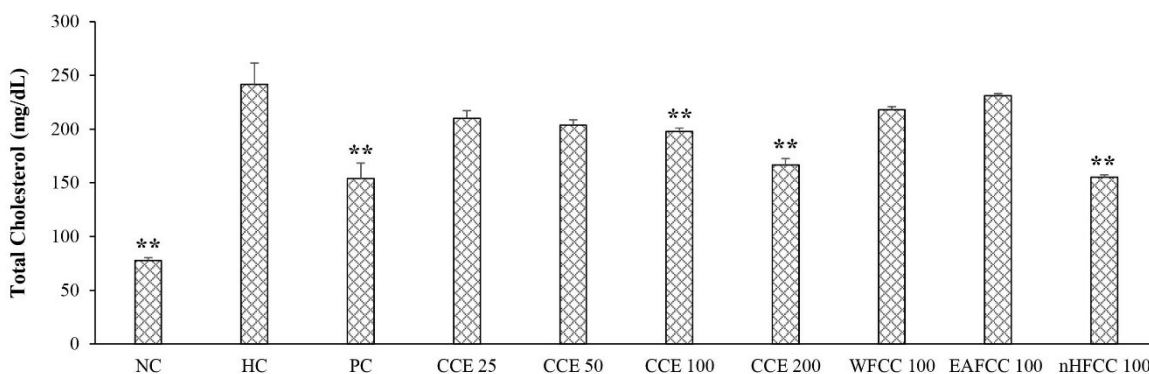


Fig.-2: Effect of Extract and Fractions of *C. costata* Leaves on Total Cholesterol. Data are presented as mean $\pm$ SEM of Four Animals in Each Group. ** $p < 0.05$ Compared to the Hyperlipidemic Control Group

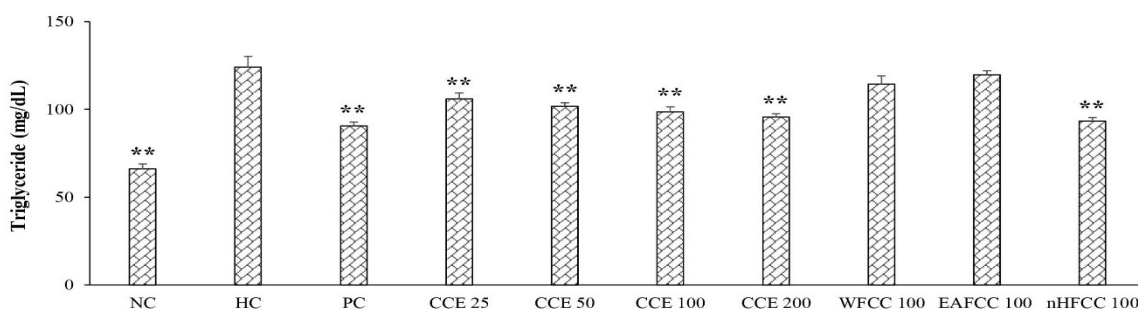


Fig.-3: Effect of Extract and Fractions of *C. costata* Leaves on Triglycerides. Data are presented as mean $\pm$ SEM of Four Animals in Each Group. ** $p < 0.05$ Compared to the Hyperlipidemic Control Group

Effect of Extract and Fractions of *C. costata* Leaves on HDL-C, VLDL-C, and LDL-C

The HDL cholesterol levels in the group induced by hyperlipidemia (HC) decreased compared to the normal group (NC). This value decreased to 23.86 ± 2.55 mg/dL compared to the normal group, which was 45.27 ± 2.35 mg/dL. In the group treated with CCE (25, 50, 100, and 200 mg/kg), the values were 33.01 ± 1.84 , 37.28 ± 1.13 , 39.08 ± 2.18 , and 45.31 ± 2.43 mg/dL, respectively. Meanwhile, in the group treated with WFCC, EAFCC and nHFCC (100 mg/kg), the values were 33.43 ± 1.82 , 31.08 ± 0.67 , and 46.81 ± 2.19 mg/dL, respectively. There was a significant increase in HDL-C values in the CCE 50, 100, and 200 mg/kg, and nHFCC 100 mg/kg treatment groups. In contrast, the positive control group (PC) also significantly increased HDL-C levels to 48.50 ± 2.18 (Fig.-4).

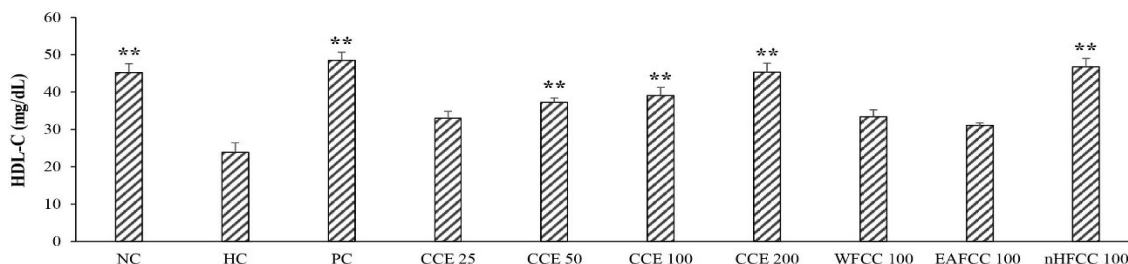


Fig.-4: Effect of Extract and Fractions of *C. costata* Leaves on HDL-C. Data are presented as mean $\pm$ SEM of Four Animals In Each Group. ** $p < 0.05$ Compared to the Hyperlipidemic Control Group

The cholesterol levels of VLDL in the group induced by hyperlipidemia (HC) increased to 24.80 ± 1.24 mg/dL compared to the normal group (NC) which was 13.23 ± 0.56 mg/dL. In the group treated with CCE (25, 50, 100, and 200 mg/kg), the values dropped significantly to 21.20 ± 0.66 , 20.36 ± 0.40 , 19.70 ± 0.60 , and 19.13 ± 0.37 mg/dL. Meanwhile, in the group treated with WFCC, EAFCC, and nHFCC (100 mg/kg), the values decreased to 22.88 ± 0.93 , 23.94 ± 0.47 , and 18.67 ± 0.41 mg/dL, respectively. There was a significant decrease in the nHFCC treatment group (100 mg/kg). In addition, the positive control group (PC) also significantly reduced the cholesterol levels of VLDL to 18.12 ± 0.44 mg/dL (Fig.-5).

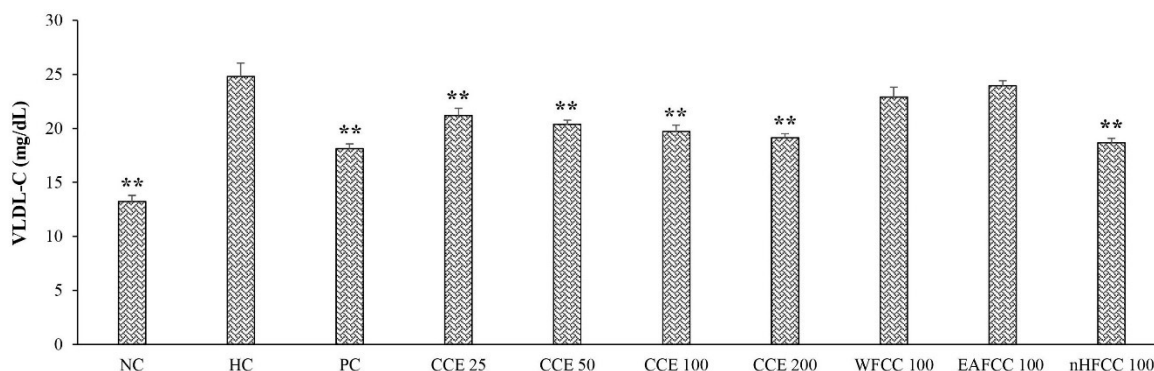


Fig.-5: Effect of Extract and Fractions of *C. costata* Leaves on VLDL-C. Data are presented as mean±SEM of Four Animals In Each Group. ** $p < 0.05$ Compared to the Hyperlipidemic Control Group

The cholesterol levels of LDL in the group induced by hyperlipidemia (HC) increased to 193.08 ± 21.41 mg/dL compared to the normal group (NC) which was 19.28 ± 4.02 mg/dL. In the group treated with CCE (25, 50, 100, and 200 mg/kg), the values decreased to 155.94 ± 7.75 , 146.21 ± 4.07 , 139.15 ± 5.17 , and 101.96 ± 8.39 mg/dL, respectively. Meanwhile, in the group treated with WFCC, EAFCC, and nHFCC (100 mg/kg), the values decreased to 161.74 ± 4.60 , 176.23 ± 2.32 , and 89.76 ± 0.50 mg/dL, respectively. There was a significant decrease in the CCE 50, 100 and 200 mg/kg, and nHFCC (100 mg/kg) treatment groups. In addition, the positive control group (PC) also significantly decrease the cholesterol levels of LDL to 87.20 ± 14.97 mg/dL (Fig.-6).

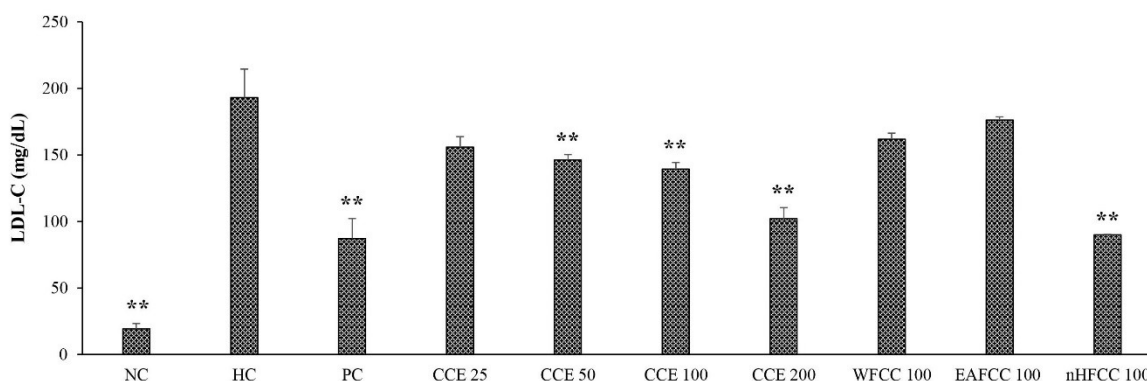


Fig.-6: Effect of Extract and Fractions of *C. costata* Leaves on LDL-C. Data are presented as the Mean±SEM of Four Animals in Each Group. ** $p < 0.05$ Compared to the Hyperlipidemic Control Group

Effect of Extract and Fractions of *C. costata* Leaves on Atherogenic Index and Coronary Risk Index

The atherogenic index in the hyperlipidemia-induced (HC) group increased to 8.41 ± 1.23 compared to the normal group (NC) which was 0.44 ± 0.11 . In the group treated with CCE (25, 50, 100, and 200 mg/kg), the values decreased significantly to 4.78 ± 0.40 , 3.92 ± 0.10 , 3.61 ± 0.31 and 2.29 ± 0.30 . Also, in the group treated with WFCC, EAFCC and nHFCC (100 mg/kg), the values decreased significantly to 4.89 ± 0.34 , 5.68 ± 0.18 , and 1.93 ± 0.09 . In addition, the positive group (PC) significantly decreased the atherogenic index to 1.83 ± 0.35 (Fig.-7).

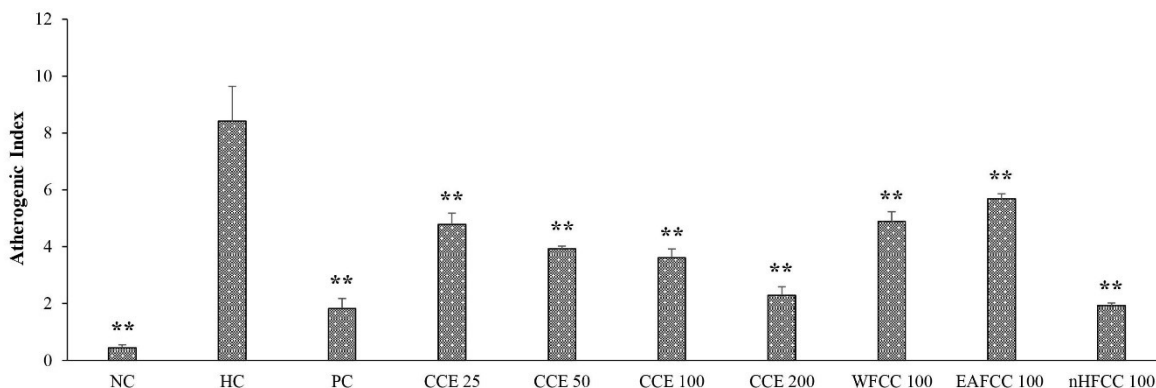


Fig.-7: Effect of Extract and Fractions of *C. costata* Leaves on Atherogenic Index. Data are presented as mean $\pm$ SEM of Four Animals In Each Group. ** $p < 0.05$ Compared to the Hyperlipidemic Control Group

The coronary risk index in the group induced by hyperlipidemia (HC) increased to 10.48 ± 1.28 compared to the normal group (NC) which was 1.73 ± 0.12 . In the group treated with CCE (25, 50, 100, and 200 mg/kg) the values decreased significantly to 6.43 ± 0.43 , 5.47 ± 0.10 , 5.12 ± 0.34 , and 3.72 ± 0.32 . Also, in the treatment group treated with WFCC, EAFCC and nHFCC (100 mg/kg), the values decreased significantly to 6.58 ± 0.36 , 7.45 ± 0.19 , and 3.33 ± 0.12 . The positive control group (PC) significantly reduced the coronary risk index to 3.21 ± 0.37 (Fig.-8).

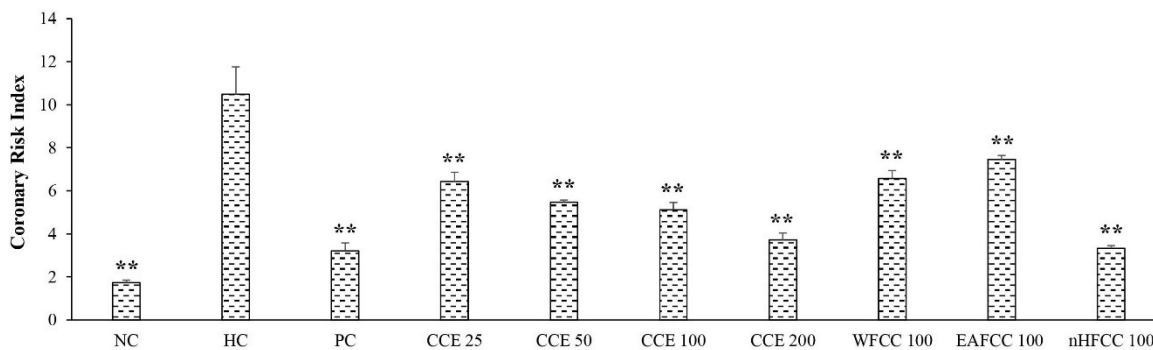


Fig.-8: Effect of Extract and Fractions of *C. costata* Leaves on Coronary Risk Index. Data are presented as mean $\pm$ SEM of Four Animals In Each Group. ** $p < 0.05$ Compared to the Hyperlipidemic Control Group

Cholesterol is an essential sterol for maintaining the integrity of cell membranes and a precursor for the formation of steroid hormones. However, high serum cholesterol increases the risk of atherosclerosis and other cardiovascular diseases. A 1% reduction in serum cholesterol reduces the risk of coronary heart disease by 2%.¹⁹ The treatment with *C. costata* leaf extract and fractions resulted in a significant reduction in the level of serum cholesterol in a rat model of hyperlipidemia induced by a high cholesterol diet. The extract and fractions are known to contain alkaloids, flavonoids, polyphenols, saponins, tannins, triterpenoids, and steroids, as well as anthraquinone glycosides. Steroid phytoconstituents are useful in treating hyperlipidemia due to their ability to mimic cholesterol in the formation of lipoproteins and chylomicrons. It has been shown that many anti-hyperlipidemic drugs have similar pharmacophores to cholesterol. The extract and leaf fractions of *C. costata* contain a high concentration of steroidal phytoconstituents and alkaloids, contributing to the hypolipidemic effect.²⁰ According to the study, *C. costata* extract was more effective at higher doses as an anti-hyperlipidemic agent against a rat model of hyperlipidemia induced by a high cholesterol diet. Meanwhile, the *n*-hexane fraction was more active as an anti-hyperlipidemic agent than the water and ethyl acetate. This is due to the phytoconstituents of steroids and alkaloids, more prevalent in the *n*-hexane fraction. In addition, bioactive compounds such as flavonoids and phenolics are reported to increase the cholesterol levels of HDL by activating the paraoxonase enzyme and protecting it from cell damage.²¹ This is shown from the HDL cholesterol profile given the extract and

the *C. costata* leaf fractions, which experienced a significant increase. Epidemiological studies showed that high HDL cholesterol levels have protection against cardiovascular diseases such as ischemic stroke and myocardial infarction.²² Meanwhile, in a previous study, *C. costata* leaf extract was a strong free radical scavenger in tests using DPPH (2,2-diphenyl-1-picrylhydrazyl).⁹ Its high antioxidant content reduced the formation of free radicals^{23,24} and inhibited the lipid peroxide process,²⁵ thereby reducing cell damage due to LDL oxidation. Also, antioxidant compounds reduce total cholesterol levels by decreasing fat absorption in the digestive system and increasing fat excretion into feces.²⁶

CONCLUSION

This study showed that the extract and fractions of *C. costata* leaves had strong anti-hyperlipidemic and anti-atherogenic effects in a rat model of hyperlipidemia induced by a high cholesterol diet. This is due to its phenolic compounds, which serve as a rap for free radicals, preventing lipid peroxidation. In addition, the content of steroid compounds in the extract and leaf fractions was also reported to have an anti-hyperlipidemic effect by mimicking cholesterol in the formation of lipoproteins and chylomicrons. Therefore, it is an important source of natural compounds for developing new treatments for hyperlipidemia and atherosclerosis, but further study is needed to determine the exact mechanism of the anti-hyperlipidemic effect of the *C. costata* leaves.

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