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Phytochemicals and Biological Activities of Ethanolic Extract of Garcinia atroviridis Leaf Grown in Indonesia

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ABSTRACT

The current investigation examined the potential antibacterial and antihyperlipidemic properties of ethanolic extract derived from the leaves of Garcinia atroviridis (EGA). The phytochemical contents of EGA were analysed through Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Gas Chromatography-Mass Spectrum (GCMS). The antioxidant properties of EGA were carried using DPPH, reducing power, and radical OH scavenging methods, whereas antibacterial activity of EGA was conducted against 4 pathogenic bacteria using agar diffusion method. To antihyperlipidemic action, High Fat Diet (HFD)-induced test hyperlipidaemic rats were given EGA at 50, 100, or 200 mg/kg orally for five weeks. Body weight changes, liver weight, serum lipid profile, and liver histology were assessed. The EGA contains phenols and flavonoids by 23.53±0.27 mg GAE/g and 20.48±0.39 mg QE/g, respectively. Around 15 compounds were identified where hexanoic acid (30.55%), 9,12,15octadecatrienoic acid (27.51%), and octadecanoic acid (15.49%) were compounds with the largest number. The EGA has antioxidant activity significantly different with ascorbic acid increasing %DPPH scavenging, %OH scavenging, and reducing power scavenging. The Minimum Inhibitory Concentration (MIC) was obtained at a concentration 10% with a clear zone diameter, while the Minimum Bactericidal Concentration (MBC) was obtained at different concentrations. This investigation was founded that EGA decreased biochemicals levels and causes liver tissue changes. Our studies demonstrate the EGA is antioxidant, antimicrobial, and antihyperlipidemic.

GRAPHICALABSTRACT GC-MS Analysis Tota phenolic content Histopathology analysis Antihyperlipidemic activity 1 Ethanolic Extract of **Biological activities** G. atroviridis leaf 2 Antibacterial activity Tota flavonoid content Antioxidant activity

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Introduction

There are at least 200 species of Garcinia dispersed over the globe, 100 of which are in Southeast Asia. The plants of this genus have culinary, medicinal, and industrial many applications. It is extremely attractive, with a dense canopy of green leaves and reddish-tinged developing leaves that are delicate. Garcinia atroviridis, commonly referred to as 'Asam Gelugur' by the indigenous population, is frequently utilized as a tart-tasting additive for enhancing the flavour of food [1]. Apart from its application as a flavouring agent, G. atroviridis is conventionally utilized in various manners to enhance well-being. Previous studies have documented the antioxidant, antibacterial, antifungal, anti-obesity, lipid metabolism, cytotoxicity, anti-inflammatory, antimalarial, and anti-nicotine stress actions of *G. atroviridis* [2, 3]. According to the report, the administration of a methanol extract obtained from G. atroviridis resulted in a reduction of cholesterol levels and aorta fat accumulation in guinea pigs that were subjected to a high-cholesterol diet [4]. Other investigations compare the antioxidant and antihyperlipidemic properties of various G. atroviridis components [5]. According to reports, the antihyperlipidemic activity of aqueous extract of G. atroviridis fruit (including seeds) was found to be highly significant and comparable to that of atorvastatin. The antioxidant activity of leaf

portion dissolved in methanol was higher than that of the other sections, which is an intriguing finding [6]. The tea production from *G. atroviridis* leaves can reduce malondialdehyde (MDA) levels, according to additional research [7].

Antioxidants are related with antimicrobial action in addition to antihyperlipidemic effects [8]. The phenol concentration of a plant material is connected to its antioxidant action. Due to their high redox potential, plant phenolic molecules such as flavonoids display antioxidant effects [9]. Many of their biological actions can be related to their antioxidant qualities, including antibacterial activity, anticarcinogenic, and antiproliferative capabilities [10]. Basri *et al.* demonstrated that ethyl acetate extract derived from G. atroviridis manifested significant antibacterial properties against seven bacterial strains and two yeast strains [11]. The precise mechanisms underlying the antioxidant and antibacterial properties of ethanolic extract remain unclear. The objective of this investigation was to assess the antihyperlipidemic and antibacterial properties of ethanolic extracts derived from the leaves of G. atroviridis.

Martials and methods

Sample preparation

The plant material of *G. atroviridis* was collected from Deli Serdang, Indonesia. The plant's leaves

underwent dehydration and were subsequently pulverized into a fine particulate matter. The powder was subjected to extraction using ethanol (v/v) and agitation overnight, followed by separation through Whatman No. 1 filter paper. The filtrates were subjected to evaporation using an evaporator, followed by lyophilization to obtain a powdered form. Subsequently, the ethanolic extract of *G. atroviridis* leaves (EGA) was stored at 20 °C until it was employed for further experimentation [12].

Assessment of total phenolic contents and total flavonoids content

The total phenolic content (TPC) of EGA was determined through spectrophotometric analysis subsequent to its reaction with the Folin-Ciocalteu reagent. The outcomes were expressed in terms of chlorogenic acid equivalents per gram of dry mass. The Total Flavonoid Contents (TFC) were determined through spectrophotometric analysis and reported as milligrams of quercetin per gram of dry weight, utilizing a previously conducted assay [13].

Phytochemicals profile using GC-MS

Using GC-MS, a phytochemical profile of EGA was determined (Organic Chemical Laboratory, UGM). To determine the bioactive compounds, researchers compared their retention times, peak areas, peak heights, and mass spectral fragmentation patterns to those of recognized chemicals listed in the NIST and Willey libraries [14].

DPPH scavenging activity

Various concentrations of the diluted extract were mixed with the DPPH working solution in ethanol to obtain a final volume of 1.0 mL. After being thoroughly mixed, each mixture was incubated at room temperature in the dark for 30 minutes before being centrifuged at 3000 rpm for 10 minutes. The measurement of optical density of each supernatant was taken at a wavelength of 517 nm [15]. Formula for DPPH percent scavenging:

> %Scavenging activity = [1 – (Asample / Acontrol)] × 100

The absorbance values at 517 nm of the control and the sample extract are denoted as Acontrol and Asample, respectively.

Hydroxyl scavenging activity

A volume of 0.5 mL of each extract was mixed with a salicylic acid-ethanol solution of concentration 9.1 mmol/L, a Fe²⁺ solution of concentration 9 mmol/L, and a volume of 3.5 mL of distilled water. The Fenton reaction was initiated using a 5 mL solution of hydrogen peroxide with a concentration of 88 mmol/L. The absorbance A1 of the reaction product was measured at 510 nm. The measurements of absorbance A2 and A3 were conducted using 0.5 mL of distilled water in lieu of the 9 mmol/L Fe²⁺ solution and the extract, respectively [16]. Formula for hydroxyl radical (OH) scavenging activity:

%Scavenging activity = $[1 - (A1 - A2) / A3] \times 100$

Reducing power activity

To evaluate the overall reducing power, 2 mL of each extract was mixed with 2.0 mL of a 2.5 mol/L phosphate buffer (PBS, pH 6.6) solution containing 1% $K_3Fe(CN)_6$ in a test tube. The mixtures were incubated for 20 minutes at 50 °C. Each combination underwent centrifugation at 3000 revolutions per minute for 10 minutes, utilizing 2.0 mL of a 10% solution of trichloroacetic acid. A volume of 2.5 mL of supernatant was mixed with an equal volume of deionized water that contained 0.1% ferric chloride. Absorbance at 700 nm was measured using spectrophotometers with distilled water serving as the blank [17].

Determination of the minimum inhibitory concentration

The pathogenic bacteria *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 19430, *Bacillus cereus* ATCC 14579, and *Staphylococcus aureus* ATCC 6538 were procured from the Biology Laboratory of the Faculty of Pharmacy at Universitas Sumatera Utara. Using the excellent method, the study employed the agar diffusion technique to determine hazardous bacteria's minimum inhibitory concentration (MIC) on

MRSA and NA bilayer media. This experiment added 50 μ L of EGA suspension with different concentrations (5%, 10%, 20%, 40%, 60%, 80%, and 100%) to individual wells. The samples were allowed to rest for 15 minutes, and then subjected to three rounds of incubation at 35 ± 2 °C for around 18-24 hours. In this investigation, a 0.2% ciprofloxacin injection was used as the positive control, while distilled water was utilized as the negative control. Distinct zones were observed in the inhibition zone surrounding the wells following incubation. The inhibition area was measured in mL using a digital calliper [18, 19]. The activity index was determined using the subsequent formula:

Activity index = (Inhibitor zone of EGA) / (Inhibitor zone of positive control (antibiotic))

Determination of minimum bactericidal concentration

From clear zone of MIC determination, the lowest bactericidal concentration was extracted and sub cultured on Trypticase Soy Agar (TSA) media. It was incubated for 18 to 24 hours at 35 ± 2 °C. The MBC value was obtained by the lowest concentration that reduced the initial bacterial population's viability by 98% to 99.9% (negative control) [20]. The decrease percentage was determined using formula:

% reduction = ((B-A)) /A × 100%

While, the log reduction can be determined by the following formula:

Log reduction = Log (B-A)

The quantity of bacterial colonies observed at each concentration and the number of colonies detected in the negative control were denoted as A and B, respectively.

Evaluation of antihyperlipidemic activity

Albino Wistar rats weighing between 120-180 g were procured from the Medan Animal Centre located in Medan, Indonesia. Throughout the trial period, the rats were provided with a conventional diet and unrestricted access to water. The specimens were placed in a regulated setting with a temperature of 25 ± 2 °C and a

relative humidity of 60% 5%. The lighting conditions were set to a 12-hour light and 12hour dark cycle. The Health Research Ethics Commission of Universitas Prima Indonesia has granted Protocol No. 059/KEPK/UNPRI/IV/2022 for the purpose of conducting experiments.

The rats were allocated into five groups randomly, with each group consisting of six rats (n = 6 per group). Each group was assigned one of the following interventions: The initial cohort was administered distilled water and a standard diet, whereas the subsequent cohort was administered distilled water and a diet high in fat (HFD). The cohorts comprising elements from groups III-V were subjected to the EGA treatment, with varying doses of 50, 100, and 200 mg/kg BW, daily. The dietary intervention utilized in the study consisted of a high-fat regimen consisting of 88% pellet diet, 10% lard, and 2% cholesterol. Blood samples were collected from the hearts of rats following a 5-week treatment period. Prior to utilization, the liver was extracted, sectioned into diminutive fragments, and cryopreserved at a temperature of -20 °C [21].

Biochemical assay

Blood samples were collected and analysed for the levels of total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, aspartate amino transferase, and alanine amino transferase. The levels of TC, TG, LDL-C, HDL-C, AST, and ALT were measured using enzymatic kits. Sigma-Aldrich is a leading global supplier of research chemicals, biochemicals, and other laboratory products [22].

Histological analysis

The liver tissues obtained from the experimental animals were fixed in 10% phosphate-buffered formalin for a duration of one day. Liver sections with 5 μ m thickness were prepared using a cryostat microtome (Leica CM1950, Germany) and subsequently stained with haematoxylin and eosin for microscopic analysis (H&E) [23].

Statistical analysis

The outcomes were presented in the form of mean values along with their corresponding standard errors of the mean. The statistical analysis involved using a one-way ANOVA test and the Tukey hsd technique, as implemented in SPSS version 22. Statistical significance was determined at a significance level of p<0.05.

Results and Discussion

Extraction yield, total phenolic, and flavonoid contents

The preparation of *G. atroviridis* leaf extract involved the utilization of maceration. A quantity of 500 g of dry powder derived from *G. atroviridis* leaf was subjected to a soaking process with 96% ethanol for a duration of three days. The resulting crude extract weighed 43.75 g, with a percentage yield of 8.75%. The present investigation yielded the total phenolic and flavonoid contents of EGA. The quantification of total phenolic content was conducted using the Folin-Ciocalteu reagent. The results were obtained using a calibration curve (with a correlation coefficient of 0.996) of gallic acid within the concentration range of 0-250 g/mL. The results were expressed as gallic acid equivalents (GAE) per gram of dry extract weight. The concentration of phenolic compounds in EGA was determined to be 23.53 ± 0.27 mg GAE/g. The flavonoids quantification in EGA was conducted using aluminium chloride and a colorimetric methodology. The outcomes were derived from the calibration curve (with a correlation coefficient of 0.9973) of quercetin, ranging from 0 to 100 g/mL, and were expressed in quercetin equivalents (QE) per gram of dry extract weight. The quantification of flavonoids in EGA yielded a value of 20.48 \pm 0.39 mg QE/g.

Phytochemicals profile of EGA

As more than 80% of the population in this developing nation uses traditional medicine, the World Health Organization (WHO) predicts that it will play a significant role in the future of healthcare there [24]. There is an increased likelihood of extinction for medicinal plants. The identification of active chemical constituents in medicinal plants is of paramount importance in verifying the scientific efficacy of traditional medicinal plants, as well as in the exploration of potential lead compounds for application in therapeutic drugs [25].

No.	RT	Name of the compound	Molecular	Molecular	Peak area
	(min)	Name of the compound	formula	weight	(%)
1	14.236	Cylopentanon-2-Carbonic Acid	C7H10O3	142	0.68
2	16.858	5,6-Digydro-4-Methoxy-2H-Pyran	$C_{6}H_{10}O_{2}$	114	0.95
3	23.875	2-Methyl-2-Penten-1,5-Dioic Acid	C8H12O4	172	2.27
4	26.193	Xanthosine	$C_{10}H_{12}N_4O_6$	284	0.91
5	26.893	Hexanoic Acid	C9H18O2	158	30.55
6	27.184	Propanoic Acid	C7H14O3	146	1.77
7	35.529	9-Octadecenoic Acid	$C_{19}H_{36}O_2$	296	0.63
8	35.692	Octadecanoic Acid	C19H38O2	298	15.49
9	36.517	Hexadecenoic Acid	$C_{16}H_{32}O_2$	256	5.59
10	39.124	9,12-Octadecadienoic Acid	$C_{19}H_{34}O_2$	294	6.87
11	39.296	9,12,15-Octadecatrienoic Acid	$C_{19}H_{32}O_2$	292	27.51
12	39.712	Pentadecanoic Acid	C17H34O2	270	1.17
13	40.026	9-Octadecenal (CAS) Octadecenyl Aldehyde	C ₁₈ H ₃₄ O	266	2.62
14	41.960	Tributyl Acetyl citrate	C20H34O8	402	1.94
15	46.955	Di-n-Octyl Phthalate	C24H38O4	390	1.05

Table 1: The GC-MS spectral analysis of ethanolic extract of *G. atroviridis* leaf

The utilization of the GC-MS technique in the examination of extracted substances may prove to be a valuable instrument in ascertaining the

levels of bioactive constituents present in botanicals utilized in the domains of cosmetology,

pharmacology, medicine, gastronomy, and forensic science [14].

Utilizing GC-MS, the present investigation sought to identify the bioactive chemicals present in EGA. The active constituents of EGA, comprising of 15 phytochemicals with biological activity, are presented in Table 1, which includes information on their retention time (RT), molecular formula (MF), molecular weight (MW), and concentration (peak area %). Among the 15 discovered chemicals, hexanoic acid (30.55%), 9,12,15octadecatrienoic acid (27.51%), and octadecanoic acid (15.49%) had the highest percentages. In addition, two additional compounds with a proportion of >5% were discovered; namely, hexadecenoic acid and 9,12-octadecadienoic acid.

Antioxidant activity of EGA

This study demonstrated the antioxidant activity of EGA through the implementation of DPPH scavenging, OH scavenging, and reducing power methodologies. The test employed ascorbic acid as a reference standard. The study evaluated the antioxidant potential of EGA by measuring its ability to reduce DPPH radicals, which was determined by observing a decrease in absorbance at 517 nm. Hydrazine was generated through the process of electron pairing facilitated by the hydrogen-donating properties of the EGA [26]. As depicted in Figure 1A, at a concentration of 100 μ g/mL, the % scavenging of EGA and ascorbic acid were 71.30 ± 0.16 and 87.71 ± 0.41, respectively (p<0.0001). This result indicates that EGA has antioxidant activity, and the scavenging activity will increase dependent on concentrations. The results depicted in Figure 1B demonstrate a positive correlation between the ascorbic acid equivalent content of the plant extract under investigation and its reduction potential. A positive control was employed using ascorbic acid, and its reducing power was measured at a concentration of 100 µg/mL, resulting in a value of 0.26 ± 0.00. At a concentration of 100 μ g/mL, the EGA exhibits a reducing power of 0.21 ± 0.01 . The statistical analysis revealed a significant difference (p<0.0001) in the reducing power between EGA and ascorbic acid. In addition, the antioxidant activity of EGA was obtained using hydroxyl scavenging activity with ascorbic acid as a positive control. Figure 1C shows the EGA increased the %scavenging depending on concentrations. The 100 μ g/mL EGA has the strongest concentration to increased %scavenging of 50.21 ± 0.77. This result was significantly different (p<0.0001) with ascorbic acid at 100 μ g/mL of 85.41 ± 1.00.

Antibacterial activity

The study assessed the minimum inhibitory concentration (MIC) of S. aureus, B. cereus, E. coli, and S. typhi at a concentration of 10%. The clear zone diameter for these microorganisms was found to be 7.27 ± 0.20 mm, 8.25 ± 0.23 mm, 7.25 \pm 0.20 mm, and 8.34 \pm 0.21 mm, respectively. The activity index values at 100% concentration for S. aureus, B. cereus, E. coli, and S. typhi of 0.60 ± 0.31, 0.57 \pm 0.78, 0.50 \pm 0.10, and 0.57 \pm 0.18 were conducted. The inhibitory zone diameter for each bacterium is presented in Table 2. The revealed a significant analysis statistical difference between means at a significance level of p<0.05.

Table 3 lists the measured Minimum Bactericidal Concentration (MBC) of EGA. The inhibitory effects of various concentrations on the growth of S. aureus, B. cereus, E. coli, and S. typhi were investigated. The results indicated that S. aureus was inhibited at a concentration of 20% with a 98.2% reduction percent, B. cereus was inhibited at a concentration of 80% with a 99.2% reduction percent, E. coli was inhibited at a concentration of 80% with a 98.5% reduction percent, and S. typhi was inhibited at a concentration of 100.0% with a 99.0%. The log reduction graph of the percentage reduction indicates that a significant reduction percentage also corresponds to a substantial log reduction. The log reduction values of S. aureus, B. cereus, E. coli, and S. typhi were 2.540, 2.215, 2.348, and 2.357, respectively.



Figure 1: The antioxidant properties of EGA and ascorbic acid are being investigated. Each value in the dataset represents the mean value along with the standard deviation, denoted as mean ± SD, and the sample size for each observation is n = 3. The three activities being referred to are DPPH radical scavenging activity, reducing power activity, and hydroxyl radical scavenging activity

Conc. (%)	S. aureus		B. cereus		E. coli		S. typhi	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI
- Control	4.21±0.00b	0.15±0.00	4.78±0.00 ^b	0.18±0.00	5.20±0.02 ^b	0.17±0.00	5.10±0.01 ^b	0.17±0.00
5%	4.32±0.00 ^{bc}	0.15±0.00	4.92±0.00 ^{bc}	0.19±0.00	5.23±0.02 ^{bc}	0.17±0.00	4.89±0.00bc	0.17±0.00
10%	7.27±0.20 ^{ab}	0.22±0.09	8.25±0.23 ^{ab}	0.24±0.01	7.25±0.20 ^{ab}	0.21±0.03	8.34±0.10 ^{ab}	0.21±0.04
20%	9.43±0.32 ^{ab}	0.28±0.12	10.13±0.10 ^{ab}	0.29±0.06	10.20±0.13 ^{ab}	0.27±0.08	9.27±0.31 ^{ab}	0.23±0.07
40%	11.57±0.25 ^{ab}	0.34±0.16	13.73±0.23 ^{ab}	0.36±0.12	12.46±0.23 ^{ab}	0.31±0.10	13.24±0.21 ^{ab}	0.39±0.09
60%	16.75±0.36 ^{ab}	0.49±0.11	15.73±0.25 ^{ab}	0.45±0.09	14.38±0.31 ^{ab}	0.37±0.12	15.24±0.41 ^{ab}	0.45±0.10
80%	17.32±0.55 ^{ab}	0.50±0.06	18.42±0.48 ^{ab}	0.52±0.12	16.22±0.25 ^{ab}	0.40±0.10	18.35±0.67 ^{ab}	0.51±0.15
100%	19.39±0.55 ^{ab}	0.60±0.31	20.32±0.67 ^{ab}	0.57±0.78	18.23±0.56 ^{ab}	0.50±0.10	20.23±0.67 ^{ab}	0.57±0.18
+ Control*	32.17±0.15ª	1.00±0.10	33.25±0.12ª	1.00±0.12	31.43±0.21ª	1.00±0.10	30.76±0.35ª	1.00±0.10

Table 2. The diameter of the zone of MIC of ethanolic extract of *G. atroviridis* leaf

Note: * A reference standard of 0.2% ciprofloxacin injections was employed. The abbreviation "IZ" refers to the Inhibitor Zone, while "AI" stands for Activity Index.

^aP-value < 0.05 (0.000), a notable disparity was observed compared to the negative control.

^bP-value < 0.05 (0.000), a notable disparity was observed in relation to the positive control.

 ^{c}P -value > 0.05 (1.000), no statistically significant difference was observed between the experimental group and negative control group.

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	S. aureus		B. cereus		E. coli		S. typhi	
Conc. (%)	% Reduction	Log	% Reduction	Log Reduction	% Reduction	Log	%	Log
		Reduction				Reduction	Reduction	Reduction
- Control	0.0%	0.000	0.0%	0.000	0.0%	0.000	0.0%	0.000
5%	15.5%	0.084	17.6%	0.074	21.8%	0.129	3.5%	0.015
10%	24.3%	0.154	20.5%	0.145	35.5%	0.250	15.2%	0.145
20%	98.2%	1.760	94.8%	1.532	95.2%	1.850	91.5%	1.750
40%	99.1%	2.037	96.5%	1.601	97.0%	1.975	92.9%	1.955
60%	99.3%	2.240	97.8%	1.678	97.8%	2.035	94.3%	2.012
80%	99.5%	2.280	99.2%	2.098	98.5%	2.220	97.5%	2.218
100%	99.7%	2.540	99.5%	2.215	99.3%	2.348	99.0%	2.357
+ Control*	100.0%	3.105	99.8%	2.460	99.7%	2.505	99.8%	2.692

Table 3: The MBC of ethanolic extract of *G. atroviridis* leaf against pathogenic bacteria

Note: * The positive control utilized in the study was the injection of Ciprofloxacin at a concentration of 0.2%.

The observed reduction in bacterial colonies at varying concentrations was found to be significantly distinct from that of the negative control.

Antihyperlipidemic activity

The antihyperlipidemic effect of EGA was seen in a rat model of hyperlipidaemia caused by a highfat diet (HFD) [27]. After 5 weeks of HFD consumption, the rats increased in body weight, and liver weight (Figure 2A and 2B). However, treatment with EGA (50, 100, and 200 mg/kg) significantly reduced the body weight after HFD consumption. The EGA at 200 mg/kg was the strongest to decrease final body weight compared with HFD group of 153.63 ± 1.40 g and 238.87 \pm 2.33 g, respectively (p<0.0001). Other concentrations of EGA also decreased the final body weight significantly different from HFD group (p<0.0001). After HFD consumption, the liver weight of HFD group was increased of 7.56 \pm 0.07 g (Figure 2B). The EGA treatment decreased the liver weight significantly different with HFD group at 50 mg/kg, 100 mg/kg, and 200 mg/kg of 5.36 \pm 0.18 g, 4.40 \pm 0.13 g, and 3.66 \pm 0.14, respectively (p<0.0001).



Figure 2: The impact of EGA on body weight (A) and liver weight and (B) subsequent to high-fat diet induction. Each datum represents a mean value with its corresponding standard deviation (n=3). The variables under investigation are the body weight and liver weight of rats

Biochemical parameter checks of hyperlipidaemic rats were conducted to perform EGA activity (Figure 3A). After HFD consumption, the total cholesterol (TC), triglyceride (TG), HDL-C, and LDL-C were increased compared with the control group (p<0.0001) of 8.27 \pm 0.15 mmol/L, 2.43 \pm 0.18 mmol/L, 2.34 \pm 0.19 mmol/L, and 10.61 \pm 0.51 mmol/L, respectively. The EGA at 200 mg/kg was the strongest concentration to decrease TC, TG, and LDL-C compared with HFD

group (significantly different, p<0.0001) of $3.25 \pm 0.05 \text{ mmol/L}$, $0.68 \pm 0.03 \text{ mmol/L}$, $2.46 \pm 0.17 \text{ mmol/L}$, and $3.12 \pm 0.08 \text{ mmol/L}$, respectively. This study also examined ALT and AST levels

following EGA at 50, 100, and 200 mg/kg (Figure 3B). ALT and AST levels were considerably reduced in the EGA group (50, 100, and 200 mg/kg) compared to the HFD group (p<0.0001).



Figure 3: Effect of EGA against biochemical values of rats after HFD induced. Each value represents a mean ± SD (n = 3). (A) TC, TG, HDL-C, and LDL-C parameters, and (B) ALT and AST parameters

To verify the hypolipidemic impact of EGA, a histopathological analysis was conducted. Liver tissue was stained with H&E to examine pathologic alterations. Figure 4A depicts that the hepatocytes of mice in the ND group lacked hepatic steatosis and possessed normal hepatic sinusoids for mass tranter. In contrast, macrovascular and microvascular steatosis were common in rats with damaged hepatic sinusoids in the HFD group (Figure 4B), as compared to the normal group. It was further demonstrated that treatment with various dosages of EGA for 5 weeks significantly suppressed these alterations in rats fed a HFD (Figures 4C, 4D, and 4E). No necrotic cells or histological indications of hepatotoxicity were seen in rats fed а conventional or HFD.

Important plant components with redox characteristics and antioxidant activity are phenolic and flavonoid compounds [28]. In this study, we were able to investigate total phenolic and flavonoid contents of EGA. The phenolic and flavonoid contents of EGA were 23.53 ± 0.27 mg GAE/g and 20.48 ± 0.39 mg QE/g, respectively. The extraction methods and solvents are responsible for dissolving the plant's endogenous components [29]. Furthermore, plant parts might be either polar or non-polar. Alcohol was chosen as the extraction solvent because phenolic and

flavonoid chemicals are more soluble in polar organic solvents than nonpolar ones [30]. Comparing the literature, the TPCs of methanolic extract and aqueous extract of G. atroviridis leaves were reported of $17.92 \pm 0.40 \text{ mg GAE/g}$ and 10.86 ± 0.31 mg GAE/g, respectively. In contrast, the TFC of the extracts of 18.97 ± 0.25 mg QE/g and 5.99 \pm 0.30 mg QE/g, respectively. As per a separate investigation, the total phenolic content (TPC) of the methanolic extract of G. atroviridis leaf was found to be 29.10 ± 4.30 mg GAE/g through the maceration process [6]. According to this report, the total phenolic content (TPC) of EGA was found to be higher than that of previous studies. However, the TFC of EGA is lower than that of the methanolic extract of G. atroviridis leaf.

Many *in vitro* assays also showed that EGA extracts had antioxidant properties, which was another interesting discovery from this study. The experimental results showed that EGA at 200 μ g/mL showed strong DPPH scavenging activity. If compared to another study, the % DPPH scavenging activity of methanolic extract of *G. atroviridis* leaf at 100 μ g/mL of 57.97% [31]. This activity was the lowest compared to what we investigated. Extract of *G. atroviridis* leaf has been shown to contain antioxidants in several prior studies, with the presences of these antioxidants

being measured by the extract's ability to convert ferric cyanide complex into ferrous form [32]. Potential antioxidant activity in a substance may be strongly correlated with its reducing power. The cessation of a radical chain reaction can be achieved through the electron donation by a sample possessing significant reducing power, leading to the formation of a stable molecule by the accepting free radical [33, 34]. The current investigation found that the EGA's reducing ability was comparable to that of previous studies, as displayed in Figure 1B [35].



Figure 4: The present study conducted histological analysis of frozen liver sections from rats using HE staining technique, with a magnification of x100. (a) The ND group, (b) The HFD group, (c) The EGA group administered with a dose of 50 mg/kg, (d) The EGA group administered with a dose of 100 mg/kg, and (e) The EGA group administered with a dose of 200 mg/kg

Previous research has shown that hydroxyl radicals, which are produced naturally, are highly reactive free radicals, and that humans lack enzymes that can neutralize them [36]. Previous research established that hydroxyl radicals are highly reactive free radicals produced in the biological system, and that human ROS lacks enzymes specifically designed to neutralize them [37]. The highly high reactivity of hydroxyl (OH) radical makes its scavenging a crucial antioxidant activity, as was shown in a previous work [38]. The conducted research indicates that the scavenging capacity of EGA towards hydroxyl radicals was observed to be directly proportional to its concentration. This study reported about antibacterial activity of EGA against pathogenic bacteria. When tested against four types of diarrhoea-causing bacteria, EGA showed antibacterial action, as indicated by the creation

of a clean zone around wells inoculated with EGA at varying doses (Tables 2 and 3) [39]. If the measured inhibition zone is less than the control sample, this does not rule out the possibility that examination the sample under possesses antibacterial properties. The MBC was ascertained through the application of a flashing technique on each zone of inhibition produced by the MIC test, as reported in [40]. The findings indicate that the concentrations of MBC test samples varied across the different bacterial strains. According to [41], the concentration at which 98.0% to 99.9% of the colony number (relative to the initial colony number) is eliminated is known as the minimal effective concentration (MBC) of an antimicrobial agent. Finally, the antihyperlipidemic activity of EGA was conducted with determined biochemical parameters and histopathological of rat's liver.

Hyperlipidaemia is characterized by elevated blood levels of fatty compounds including triglycerides and lipoproteins [42]. Because clogged veins and arteries can slow down metabolic processes, a rise in cholesterol levels can lead to cardiovascular disease [27]. High-fat diets and oxidative stress are linked to the hyperlipidaemia development. Regarding their antioxidant and lipid-lowering properties, there is a growing interest in plants and natural products derived from them [43, 44]. The lipidlowering effect of EGA (50, 100, and 200 mg/kg) was demonstrated using a HFD induced hyperlipidaemic rats. Similar lipoprotein and bile acid metabolic processes exist in rats and humans. We investigated the EGA effect on the blood lipids of rats and found that it decreased TC, TG, and LDL-C. Recently, the in vitro antihyperlipidemic efficacy of EGA leaves was found. Previously, the fruit of G. atroviridis was studied for its antihyperlipidemic action. With a cholesterol-rich diet, the methanolic extract of G. atroviridis fruit lowered the serum lipid profile. Compared to the high cholesterol diet group, histological investigations revealed a reduction in fat deposition in the aorta of animals given extract and fed a high cholesterol diet [45]. The extract contains octadecadienoic acid, which is also present in EGA, as presented in (Table 1). Octadecadienoic acid, hexadecenoic acid, and oleic acid were found to have a role in the antihyperlipidemic action. Nonetheless, it is plausible that such an outcome could be attributed to the existence of alkaloids, phenols, flavonoids, and tannins [46, 47].

Conclusion

To summarize, the EGA exhibits numerous bioactive components that hold potential as sources of antioxidant, antibacterial, and antihyperlipidemic properties. Additional research and analysis are necessary to verify the antibacterial and antihyperlipidemic properties attributed to EGA. Collectively, the evidence suggests that EGA possesses the capacity to be employed in the lipids regulation.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the article and agreed to be responsible for all the aspects of this work.

Conflict of interest

We have no conflicts of interest to disclose.

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