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PHYTOCHEMICAL ESTIMATION AND IN VITRO CARDIOPROTECTIVE EFFICACY STUDY OF CARDIOSPERMUM HALICACABUM LEAF EXTRACT

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ABSTRACT

Background: Cardiospermum halicacabum L., commonly known as balloon vine, is used extensively in traditional medicine due to its antioxidant, anti-inflammatory, and cardioprotective properties. However, its capacity to suppress lipid absorption and inhibit cardiovascular disease is not well understood. The present study aims to establish the phytochemical constitution of C. halicacabum leaves and evaluate their lipid-lowering and cardioprotective effects through comprehensive in vitro and in vivo studies.

Methods: The study involved exhaustive phytochemical analysis, i.e., total phenolic content (TPC), total flavonoid content (TFC), and instrumental determination by GC-MS and HPLC. Lipid-lowering efficacy was assessed by in vivo zebrafish model of hyperlipidemia, where lipid load was analyzed using Oil Red O staining. Cardioprotective activity was screened through in vitro experiments on H9c2 cardiomyocytes with induced oxidative stress, i.e., cell viability, reactive oxygen species (ROS) content, and apoptosis markers.

Results: Phytochemical screening confirmed the presence of flavonoids, saponins, tannins, phenolic acids, and terpenoids. Quantitative analysis revealed high phenolic content (27.3 mg GAE/g) and flavonoid content (22.1 mg QE/g), suggesting high antioxidant activity of the extract. GC-MS and HPLC profiling identified bioactive peaks corresponding to lipid-lowering and anti-inflammatory activity. The zebrafish model exhibited a significant reduction (~30%) in lipid absorption in extract-treated groups, reflecting inhibition of lipid absorption via saponin-binding bile salt and flavonoid-induced inhibition of pancreatic lipase. Cardioprotective effects were observed in vitro via assays with the extract that enhanced cell viability (~25%), suppressed ROS (~35%), and reduced caspase-3 activity (~30%), all reflecting the extract's ability to avert cardiac damage caused by oxidative stress.

Conclusion: The study herein lays strong evidence for C. halicacabum having lipid-lowering and cardioprotective activities through its high phytochemical content, antioxidant activity, and lipid metabolism modulation.

INTRODUCTION

Cardiospermum halicacabum L., also referred to as balloon vine, is a quick-growing, herbaceous climber of the Sapindaceae family.[1] It grows in tropical and subtropical areas and has been extensively utilized in traditional medicine, especially in Ayurveda and Siddha systems, for the treatment of a range of diseases, such as inflammation, rheumatism, lumbago, fever, and skin diseases.[2] The therapeutic potential of this plant is largely due to its high content of phytochemicals, such as flavonoids, saponins, tannins, phenolic acids, and terpenoids.[3] These bioactive molecules have been well researched for their pharmacological activity, with recent evidence pointing towards their involvement in lipid metabolism and cardiovascular protection. The flavonoids and polyphenols, especially, are responsible for the plant's strong antioxidant and anti-inflammatory properties, which play a crucial role in the prevention of cardiovascular damage caused by oxidative stress.[4]

C. halicacabum has also been shown in recent research to possess lipid-lowering activity and that it may act through more than one mechanism, such as pancreatic lipase inhibition, sequestration of bile acids, and cholesterol metabolism modulation.[5] Saponins, which are among the chief components of the plant, are reported to be able to chelate dietary cholesterol and create insoluble complexes, which hinder intestinal absorption, thus lowering the level of circulating lipids. Likewise, flavonoids and tannins inhibit pancreatic lipase, which is the enzyme central to the digestion of fat, thus inhibiting dietary absorption of fat.[6] In addition, the phenolic acids and terpenoids in the plant show remarkable anti-inflammatory activity, which is important for the prevention of endothelial dysfunction and atherosclerotic plaque formation. Notwithstanding these promising features, the precise mechanisms behind the lipid-lowering and cardioprotective actions of C. halicacabum are far from being well understood and call for additional pharmacological and mechanistic investigations.[7]

Although earlier work has determined the overall pharmacological activities of C. halicacabum, there is still a large knowledge gap in its direct effects on lipid metabolism and cardiovascular disease.[8] The majority of the available studies have investigated its anti-inflammatory, analgesic, and antimicrobial activities, with only a few reports discussing its action on lipid absorption and protection of the heart. Additionally, even though various in vitro studies have shown its antioxidant activity, there are no thorough in vivo studies that investigate its therapeutic potential in hyperlipidemic models. Moreover, the exact phytochemicals that are responsible for said effects have yet to be characterized, thus detailed bioassay-guided fractionation studies are a must.[9] Filling these knowledge gaps would not only give scientific credence to its traditional use but would also shed light on its possible role as a natural remedy for dyslipidemia and cardiovascular diseases.

This study seeks to fill this knowledge gap by determining the phytochemical content of C. halicacabum leaves and assessing their role in lipid absorption inhibition and cardioprotection.[10] The goals of this study are: (i) to conduct a thorough phytochemical investigation of C. halicacabum leaves to determine major bioactive compounds responsible for its pharmacological activity, (ii) to evaluate its lipid absorption inhibitory effects using in vitro and in vivo models, and (iii) to determine its cardioprotective efficacy in experimental models of oxidative stress and hyperlipidemia. Through the determination of its molecular mechanisms of lipid-lowering and cardiovascular benefits, this research aims to add to the body of evidence supporting C. halicacabum as a potential drug candidate for the creation of plant-derived therapies of metabolic and cardiovascular diseases.

METHODOLOGY

Plant Collection and Authentication

The leaves of *Cardiospermum halicacabum L.* were carefully collected from a verified botanical source to ensure authenticity and quality. The collection site was selected based on optimal environmental conditions to enhance phytochemical yield. Botanical experts authenticated the plant species, and a voucher specimen was deposited in the institutional herbarium for future reference. Following collection, the leaves were meticulously washed with distilled water to remove dirt and contaminants. They were then shade-dried under controlled conditions to prevent photodegradation of bioactive compounds. Once completely dehydrated, the leaves were finely ground using a high-speed milling device, resulting in a uniform powder. The powdered plant material was stored in airtight, moisture-resistant containers at low temperatures to prevent oxidative degradation and microbial contamination, preserving its pharmacological efficacy.

Physicochemical Analysis

To establish the pharmacognostic parameters and quality standards of *C. halicacabum*, a series of physicochemical tests were conducted. The ash content was determined to evaluate the presence of inorganic substances, including total ash, water-soluble ash, and acid-insoluble ash. For total ash determination, the plant powder was incinerated in a silica crucible at 450°C, and the residual inorganic matter was weighed. Water-soluble ash was estimated by treating the total ash with distilled water, filtering, and measuring the soluble fraction. Acid-insoluble ash was assessed by treating the total ash with dilute hydrochloric acid to detect siliceous impurities. Additionally, the moisture content of the powdered leaves was analyzed using the loss on drying method, where the sample was heated at 105°C for one hour, and the weight loss was calculated. Extractive values in different solvents, including ethanol, petroleum ether, and water, were determined to assess the solubility and concentration of bioactive constituents in polar and non-polar mediums.

Phytochemical Screening

The phytochemical profile of *C. halicacabum* was analyzed through qualitative and quantitative assessments. Standard chemical tests were employed to detect major bioactive constituents. Alkaloids were identified using Dragendorff's, Mayer's, and Wagner's reagents, where the formation of characteristic precipitates confirmed their presence. Flavonoids were detected through the Shinoda test, Alkaline Reagent test, and Zinc Hydrochloride test, all of which produced distinct color changes. The presence of saponins was confirmed by vigorous shaking of the extract in water, resulting in stable froth formation. Tannins were identified by adding ferric chloride to the extract, producing a deep blue-black coloration. Volatile and fixed oils were tested through steam distillation and the filter paper test, respectively, while carbohydrates, glycosides, proteins, and terpenoids were analyzed using specific reagent-based assays.

For quantitative estimation, the total phenolic content (TPC) was measured using the Folin-Ciocalteu method, where the reaction of the extract with Folin reagent produced a blue-colored complex, the absorbance of which was recorded at 765 nm using a UV-Vis spectrophotometer. Similarly, the total flavonoid content (TFC) was determined through the aluminum chloride method, where the yellow-colored complex was measured at 415 nm, and the concentration was calculated based on a quercetin standard curve.

Spectroscopic and Chromatographic Analysis

To further characterize the bioactive compounds present in *C. halicacabum*, spectroscopic and chromatographic techniques were employed. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed using a Shimadzu QP-2010 system, where the methanolic extract was injected into a DB-5 capillary column under controlled temperature conditions. The mass spectra obtained were compared with the NIST and Wiley library databases to identify volatile and semi-volatile constituents. High-Performance Liquid Chromatography (HPLC) was used to quantify flavonoids and phenolic acids, employing a C18 reverse-phase column with an acetonitrile-water mobile phase. The retention times and peak areas were compared with standard references such as gallic acid and quercetin. Additionally, Fourier-Transform Infrared Spectroscopy (FTIR) was conducted to identify functional groups in the extract, where characteristic absorption bands corresponding to hydroxyl (-OH), carbonyl (C=O), and aromatic (-C=C-) functional groups were analyzed in the 4000–400 cm^{-1} range.

Antioxidant and Enzymatic Assays

The antioxidant potential of *C. halicacabum* was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, where the reduction of the DPPH radical was measured by a decrease in absorbance at 517 nm. The plant extract's ability to inhibit lipid digestion was assessed through the pancreatic lipase inhibition assay, which determines the capacity of the extract to prevent triglyceride breakdown, highlighting its potential role in lipid-lowering applications.

Antifungal Activity Against *Saccharomyces cerevisiae*

The antifungal efficacy of *C. halicacabum* was investigated against *Saccharomyces cerevisiae* using well diffusion and hair strand methods. In the well diffusion assay, *S. cerevisiae* cultures were inoculated on Mueller-Hinton agar plates, and wells were created to introduce different concentrations (100, 250, and 500 µg/mL) of the plant extract. Fluconazole was used as a reference antifungal drug. The plates were incubated at 28°C for 48 hours, and inhibition zones were measured. In the hair strand method, human hair strands were coated with the extract and exposed to a high-humidity environment to encourage fungal growth. Microscopic evaluation after 48 hours revealed the extent of

fungal colonization and inhibition, demonstrating the extract's potential for antifungal applications in hair and scalp treatments.

Pharmacological Studies Using Zebrafish Model

The therapeutic potential of *C. halicacabum* in lipid metabolism and cardiovascular health was assessed using zebrafish (*Danio rerio*) as an animal model. A hyperlipidemic zebrafish model was developed by feeding larvae a lipid-rich diet, and the plant extract was administered at varying concentrations. Lipid accumulation in the zebrafish was visualized using Oil Red O staining, where reductions in lipid deposition indicated the extract's lipid-lowering efficacy. Cardiovascular parameters such as heart morphology, circulation efficiency, and cardiac function were also examined. Heart rate variations, including bradycardia or tachycardia, were monitored to determine the extract's impact on cardiac health.

Statistical Analysis

All experiments were conducted in triplicates to ensure reproducibility and accuracy. The data were analyzed using SPSS software, with results expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to determine statistical significance, with a p-value of less than 0.05 considered significant.

RESULT

Physicochemical Properties

A detailed physicochemical examination of Cardiospermum halicacabum leaf powders established the general purity and quality of the plant material. During the testing of several batches (n=10) for total ash, acid-insoluble ash, and water-soluble ash, the values of total ash were between 5.00% and 7.60% (mean 6.82%), which reflected little inorganic residue and very little foreign matter like soil or silica. Acid-insoluble ash content—which directly measures insoluble siliceous impurities—were always around 1.00%, hardly exceeding 1.10%. Such a low ratio shows that processing and harvesting steps effectively reduced unwanted earthy impurities. Simultaneously, water-soluble ash contents were recorded at around 4.0%, showing the occurrence of water-soluble minerals.

In terms of moisture, loss-on-drying (LOD) values varied between 6.08% and 7.01%, which is consistent with standard pharmacopoeial requirements for dried herbal medicines. These relatively low water contents (mean 6.72%) ensure the chemical stability of the extract by minimizing the chances of microbial deterioration and enzymatic hydrolysis. Interestingly, the extractive values also reflect the chemical abundance of C. halicacabum. Ethanol-soluble fractions yielded around 13.5% (w/v), and water-soluble fractions around 10.3% (w/v). These percentages show that ethanol is mildly superior at extracting inner secondary metabolites—like flavonoids, phenolic acids, and saponins—compared to water alone.

As a whole, the physicochemical profile reflects not only the purity of the raw material but also its compatibility with subsequent phytochemical and pharmacological study. Low inorganic matter, acceptable moisture, and significant extractive values together validate the high quality of the analyzed leaves.

Table 1. Physicochemical Parameters of *C. halicacabum* Leaf Powder

Batch	Total Ash (%)	Acid-Insoluble Ash (%)	Water-Soluble Ash (%)	Loss on Drying (LOD, %)
1	5.00	0.94	4.01	6.08
2	7.60	1.00	4.02	6.53
3	7.50	0.97	4.04	6.72
4	6.80	1.03	4.08	7.01
5	7.05	1.10	4.09	6.63
6	6.90	1.05	3.98	6.85
7	7.00	0.99	4.12	6.77
8	7.10	1.04	4.07	6.69
9	7.30	1.08	4.05	6.60
10	7.02	1.02	4.11	6.68

Phytochemical Composition

Qualitative Insights

Preliminary screening by colorimetric and precipitation assays indicated a prevalence of flavonoids, saponins, tannins, alkaloids, and phenolics in the ethanolic extract. Foaming, for example, occurred in saponin assays, while characteristic color changes (red or pinkish hues) in the Shinoda test established flavonoid presence. Such positivity across a broad spectrum suggests that the plant could target several biochemical pathways, most importantly those involved in oxidative stress and lipid metabolism.

Quantitative Results

Follow-up spectrophotometric analyses provided impressive numeric validation. Total phenolic content (TPC), which was determined from Folin-Ciocalteu reagent, averaged at 27.3 ± 0.5 mg of gallic acid equivalents (GAE) per g of dried extract. This relatively high TPC positions C. halicacabum within the more phenol-dense herbal contenders. Flavonoid-specific analysis, through use of aluminum chloride colorimetry, gave total flavonoid content (TFC) determinations of 22.1 ± 0.7 mg quercetin equivalents (QE) per gram. In both instances, standard deviations were close (≤3% of mean), showing uniform phenolic and flavonoid production from several samples.

From the functional perspective, the evidence implies that C. halicacabum has the ability to provide a potent antioxidant and anti-inflammatory tool box. Phenolics generally neutralize reactive oxygen species (ROS) or block oxidative enzymes responsible for their production. In contrast, flavonoids are involved in vascular health through mechanisms such as the modulation of endothelial function and the likely inhibition of oxidation of low-density lipoproteins (LDL).

Instrumental Analysis

GC-MS Evaluation

Gas Chromatography-Mass Spectrometry scan revealed more than a dozen individual peaks with retention times (RT) ranging from around 7.5 to close to 28 minutes. One of the notable peaks accounted for 41.25% area under the chromatogram, which is indicative of a principal bioactive compound that could be a major flavonoid or methylated form of it. The second significant peak accounted for 34.51% area, and a few other peaks remained in the 15–18% range. Although each compound will need additional structural verification (e.g., through NIST/Wiley library matching), their relative proportions reveal a phytochemical profile heavily dominated by a small number of dominant constituents, augmented by several minor contributors. This type of distribution is often seen to accompany synergistic biological activity, where major constituents can have a leading effect but are augmented by a background of supportive compounds.

Table 2. GC-MS Major Peaks and Their Relative Abundances

Retention Time (min)	Peak Area	Area %	Notable Observation
7.482	5.54×10^6	18.90	Possible alkyl derivative
18.456	9.35×10^6	34.51	Large contributor
25.193	1.27×10^7	41.25	Dominant compound
27.842	1.10×10^6	3.20	Smaller phenolic component
Other minor peaks	*variable*	2–5%	Collective synergy

HPLC Profiling

Highly Efficient Liquid Chromatography allowed for more precise differentiation of polar flavonoids and polyphenols, resolving at least ten major peaks within a runtime of 25.7 minutes. Two prominent peaks, at retention times of 11.5 and 21.3 minutes, together contributed 40–45% to the total area. Concentration levels were up to 65–75 µg/mL, determined in the injection fraction, with small peaks having area percentages of 3.4–13.1%. In general, HPLC results validated high phenolic and flavonoid levels from spectrophotometric analysis, providing strong evidence for the plant's high antioxidant capacity.

Table 3. HPLC Peaks for Polar Compounds (Ethanolic Extract)

Peak	Retention Time (min)	Peak Area (mAU)	Approx. Concentration (µg/mL)	Area %
1	2.50	15,326	12.56	3.40
2	8.80	27,964	24.91	7.12
3	11.50	65,789	56.78	20.10
4	14.70	33,112	31.05	10.12
5	16.25	19,674	16.98	6.00
6	18.10	26,505	24.40	8.05
7	19.40	22,304	20.60	6.48
8	21.30	75,389	64.89	23.00
9	23.90	16,456	14.75	5.02
10	25.70	12,480	11.22	3.71

Elemental Composition by EDS

Energy-Dispersive X-Ray Spectroscopy revealed an organic matrix primarily composed of carbon (≥60% by weight) and oxygen (~30% by weight). Additionally, analysis revealed moderate intensities of potassium (~1.35% by weight) and calcium (reported intensities ~5,000 counts). Both ions have been reported to facilitate physiological functions, such as cardiac electrical conduction and bone metabolism, that could justify part of the plant's ethnomedicinal fame in maintaining circulatory and musculoskeletal wellness.

Pharmacological Assessment in Zebrafish

Acute Toxicity Profile

Toxicity levels were assessed by treating zebrafish embryos and larvae with extract levels ranging from 0.5–2.0 µg/mL. At 0.5 µg/mL, survival was virtually 100%, and at 2.0 µg/mL, it was about 78%. While slight developmental malformations (like minor pericardial edema or curvature of the tail) increased in frequency above 1.5 µg/mL, the extract generally was barely toxic in the subtoxic concentration. These embryo-larval zebrafish assays thus identify a relatively safe dosing interval for following lipid-modulation or cardio-protection experiments.

Lipid-Lowering Activity

A hyperlipidemic zebrafish model was obtained by feeding fish diets supplemented with high-lipid diet for 5–7 days. C. halicacabum extract-treated zebrafish larvae showed about 20–30% decreased lipid staining, as revealed by Oil Red O visualization. The decrease suggests that the plant compounds (presumably the saponins, which have the potential to inhibit pancreatic lipase or chelate bile salts) effectively suppress fat absorption in vivo. Even at relatively modest exposure concentrations (approximately 1.0 µg/mL), lipid deposition was remarkably lower than in untreated hyperlipidemic controls. Visual and quantitative differences together highlight the leaf extract's ability to control lipid homeostasis.

Table 4. Lipid-Lowering Efficacy in Hyperlipidemic Zebrafish

Treatment Group	Mean Lipid Staining Intensity (AU)	% Reduction vs. Untreated
Normal Diet (Control)	1.00 ± 0.05	-
High-Lipid Diet (Untreated)	2.55 ± 0.10	0
High-Lipid + Extract (0.5)	1.80 ± 0.07	~29
High-Lipid + Extract (1.0)	1.72 ± 0.08	~33
High-Lipid + Extract (1.5)	1.67 ± 0.06	~35

Cardiovascular Indices

Cardiovascular improvements—particularly in the stressed or hyperlipidemic state—are a powerful indicator of cardioprotection. Plant extract-treated zebrafish had heart rates close to normal (approximately 120–140 bpm in larvae), compared with untreated hyperlipidemic animals that tended towards mild tachycardia (>150 bpm). In addition, histological analyses of zebrafish hearts indicated lower inflammatory infiltration and reduced pericardial swelling in the treated group. These findings collectively place C. halicacabum as a promising natural option for athero-sclerotic onset prevention and endothelial damage, possibly by regulating oxidative stress mechanisms and stabilizing lipid levels.

In Vitro Cardiomyocyte Protection

Table 5. In Vitro Cardiomyocyte Protection (H9c2 Cell Line)

Condition	Cell Viability (MTT, %)	ROS Levels vs. Control (%)	Caspase-3 Activity (%)	LDH Leakage (%)
Untreated Control	100	100	100	100
H_2O_2-Induced Stress (No Extract)	62 ± 3	185 ± 8	150 ± 5	145 ± 6
H_2O_2 + Extract (Pre-Treatment)	78 ± 4	120 ± 5	110 ± 4	115 ± 3
Doxorubicin-Induced Toxicity (No Extract)	55 ± 2	210 ± 9	155 ± 7	138 ± 5
Doxorubicin + Extract (Pre-Treatment)	75 ± 5	135 ± 6	125 ± 3	118 ± 4

Defining the mechanistic basis of cardioprotection, in vitro models employed H9c2 rat cardiomyoblasts treated with oxidative stressors (e.g., 100 µM hydrogen peroxide for 4 hours) or chemotoxic drugs such as doxorubicin. Cells co-incubated with C. halicacabum extract showed a roughly 25% greater viability compared to untreated controls, according to MTT readings. Reactive oxygen species (ROS) content, as detected by fluorescent DCF-DA probes, decreased by ~35%, and caspase-3 activation decreased by ~30%, in favor of an anti-apoptotic effect of the phytochemicals. Additionally, lactate dehydrogenase (LDH) leakage, a marker of membrane damage, was decreased by 20–25% in the plant-treated group. These cell-level findings closely complement the in vivo lipid-lowering and anti-inflammatory outcomes derived from zebrafish tests.

DISCUSSION

This present investigation of Cardiospermum halicacabum highlights the cardioprotective and lipid-lowering value of the plant in keeping with its ancient heritage as a medication in various traditional medicine systems. High phenol content (~27 mg GAE/g) and flavonoid content (~22 mg QE/g) in ethanolic leaf extract, documented here, are consistent with work by Mohaddesi, B., (2016), says that identified Cardiospermum halicacabum L. have several methods of phytoconstituents such as alkaloids, flavanoids, and tannins that may assist in improved

identification and standardization of their medicinal value. [11]. Saponins also cropped up as primary constituents, in agreement with broader reports that these compounds sequester bile salts and suppress pancreatic lipase, thereby reducing plasma lipid accumulation [12].

Extract-treated groups in the hyperlipidemic zebrafish model demonstrated a ~30% reduction in lipid staining versus untreated fish, indicating potent inhibition of dietary fat absorption. Previous botanically focused research on hyperlipidemic animal models has also shown flavonoid-saponin synergy as a primary factor in reducing LDL oxidation and atherosclerotic plaque deposition [13,14]. Aside from lipid markers, metabolic and cellular experiments in vitro with H9c2 cardiomyoblasts demonstrated a substantial (~25%) preservation of cell viability during conditions of oxidative stress. These data align with other antioxidant-rich plant materials that block reactive oxygen species (ROS) and protect against caspase-mediated apoptotic events [15]. Ethanol extract of Cardiospermum halicacabum L. blocks inflammation through the inhibition of COX-2, TNF-alpha, iNOS, and NF-kappaB expression in RAW264.7 cells. [16].

There are several lines of evidence that substantiate the suggestion that C. halicacabum exerts a multifactorial cardiovascular effect through the synergistic action of antioxidant, anti-inflammatory, and lipid-modulatory activities. Sun et al. reported the presence of diverse triterpenoid saponins in the plant, some of which were correlated with improved lipid profiles in arthritic and metabolic rodent models [17]. Current zebrafish results add to this insight by providing quantifiable, visible evidence (by Oil Red O staining) of decreased lipid uptake, therefore supporting a direct impact on fat uptake by the intestines. Further, in vitro inhibition of ROS and preservation of cardiomyocyte membrane integrity are a protective action against myocardial cells. These trends are in line with the broader polyphenolic synergy viewpoint, whereby various compounds synergistically suppress oxidative stress, maintain vascular reactivity, and inhibit inflammatory signals [18].

Overall, the findings suggest that C. halicacabum employs a rich combination of phenolics, flavonoids, and saponins to guard against dyslipidemia and ensuing oxidative insult—factors at the center of advancing cardiovascular diseases. Nevertheless, while the zebrafish models and cell-based approach yield high value for translation, clinical validation cannot yet be ruled out. Dose-response relationship studies, isolation of the active ingredient, and safety factor investigations in humans will ascertain whether the extract may be utilized as an adjunct treatment or alternative medication for hyperlipidemia as well as related cardiovascular diseases.

CONCLUSION

By extensive phytochemical analysis, the present research validated that leaves of C. halicacabum contained flavonoids, saponins, tannins, phenolic acids, and terpenoids, which are biologically active principles responsible for regulating lipid metabolism as well as modifying oxidative stress—bioactive markers. Quantitative analysis further upheld its exceptionally strong antioxidant activity wherein total phenolics were estimated about 27.3 mg GAE/g, and total flavonoids constituted 22.1 mg QE/g, attesting the pharmacological importance of the free radical scavenging potential of the plant. The pharmacological tests, such as zebrafish hyperlipidemia models and in vitro cardiomyocyte protection tests, gave direct proofs of C. halicacabum's inhibition of lipid absorption and cardiovascular effects. The zebrafish model demonstrated a remarkable decrease (~30%) of lipid accumulation after extract treatment, implying a potent inhibitory effect on dietary fat digestion, possibly mediated by saponins' bile salt binding and flavonoids' inhibitory effect on pancreatic lipase. Also, in vitro experiments illustrated that C. halicacabum extract abated oxidative stress-induced cardiomyocyte injury, maintaining cell viability by ~25% and lowering reactive oxygen species (ROS) content by ~35% and apoptotic markers like caspase-3 by ~30%. These results show that the extract provides protection against cardiac dysfunction, which is associated with oxidative stress, an important determinant of atherosclerosis and other cardiovascular disorders. Instrumental studies, such as GC-MS and HPLC, also profiled the chemical composition of the plant, isolating major phytochemical constituents with potent lipid-lowering and anti-inflammatory activities. The occurrence of prominent peaks for flavonoids and phenolic acids supports their synergistic action in regulating lipid metabolism and protecting against oxidative stress. Elemental analysis by EDS also validated the occurrence of critical minerals like potassium and calcium, which are supportive in cardiovascular function.

Although these results are encouraging, some limitations should be mentioned. Although the zebrafish model is very informative regarding lipid metabolism and cardiovascular protection, the findings should be replicated in mammalian models and human clinical trials to conclusively determine C. halicacabum's efficacy and safety as a therapeutic agent. Additionally, bioassay-guided fractionation studies should be conducted to determine the specific phytochemicals accountable for its lipid-lowering and cardioprotective activities. Future studies are also needed to investigate the molecular basis of its bioactivity, especially its purported function in AMPK signaling modulating, cholesterol homeostasis, and inflammation pathways.

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