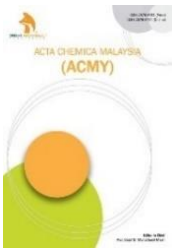
ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2576-6724 (Print)
ISSN: 2576-6732 (Online)
CODEN: ACMCCG

Acta Chemica Malaysia (ACMY)

DOI: http://doi.org/10.26480/acmy.01.2026.47.51



RESEARCH ARTICLE

HYPERCHOLESTEROLEMIC ACTIVITIES AND STRUCTURAL ELUCIDATION OF THE ISOLATE FROM HEXANE EXTRACT OF *Acalypha wilkesiana* LEAF

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ARTICLE DETAILS

Article History:

Received 11 February 2026
Revised 20 March 2026
Accepted 27 April 2026
Available online 08 May 2026

ABSTRACT

Acalypha wilkesiana Hoffmanni is a medicinal plant known to contain several bioactive secondary metabolites with reported therapeutic potential. This study investigated the antihypercholesterolemic activity and structural characterization of compounds isolated from the hexane leaf extract of *A. wilkesiana*. Hypercholesterolemic rats exhibited elevated total cholesterol and low-density lipoprotein (LDL) levels compared to treated groups. Treatment with the isolated compound significantly reduced total cholesterol and LDL levels ($P < 0.05$) relative to untreated hypercholesterolemic rats. In addition, antioxidant enzyme activities, including glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), were significantly increased, while malondialdehyde (MDA) levels were reduced ($P < 0.05$). Spectroscopic analysis revealed characteristic infrared absorption peaks at 1019.3, 1101.3, 1116.47, 1248.74, 1462.44, 1721.48, and 2859.17 cm^{-1}. Mass spectrometry showed a molecular ion peak at m/z 406 (M+2), corresponding to a molecular formula of $C_{25}H_{42}O_4$. Based on spectral data and comparison with literature, the compound was identified as ursodeoxycholic acid methyl ester. The isolated compound from *A. wilkesiana* demonstrated significant antihypercholesterolemic and antioxidant effects, suggesting its potential as a candidate for further drug development.

KEYWORDS

Hypercholesterolemia, *Acalypha wilkesiana*, Lipid Profile, Oxidative Stress, Antihypercholesterolemic activities, Green Acalypha, Structural elucidation and characterisation.

1. INTRODUCTION

Acalypha wilkesiana is an ornamental medicinal plant known for its diverse leaf colouration, ranging from red, copper, bronze, purple, white, to green varieties. The present study focuses specifically on the green variety of the plant. *Acalypha wilkesiana* has been widely documented in ethnomedicine for its therapeutic potential in the management of various diseases. Traditional preparations of the plant include the use of leaf ointments derived from powdered leaves for the treatment of fungal skin infections (Iyekowa et al., 2016). In some communities, the young shoots are consumed as vegetables, while other parts of the plant have been reported to possess toxic properties. The plant has also been associated with a wide range of pharmacological activities, including antibacterial, antifungal, abortifacient, and antinematodal effects (World Health Organization, 2009; Sofowora, 2013).

Various traditional applications have been reported, including the use of leaf extracts for the treatment of diarrhoea and dysentery, and as part of herbal preparations used in the management of malaria, fever, pneumonia, and pain. Inhalation therapy involving vapour from hot water infusions of *Acalypha wilkesiana* combined with other medicinal plants has also been documented in ethnomedicinal practice (World Health Organization, 2009). Furthermore, it has been used in the treatment of *Pityriasis versicolor*, a superficial fungal infection associated with skin discoloration (Oyelami et al., 2003).

Phytochemical investigations have revealed the presence of bioactive compounds such as gallic acid, quercetin, and geramine in the plant (Adesina et al., 2000). In addition, studies have reported the presence of saturated fatty acids with diverse biological activities in its oil profile (Oguoma et al., 2023). In Southern Nigeria, the leaves are consumed as vegetables in traditional management of hypertension, and experimental studies have shown effects on electrolyte balance and metabolic parameters in animal models, supporting its nutraceutical potential (Ikewuchi et al., 2008; Ikewuchi et al., 2009a).

Cardiovascular diseases remain a leading cause of mortality worldwide, with hypercholesterolemia being a major contributing risk factor (World Health Organization, 2023). Elevated levels of low-density lipoprotein (LDL) and oxidative stress are strongly associated with the development of atherosclerosis and related complications (Libby, 2012).

Plant-derived compounds have been widely investigated as potential alternatives for managing lipid disorders due to their relatively lower side effects compared to synthetic drugs. Despite these reported pharmacological and nutritional properties, limited studies have focused on the isolation and structural characterization of bioactive compounds responsible for its antihypercholesterolemic activity, particularly from the hexane fraction of the green variety. Therefore, this study investigates the antihypercholesterolemic activity and structural elucidation of isolated compounds from the hexane extract of green *Acalypha wilkesiana*.

Quick Response Code	Access this article online	
	Website: www.actachemicamalaysia.com	**DOI:** 10.26480/acmy.01.2026.47.51

Cite the Article: Joy O. Oyebisi, Osaro Iyekowa, Amowie P. Oviawe, Ayodele Fatona, and Silvanus O. Innih (2026). Hypercholesterolemic Activities And Structural Elucidation Of The Isolate From Hexane Extract Of *Acalypha Wilkesiana* Leaf. *Acta Chemica Malaysia, (ACMY),* 10(1): 47-51.

Plate 1: Green Acalypha (*Alcalypha Wilkesiana*)

2. Materials and Methods

2.1 Experimental Animals

Adult Wistar rats (136 ± 4.1 g) were used in this study. The animals were acclimatized for one week prior to experimentation and maintained under standard laboratory conditions with access to feed (Premier Feed Mills Ltd., Edo State, Nigeria) and water ad libitum. Animal handling followed internationally accepted guidelines, and ethical approval was obtained from the Ethics Committee for Experimental Animals, University of Benin (EC/FP/019/07).

2.2 Plant Collection, Extraction, and Isolation

Fresh leaves of *Acalypha wilkesiana* were collected from Iguosa community, Benin City, and authenticated by Prof. J. F. Bamidele, Department of Plant Biology and Biotechnology, University of Benin. The leaves were air-dried for three weeks and pulverized. A total of 200 g of powdered sample was extracted by maceration for 72 hours. The extract was concentrated under reduced pressure. Fractionation and isolation were performed using solvent partitioning, vacuum liquid chromatography, preparative thin-layer chromatography, and column chromatography.

2.3 Characterization of Isolate

Structural characterization was carried out using the following techniques:

Nuclear Magnetic Resonance (NMR): ^{1}H and ^{13}C spectra were recorded using a Bruker AV600 (600 MHz) spectrometer in $CDCl_3$.

Mass Spectrometry (MS): Analysis was performed using LC-ESI/APCI Triple Quadrupole and Q-TOF instruments.

Fourier Transform Infrared Spectroscopy (FTIR): Spectra were obtained using a Thermo Scientific Nicolet 6700 FT-IR with ATR accessory.

UV–Visible Spectroscopy: Absorbance was measured using a Cary 100 Bio UV-Vis spectrophotometer.

2.4 Experimental Design

A total of 30 rats were randomly divided into six groups (A–F), with five rats per group:

Group A: Control (distilled water, 5 mL/kg/day)

Group B: Tween 80 (5%)

Group C: Hypercholesterolemic model induced with margarine (20 mg in vegetable oil)

Group D: Isolate only (50 mg/kg)

Group E: Hypercholesterolemic + atorvastatin (10 mg/kg after induction)

Group F: Hypercholesterolemic + isolate (50 mg/kg after induction)

Induction of hypercholesterolemia was maintained for 60 days. At the end of the experimental period, animals were sacrificed under chloroform anesthesia, and blood and cardiac tissues were collected for biochemical analysis.

2.5 Administration of Isolate

The isolated compound was administered orally using an orogastric tube under careful handling to avoid injury.

2.6 Biochemical Analysis

2.6.1 Lipid Profile

Serum was obtained after centrifugation of clotted blood. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were analyzed using Randox diagnostic kits (UK). LDL was calculated using Friedewald's formula:

$$LDL = TC - (TG/5 + HDL)$$

2.7 Oxidative Stress Markers

Glutathione Peroxidase (GPx): Assayed using pyrogallol oxidation method (Innih et al., 2022).

Superoxide Dismutase (SOD): Based on inhibition of adrenaline auto-oxidation (Omage et al., 2021).

Catalase (CAT): Determined via hydrogen peroxide decomposition method (Omage et al., 2021).

Malondialdehyde (MDA): Measured using TBARS assay (Buege and Aust, 1978).

Absorbance readings were taken using a UV spectrophotometer at appropriate wavelengths for each assay.

2.8 Statistical Analysis

Data were expressed as mean ± SEM (n = 5). Statistical significance was determined using Student's t-test in SPSS version 17. Differences were considered significant at $P < 0.05$.

3. Results

3.1 Lipid Profile for the Isolate

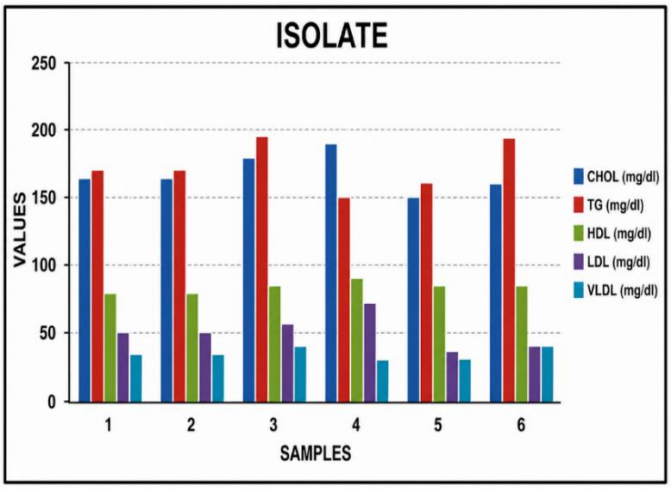


Figure 1: Lipid Profile for the Isolate $P < 0.05$ indicates a significant difference.

3.2 Oxidative Stress Markers Results

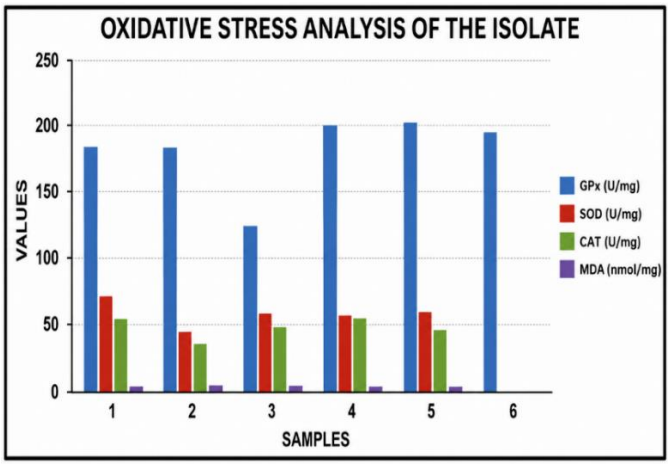


Figure 2: Oxidative Stress Result for the Isolate $P < 0.05$ indicates a significant difference.

3.3 Spectroscopic Results

The FT-IR spectra analysis is presented in table 1 below;

Table 1: FT-IR Band for the Isolate

S/N	Peak Frequency	Bond	Functional Group Suspected
1	1019.3	C-O Stretch	Alcohol (RCH2-OH)
2	1101.3	C-O Stretch	Ether
3	1116.47	C-O Stretch	Alcohol
4	1248.74	C-O Stretch	Ether
5	1462.44	C-H Bend	Alkyl groups
6	1721.48	C=O Stretch	Ester
7	2859.17	C-H Bend	Alkyl groups

3.4 UV/Vis of Isolated Compound Result

The UV-VIS spectra analysis is presented in table 2

Table 2: UV Absorption Maximum of Sample B Isolate Maximum Wavelength

	Emax	Absorbance	Peak	Chromophore Detected
<280 (Sample B)	35,000	0.2	Sharp	C=C, C=O

3.5 ^{1}HNMR and ^{13}CNMR of the Isolated Compound

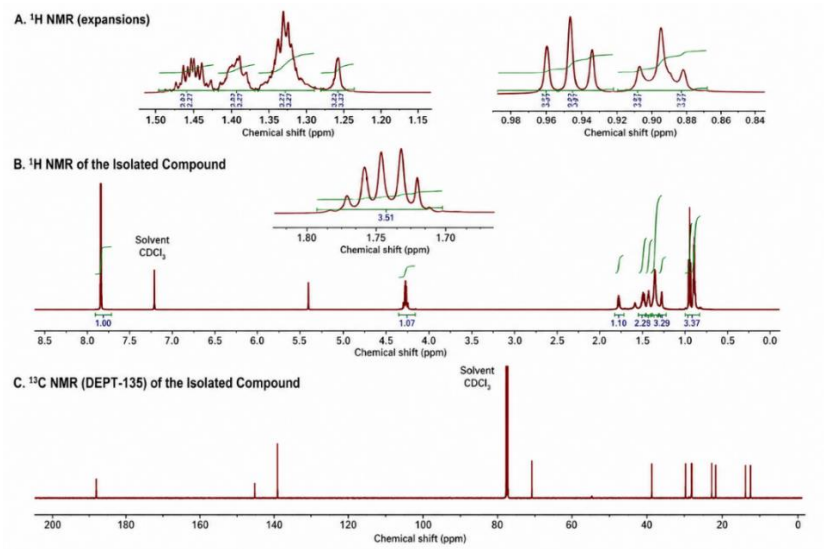


Figure 3: ^{1}HNMR and ^{13}CNMR of the isolated Compound

The ^{1}HNMR and ^{13}CNMR Chemical Shift Values of Isolated Compounds Compared with those obtained from a steroid skeleton of cholic acid literature. Barnes and Geckle (1982) is analysed in table 3;

Table 3: 1HNMR and 13CNMR Chemical Shift Values of Isolated Compound

Position	δH (^{1}H NMR)	δ^{13}C NMR	Literature δH (^{1}H NMR)	Literature δ^{13}C NMR
C1 (CH_2)	1.67	11.3	1.81	36.1
C2 (CH_2)	1.54	14.9	1.62	31.0
C3 (CH)	3.54	65.0	3.47	71.9
OH	7.25	14.9	–	–
C4 (CH_2)	1.54	53.0	1.55	38.6
C5 (CH)	1.40	39.0	1.47	42.4
C6 (CH_2)	1.75	53.0	1.60	38.0
C7 (CH)	4.25	67.0	3.49	72.1
OH	7.25	–	–	–
C8 (CH)	1.41	34.0	1.45	43.4
C9 (CH)	1.52	32.1	1.48	40.7
C10 (C)	–	30.1	–	35.1

Table 3 (Cont): 1HNMR and 13CNMR Chemical Shift Values of Isolated Compound				
Position	**δH (^{1}H NMR)**	**δ^{13}C NMR**	**Literature δH (^{1}H NMR)**	**Literature δ^{13}C NMR**
C11 (CH_2)	1.47	24.5	1.34	22.4
C12 (CH_2)	1.19	22.5	2.30	41.5
C13 (C)	–	40.1	–	44.8
C14 (CH)	1.10	54.1	1.09	56.5
C15 (CH_2)	1.90	28.0	1.46	27.9
C16 (CH_2)	1.75	29.5	1.86	29.6
C17 (CH)	1.64	54.1	1.55	57.4
C18 (CH_3)	0.86	11.1	0.71	12.7
C19 (CH_3)	0.88	23.5	0.94	24.0
C20 (CH)	1.50	33.5	1.44	36.6
C21 (CH_3)	0.85	21.4	0.96	19.0
C22 (CH_2)	1.25	30.3	1.32	32.3
C23 (CH_2)	2.35	32.0	2.21	32.0
C24 (C=O)	–	167.1	–	178.2
C25 (CH_3)	1.50	136.0	–	–

3.6 Mass Spectra Result

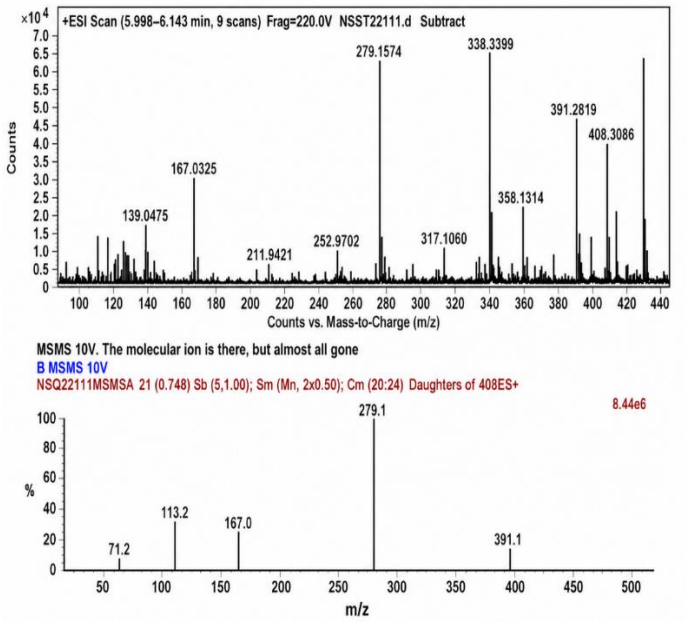


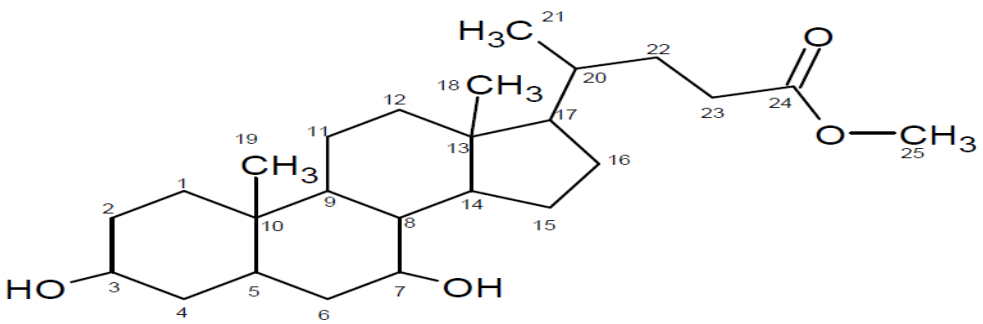
Figure 4: ESI-Mass and MSMS Spectra

4. Discussion

Hypercholesterolemia is a major risk factor for cardiovascular diseases, including coronary heart disease, stroke, and peripheral vascular disorders (Peters et al., 2016). It is also frequently associated with metabolic conditions such as hypertension and diabetes mellitus (WebMD, 2017). In this study, administration of the isolated compound from the hexane extract of *Acalypha wilkesiana* resulted in a significant reduction ($P < 0.05$) in total cholesterol and low-density lipoprotein (LDL) levels compared to the negative control group, while high-density lipoprotein (HDL) levels were not significantly affected. This suggests a selective lipid-lowering effect, particularly on atherogenic lipoproteins. Furthermore, treatment with the isolate enhanced antioxidant defense mechanisms, as evidenced by increased activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), alongside a reduction in malondialdehyde (MDA) levels.

This indicates that the compound may also mitigate oxidative stress, which is closely linked to lipid peroxidation and cardiovascular risk. The isolated compound was obtained as a pale-yellow oily substance with a boiling range of 245–247°C. It was soluble in non-polar and moderately polar solvents such as hexane and ethyl acetate (2:1), suggesting a largely lipophilic character. Spectroscopic analysis provided evidence for its structural identity. The FTIR spectrum revealed characteristic absorptions corresponding to hydroxyl (C–O), aliphatic C–H bending, and ester carbonyl (C=O) functional groups. UV analysis showed absorption at 280 nm, indicating the presence of a conjugated carbonyl system. ^{1}H-NMR analysis displayed signals consistent with multiple methyl and methylene protons, while ^{13}C-NMR confirmed the presence of 25 carbon atoms, including methyl, methylene, methine, quaternary, and carbonyl carbons.

These features are consistent with a steroidal skeleton.Mass spectrometry revealed a molecular ion peak at m/z 406 (M+2), with major fragment ions supporting a molecular formula of $C_{25}H_{42}O_4$. Comparison with literature data indicated close similarity to cholic acid derivatives (Barnes and Geckle, 1982). The combined spectroscopic evidence suggests that the isolated compound is an ester derivative of cholic acid, specifically ursodeoxycholic acid methyl ester. The observed biological activity may be attributed to the steroidal framework of the compound, which is known to influence lipid metabolism and oxidative stress pathways. This supports the potential of *Acalypha wilkesiana* as a source of bioactive compounds for the management of hypercholesterolemia. Based on the combined spectroscopic data and comparison with literature, the proposed structure and fragmentation pattern of the isolated compound are presented below.



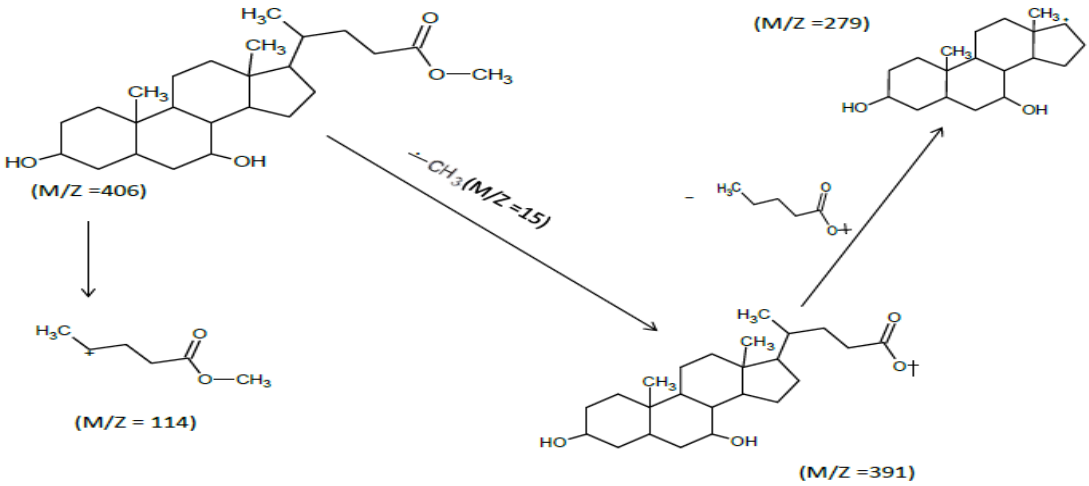


Figure 5: Proposed Structure and Fragmentation

5. Conclusion

The isolated compound was identified as ursodeoxycholic acid methyl ester, a derivative of cholic acid. The findings of this study demonstrate that the hexane extract of *Acalypha wilkesiana* contains bioactive constituents with significant antihypercholesterolemic activity. This suggests that the plant possesses notable therapeutic potential and may serve as a promising candidate for further drug development.

Conflict of Interest

The authors declare no conflict of interest.

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