

Isolation, Characterization, and Evaluation of Anticancer and Antioxidant Bioactive Compounds from *Calophyllum inophyllum* Leaves

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Abstract

Calophyllum inophyllum L., a tropical evergreen tree from the Guttiferae family, has been traditionally used for its medicinal properties, including the treatment of inflammation, infections, and cancer. This study aimed to isolate and characterize bioactive compounds from *C. inophyllum* leaves and evaluate their anticancer and antioxidant potential. Methanol extraction of the leaves yielded a crude extract, which was partitioned into *n*-hexane, chloroform, and *n*-butanol fractions. Phytochemical screening revealed the presence of carbohydrates, alkaloids, saponins, flavonoids, tannins, and terpenoids, with the *n*-butanol fraction exhibiting the highest total phenolic (32.5 µg/g GAE) and flavonoid (130.42 µg/g QE) content. Column chromatography and spectroscopic techniques (¹H NMR, ¹³C NMR, HMBC, HSQC) were employed to isolate and characterize two major compounds: friedelin, a triterpenoid from the *n*-hexane fraction, and quercitrin, a flavonoid glycoside from the *n*-butanol fraction. The *n*-hexane fraction demonstrated significant cytotoxic activity against human H460 lung cancer cells (IC₅₀ = 16.0 µg/mL), while the *n*-butanol fraction exhibited potent antioxidant activity (IC₅₀ = 13.3 µg/mL) in the DPPH radical scavenging assay. These findings validate the traditional use of *C. inophyllum* in cancer treatment and highlight its potential as a source of bioactive compounds for drug development, particularly in anticancer and antioxidant therapies.

Keywords: *Calophyllum inophyllum*, friedelin, quercitrin, anticancer, antioxidant, cytotoxicity.

1. Introduction

Calophyllum inophyllum L., a member of the Guttiferae (or Clusiaceae) family, is a tropical evergreen tree widely distributed in coastal regions of Africa, Asia, and the Pacific. Commonly

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known as Alexandrian laurel, this plant has been traditionally used in various cultures to treat a range of ailments, including inflammation, rheumatism, skin infections, and cancer. Its medicinal properties are attributed to its rich phytochemical composition, which includes secondary metabolites such as xanthenes, coumarins, flavonoids, and triterpenoids. These compounds have been extensively studied for their pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Taher *et al.*, 2005; Shanmugapriya *et al.*, 2016).

Cancer remains one of the leading causes of death worldwide, and the search for effective and safe anticancer agents from natural sources has gained significant attention. Natural products, particularly plant-derived compounds, have shown promise in cancer therapy due to their diverse chemical structures and biological activities. Among these, triterpenoids and flavonoids have been widely studied for their anticancer and antioxidant properties. Friedelin, a pentacyclic triterpenoid, has demonstrated antiproliferative activity against various cancer cell lines, while quercitrin, a flavonoid glycoside, is known for its potent antioxidant effects (Laure *et al.*, 2005; Taher *et al.*, 2005). These compounds not only inhibit cancer cell proliferation but also mitigate oxidative stress, which plays a critical role in the pathogenesis of many diseases, including cancer.

Despite the traditional use of *C. inophyllum* in treating various ailments, there is a need for systematic scientific validation of its bioactive compounds and their pharmacological potential. This study aims to isolate, characterize, and evaluate the anticancer and antioxidant potential of bioactive compounds from the leaves of *C. inophyllum*. Specifically, the *n*-hexane and *n*-butanol fractions of the methanol extract were investigated for their cytotoxic and antioxidant activities.

2. Materials and Methods

3.1 Plant Material and Extraction

Fresh leaves of *C. inophyllum* were collected from Imo State, Nigeria, and authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The leaves were shade-dried, pulverized, and extracted using cold maceration with methanol. Approximately 750 g of powdered leaves were soaked in methanol for 72 hours, with occasional stirring. The extract was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C to obtain the crude methanol extract. The concentrated extract was further partitioned sequentially with *n*-hexane, chloroform, and *n*-butanol to obtain three fractions: *n*-hexane, chloroform, and *n*-butanol.

2.1 Phytochemical Screening

Qualitative and quantitative phytochemical analyses were conducted to identify the presence of carbohydrates, alkaloids, saponins, flavonoids, tannins, terpenoids, and steroids in the fractions. Standard methods described by Evans (2002), Sofowora (1993), and Harborne (1988) were used for the qualitative screening. For quantitative analysis, the total phenolic content (TPC) was determined using the Folin-Ciocalteu method, while the total flavonoid content (TFC) was measured using the aluminum chloride method. Gallic acid and quercetin were used as standards for TPC and TFC, respectively.

2.2 Isolation and Purification of Bioactive Compounds

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The n-hexane and n-butanol fractions were subjected to column chromatography for the isolation of compounds. Silica gel (60–120 mesh) was used as the stationary phase, and a gradient of solvents (hexane, ethyl acetate, chloroform, and methanol) was used as the mobile phase. Fractions with similar thin-layer chromatography (TLC) profiles were pooled and further purified using Sephadex LH-20 gel filtration chromatography. The isolated compounds were characterized using spectroscopic methods, including ^1H NMR, ^{13}C NMR, and 2D NMR analysis like the HMBC, HSQC and others.

2.3 Antioxidant Activity

The antioxidant activity of the fractions was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay as described by Susanti et al. (2007). Briefly, 2.0 mL of 0.1 mM DPPH solution was mixed with 0.9 mL of Tris-HCl buffer (pH 7.4) and 0.1 mL of the test sample. The mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 517 nm using a UV-visible spectrophotometer. The percentage inhibition of DPPH radicals was calculated, and the IC_{50} values were determined.

2.4 Cytotoxicity Assay

The cytotoxic activity of the fractions was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay described by Mosmann (1983) on human H460 lung cancer cells. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were seeded in a 96-well microplate at a density of 1×10^4 cells/mL and incubated for 24 hours. The test samples were added at concentrations ranging from 3.13 to 100 $\mu\text{g/mL}$, and the cells were incubated for 72 hours. After incubation, 20 μL of MTT solution (5 mg/mL) was added to each well, and the cells were further incubated for 4 hours. The formazan crystals formed were dissolved in dimethyl sulfoxide (DMSO), and the absorbance was measured at 540 nm using a microplate reader. The percentage of cell viability and IC_{50} values were calculated.

2.5 Statistical Analysis

All experiments were performed in triplicate, and the data were expressed as mean $\pm$ standard error of the mean (SEM). Regression analysis was used to calculate the IC_{50} values from the dose-response curves using Microsoft Excel 2016.

3. Results

3.1 Phytochemical Screening

The qualitative phytochemical analysis of the methanol extract of *C. inophyllum* leaves revealed the presence of carbohydrates, alkaloids, saponins, flavonoids, tannins, and terpenoids. These findings are consistent with previous studies that have reported the presence of diverse secondary metabolites in *C. inophyllum* (Saravanan *et al.*, 2015; Indrakumar *et al.*, 2012). The quantitative analysis showed that the n-butanol fraction had the highest total phenolic content (32.5 $\mu\text{g/g}$ GAE) and total flavonoid content (130.42 $\mu\text{g/g}$ QE), indicating its potential as a rich source of antioxidants. The calibration curves for Gallic acid and quercetin were presented in Figure 1. The results of the qualitative and quantitative phytochemical screening are summarized in Table 1 and Table 2, respectively.

Table 1: Qualitative Phytochemical Analysis of *C. inophyllum* crude methanol Leaves Extract and its Fractions

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Phytochemicals	Crude Extract	n-Hexane Fraction	Chloroform Fraction	n-Butanol Fraction
Carbohydrates	+	+	+	+
Alkaloids	+	-	-	+
Saponins	+	+	+	+
Flavonoids	+	-	-	+
Tannins	+	-	-	+
Terpenoids	+	+	+	-
Steroids	+	+	+	+
Anthraquinone	-	-	-	-
Glycosides	-	-	-	-
Cardiac Glycosides	-	-	-	-

Key: + = Present, - = Absent.

Table 2: Quantitative Phytochemical Analysis of *C. inophyllum* crude methanol Leaves Extract and its Fractions

Fraction	Total Phenolic Content (µg/g GAE)	Total Flavonoid Content (µg/g QE)
Crude Extract	25.0	85.0
n-Hexane	12.53	12.41
Chloroform	14.0	70.42
n-Butanol	32.5	130.42

Key: GAE = Gallic Acid Equivalent, QE = Quercetin Equivalent.

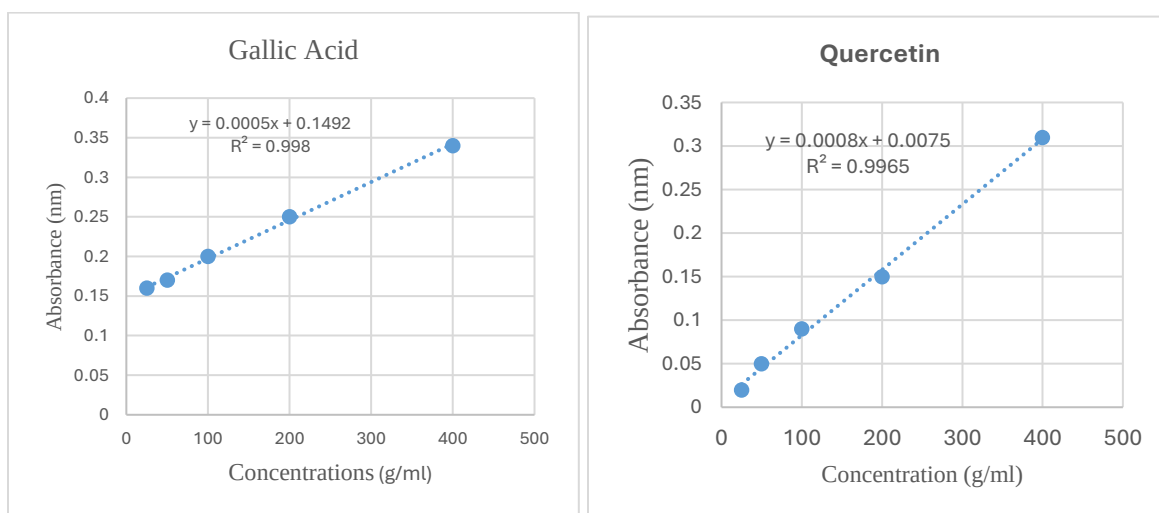


Figure 1: Calibration curves gallic acid and quercetin

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3.2 Isolation of Bioactive compounds

3.2.1 Isolation of Compound 1

Isolation of Compound 1

Approximately 20 g of the *n*-Hexane fraction was subjected to gravity column chromatography using silica gel as the stationary phase. A gradient mobile phase of increasing polarity (*n*-Hexane, *n*-Hexane/Ethyl acetate, Ethyl acetate, Ethyl acetate/Methanol, and Methanol) was employed. Fractions with similar TLC profiles were pooled, yielding 21 fractions (A1–A21). TLC analysis was performed using *n*-Hexane/Ethyl acetate (9:1) as the solvent system throughout the isolation process. Fractions A6 and A7, which showed single spots with minimal impurities on TLC, were combined and further purified using a 4.0 cm diameter silica gel column. Elution was carried out with a gradient of *n*-Hexane, *n*-Hexane/Ethyl acetate, and Ethyl acetate, resulting in 30 subfractions (A6-1 to A6-30). Subfractions A6-6 and A6-7, displaying a single spot with an *R_f* value of 0.80 (in *n*-Hexane/Ethyl acetate, 9:1), were combined and subjected to gel filtration chromatography using Sephadex LH-20. Fractions with single spots were pooled and washed with analytical-grade acetone, yielding white needle-like crystals identified as Compound 1 with a final yield of approximately 15 mg. The purity of Compound 1 was confirmed by TLC and NMR spectroscopy.

Isolation of Compound 2

Approximately 30 g of the *n*-butanol fraction was subjected to gravity column chromatography using silica gel as the stationary phase. A gradient mobile phase of increasing polarity (Chloroform, Chloroform/Methanol, and Methanol) was employed. Fractions with similar TLC profiles were pooled, yielding 9 fractions (A1–A9). Fraction A7, which showed distinct spots on TLC with characteristic *R_f* values (in Ethyl acetate: Chloroform: Methanol: Water, 15:8:4:1), was selected for further purification. It was subjected to a 2.5 cm diameter silica gel column and eluted with a gradient of Chloroform, Chloroform/Methanol, and Methanol, yielding 33 subfractions (A7-1 to A7-33). Subfraction A7-29, appearing as a brown gummy powder, showed three distinct spots on TLC with *R_f* values of 0.32, 0.67, and 0.58 (in Ethyl acetate: Chloroform: Methanol: Water, 15:8:4:1). Subfraction A7-29 was further purified using gel permeation filtration with Sephadex® LH-20 as the stationary phase and eluted with 100% methanol, resulting in 12 subfractions. Fraction 10 was further purified using preparative TLC with a solvent system of Ethyl acetate: Chloroform: Methanol: Water (15:8:4:1) on a 20 cm × 20 cm plate, yielding three compounds (2, 3, and 4). Compound 2, with a final yield of approximately 12 mg, showed an *R_f* value of 0.67 (in Ethyl acetate: Chloroform: Methanol: Water, 15:8:4:1) and was isolated for structural characterization using NMR spectroscopy.

3.3 Characterization of the Isolated Compounds

The structural elucidation of compounds 1 and 2, isolated from the *n*-hexane and *n*-butanol fractions of *Calophyllum inophyllum*, respectively, was accomplished using comprehensive NMR analysis. This included ¹H NMR, ¹³C NMR, DEPT-135, HMBC, and HSQC spectra. The NMR data were cross-referenced with literature values to confirm the structures of the isolated compounds: friedelin (from the *n*-hexane fraction) and quercitrin (from the *n*-butanol fraction).

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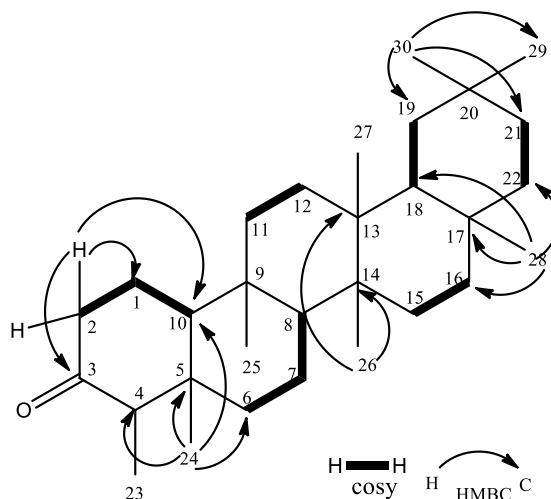
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3.3.1 Compound 1: Friedelin

The ^1H NMR spectrum (850 MHz, CDCl_3) of compound 1 displayed signals corresponding to eight methyl groups, with chemical shifts ranging from δH 0.715 to 1.197 ppm, characteristic of the methyl groups in friedelin. Methylene (CH_2) protons were observed between δH 1.2 and 1.5 ppm, consistent with the aliphatic chain of the triterpenoid structure. Methine protons were identified at δH 2.234 ppm (C-4), δH 1.410 ppm (C-8), δH 1.521 ppm (C-10), and δH 1.490 ppm (C-18). The ^{13}C NMR (213 MHz, CDCl_3) spectrum revealed the presence of 30 carbons, including a carbonyl carbon resonating at δC 213.34 ppm, indicative of the ketone group in friedelin. The DEPT-135 spectrum further confirmed 23 carbons, comprising 8 methyl carbons, 4 methine carbons, and 11 methylene carbons, with the remaining 7 quaternary carbons (C-3, C-5, C-9, C-13, C-14, C-17, and C-20). The ^1H and ^{13}C NMR data aligned with literature values for friedelin ($\text{C}_{30}\text{H}_{50}\text{O}$) as reported by Abbas *et al.*, (2007) and Mann *et al.*, (2011), confirming its structure. Friedelin, a triterpenoid (Figure 2), was further characterized using 1D and 2D NMR (DEPT-135, H-H-COSY, HSQC, and HMBC) analyses (Figures 3a-3f). Table 3 summarizes the chemical shift values of friedelin, with comparisons to literature data.



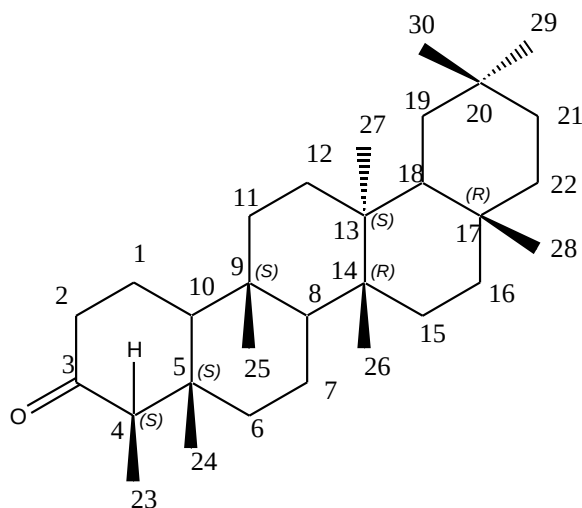


Figure 2: ^1H - ^1H COSY and HMBC correlations of compound 1 (Friedelin)

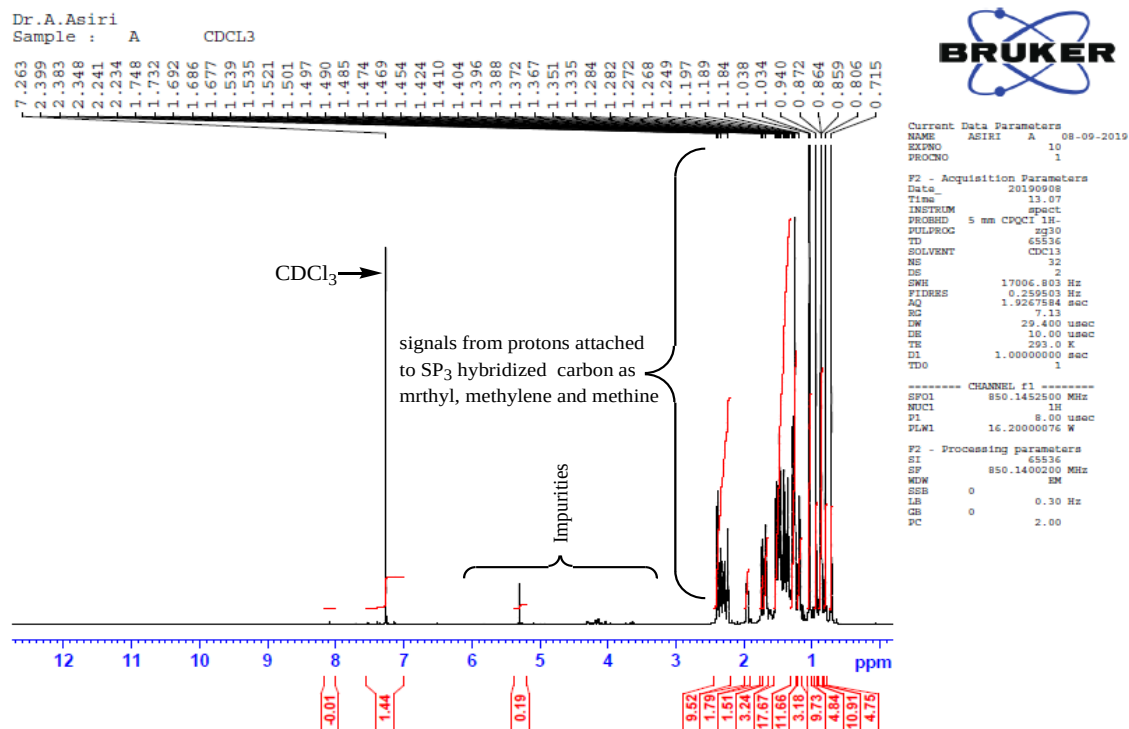


Figure 3a: ^1H NMR spectrum of compound 1

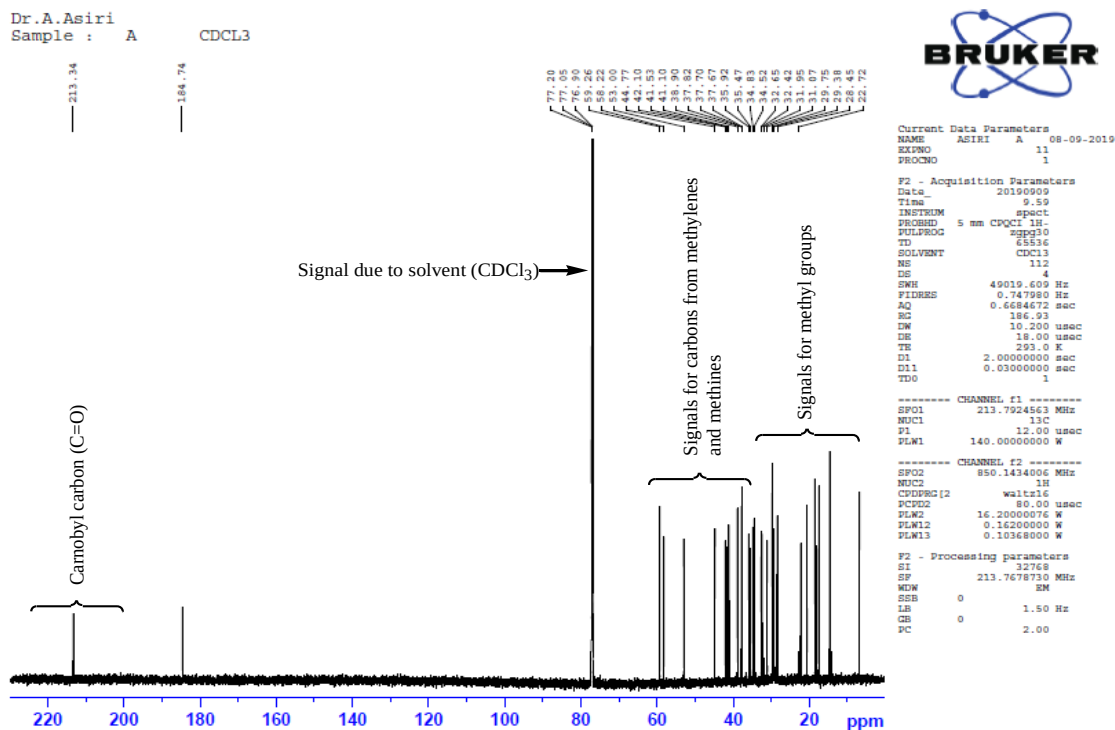


Figure 3b: ¹³C NMR spectrum of compound 1

Sample : A CDCL₃

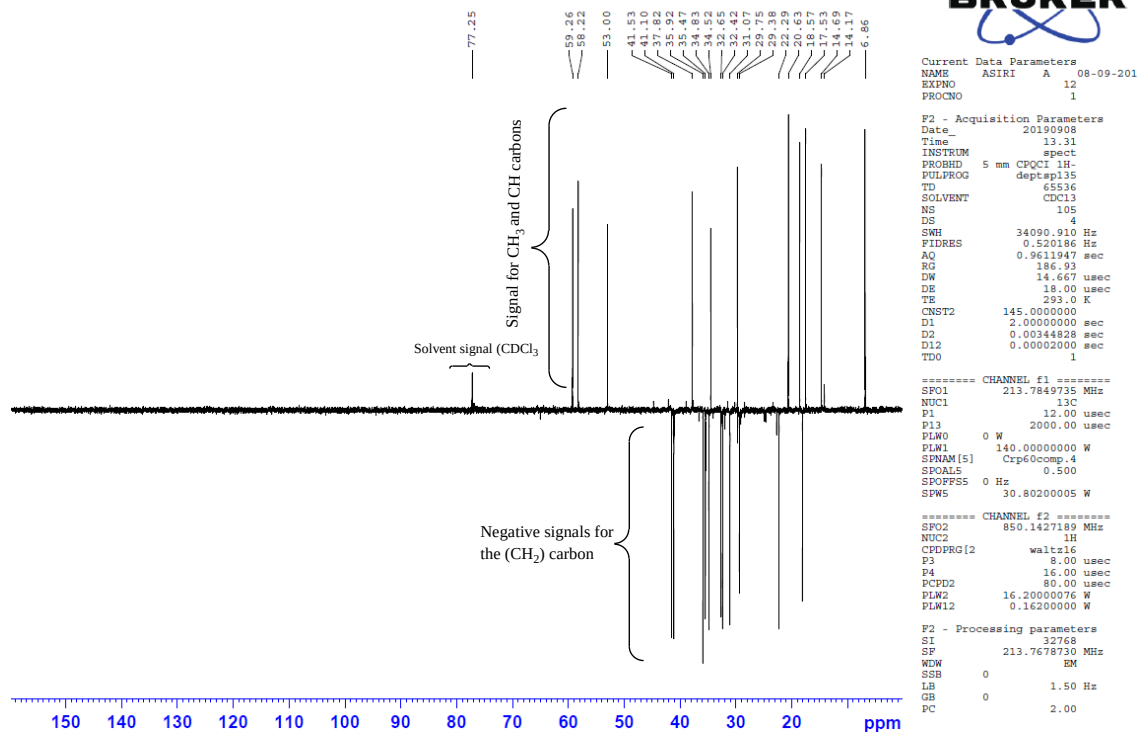


Figure 3c: ¹³C DEPT 135 spectrum of compound 1

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Dr.A.Asiri
Sample : A

CDCL₃

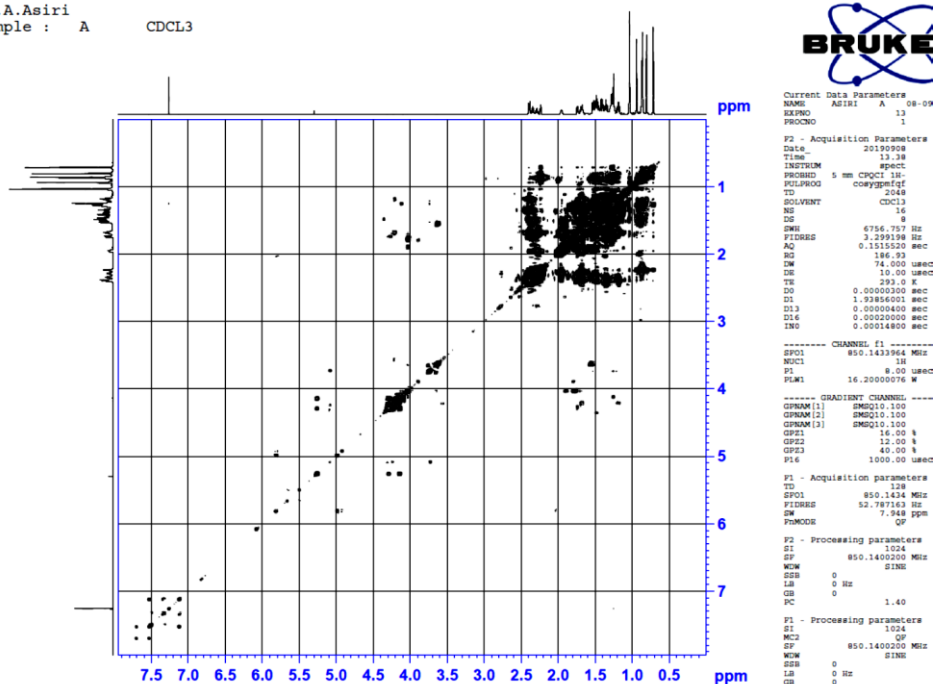


Figure 3d: ¹H-¹H Cosy spectrum of compound 1

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Dr.A.Asiri
Sample : A

CDCL₃

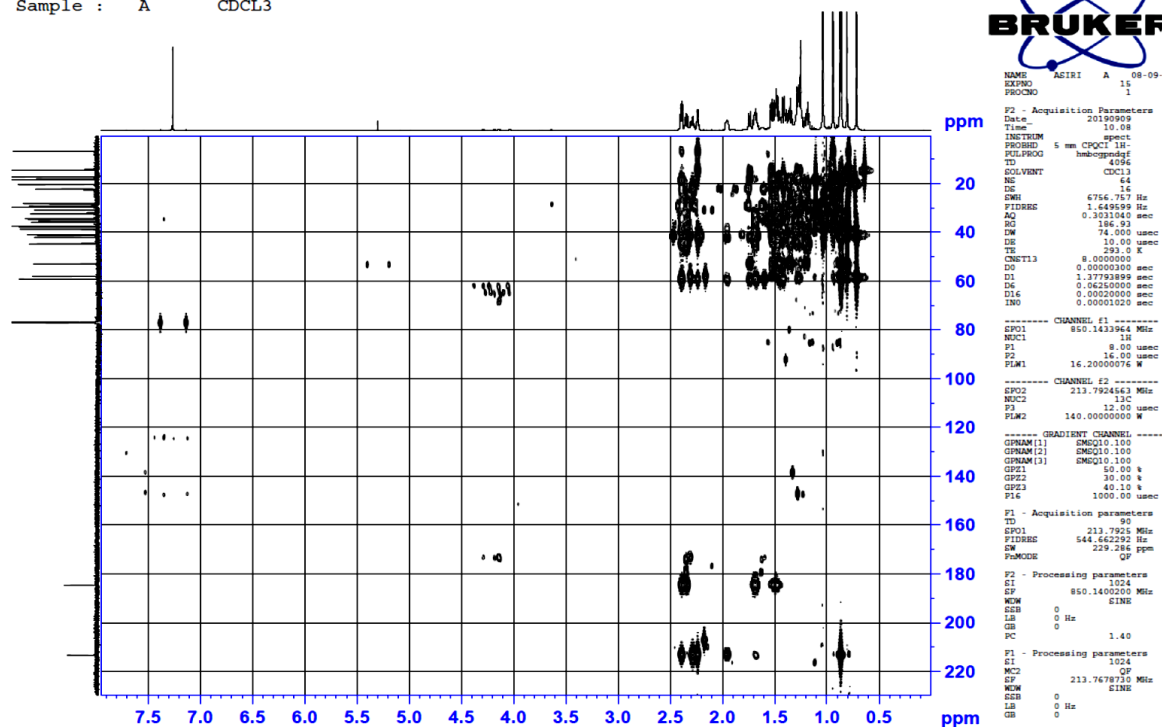


Figure 3e: HMBC spectrum of compound 1

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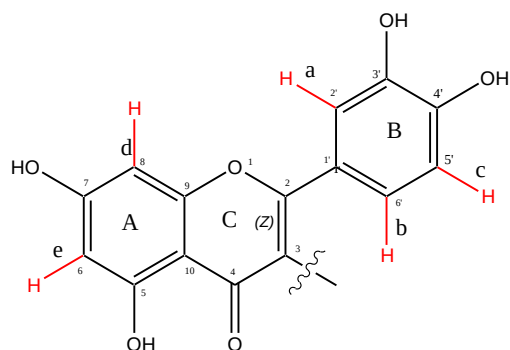


Figure 4: Structure of the aglycon flavanol

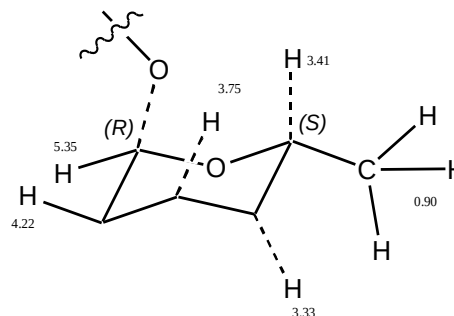


Figure 5: Structure of the rhamnose moiety

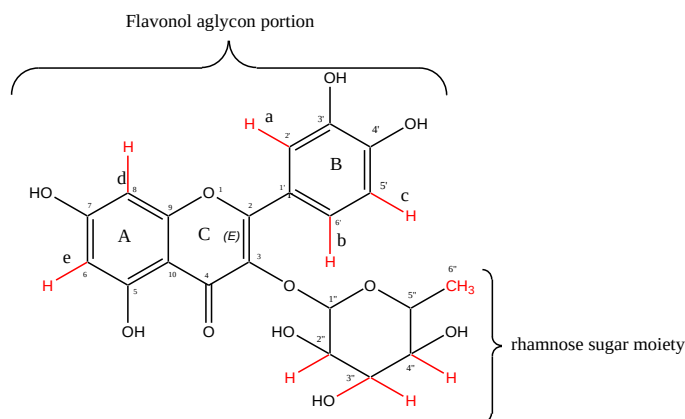


Figure 6: Structure of Quercitrin (Quercetin 3-O-rhamnoside)

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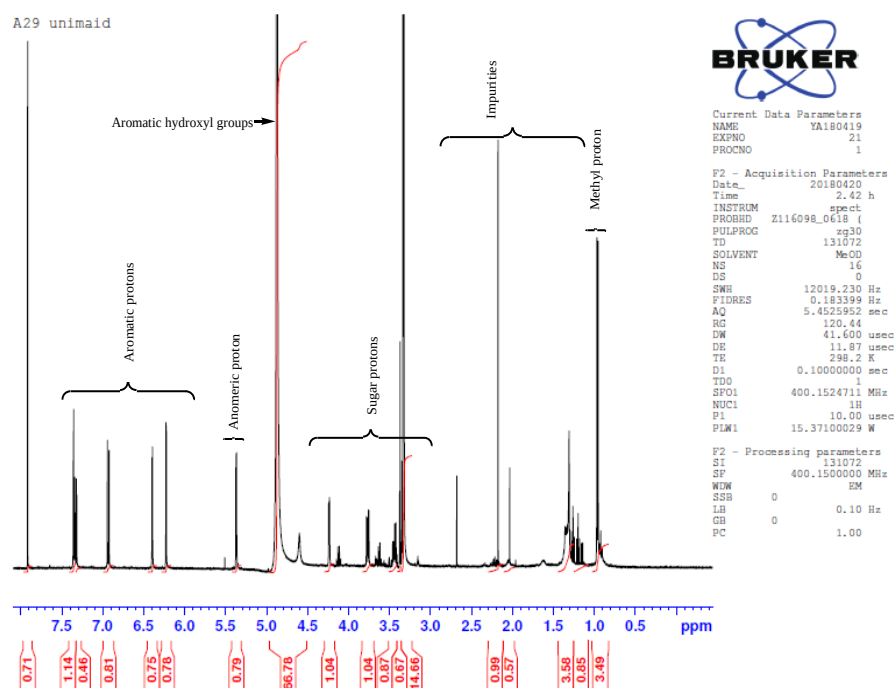


Figure 7a: ^1H NMR of compound 2

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Figure 7c: HMBC spectrum for compound 2

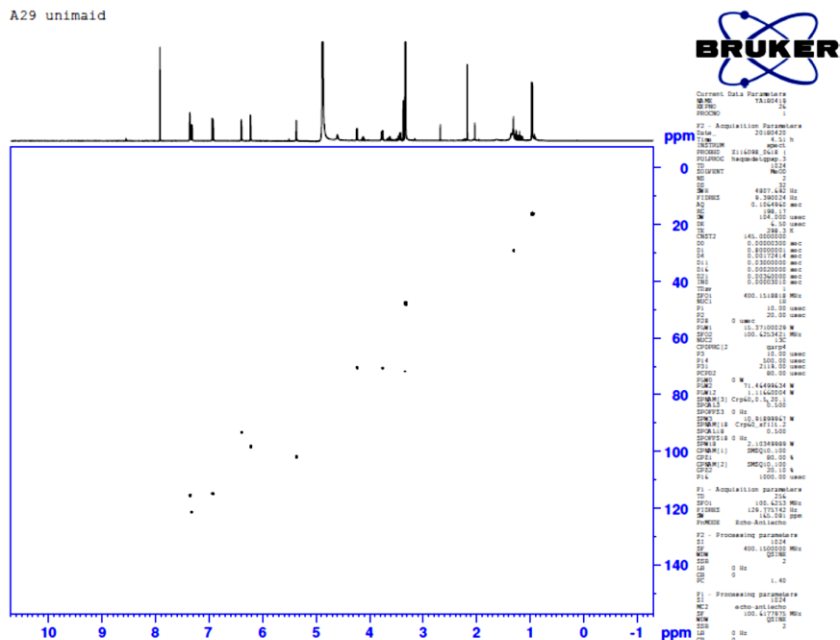


Figure 7d: HSQC spectrum for compound 2

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Table 3 Comparison of chemical Shift data obtained from Compound-1 with Literature data of friedelin

Position	¹³ C NMR chemical shifts (ppm)			δ ¹ H NMR chemical shifts (ppm)			Types of carbon/proton
	δ 1	δ α	δ β	δ 1	δ α	δ β	
1	22.29	22.3	22.3	1.677, 1.951	1.71 1.97	1.72 1.95	CH ₂
2	41.53	41.6	41.5	2.399, 2.383	2.41 2.31	2.37 2.26	CH ₂
3	213.34	213.4	231.2	Nil	-	-	C=O
4	58.22	58.3	58.2	2.234	2.28	2.26	CH
5	42.10	42.2	42.1	Nil	-	-	C
6	41.10	41.4	41.1	1.748, 1.272	-	-	CH ₂
7	18.10	18.3	18.2	1.404, 1.424,	-	-	CH ₂
8	53.00	53.1	53.2	1.410	-	-	CH
9	35.47	37.4	37.5	Nil	-	-	C
10	59.26	59.5	59.5	1.521	-	-	CH
11	34.83	35.6	35.7	1.284,	-	-	CH ₂
12	29.38	30.5	30.6	1.367, 1.677	-	-	CH ₂
13	38.90	39.7	39.8	Nil	-	-	C
14	37.67	38.3	38.4	Nil	-	-	C
15	31.95	32.4	32.8	1.485, 1.268	-	-	CH ₂
16	35.47	36.0	36.1	1.539, 1.35	-	-	CH ₂
17	28.45	30.0	30.1	Nil	-	-	C
18	44.77	42.8	42.8	1.49	-	-	CH
19	34.52	35.3	35.4	1.22, 1.37	-	-	CH ₂
20	28.48	28.2	28.3	Nil	-	-	C
21	32.42	32.8	32.5	1.249	-	-	CH ₂
22	37.70	39.	39.3	2.383	-	-	CH ₂
23	6.86	7.0	6.8	0.864	0.92	0.86	CH ₃
24	14.69	14.6	14.7	0.715	0.75	0.70	CH ₃
25	17.53	17.9	18.0	0.859	0.9	0.84	CH ₃
26	20.63	20.6	20.3	0.806	1.03	0.93	CH ₃
27	18.57	18.6	18.7	1.034	1.07	1.03	CH ₃
28	31.07	32.1	32.1	1.038	1.2	1.16	CH ₃
29	32.65	35.0	35.1	1.184	0.98	0.98	CH ₃

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30	29.75	31.8	31.9	0.940	1.02	0.98	CH ₃
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δ 1= compound-1, δ α = Abbas *et al.*, 2007, δ β = Mann *et al.*, 2011

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Table 4 Comparison of chemical Shift data obtained from Compound-2 with Literature data of Quercitrin

Position	$\beta 1$		$\beta 2$		Carbon Type
	^{13}C NMR (ppm)	^1H NMR (ppm)	^{13}C NMR (ppm)	^1H NMR (ppm)	
2	158.5	-	157.2	-	C
3	136.5	-	134.9	-	C
4	179.7	-	178.3	-	C
5	163.3	-	161.9	-	C
6	98.44	6.20	98.4	6.25	CH
7	166.0	-	161.9	-	C
8	93.32	6.40	98.4	6.36	CH
9	159.0	-	158.0	-	C
10	105.5	-	104.5	-	C
1'	121.6	-	121.6	-	C
2'	115.4	7.35	115.0	7.33	CH
3'	146.5	-	146.1	-	C
4'	149.0	-	148.5	-	C
5'	114.5	6.90	115.6	6.91	CH
6'	121.5	7.30	121.5	7.30	CH
1''	102.2	5.35	102.2	5.35	CH
2''	70.6	4.22	70.8	4.20	CH
3''	70.7	3.75	70.7	3.70	CH
4''	71.9	3.42	71.9	3.34	CH
5''	70.5	3.33	70.6	3.43	CH
6''	17.0	0.90	16.3	0.90	CH ₃

Key: ($\beta 1$)= compound-1, ($\beta 2$)= Utari *et al.*, (2019)

3.4 Antioxidant Activity

The results of the in-vitro antioxidant activity assay (Table 5) demonstrate the concentration-dependent DPPH radical scavenging activity of *Calophyllum inophyllum* fractions and the crude extract. The *n*-Butanol fraction (BF) exhibited the highest antioxidant activity with an IC₅₀ value of 13.3 $\mu\text{g/mL}$, followed by the chloroform fraction (CF, IC₅₀ = 34.3 $\mu\text{g/mL}$) and the crude methanol extract (CRF, IC₅₀ = 23 $\mu\text{g/mL}$). The *n*-Hexane fraction (HF) showed the least antioxidant activity (IC₅₀ = 83.6 $\mu\text{g/mL}$). In comparison, the positive control, ascorbic acid (AA), displayed the strongest antioxidant activity with an IC₅₀ of 6.9 $\mu\text{g/mL}$.

Table 5: Results of *In-vitro* Antioxidant Activities of the Leaves of *C. inophyllum*

Concentration ($\mu\text{g/mL}$)	% DPPH Scavenging Activity				
	HF	CF	BF	CRF	AA
6.25	18	32	40	30	45
12.5	24	37	51	49	52
25	30	49	60	55	63
50	46	64	70	72	78
100	52	79	87	83	96
IC₅₀ ($\mu\text{g/mL}$)	83.6	34.3	13.3	23	6.9

Key: HF = n-Hexane fraction, CF = Chloroform fraction, BF = n-Butanol fraction, CRF = Crude methanol extract, AA = Ascorbic acid (positive control).

3.5 Cytotoxicity Assay

The *in-vitro* cytotoxicity assay results (Table 6) demonstrate the concentration-dependent cytotoxic effects of *Calophyllum inophyllum* fractions against human H460 lung cancer cells, with the *n*-hexane fraction showing the most potent activity ($\text{IC}_{50} = 16.0 \mu\text{g/mL}$), followed by the chloroform fraction ($\text{IC}_{50} = 23.0 \mu\text{g/mL}$) and the crude extract ($\text{IC}_{50} = 30.0 \mu\text{g/mL}$). In comparison, the positive control, 5-Fluorouracil (5FU), exhibited the highest potency with an IC_{50} of $2.7 \mu\text{g/mL}$, underscoring its efficacy as a standard chemotherapeutic agent. The dose-dependent cell viability of H460 lung cancer cells at different concentrations of 5-Fluorouracil and extract fractions, as determined by the MTT assay, is illustrated in Figure 8.

Table 6: *In-vitro* Cytotoxicity Assay Results (MTT Assay)

Concentration ($\mu\text{g/mL}$)	Percentage cell viability (%)				
	5FU (%)	Crude Extract	n-Hexane fraction	Chloroform fraction	n-Butanol fraction
0.1	78.88	-	-	-	-
1	79.45	93.64	86.82	97.83	93.57
3	46.83	91.26	83.73	97.72	80.51
10	29.96	83.07	76.51	71.01	78.09
30	29.64	50.82	5.26	42.9	72.79
100	-	14.6	4.12	7.83	64.59
IC₅₀ ($\mu\text{g/mL}$)	2.7	30.0	16.0	23.0	>65

Key: (IC_{50})= concentration at 50% inhibition, (5FU)= 5-Fluorouracil, (PCV%)= percentage cell viability

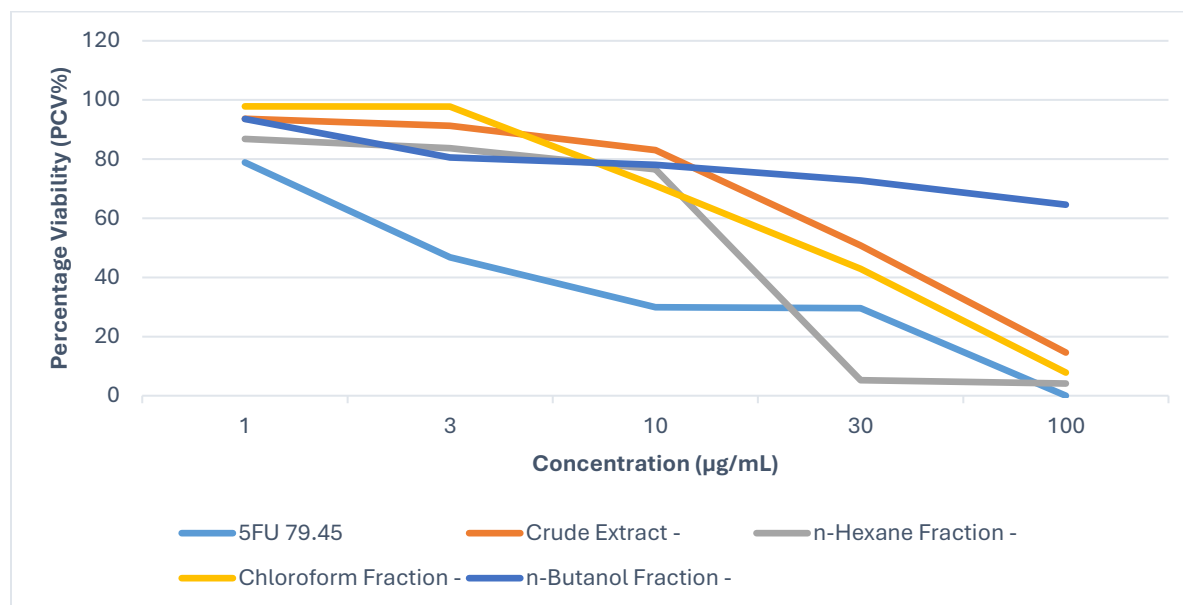


Figure 8: Percentage Viability of H460 Lung Cancer Cells at Different Concentrations of 5-Fluorouracil and Extract Fractions Using the MTT Assay.

4. Discussion

The findings of this study provide significant insights into the phytochemical composition and pharmacological potential of *Calophyllum inophyllum*. The qualitative and quantitative phytochemical analyses confirmed the presence of diverse secondary metabolites, including carbohydrates, alkaloids, saponins, flavonoids, tannins, and terpenoids, in the methanol extract and its fractions. Notably, the *n*-Butanol fraction exhibited the highest total phenolic and flavonoid content, aligning with its potent antioxidant activity ($IC_{50} = 13.3 \mu\text{g/mL}$). This is consistent with previous studies that have highlighted the antioxidant properties of phenolic compounds and flavonoids, which are known to scavenge free radicals and mitigate oxidative stress-related diseases (Taher *et al.*, 2005; Shanmugapriya *et al.*, 2016).

The isolation and structural elucidation of friedelin and quercitrin from the *n*-Hexane and *n*-Butanol fractions, respectively, underscore the medicinal potential of *C. inophyllum*. Friedelin, a pentacyclic triterpenoid, demonstrated significant cytotoxic activity against human H460 lung cancer cells, with an IC_{50} value of $16 \mu\text{g/mL}$. This finding is supported by earlier studies that have reported the antiproliferative effects of friedelin against various cancer cell lines, suggesting its potential as a lead compound for anticancer drug development (Laure *et al.*, 2005; Li *et al.*, 2010). In comparison, the positive control, 5-Fluorouracil (5-FU), exhibited an IC_{50} value of $2.7 \mu\text{g/mL}$, highlighting its superior potency as a standard chemotherapeutic agent. The *n*-hexane fraction demonstrated significant cytotoxic activity, with an IC_{50} value of $< 30 \mu\text{g/mL}$, which meets the American National Cancer Institute (NCI) criteria for promising anticancer candidates (Shoemaker, 2006). This activity is attributed to the presence of friedelin, a bioactive compound known for its anticancer properties. These findings further validate the *n*-hexane fraction's

potential for drug development and warrant further investigation into its mechanisms and therapeutic applications.

On the other hand, quercitrin, a flavonoid glycoside, exhibited strong antioxidant activity, with an IC_{50} value of 13.3 $\mu\text{g/mL}$ in the DPPH assay. This aligns with the well-documented role of flavonoids in reducing oxidative damage and inflammation, further supporting the traditional use of *C. inophyllum* in treating oxidative stress-related ailments (Taher *et al.*, 2005; Dutta and Ray, 2014). The *n*-butanol fraction's high antioxidant activity can be attributed to its rich phenolic and flavonoid content, making it a valuable source of natural antioxidants.

The differential bioactivity of the fractions highlights the importance of targeted isolation and characterization of bioactive compounds from medicinal plants. The *n*-hexane fraction, containing friedelin, showed potent cytotoxic effects, while the *n*-butanol fraction, rich in quercitrin, demonstrated strong antioxidant properties. These findings not only contribute to the scientific understanding of the pharmacological properties of *C. inophyllum* but also underscore the importance of exploring natural products for drug discovery.

5. Conclusion

This study demonstrates the potential of *Calophyllum inophyllum* as a valuable source of bioactive compounds with significant anticancer and antioxidant properties. The isolation of friedelin and quercitrin from the leaves of *C. inophyllum* validates its traditional use in cancer treatment and oxidative stress management. Friedelin's potent cytotoxic activity against lung cancer cells ($IC_{50} = 16 \mu\text{g/mL}$) and quercitrin's strong antioxidant effects ($IC_{50} = 13.3 \mu\text{g/mL}$) highlight their potential as lead compounds for the development of novel therapeutic agents. These findings not only contribute to the scientific understanding of the pharmacological properties of *C. inophyllum* but also underscore the importance of exploring natural products for drug discovery. Further studies, including *in vivo* experiments and clinical trials, are recommended to fully elucidate the therapeutic potential of these compounds and their mechanisms of action. Overall, this research reinforces the significance of medicinal plants in modern drug development and provides a foundation for future investigations into the bioactive constituents of *C. inophyllum*.

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Conflict of interest

The authors declare no conflict of interest.

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