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CHROMATOGRAPHIC ISOLATION AND SPECTROSCOPIC CHARACTERIZATION OF QUERCETIN FROM *Cyathea nilgirensis* Holttum AND EVALUATION OF ITS CYTOTOXIC POTENTIAL AGAINST DALTON'S LYMPHOMA ASCITES CELLS

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ABSTRACT

Flavonoids are important secondary metabolites widely distributed in plants and are known for their diverse biological activities, including antioxidant, anti-inflammatory, and anticancer effects. Exploration of medicinal plants as sources of structurally diverse and biologically active flavonoids continues to provide valuable opportunities for the discovery of new therapeutic candidates. In the present study, a flavonoid compound was isolated from *Cyathea nilgirensis* Holttum and evaluated through integrated phytochemical, spectroscopic, and biological investigations. The crude plant extract was subjected to successive purification using chromatographic techniques, including column chromatography and thin-layer chromatography, to obtain the purified constituent. Structural identification of the isolated compound was achieved through a combination of UV–Visible, FT-IR, mass spectrometry, ^{1}H NMR, ^{13}C NMR, and HMBC analyses. The spectral data confirmed the compound as quercetin, a polyhydroxylated flavonol characterized by hydroxyl substitutions at the 3,5,7 and 3′,4′ positions of the flavonoid nucleus. The cytotoxic potential of the isolated flavonoid was examined against Dalton's lymphoma ascites cells using the Trypan blue exclusion method. The results revealed a dose-dependent reduction in cell viability, demonstrating the inhibitory effect of the compound on tumour cell proliferation. The present findings highlight *C. nilgirensis* as a promising source of bioactive flavonoids and provide experimental evidence supporting the potential anticancer relevance of the isolated quercetin derivative. Further studies focusing on molecular mechanisms, apoptotic signalling pathways and in vivo efficacy are required to establish its therapeutic potential.

Keywords: *Cyathea Nilgirensis* Holttum, Polyhydroxylated Flavonol, Flavonoid Isolation, Spectroscopic Characterisation, Chromatographic Separation, Dalton's Lymphoma Ascites Cells, Cytotoxicity, Anticancer Activity.

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INTRODUCTION

Flavonoids are a great class of plant-derived polyphenolic secondary metabolites extensively scattered across plant species.[1–3] Over 6,500 structures have been identified, reflecting their significant chemical diversity and biological importance. In plants, flavonoids play essential protective roles against biotic stresses such as pathogens and herbivores, as well as abiotic factors including UV radiation, oxidative stress, and drought.[4] They also function as signalling molecules in plant–microbe interactions and symbiosis.[5] These compounds contribute to the pigmentation of flowers and fruits, supporting pollination and seed dispersal.[6,7] Their antioxidant activity is mainly attributed to hydroxyl groups, which enable effective scavenging of reactive oxygen species, along with enhancement of cellular detoxification systems.[8–10] Flavonoids exhibit diverse pharmacological properties, including antimicrobial, cardioprotective, anticancer, and anti-ageing effects.[11] Their anticancer potential involves modulation of apoptosis, cell cycle progression, and tumour suppression pathways.[12] In plant systems, they also act as

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UV filters, drought protectants, and allelopathic agents.[13–15] Structurally, flavonoids possess a C6–C3–C6 backbone with two aromatic rings linked by a heterocyclic ring.

Their extraction efficiency depends on solvent properties such as polarity and pH, which influence stability and yield. Common solvents include methanol, ethanol, and acetone, while techniques like ultrasound-assisted extraction improve recovery. Acidified conditions further enhance stability by minimising oxidative degradation.[16,17] Studies on the genus Cyathea have reported flavonoids such as kaempferol, cyrtominetin, farrerol, and vitexin, along with newer acylated derivatives.[18,19] In recent years, plant-derived materials have also been explored for their applications in corrosion protection, nanomaterial production and environmental remediation.[20–22] These findings highlight the value of exploring different plant species as sources of useful natural compounds and sustainable materials. In this study, flavonoids were isolated from *Cyathea nilgirensis* Holttum using optimised methods and evaluated for anticancer activity, highlighting their potential as therapeutic agents.

EXPERIMENTAL

Plant Material Collection and Preparation

The fronds of *Cyathea nilgirensis* Holttum were collected, shade-dried, and pulverized into a fine powder to facilitate efficient extraction of phytoconstituents. The powdered material was subjected to sequential solvent extraction using ethanol to recover flavonoid-rich fractions. The extraction process was carried out through repeated cycles to ensure effective dissolution of bioactive constituents. The obtained ethanolic extract was further subjected to liquid–liquid fractionation using ethyl acetate to enrich flavonoid components based on their polarity characteristics. The separated fraction was filtered to remove insoluble impurities and concentrated under reduced pressure to obtain the crude flavonoid-rich extract. The concentrated extract was subsequently used for chromatographic purification and further biological evaluation.

Isolation of Flavonoid

Column Chromatography

A glass column (150 × 5 cm) was cleaned, rinsed with suitable solvents, and air-dried. A glass wool plug was placed at the base, and the column was packed with silica gel slurry (40 µm) in petroleum ether to form a uniform stationary phase. Proper packing was ensured by gentle tapping and removal of air bubbles to avoid channeling. The plant extract was carefully loaded onto the silica bed without disturbance. Elution was performed using an optimized polarity-gradient solvent system for effective flavonoid separation. Fractions were collected sequentially and monitored, with details provided in Table-1. The overall setup and workflow are shown in Fig.-1.

Table-1: Details of column chromatography

S. No.	Column size	Stationary phase	Sample mobile phase	Fractions collected	Chemical test
1.	150×5 cm	Silica gel (40 µ)	$CHCl_3$:CH_3OH (7:1)	Yellow green fraction	Positive with Shinoda test
Flavonoid Test (Shinoda test)					0.5 ml sample + ethanol + warmed + filtered + Three pieces of magnesium chips +0.5ml of conc. HCl. Orange-red precipitate appeared.

Thin layer Chromatography

Pre-cleaned glass plates (20 × 5 cm) were coated with a uniform silica gel slurry (1:2, silica: water) and stimulated at 110 °C for 30 min. After chilling in a desiccator, the sample was spotted ~0.75 cm from the base using a capillary tube. The plate was developed in a pre-saturated chamber with the selected eluent, allowing solvent ascent by capillary action to separate flavonoids. After development, the plate was dried and analyzed. Chromatographic conditions are given in Table-2, and the setup is shown in Fig.-2.

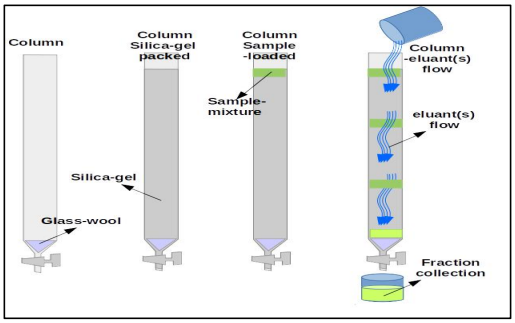


Fig.-1: Column Chromatographic Illustration

Table-2: Details of Thin-Layer Chromatography

S. No.	Plate size	Stationary phase	Mobile phase	Sample	Spot developed and Rf value	Chemical test
1.	20×5 cm	Silica gel (100 µ)	n-BuOH:AcOH: H_2O (4:1:5)	Plant extract	Yellow spot (0.95)	Positively tested with ammonia

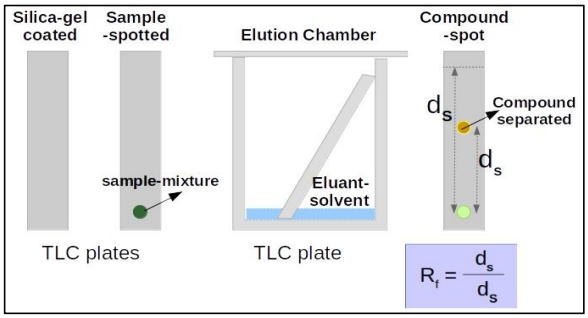


Fig.-2: Thin Layer Chromatographic Illustration

Structural Elucidation of the Isolated Flavonoid

The purified flavonoid was recrystallized using acetone and subjected to basic physicochemical analysis. Melting point (Mettler Toledo MP 50) and optical rotation (Digital Polarimeter CDP 001) were recorded, along with preliminary tests to identify functional groups and structural features. Further characterization employed UV–Vis (Perkin Elmer Lambda 35, ethanol), FT-IR (Perkin Elmer Spectrum, KBr pellet), and NMR (^{1}H and ^{13}C, BrukerAvance III HD 400 MHz, DMSO-d_6 , TMS). Mass analysis (SCIEX 4500 MD) confirmed molecular weight and fragmentation. Combined data enabled structural identification.

Evaluation of Anticancer Activity

The cytotoxic effect was determined by the Trypan blue exclusion method using Dalton's Lymphoma Ascites (DLA) cells. Viable cells retained their clear appearance, while damaged or dead cells incorporated the dye and showed blue staining (1×10^6/0.1 mL) were treated with flavonoid (10–200 µg/mL) and adjusted to 1 mL with PBS; untreated cells served as control. After incubation at 37°C for 3h, Trypan blue (0.1 mL, 1%) was added, and cells were counted using a hemocytometer. Live and dead cells were recorded separately, and cytotoxicity was calculated. Results showed dose-dependent cytotoxic activity of the flavonoid.

RESULTS AND DISCUSSION

The compound showed a melting point of 298.5 ± 3 °C, indicating good purity, and was soluble in chloroform, acetone, and ethanol, suggesting moderate polarity. Ferric chloride produced a blue coloration (Fig.-3), confirming phenolic –OH groups. Decolorization of Baeyer's reagent indicated unsaturation, and a positive Borche's test confirmed a carbonyl group. These findings supported its flavonoid nature, later verified by spectral analysis.

UV Visible Spectral Analysis

Flavonoids exhibit characteristic UV absorption because of their extended conjugated aromatic structure. Absorption at 240-280 nm is attributed to the benzoyl moiety of ring A, whereas bands between 300 and

380 nm originate from the cinnamoyl moiety of ring B together with the conjugated carbonyl group. Substituents like –OH and –OCH_3 influence these electronic transitions. The UV-Visible spectrum (Fig.-4) displayed three characteristic bands: 371.55 nm (cinnamoyl system, ring B), 256.30 nm (benzoyl system, ring A), and 206.35 nm (ring C, $\pi \rightarrow \pi^*$ transition). These confirm a conjugated flavonoid structure.

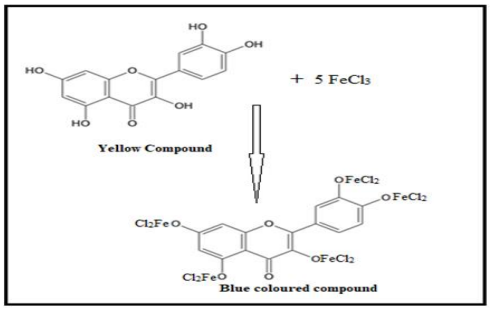


Fig.-3: Neutral Ferric Chloride Test

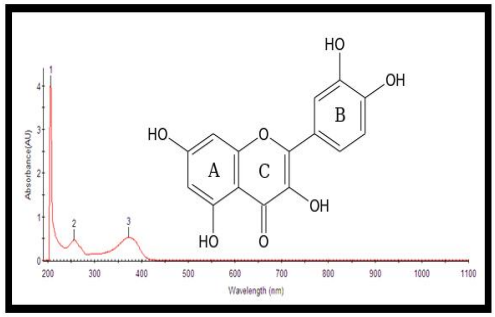


Fig.-4: U-V Spectra of the Flavonoid compound

FT- IR Spectral Analysis

Figure-5 presents the FT-IR spectrum of the isolated flavonoid. A broad, intense absorption at 3392.47 cm^{-1} corresponds to O–H stretching. The bands at 1367.34 and 1244.37 cm^{-1} are assigned to O–H bending and C–O stretching vibrations, while the absorption at 638.71 cm^{-1} is attributed to out-of-plane O–H bending. A weak band at 3083.47 cm^{-1} indicates aromatic C–H stretching. The bands observed at 1616.35, 1561.45, and 1513.47 cm^{-1} are characteristic of aromatic C=C stretching vibrations, whereas the band at 1656.45 $cm^{-1}$is assigned to alkenic C=C stretching. In addition, the absorptions at 808.08 and 788.27 cm^{-1} correspond to out-of-plane aromatic C–H bending. A prominent band at 1683.27 cm^{-1} confirms the presence of the carbonyl (C=O) group associated with the flavonoid C-ring.

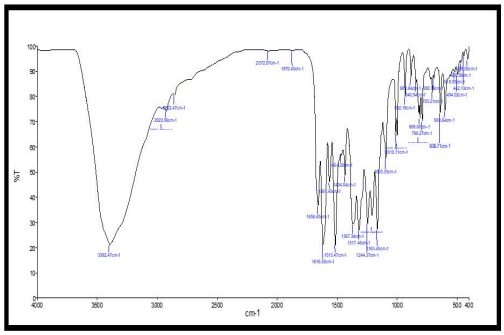


Fig.-5: FT- IR Spectra of the Flavonoid compound

^{1}H- NMR spectral Analysis

Figure-6 shows the ^{1}H-NMR spectrum of the isolated flavonoid-type compound. The chemical shift values 6.189 ppm, 6.411 ppm and 7.676 ppm singlet (1H) is observed for deshielded three aromatic protons -(CH=CR)-) without any neighbouring proton. The chemical shift values 6.895 ppm and

7.552 ppm doublet (1H), *J* 8 Hz, represent the significantly deshielded two aromatic protons with one neighbouring proton (-(CH=CH)-). The chemical shift signal patterns 9.327 ppm, 9.384 ppm and 9.622 ppm singlet (1H) represents Highly deshielded hydroxyl protons attached to the aromatic ring(s) in three different positions (-(C=C[OH])- of Ar ring), chemical shift value 10.819 ppm singlet (1H) represents the hydroxyl proton of the alkenic part, that is highly deshielded due to the closer proximity of carbonyl functionality [-(C=C[OH])-C=O], and 12.496 ppm singlet (1H) confirms the hydroxyl proton of the aromatic ring, that is highly deshielded due to the closer proximity of carbonyl functionality z[-(C=C[OH])Ar-C=O].

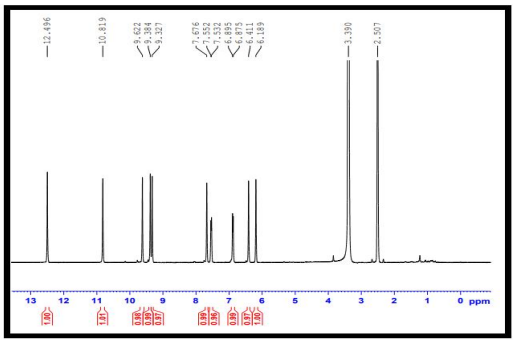


Fig.-6: H-NMR Spectra of the Flavonoid Compound

^{13}C- NMR Spectral Analysis

Figure-7 shows the ^{13}C -NMR spectrum of the isolated flavonoid compound. The chemical shift values 93.81, 98.63, and 103.46 ppm represent the three observably deshielded carbon atoms of the aromatic ring and the chemical shift values 156.58, 164.33 and 161.17 ppm represent the three significantly deshielded carbon atoms with electronegative groups in the aromatic ring. The chemical shift values 122.40 and 136.19 ppm represent the two highly deshielded alkenic carbons with electronegative groups. The chemical shift at 176.29 ppm represents the highly deshielded carbonyl carbon. The chemical shift values 115.50, 116.05, and 120.44 ppm represent remarkably deshielded carbons of the aromatic ring. The chemical shift values 145.51, 147.25, and 148.15 ppm represent three carbons of the same aromatic ring, significantly deshielded due to the attachment of electronegative groups.

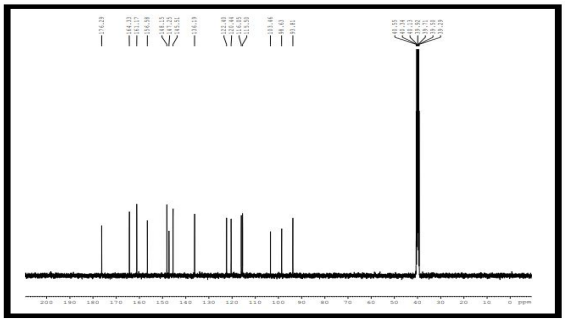


Fig.-7: C-13-NMR Spectra of the Flavonoid Compound

Anticancer Activity of the Flavonoid Compound

The anticancer potential of the isolated flavonoid was assessed against Dalton's Lymphoma Ascites (DLA) cell lines using concentrations ranging from 10 to 200 µg/mL, with cyclophosphamide serving as the standard control. The untreated control showed no inhibition, while treated samples exhibited a clear dose-dependent increase in cytotoxicity (Table-3, Fig.-8). Cancer is a complex disorder involving abnormal cellular growth, metabolic reprogramming, evasion of programmed cell death, and the ability to invade and spread to distant tissues.[23,24] These features are closely associated with oxidative stress, mitochondrial dysfunction, and dysregulated reactive oxygen species (ROS).[25–27] Flavonoids exert anticancer effects by modulating these pathways, including regulation of ROS levels, cellular proliferation control through cell cycle regulation and stimulation of programmed cell death pathways.[28]

While flavonoids generally exhibit antioxidant activity in healthy cells, they may promote oxidative stress responses within cancer cells, elevating ROS beyond a critical threshold and triggering cell death.[29–31] They also influence detoxification enzymes and inhibit tumour-promoting mechanisms. The observed dose-dependent inhibition of DLA cells indicates that the isolated flavonoid effectively disrupts cancer cell survival, likely through ROS-mediated apoptosis and metabolic interference, indicating its potential as a valuable candidate for future anticancer therapeutic development.[32,33]

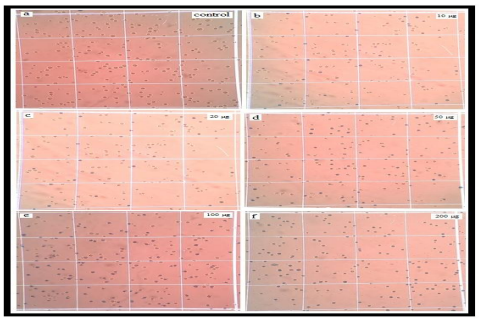


Fig.-8: Anticancer Activity of Flavonoid Extract of CyatheanilgirensisHolttum (a) Control, (b) 10 µg, (c) 20 µg, (d) 50 µg, (e) 100 µg f 200 µg

Table-3: Anticancer Activity Results of Isolated Flavonoid

Cell line	Type of Extract	Control (100 live cells)	Anticancer Results for various concentrations				
			Concentration µg /ml				
			10	20	50	100	200
	Flavonoid extract	100	19	34	72	80	98

CONCLUSION

The present study successfully achieved the isolation and structural elucidation of a bioactive flavonoid from *Cyathea nilgirensis* Holttum through chromatographic separation and advanced spectroscopic investigations. The combined UV–Visible, FT-IR, NMR, mass spectrometric, and HMBC analyses confirmed the isolated compound as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (quercetin). The purified flavonoid exhibited a concentration-dependent cytotoxic response against Dalton's lymphoma ascites cells, with the highest inhibitory effect observed at 200 µg/mL, demonstrating its potential to suppress tumour cell viability. The observed activity suggests that the compound may exert its biological effects through modulation of cellular oxidative balance and apoptosis-associated pathways. This investigation highlights *C. nilgirensis* as a valuable source of naturally occurring flavonoids and provides preliminary evidence supporting the anticancer potential of the isolated compound. Further studies involving detailed molecular mechanism analysis, evaluation of oxidative stress markers, in vivo validation, and bioavailability enhancement strategies are recommended to establish its therapeutic significance and facilitate future drug development approaches.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

This work is related to *SDG-3: Good Health and Well-Being*. All authors contributed to the manuscript, participated in its review and editing, and approved the final version for publication. The authors' research profiles can be accessed through their ORCID records:

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