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UNVEILING THE MEDICINAL POTENTIAL OF *MEMECYLON MALABARICUM* (C.B. CLARKE) COGN.: PHYTOCHEMICAL INSIGHTS AND CYTOTOXIC PROFILING FOR THERAPEUTIC ADVANCEMENTS

Medicinal Plants

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ABSTRACT

Memecylon malabaricum (C.B. Clarke) Cogn., recognized for its medicinal potential, has undergone phytochemical analyses revealing diverse bioactive compounds. This study aims to delve into its flavonoid composition and evaluate the cytotoxic effects on HCT-116 and U87 cells through MTT assays. The exploration of flavonoids and their concentrations in various fractions, coupled with an assessment of cytotoxicity, holds promise for pharmaceutical applications and therapeutic advancements. Phytochemical analysis of *M. malabaricum* involved total flavonoid content was assessed using the aluminum chloride colorimetric method. UV-VIS spectrophotometric analysis determined flavonoid-specific absorption peaks. FT-IR spectroscopy identified functional groups. MTT assays assessed cytotoxic effects on HCT-116 and U87 cell lines. *M. malabaricum* extracts, devoid of alkaloids and anthraquinones, exhibited the presence of coumarin, flavonoids, glycosides, phenol, tannins, and terpenoids. Fractionation revealed the flavonoid concentration (228 µg QE/mg), UV-VIS spectrophotometry confirmed flavonoid-specific absorption peaks at 276 nm and standard catechin 279.2 nm, while FT-IR spectroscopy identified key functional groups. These flavonoid extract induced dose-dependent cytotoxicity in HCT-116 (IC50: 491.1 µg/5µl) and U87 (IC50: 100 µg/5µl) cells within 24 hours, showcasing specific effects and validated by the positive control, 10 µg/5µl Cisplatin. Flavonoid extract of *M. malabaricum* exhibits a rich phytochemical profile, emphasizing flavonoids with potential medicinal implications. The distinct concentrations of flavonoids across fractions highlight the need for further exploration of specific bioactive compounds. The cytotoxic effects on HCT-116 and U87 cells underscore its dose-dependent impact, presenting avenues for future investigations into the extract's therapeutic applications.

KEYWORDS

Memecylon malabaricum, UV-VIS spectroscopy, FT-IR spectroscopy, MTT assay.

INTRODUCTION

Memecylon malabaricum (C.B. Clarke) Cogn., a member of the Melastomataceae family, stands as a botanical entity of notable interest owing to its traditionally recognized medicinal potential. Indigenous to the Western Ghats in India, this plant has been historically employed in traditional medicine for its diverse therapeutic properties (Murugan and Gopalan, 2006). Previous investigations into *M. malabaricum* have unveiled a rich phytochemical profile, indicating the presence of various bioactive compounds. However, a focused exploration into specific constituents, such as flavonoids, and their potential medicinal implications is warranted (Viswanathan and Manikandan, 2001).

Flavonoids, a subgroup of polyphenolic compounds, are widely acknowledged for their diverse biological activities, including antioxidant, anti-inflammatory, and anticancer properties. Despite the well-documented significance of flavonoids in medicinal plants, there exists a gap in the literature regarding the detailed analysis of flavonoid composition in *M. malabaricum*. Understanding the specific flavonoids present and their concentrations within the plant extract is crucial for unraveling the therapeutic potential associated with these bioactive compounds (Vivek *et al.,* 2014).

Moreover, the investigation into the cytotoxic effects of *Memecylon malabaricum* extract on HCT-116 cells serves as a pivotal aspect of this study. The HCT-116 cell line, derived from human colorectal carcinoma, is a widely utilized model for assessing the anticancer potential of natural compounds. Previous studies have demonstrated the cytotoxic effects of various plant extracts on cancer cell lines, making it imperative to explore the impact of *M. malabaricum* on HCT-116 cells (Chaudhary *et al.,* 2017).

This study aims to bridge the existing gaps in the knowledge regarding *M. malabaricum* by conducting a comprehensive phytochemical analysis, with a specific focus on flavonoid composition. Additionally, the investigation seeks to elucidate the cytotoxic effects of the extract on HCT-116 Human colorectal carcinoma and U87 (Human Glioblastoma cancer) cell lines, providing valuable insights into its potential as a source of bioactive compounds with therapeutic applications. The outcomes of this research contribute not only to the understanding of *M. malabaricum* chemical constituents but also to its potential role in developing novel pharmaceutical interventions for various health conditions.

MATERIALS AND METHODS

Collection of plant material

Memecylon malabaricum (C.B. Clarke) Cogn leaves were collected from the Charaka Nagar area in Hosanagara taluk, Shivamogga district, Karnataka, India. The collection site is situated at the geographical center of the Karnataka state. The plant was identified and validated by Dr. Pusphalatha, a plant taxonomist at the Department of Botany, Sahyadri Science College, Kuvempu University, Shivamogga, India.

Extraction Of Phytochemicals

Fresh leaves of *M. malabaricum*. were meticulously separated, washed, and subsequently shade dried at room temperature. The dried leaves were crumbled using a blender to facilitate further processing. The resulting material was then ground into a coarse powder using a grinder and sieved to achieve a uniform particle size. The weighed amount of 20 grams of this coarse powder was used in the subsequent extraction process. The extraction involved combining the coarse powder with 200ml of prechilled methanol in a beaker. The sonication process, utilizing a 13mm-diameter sonicator probe operating at a frequency of 20 kHz, was conducted at 20 °C for 30 minutes per cycle, employing a pulse mode of 3 seconds on and 1 second off, and three cycles were completed. Following sonication, the extract underwent centrifugation at 3000g for 10 minutes in a cooling centrifuge machine

maintained at 20°C. The separated supernatant was collected in a 250ml glass beaker, allowed to dry at ambient temperature, and the resulting residue was stored in a 5ml microcentrifuge tube at 4-8°C for subsequent analysis and screening (Laghari *et al.,* 2011).

Preliminary Phytochemical Screening

A 2.5 ml aqueous extract of *M. malabaricum* underwent various tests for phytochemical constituents. The Foam Test revealed stable foam formation, indicative of saponins. Lieberman's Test displayed a colour change from violet to blue to green, suggesting the presence of steroidal nuclei in glycosides. Coumarin was identified by a yellow colour upon the addition of alcoholic sodium hydroxide. Mayer's Test confirmed alkaloids with a yellow precipitate. The Lead Acetate Test exhibited a yellow precipitate for flavonoids. Tannins were detected through the Ferric Chloride Test, producing a green precipitate. Terpenoids were identified by a greyish colour in Salkowski's Test. Steroids were confirmed by a red colour in the Steroids Test. Anthraquinones were indicated by a bright pink colour in Bontrager's Reaction. Phenols displayed a blue or green colour in the Ferric Chloride Test. The Borutrager's Test for anthraquinones resulted in a pink or deep red colour. Control tests were performed using chloroform and ammonia solution (Hanoon *et al.*, 2019).

Determination Of Total Flavonoids

For the determination of total flavonoid content in each fraction of *M. malabaricum*, 1 mg of the solubilized fraction in 2 mL of deionized water was employed. The aluminum chloride colorimetric method was utilized for this purpose (Lin and Tang, 2007). The fractions were mixed with 0.1 mL of 10% aluminum chloride hexahydrate, 0.1 mL of 1 M potassium acetate, and 2.8 mL of deionized water. Following a 40-minute incubation at room temperature, the absorbance of the reaction mixture was measured spectrophotometrically at 415 nm. Quercetin was used as the standard within a concentration range of 0.005 to 0.1 mg/mL. The total flavonoid content was expressed as micrograms of quercetin equivalents (QE) per milligram of fraction.

UV-VIS Spectrophotometric Analysis

M. malabaricum Leaf extracts underwent UV-VIS spectrophotometric analysis using a single-beam UV-VIS spectrophotometer (Systronics-119). The scans were performed within the wavelength range of 200-800 nm, employing a scan speed of 400 nm/min, as detailed in previous studies (Harborne, 1987).

Fourier-transform Infrared Spectrometer (FT-IR) Analysis

This study employed a Fourier-transform infrared spectrometer (FT-IR) to assess the structural characteristics of lyophilized *M. malabaricum* extracts. The lyophilization process enhanced spectral band intensity and minimized interference from water and organic solvents. FT-IR spectra were recorded using a Bruker alpha Eco-ATR Optics system with a (ZnSe) reflection crystal at ambient temperature (20 °C). Spectra were acquired in the range of 4000 to 600 cm^-1 using OPUS software (v. 5.5, Bruker Optics, Germany) (Antil, 2019).

Cytotoxicity by MTT Assay

Leaf phytochemical extract of *M. malabaricum* were prepared by dissolving 1gm of the test sample in 10ml of methanol, followed by sterilization through filtration using a 0.22µm syringe filter. Serial dilutions of the drug concentrations were prepared in MEM supplemented with 10% FBS (Complete medium). HCT-116 cells at passage number P21 obtained from NCCS Pune, India, were cultured in MEM with 10% FBS, and their viability was assessed after 24 hours of exposure to different concentrations of *M. malabaricum* extracts. The MTT solution, prepared by dissolving 500 mg MTT powder in 100 mL PBS, was utilized for viability measurements at 570 nm using an ELISA plate reader (Bio-base). The cell culture setup involved seeding 84,000 live cells into a 96-well plate, and after a 24-hour incubation, the cells were exposed to various concentrations of the test compound. The incubation period lasted for 24 hours, and formazan crystals were dissolved using DMSO before measuring the optical density at 570nm. The percentage of cell survival was calculated using the formula (OD of treated well [−blank])/(mean OD of control well [−blank]) ×100. The experiment was performed in triplicate to ensure reliability and reproducibility of the results. The overall protocol adhered to standard laboratory practices and employed essential equipment, including a plate shaker, pipettes, a Class 2B hood, a benchtop centrifuge, a microplate reader, and a CO_2 incubator, to facilitate a comprehensive assessment of the cytotoxic effects of *M. malabaricum* extracts on HCT-116 cells (Twentymanand Luscombe, 1987).

RESULTS

Phytochemical Analysis

The phytochemical analysis of *Memecylon malabaricum* (C.B. Clarke) Cogn. extract revealed the presence of certain bioactive compounds. The extract tested negative for alkaloids and anthraquinones. However, coumarin, flavonoids, glycosides, phenol, tannins, and terpenoids were detected in the extract. The presence of coumarin, flavonoids, glycosides, phenol, tannins, and terpenoids suggests the potential medicinal value of the extract, as these compounds are often associated with diverse therapeutic properties. Notably, the absence of alkaloids and saponins in the extract indicates a specific chemical profile, laying the groundwork for further investigations into the pharmacological activities of *M. malabaricum*. Results are summarized in Table 1.

Table 1: Results Of Phytochemical Preliminary Tests For *M. Malabaricum* Leaf Extract

Phytochemical	Results
Alkaloids - Mayer's test	–
Anthra Quinones - Bontrager's reaction for free anthraquinones	–
Coumarin	+
Flavonoids - Lead acetate test	+
Glycosides - Lieberman's test and Coumarin test	+
Phenol - Ferric chloride test	+
Saponins	–
Steroids	–
Tannins - Ferric chloride test	+
Terpenoids - Salkowski's test	+

(+ high presence, - indicates the absence)

Total Flavonoid Content

The quantification of total flavonoid content in various fractions of *M.malabaricum* leaf extract was conducted utilizing the aluminum chloride colorimetric method, as described by Lin and Tang (2007). The results, expressed in micrograms of quercetin equivalents (QE) per milligram of fraction, revealed a concentration of 228 µg QE/mg in the *M. malabaricum* leaf extract.

UV-VIS Spectrophotometric Analysis

UV analysis of *M. malabaricum* leaf extract revealed flavonoid-specific absorption peaks at 276 nm and standard catechin 279.2 nm. These findings indicate the presence of flavonoids, providing crucial insights into the extract's chemical composition and suggesting potential bioactive properties associated with these compounds.

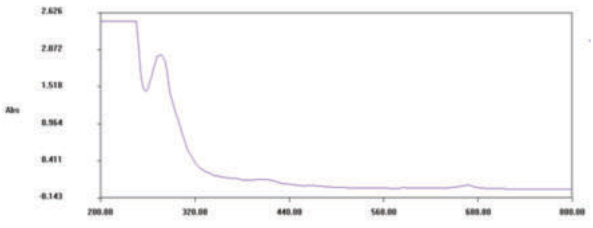

Figure 1(a): UV- Visible spectrometric analysis of *M. malabaricum* leaf extract

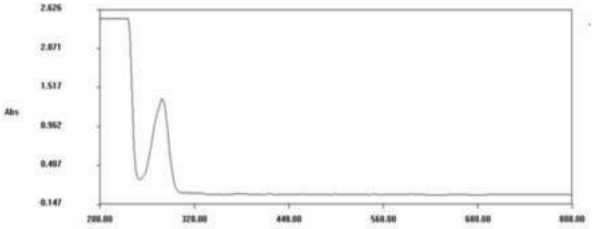

Figure 2(b): UV- Visible spectrometric analysis of standard Catechin

Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

In this investigation, Fourier Transform Infrared (FT-IR) spectroscopy served as a crucial tool for the identification of functional groups within the active components of *M. malabaricum* leaf extracts. The FT-IR spectrum allowed for the discernment of specific functional groups by analyzing peak values within the infrared radiation region(Malla *et al*., 2023). Upon introduction of the extract into the FT-IR instrument, the functional groups of its components were effectively separated

based on their respective peak ratios. The subsequent FT-IR analysis yielded compelling results, confirming the presence of distinct functional groups, including N-H, O-H, C=C, C-H, C-O, and CH3 (Figure 2 and Table 4). These findings underscore the utility of FT-IR spectroscopy as a reliable and sensitive method for detecting the biomolecular composition of *M. malabaricum* extracts.

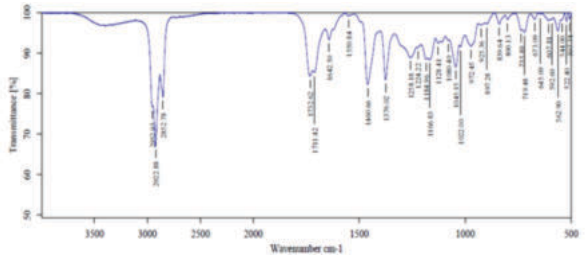

Figure 2: FT-IR Spectrum Of *M. malabaricum* Leaf Extracts For Functional Group Identification

Table 2: FT-IR Analysis of *M. malabaricum* Leaf Extracts for Functional Group Identification

Band	Wave numbers range cm-1	Band location cm-1	Band interaction	Band assignments	Possible compound
A	400-800	673.091, 607.8053, 562.8993, 522.404 507.7441	Stretch	C-Br	Akyl halides
B	700-800	719.4796	Stretch	C-Cl	Akyl halides
C	800-1100	972.4466, 839.6376, 800.1259, 719.4796, 673.091, 607.8053, 562.8993, 522.404, 507.7441, 925.3555, 897.2759, 1045.1548, 1022.0031, 1080.3988 1224.2237	Stretch	C-O	Alcohol
D	1100-1200	1184.9573, 1166.8333 1128.4057	Stretch	C-O	Ester
E	1100-1500	1460.6602, 1376.0231 1258.1804,1224.2237	Bend	C-H	Alkanes
F	1500-1650	1550.8416	Bend	N-H	Secondary amines
G	2000-2800	2852.7793	Stretch	C-H	Alkane
H	2800-2900	2852.7793	Stretch	C-H	Alkane
I	2900-3000	2952.9504 2922.8843	Stretch	C-H	Alkane

Cytotoxicity by MTT Assay

In this study, the cytotoxic effects of *M. malabaricum* extracts were comprehensively evaluated using the MTT assay in two distinct cell lines, HCT-116 and U87. Notably, the IC50 values differed significantly between the two cell lines, with HCT-116 exhibiting an IC50 of 491.1 µg/5µl and U87 displaying a higher sensitivity with an IC50 of 100 µg/5µl. Both cell lines demonstrated a clear dose-dependent induction of cell death within 24 hours, with the highest rates observed at 500 µg/5µl and minimal effects at 0.00 µg/5µl. Importantly, the specificity of the observed cytotoxic effects was emphasized by the lack of significant cell death in the vehicle control (5µg/100 µl methanol v/v) and media control groups in both cell lines. Additionally, the positive control, 10 µg/5µl Cisplatin, induced dose-dependent cell death in both HCT-116 and U87.

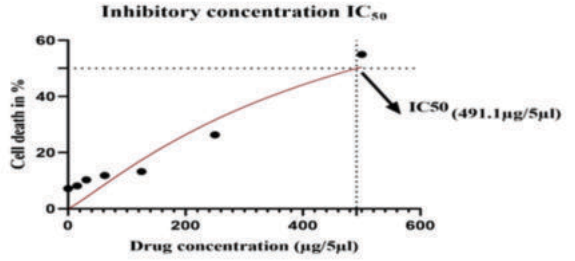

Figure 3: IC 50 graph of HCT-116 against MMLE (*Memecylon malabaricum* Leaf Extract) Dose-dependent cytotoxic effects.

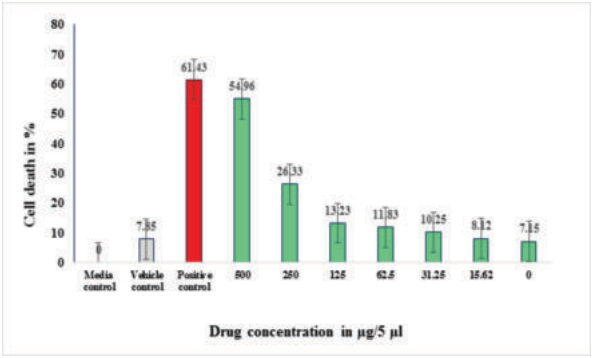

Figure 4: Dose-Dependent Cytotoxic Effects of *Memecylon malabaricum* leaf extract (500µg /5µl to 00µg /5µl) on HCT-116 Cells.

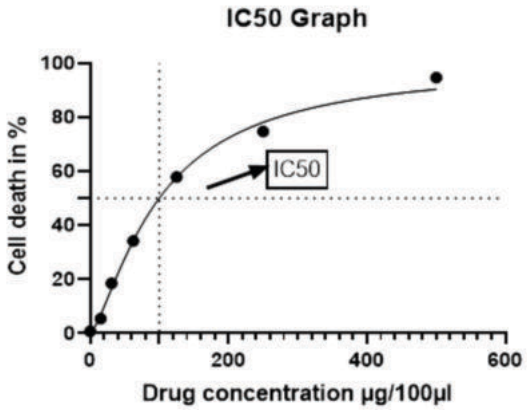

Figure 5: IC 50 graph of U87 against *Memecylon malabaricum* leaf extract. Dose-dependent cytotoxic effects

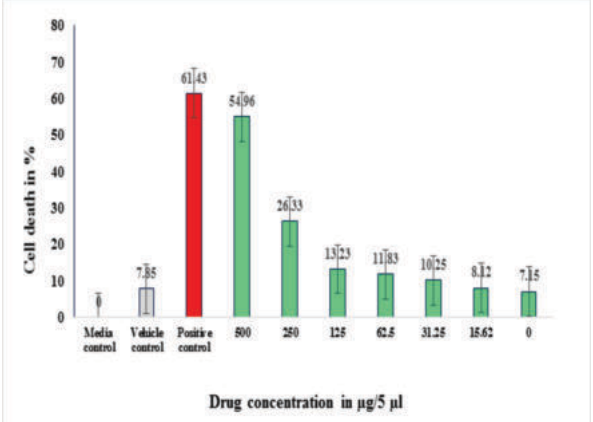

Figure 6: Dose-dependent Cytotoxic Effects of *Memecylon malabaricum* leaf extract (500µg /5µl to 00µg /5µl) on U87 Cells.

DISCUSSION

The comprehensive phytochemical analysis of *Memecylon malabaricum* (C.B. Clarke) Cogn extract has shed light on its diverse chemical composition, revealing the presence of coumarin, flavonoids, glycosides, phenol, tannins, and terpenoids. These findings align with the plant's traditional medicinal use, as these compounds are often associated with various therapeutic properties. The absence of alkaloids and saponins in the extract further refines its chemical profile. Notably, flavonoids, a subgroup of polyphenolic compounds, have been identified as major constituents, prompting a closer examination of their potential medicinal implications. The investigation of *Memecylon randerianum* leaves' methanolic extract revealed a diverse phytochemical composition, including carbohydrates, reducing sugars, alkaloids, phenols, flavonoids, cardiac glycosides, steroids, phytosterols, terpenoids, diterpenoids, and coumarins (Hegde and Hungund, 2021). Quantitative analysis demonstrated substantial levels of total phenolics (11.95mg GAE/g), flavonoids (20.34mg QE/g), and alkaloids (316.68mg AE/g). Notably,

the extract displayed potent antimicrobial activity against *Escherichia coli, Staphylococcus aureus,* and *Candida albicans,* along with noteworthy antioxidant, antidiabetic, and anticancer properties. comparison, *M. malabaricum* extracts, devoid of alkaloids and anthraquinones, exhibited the presence of coumarin, flavonoids, glycosides, phenol, tannins, and terpenoids. Flavonoid concentration was quantified at 228 µg QE/mg.

Further, UV analysis of *M. malabaricum* leaf extract provided insights into its flavonoid composition, revealing absorption peaks at 276 nm. The congruence of these peaks with the standard catechin at 279.2 nm further strengthens the evidence for the presence of flavonoids. This analytical approach enhances our understanding of the extract's chemical composition and suggests potential bioactive properties associated with these compounds (Manjunatha *et al*., 2023).

FT-IR analysis emerged as a pivotal tool in identifying the functional groups within *M. malabaricum* leaf extracts. The distinct functional groups, including N-H, O-H, C=C, C-H, C-O, and CH3, unravel the intricate molecular composition of the extract. The FTIR spectral bands allowed for detailed insights, enriching our understanding of the chemical makeup and providing a molecular signature for *M. malabaricum*. The band assignments in the FTIR analysis further elucidated the interaction and possible compounds associated with different bands. The stretches and bends corresponding to various functional groups, such as C-Br, C-Cl, C-O, C-O (Ester), C-H (Alkanes), N-H (Secondary amines), among others, offer a comprehensive view of the extract's biomolecular complexity.

The investigation into the cytotoxic effects of *M. malabaricum* extracts using the MTT assay revealed distinct responses in HCT-116 and U87 cell lines. The IC50 values reflected a notable difference, with HCT-116 exhibiting a higher resistance at 491.1 µg/5µl compared to the heightened sensitivity of U87 with an IC50 of 100 µg/5µl. This variability underscores the divergent susceptibility of different cell lines to the tested extracts, a crucial consideration for potential therapeutic applications. Both cell lines exhibited a dose-dependent induction of cell death within 24 hours, with the highest observed rates at 500 µg/5µl, indicating a concentration-dependent impact on viability. Importantly, minimal effects were noted at 0.00 µg/5µl, reinforcing the specificity of the observed cytotoxic effects associated with *M. malabaricum* extracts. The robustness of these findings is underscored by the control groups' outcomes. The vehicle control (5µg/100 µl methanol v/v) and media control groups demonstrated no significant cell death, emphasizing the specificity of the observed cytotoxic effects attributable to *M. malabaricum* extracts. Furthermore, the positive control, 10 µg/5µl Cisplatin, exhibited dose-dependent cell death in both HCT-116 and U87, validating the experimental setup and reaffirming the potential of *M. malabaricum* extracts in inducing comparable cytotoxic effects.

These results and their contextualization contribute valuable insights into the differential cytotoxicity of *M. malabaricum* extracts in distinct cell lines, laying the groundwork for further exploration of its therapeutic potential and underlying mechanisms.

In the context of the referenced studies on *Memecylon edule* HL-60 cell line with IC50 value of 183.44 µM/ml and U-937 with low IC50 value of 76.27 µM/ml (Srinivasan *et al*., 2016), *Memecylon sisparense Gamble* (MSG) IC50 value is 48.40 ± 1.68 µg/ml show on MDA-MB-231 cell line (Uppu *et al*., 2024), and compounds isolated from *Elaeagnus indica* and *Memecylon edule* ethyl acetate extract and rutin displayed dose-dependent anti-proliferative activity against U-937 cell line IC50 value is 297.42±1.64μmol/mL and HT-60 cell line IC50 value is 486.15±2.74μmol/mL (Srinivasan *et al*., 2020), the discussion section provides a comparative analysis with the findings on *M. malabaricum* extracts. MTT assays conducted on *Memecylon edule* revealed varying antioxidant potential in different solvent crude leaf extracts, particularly noting antiradical activity in the ethyl acetate extract. The present study on *M. malabaricum* extracts showcased significant cytotoxicity against HCT-116 and U87 cells, suggesting potential anticancer properties.

CONCLUSION

M. malabaricum extracts, rich in flavonoids and other phytochemicals, exhibited potent cytotoxicity in HCT-116 (IC50: 491.1 µg/5µl) and U87 (IC50: 100 µg/5µl) cells. The absence of alkaloids and anthraquinones was noted. Fractionation revealed the highest flavonoid content (228 µg QE/mg). UV-VIS spectrophotometry confirmed flavonoid-specific absorption peaks, while FT-IR spectroscopy identified key functional groups. The specificity of cytotoxic effects, along with the validation through Cisplatin as a positive control, underscores the potential therapeutic value of *M. malabaricum* extracts, offering promising avenues for future pharmaceutical exploration.

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