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Bioactive Damnacanthal from Morinda citrifolia: Isolation, Purification, and Cytotoxic Assessment

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Abstract
This study reports the extraction, characterization, and in vitro cytotoxic evaluation of damnacanthal, a bioactive anthraquinone, from the fruit of Morinda citrifolia (Noni). Soxhlet extraction with ethanol followed by column chromatography yielded purified fractions, whose identity was confirmed by thin-layer and paper chromatography against standard values. Spectroscopic analyses further substantiated structural features: UV–Vis spectroscopy revealed characteristic transition peaks consistent with extended conjugation within the anthraquinone core, while IR spectroscopy confirmed the presence of key functional groups. Cytotoxicity was assessed against Dalton's Lymphoma Ascites (DLA) cells, where damnacanthal exhibited a strong dose-dependent response. Cell death ranged from 20.9% at 20 µg/mL to 98.2% at 200 µg/mL, with an estimated IC_{50} of 50–75 µg/mL. These findings highlight the potent cytotoxic activity of damnacanthal at relatively low concentrations and suggest its promise as a lead candidate for anticancer drug development, particularly against lymphoma. Further mechanistic and translational studies are warranted to establish its therapeutic potential.

Keywords: *Damnacanthal; Morinda citrifolia;Cytotoxicity; Dalton's Lymphoma Ascites cells*

1. Introduction
Natural products have always been the vital source for drug discovery, with most contemporary therapeutic drugs being directly or indirectly obtained from phytochemicals. Among the vast array of plants that have been investigated, Morinda citrifolia (Noni) has found vigorous use in traditional medicine in Polynesian, Indian, Chinese, and Southeast Asian cultures owing to its wide range of therapeutic activities[1]. The plant parts and fruit contain rich bioactive constituents like phenolics, alkaloids, and anthraquinones like damnacanthal, morindone, and morindin. Figure 1 shows (A) Noni Plant and Fruit (B)

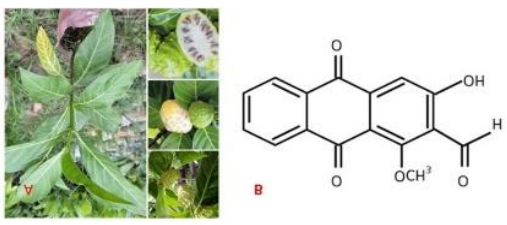

Figure 1 (A) Noni Plant and Fruit (B)

1.1.Damnacanthal
Damnacanthal(3-hydroxy-1-methoxy-anthraquinone -2- aldehyde) is an anthraquinone derivative initially isolated from the roots of M. citrifolia. It has been found to exhibit anticancer, antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory activities[2]. Earlier studies have pointed towards its activity in inhibiting tyrosine kinases, Ras oncogene signal transduction, and NF-κB signaling, as well as inducing apoptosis in cancer cell lines[3]. All these findings notwithstanding, there is still a need for systematic extraction, characterization, and assessment of its cytotoxic efficacy in certain cancer models. The current research focused on isolating damnacanthal from fruit of M. citrifolia, identifying its phytochemical and spectroscopic properties, and investigating it's in vitro cytotoxic effect against Dalton's Lymphoma Ascites (DLA) cell lines. [1]

2. Materials and Methods
2.1.Plant Material and Extraction
Fruits of M. citrifolia were harvested, washed, and

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extracted using Soxhlet extraction in the presence of ethanol as the solvent. The extract was rotary evaporated to get the crude extract. [2]

2.2.Purification of Damnacanthal

Column chromatography was conducted employing silica gel of mesh size 60–120 as stationary phase and a mixture of ethanol and water as mobile phase. The fractions thus obtained were additionally examined with thin-layer chromatography (TLC) and paper chromatography. [3]

2.3.Spectroscopic Characterization

UV–Visible spectroscopy was employed to measure the λmax values of the extract, establishing representative absorption peaks characteristic of its conjugated system [4]. Infrared (IR) spectroscopy was further utilized to detect functional groups, thereby supporting structural elucidation and confirming the presence of key chemical functionalities[5].

2.4.In Vitro Cytotoxicity Assay

Cytotoxicity against Dalton's Lymphoma Ascites (DLA) cells was tested[6]. Tumor cells were collected from the peritoneum of tumor-bearing mice, and PBS was used to wash them before treating them with varying concentrations of damnacanthal (20–200 µg/mL). Cell viability was measured using the trypan blue exclusion method. [4] [9]

3. Results

3.1. Extraction and Purification

Ethanolic Soxhlet extraction gave crude extracts with a dark yellow to orange-brown color. Column chromatography gave clear-cut fractions, and TLC and paper chromatography established the existence of damnacanthal. The Rf values obtained (0.55 in TLC, 0.56 in paper chromatography) agreed with the given standard (0.50), establishing compound identity. [5]

3.2. UV–Visible Spectroscopy

The UV–Vis spectrum showed intense absorption bands at 221.5–239.3 nm corresponding to π→π* transitions in the conjugated anthraquinone nucleus[7]. Further peaks at 305.9, 340.2, and 364.4 nm indicated extended conjugation and n→π* transitions, substantiating damnacanthal's structural properties. Figure 2 shows UV Spectrum of Damnacanthal

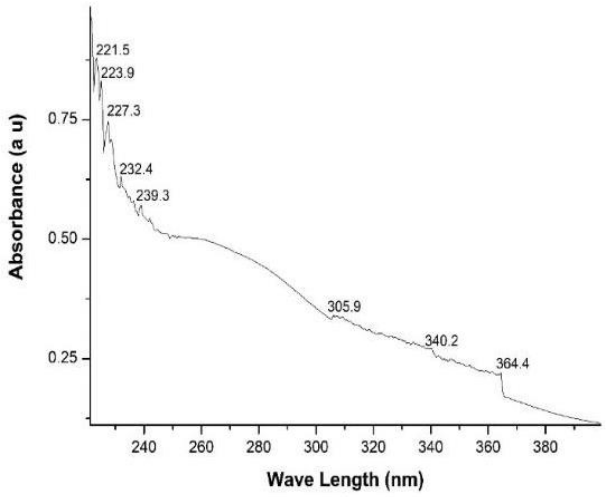

Figure 2 UV Spectrum of Damnacanthal

3.3. IR Spectroscopy

The IR spectrum had characteristic absorption bands: 1660–1685 cm^{-1} (C=O stretch), 1580–1620 cm^{-1} (aromatic C=C stretch), and 3200–3500 cm^{-1} (O–H stretch), typical of anthraquinone functional groups [8].

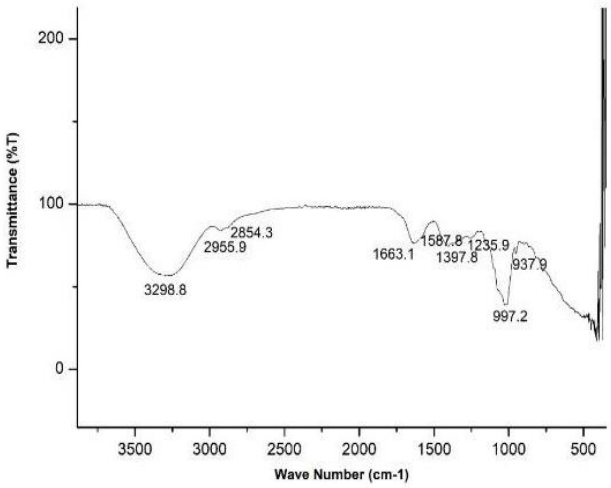

Figure 3 IR Spectrum of Damnacanthal

3.4. In Vitro Cytotoxicity

Damnacanthal caused dose-dependent cytotoxicity against DLA cells. Cell mortality ranged from 20.9% at 20 µg/mL to 98.2% at 200 µg/mL. The IC_{50} value was estimated to be between 50–75 µg/mL, which indicates strong cytotoxic activity at relatively low doses [9]. Figure 4 shows In Vitro Cytotoxicity Study of Damnacanthal against DLA

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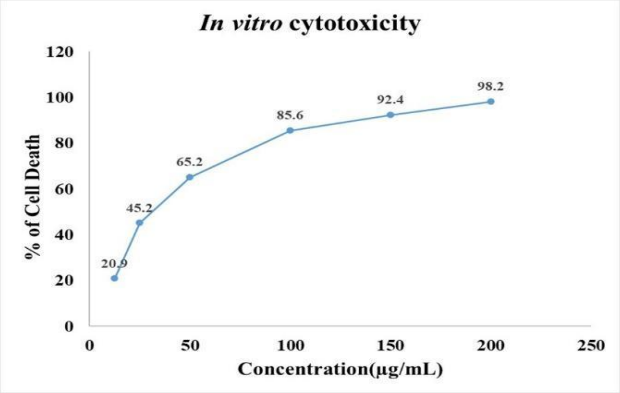

Figure 4 In Vitro Cytotoxicity Study of Damnacanthal against DLA

Discussion

The efficient isolation and identification of damnacanthal from M. citrifolia fruit confirm earlier reports of anthraquinones as predominant phytochemicals in Noni. Conjugated systems and functional groups characteristic of the anthraquinone structure were confirmed by spectroscopic analyses in accordance with previous studies. The cytotoxicity assays showed significant dose-dependent activity against DLA cells, with effective cell killing at levels greater than 50 µg/mL. The IC_{50} (50–75 µg/mL) is consistent with similar previously described cytotoxic activities of damnacanthal in other cancer cell lines. Such results indicate that damnacanthal is cytotoxic possibly through mechanisms involving the induction of apoptosis, generation of ROS, and suppression of oncogenic pathways. In comparison to traditional chemotherapeutics, damnacanthal holds potential as a naturally occurring compound with significant anticancer activity. Yet, much work needs to be done to assess its selectivity for cancer cells, cytotoxicity on normal cells, and explicit mechanism(s) of action. Future studies should involve sophisticated spectroscopic analyses (^{1}H NMR, ^{13}C NMR, MS, and XRD), molecular docking, and in vivo efficacy tests. [6]

Conclusion

This work effectively showed the extraction, purification, and characterization of damnacanthal from Morinda citrifolia fruit by Soxhlet extraction, column chromatographic procedures, and spectroscopic analyses. Thin-layer and paper chromatographic identifications showed the existence of damnacanthal with Rf values that were very close to standard references, maintaining accuracy in the identification of compounds. UV–Vis and IR spectral investigations corroborated the structural characteristics of damnacanthal with the detection of extended conjugation, carbonyl and hydroxyl groups, and aromatic ring vibrations typical of anthraquinone derivatives. The in vitro cytotoxicity tests showed that damnacanthal causes potent dose-dependent cytotoxicities against Dalton's Lymphoma Ascites (DLA) cells with an IC_{50} value estimated at 50–75 µg/mL. The observation indicates that damnacanthal possesses strong anticancer properties at relatively low concentrations. The almost complete cell killing at 200 µg/mL demonstrates the potency of this compound relative to crude extracts described in previous research. These findings contribute to the increasing body of evidence that damnacanthal can also serve as a natural lead compound with oncology therapeutic significance. Notably, its cytotoxicity on lymphoma cells shows promise for the potential development of damnacanthal-formulated medications for hematologic cancers. However, further studies are needed to assess its toxicity against normal cells, mode of action, and therapeutic index in vivo. Overall, this work lays a solid groundwork for future pharmacological studies of damnacanthal as a potential anticancer drug. [7]

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